Habitat Use and Behaviour of Lemon Sharks (*Negaprion brevirostris*) at a Sub-Tropical Nursery Site in the Bahamas

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This thesis is dedicated to my Grandparents, Joyce and John Swanborough, Judy and Bill Bullock, to whom I owe a very happy childhood.

“The scientist does not study nature because it is useful, he studies it because he delights in it, and he delights in it because it is beautiful. If nature were not beautiful it would not be worth knowing, and if nature were not worth knowing, life would not be worth living.”

— *Henri Poincare*

Table of Contents

Figures x

Tables xii

Abstract xiii

Acknowledgments xiv

Chapter 1 – General Introduction 1

- **1.1 Behaviour, activity and habitat use of sharks** 2
- **1.2 Sharks in coastal ecosystems** 6
- **1.3 Aim, Objectives and Hypotheses** 8
- **1.4 Selection of study species and site** 10
- **1.5 Methods to observe and study behaviour, activity and habitat use** 12
- **1.6 Methodological design and thesis structure** 16

Chapter 2 – General Methods 17

- **2.1 Study Site** 17
- **2.2 Tag Package Construction and Attachment** 18
- **2.3 Shark Capture** 20
- **2.4 Captive Trials** 21
- **2.5 Telemetry** 22
  - 2.5.1 Passive receiver array 22
  - 2.5.2 Active tracking 23
- **2.6 Recapture** 24
- **2.7 Baited Remote Underwater Video** 24
- **2.8 Data analysis** 27
  - 2.8.1 Tracking data analysis 27
  - 2.8.2 Accelerometer data analysis 27

Chapter 3 – Measuring the Behavioural Impacts of Externally Mounted Tag Packages and Capture Techniques on Young Lemon Sharks* 30

- **3.1 Introduction** 30
- **3.2 Materials and Methods** 32
  - 3.2.1 Tag weight 32
  - 3.2.2 Captive trials 33
  - 3.2.3 Free-range shark tagging 34
  - 3.2.4 Data analysis 35
- **3.3 Results** 35
- **3.4 Discussion** 40

3.4.1 Effects of tag presence 40

3.4.2 Rate of recovery from tagging..40

3.4.3 Post-release behaviour...41

3.5 Conclusions 42

Chapter 4 – A Quantitative Understanding of Habitat Use, Activity and Behaviour in a Site-attached, Coastal Shark Species (*Negaprion brevirostris*) 43

4.1 Introduction 43

4.2 Methods...45

4.2.1 Captive Ethogram trials..46

4.2.2 Wild shark tagging 46

4.2.3 Data analysis 47

4.3 Results 49

4.3.1 Captive trials 49

4.3.2 Wild trials...51

4.4 Discussion..60

4.4.1 Patterns of space use 60

4.4.2 Activity and specific behaviour...60

4.4.3 Environmental drivers 61

4.5 Conclusions 63

Chapter 5 – Understanding the Foraging Ecology of Nursery Bound Lemon Sharks (*Negaprion brevirostris*): Assessing the Effects of Prey Availability and Environmental Variability on Habitat Use Patterns and Foraging Effort 64

5.1 Introduction..64

5.2 Materials and Methods 67

5.2.1 Captive trials - delineation of burst swimming behaviour 67

5.2.2. Captive trials - identification of feeding events from acceleration data……………………..…..67

5.2.3 Data Analysis 68

5.3 Results 70

5.3.1 Captive trials 70

5.3.2 Wild shark foraging behaviour..74

5.3.3 Distribution and Abundance of Faunal Species 77

5.3.4 Shark-prey interactions 83

5.4 Discussion..85

5.4.1 Foraging and prey handling..85

5.4.2 Relationships with faunal communities...86

5.4.3 Relationships with environmental conditions 87

5.5 Conclusions 88

Chapter 6 – Ontogenetic Shifts in Habitat Use, Activity and Behaviour of Lemon Sharks *Negaprion brevirostris* at a Sub-Tropical Nursery Site 89
6.1 Introduction 89
6.2 Materials and Methods 92
6.2.1 Data analysis 92
6.3 Results 93
6.4 Discussion 101
6.4.1 Ontogenetic changes in habitat use 101
6.4.2 Ontogenetic changes in activity and behaviour 102
6.5 Conclusions 104
Chapter 7 – General Discussion 105
7.1 Summary of research findings 105
7.2 Intrinsic Value of the Nursery Habitat and Impacts of Environmental Perturbation and Habitat Loss 116
7.3 Limitations of this study and suggested directions for future work and research 120
7.3.1 Logistical constraints 120
7.3.2 Technology and methods 121
7.3.3 Foraging ecology 122
7.3.4 Other concepts and directions 123
7.4 Conclusions 125
References 127

Figures

Fig 1.1 Map of the known global range of the lemon shark. Accessed via iucnredlist.org on 16/01/2019 (International Union for Conservation of Nature (IUCN). ***Negaprion brevirostris*****. The IUCN Red List of Threatened Species. IUCN 2015-3)**……………………………………..………11

Fig. 2.1 Map of Bimini, Bahamas (25°44' N, 79° 16' W). The dashed line box represents the site at which captive trails were conducted and the solid line box represents the site at which free-ranging trials were conducted...17

Fig. 2.2 a) Image of an accelerometer/acoustic transmitter tag package with PT-04 transmitter epoxy fastened to the G6A accelerometer b) Tag package attached to the first dorsal fin of a shark, oriented to align the horizontal acceleration plane with the posture of the animal...........................19

Fig. 2.3 Axial orientation of dynamic acceleration for fin-attached tags....19

Fig. 2.4 Experimental setup for semi-captive trials……..22

Fig. 2.5 Positions of SUR units within the study site. Each unit with a designated ID number. Units are set up to overlap, any sharks undetected by the array can be assumed to be occupying waters

Fig. 2.6 BRUV rig setup. Rebar steel frame with attached Go-Pro Hero 2 in underwater housing, with 800g of chum in a mesh chum bag, inside a steel cage...23

Fig. 2.7 Map of the Bimini Islands with high and low tide sampling areas for the BRUVs field work (low tide profile overlaid on high tide). Random points were selected within these profiles for BRUV deployments. 25

outside of the Bonefish Hole area...26

Fig. 2.8 Spectrogram of z axis dynamic acceleration for a full 6 h captive observation trial (for trial shark 15C), generated from continuous wavelet transformation in Ethographer……….…………29

Fig. 3.1 Comparisons of mean totals ± SE for chafing and resting (n = 10) and swimming and tailbeats (n = 7), C = control, T= tagged. There were no significant differences found between control and tagged sharks for any indicator (Wilcoxon signed rank test, $P > 0.05$).........................36

Fig. 3.2 Acceleration profiles for all free-ranging sharks, displaying overall dynamic body acceleration (ODBA) over the first 30 min of data logging after release from the tagging vessel...377

Fig. 3.3 Map displaying tracking coordinates for each shark upon release. Start = location of capture and tagging. End = location at which track was terminated...39

Fig. 3.4 Scatter plot displaying mean distance-to-shore (± SE), per 5 min interval. Release = mean total distance from shore for all sharks, over a 1 h period, immediately after release. Control = mean total distance from shore for all sharks, over a 1 h period, between 24 h and 36 h after release. 39

Fig. 4.1 Map displaying locations of environmental sampling across the study site. Water temperature was recorded at each location. Locations were selected randomly using a random-points generator in ArcMap v 10.0. 50

Fig. 4.2 Acceleration profile and associated masked clusters for specific behaviours, a) time-series of 6 h captive observation trial for shark 15C, the grey box identifies a section of the time-series zoomed and presented in b) showing transition between behavioural states, confirmed as accurate by direct observation records....51

Fig. 4.3 Mean percentage total detections among SUR units, for all sharks, a) representing detections during high tide phase and b) representing detections during low tide phase. SURs with no detections recorded are not displayed..54

Fig. 4.5 non-metric MDS plots for ODBA across all sharks. a) shows colour designations of tidal phase for each sample (H=high, L=low, M=mid), b) shows colour designations for diel phase for each sample (D=day, DA=dawn, DU=dusk, N=night)...54

Fig. 4.6 PCO plots for all temperature and depth data for all sharks. a) shows colour designations of tidal phase for each sample (H=high, L=low, M=mid), b) shows colour designations for diel

phase for each sample (D=day, DA=dawn, DU=dusk, N=night). Vectors show trends in data relative to individual variables... 56
Fig. 4.7 Bar-plot displaying mean percentage time spent resting and bursting, for all sharks, across tidal phase. H = high, M = mid and L = low tide phase... 56
Fig. 4.8 Bar-plot displaying mean percentage time spent resting and bursting, for all wild sharks, across the diel phase. DA =dawn, D = day, DU = dusk and N = night. 57
Fig. 4.9 Scatter plots displaying temperature data at each sampling location within the study site, at differing distances from the shoreline. a) High tide sample locations, b) low tide locations. Solid lines represent the regression line for the associated linear regression model. Data show no association between water temperature and distance-to-shore……………………………………57
Fig. 5.1 A segment of the acceleration time-series for a captive trial (Shark 15C) at the introduction of a prey food item, showing differences in acceleration signatures of bursting behaviour and prey handling behaviour.... 71
Fig. 5.2 Example segments of the acceleration time-series for a captive trial (Shark 15C) at the introduction of fright stimuli. 72
Fig. 5.3 Bar plots displaying mean foraging events for all sharks across entire deployment duration: a) showing differences between day, dawn, dusk and night, b) showing differences between high, mid and low tide... 72
Fig. 5.4 Mean number of prey handling events over entire deployment duration for all sharks, tested across tidal phase. Letters denote groupings from Mann-Whitney post-hoc pairwise tests. 74
Fig. 5.5 Mean number of individuals recorded for each of the significantly contributing faunal groups. ME and NME show differences in abundances between habitat types. Only Gerreidae are distribution evenly among ME and NME zones.... 79
Fig. 5.6 Non-metric multi-dimensional scaling (MDS) plot of abundance data for all teleost family groups, sorted by habitat type (ME vs NME)... 80
Fig. 5.7a - f Displays maps of abundance and distribution for the most prevalent and relevant teleost species, as relates to lemon shark prey. All grey coloured 'x' marks represent BRUV deployment sites for which no individuals of that particular family group were recorded. 81
Fig. 5.8 Principal component analysis (PCA) of environmental data for all BRUVs, sorted by habitat type (ME vs NME). Vectors for each variable are labelled... 82
Fig. 5.9 Map of prey distributions relative to shark locations. Blue markers represent BRUV deployment locations, size of marker relative to MaxN Gerreidae. Orange markers represent SUR sites, size of marker relative to total detections of all sharks throughout the low tide period. 83
Fig. 6.1 Minimum convex polygons (MCPs) representing area of use for each actively tracked shark. MCP was calculated for 14 of 19 total wild deployments, each with at least one h of tracking data. Date of deployment is included for each shark with multiple deployments over the study...... 97
Fig. 6.2a-h Simple linear regression models for all variables tested against size of shark. Each circle marker represents a value for each shark and solid lines represent linear regression lines (summary in table 6.2). Size (x-axis label) = total length of sharks. 99
Fig. 6.3 a) Mean ODBA values at different tidal phases (high, mid and low) for each shark; b) Mean ODBA values at different diel phases (dawn, day, dusk and night) for each shark. Sharks displayed in increasing size order from left to right. 99
Fig 7.1 Theoretical model explaining spatio-temporal activity and behaviour relative to the threat of predation (by author, 2018)………………………………………………………………………………114
Fig. 7.2 Theoretical model exploring the consequences of environmental perturbation due to coastal development, based on the behavioural understanding gained from this research and the known perturbations experienced in neighbouring habitat (by author, 2018). 118

Tables

Table 3.1 Summary data f............or sharks caught and used in captive trials. Shark denoted by PIT (passive integrated transponder) tag number. TL = total length of the shark, M = male, F = female. 31

Table 3.2 Summary data for sharks used in wild deployments. Shark denoted by PIT (passive integrated transponder) tag number. Handling duration derived from the time a shark became hooked to the time of its release from the boat. M = male, F = female. 37

Table 3.3 Spearman rank correlation between distance-to-shore and time. *P* = result from test over 1 h release period and *P* (control) = result from test over 1 h control period....................38

Table 4.1 Summary information for all sharks caught and tested in captive ethogram trials over the course of the study. TL = total length. M = male, F = female 50

Table 4.2 Summary information for all sharks caught and tracked for wild trials. Greyed individuals were not used in data analysis due to either loss or failure of tag package. Acoustic tag and frequency relate to the ping codes of acoustic transmitters. TL = total length. M = male, F = female....................53

Table 4.3 Results for PERMANOVA tests of square root transformed ODBA data, based on Bray-Curtis similarities and normalised environmental (temperature and depth) data based on Euclidean distance. Two-way tests of two conditions (tidal and diel phase) were conducted in each instance. 55

Table 5.1 Summary data for sharks caught and tested in captive foraging trials. A range of sizes were tested to account for changes in swimming kinematics. TL = total length. M = male, F = female.....................69

Table 5.2 Summary of estimated foraging events recorded by accelerometers. *P* column refers to the p-value output from Chi^2 - tests. Values with $P < 0.05$ are highlighted grey, indicating a significant variance between data groups.....................75

Table 5.3 Summary of all identified teleost fishes from 140 BRUV deployments, organised alphabetically by family. All species names taken from the World Register of Marine Species (WoRMS Editorial Board, 2016).....................78

Table 5.4a, b and c Teleost families identified by similarity percentages (SIMPER) for habitat type (a = NME, b = ME, c = Comparisons). 79

Table 5.5 Wilcoxon-Mann-Whitney rank sum test results for comparisons of environmental data.79

Table 5.6 Kruskal-Wallis tests for PAS and PAS-G of tracking points at different tidal phases. Coloured blocks represent groupings from post-hoc Mann-Whitney pairwise tests. 83

Table 6.1 Summary information for all sharks tested in this study. Distance-to-shore, ODBA, % resting, % bursting, temperature and depth data area all mean values calculated for each individual. TL = total length. Shark ID codes d2* and d3* refer to second and third deployments of the same shark. 95

Table 6.2 Results from linear regression models for each variable tested against size of shark. 95

Table 6.3 Summary of tidal differences in mean distance-to-shore values for different sharks. Different colours represent statistical differences between groups, identified by post hoc tests. H = high tide phase, M = mid tide phase, L = low tide phase.....................95

Table 6.4 t-test results comparing relative abundance data for fish groups between habitat-type. ME = Mangrove-edge, NME = Non-mangrove-edge.....................96

Abstract

Understanding how animals behave in their natural environment provides important insights into their ecology and is valuable for species conservation. For many aquatic species, observation of behaviour is difficult and research has in the past largely focused on understanding space use and movement patters. However, a more comprehensive ecological understanding requires knowing not just where animals are within their habitat but what they do whilst there and why. With the emergence of new technologies capable of obtaining more accurate temporal behavioural data, pathways to a more complete understanding can be forged. This research used a series of semi-captive experimental trials, tagging and tracking of wild animals, environmental sampling and prey species mapping to observe the spatio-temporal behavioural routine of young lemon sharks using a nursery habitat in the Bahamas. Capture and handling practices were found to minimally impact the behaviour of young lemon sharks and individuals recovered quickly, upon release. Periods of refuging behaviour were exhibited by sharks, post-release, but in all instances this behavioural response was over a short timeframe (< 1 h). Wild lemon sharks were found to display repeatable patterns in space use, activity and specific behaviours within the nursery habitat. Supporting existing research, movement within the habitat was shown to be, in part, driven by tidally mediated predation threat, forcing most sharks to move closer to shore at times of increased threat. Furthering existing research, findings here uncover a behavioural strategy in these sharks to trade-off predation risk with foraging and bioenergetic management. Accelerometer-derived measurements of activity and specific behaviour, alongside prey abundance mapping, show that sharks increase swimming activity and foraging behaviour at times of lower predation risk in areas further from shore, coinciding with increased abundances of preferred prey and potentially favourable environmental conditions. At times of higher predation threat, sharks use nearshore areas, decrease swimming activity and exhibit resting behaviour. Findings also demonstrate changes in space use, activity and specific behaviour of sharks as they grow and predation risk decreases. This research elucidates the complete spatio-temporal behavioural routine of young sharks using a nursery site and furthers understanding of the critical components these habitats provide to support the survival of sharks to adulthood.

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Chapter 1 – General Introduction

It has long been established that the study of animal behaviour should play an important role in conservation biology (Caro and Sherman, 2013) and over recent years burgeoning literature evidences the synergy between these fields (Buchholz, 2007; Berger-Tal et al., 2011; Candolin & Wong, 2012; Lechner et al., 2015). By necessity, behavioural and ecological research has increasingly been considered in terms of its application to conservation (Buchholz, 2007). Indeed, the usefulness of connectivity modelling and population viability analyses for conservation planning are greatly increased with inclusion of fine-scale behavioural data (Brook et al., 2000; Berger-Tal et al., 2011) and failing to properly include behavioural data can even lead to failure in some management programmes (Knight 2001).

Behavioural ecology helps to characterise and predict behaviour in animals, to measure animal's interactions with their environments and define their needs from those environments. In this way, the study of animal behaviour directly informs conservation (Berger-Tal et al., 2011). How an animal behaves within its environment contributes to the fitness and survival. Patterns in activity and behaviour can be defined as adaptations by an animal in response to ecological pressures (de Matos Dias et al., 2018). Decisions made by an animal in space and time, weighing costs and benefits of different interconnecting behaviours, impact its fitness and survival. Where and how long an animal searches for food, for example, is driven by a series of decisions linked to relative costs and benefits of expressing that behaviour, in that place, over that timeframe (Lima 1998b).

It then stands to reason that the more fine-scale our understanding of animal behaviour, the more accurately we can measure decision trade-offs, needs and survivability. Through this we improve our ability to model the impacts of changes to routine behaviour and inform effective conservation. For those species most acutely impacted by human induced perturbations, research into fine-scale behavioural patterns should therefore be a priority.

Elasmobranch fishes, comprising sharks (Selachii), skates and rays (Batoidea), occur in all oceans, throughout a diverse range of coastal, demersal and pelagic habitats (Compagno, 1990). Sharks are slow growing, mature late and have generally low fecundity. These life history traits make them susceptible to overfishing (Simpfendorfer & Kyne, 2009). The impact of fishing on elasmobranch stocks has become a global issue, with many elasmobranch species now subjected to intense fishing pressures, causing steep population declines (Stevens et al., 2000, Ferretti *et* al., 2010; Dulvy et al., 2014). As well as the prolific loss of sharks to global fishing effort, there is equally a concern for the impacts of habitat loss to sharks at key life stages. The loss of, or alteration to, marine coastal habitat due to anthropogenic pressures has increased over time, and these changes can have consequences to the survival and genetic variance of shark populations (Heupel et al., 2007; Airoldi et al., 2008; DiBattista et al., 2011). Sharks and their relatives often occupy key predatory roles within the environments they inhabit, exerting top-down control on meso-predator populations and helping to maintain ecosystem dynamics and function (Block et al., 2011). Losing key predators from an ecosystem can lead to meso-predator release and trophic cascades, which in turn cause trophic downgrading in marine ecosystems (Baum & Worm, 2009; Estes et al., 2011; Heupel et al., 2014). Rapid declines reported for some shark species and their related ecological impacts have motivated questions regarding the potentially fundamental role that sharks play in ocean ecosystems (Ferretti et al., 2010). Exploring how sharks use their environment and understanding their requirements from those environments is necessary to inform effective management and conservation policy. To achieve these goals there is the need to study patterns in behaviour, activity and habitat use of sharks.

1.1 Behaviour, activity and habitat use of sharks

A wide range of biological and environmental variables influence how sharks use their environment and behave within it. Some of the first studies into patterns of activity in sharks were laboratory studies assessing behavioural changes across diel cycles. Casterlin and Reynolds (1979) using an electric shuttlebox, in which activity was monitored by photocells, found smooth dogfish *Mustelis canis* (Mitchill, 1815) held in a lab aquarium under an artificial photoperiod were principally nocturnal. Similarly, Nelson and Johnson (1970) found horn sharks *Heterodontus francisci* (Girard, 1855) and swell sharks *Cephaloscillium ventriosum* (Garman, 1880) exhibited nocturnal activity in both a laboratory-controlled environment and in their natural habitat.

The lemon shark *Negaprion brevirostris* (Poey, 1868) has been a comparatively well-documented species. Nixon and Gruber (1988) investigated the diel activity patterns of lemon sharks using an annular respirometer, subjected to artificial 12 h illumination. Levels of activity of three sharks in eight, 24 h experiments increased at the onset of darkness, and thus suggested lemon sharks to be nocturnal. Drawing accurate and valid results on natural patterns of activity, however, requires field-based studies (Gruber et al., 1988). Yet studying free-swimming sharks in their natural state is difficult, primarily due to the concealing nature of the natural underwater environment. On land it is easier to observe animals directly; doing so underwater, whilst remaining unobtrusive, often limits studies (Nelson, 1977). Consequently, many studies of shark behavior and habitat use at liberty rely on telemetric tools to track sharks. Acoustic telemetry is by far the most prevalent method used to observe movement behaviour in sharks. Studies using this technology have, for example, documented patterns in habitat use, site preference, home ranging and swimming speeds of free-ranging shark species. For example, in lemon sharks, Gruber et al. (1988) and Morrisey & Gruber (1993a) investigated patterns of activity and habitat use, Morrisey & Gruber (1993b) studied home range size and Sundstrom and Gruber (1998) used speed-sensing transmitters to construct a bioenergetics model. In other species, Heupel & Heuter (2001) tracked blacktip shark movements, Knip et al. (2011) observed ontogenetic changes in habitat use and movement of pigeye sharks *Carcharhinus amboinensis* (Muller & Henle, 1839), Speed et al. (2011) considered the movement of multiple species using a reef and Cagua et al. (2015) revealed cryptic residency in whale sharks *Rhincodon typus* (Smith, 1828).

Many studies have documented changes in shark behaviour over the diel cycle. For example, Holland et al. (1993) found that juvenile scalloped hammerheads *Sphyrna lewini* (Griffith & Smith, 1884) had a much greater area of occupancy at night than during the day. Gruber et al. (1988) found east to west diel movements in lemon sharks using an enclosed lagoon habitat, and more recently Gleiss et al. (2013) used accelerometers to show whale sharks display crepuscular patterns in locomotory activity. Longer-term factors are also important considerations, potentially revealing patterns otherwise unobserved in shorter-term studies. Sharks can also change their behaviour and movements over ontogenetic or seasonal scales (e.g. Heupel & Heuter, 2001; Taylor & Bennett, 2013). Such factors as those discussed, describing changing patterns in behaviour and movements, often do not address why such patterns are observed. A range of biological and environmental factors drive behaviour in sharks and their influences vary greatly between species, habitat type and life stage.

Environmental factors driving behaviour include temperature, tidal flow, salinity, dissolved oxygen, depth and bottom type (Heupel, 2007; Heupel & Simpfendorfer, 2008; Heithaus et al., 2009; Matich & Heithaus, 2012). Temperature is a particularly important parameter for many sharks as most species cannot survive in a full range of temperatures (Simpfendorfer, 2004). DiGirolamo et al. (2012) found that juvenile lemon sharks selected for different temperatures at different times of day, choosing to occupy the warmest available areas during the middle of the day, moving to areas of cooler temperatures in evenings or mornings. This was considered to be linked to key daily activities such as prey acquisition and predator avoidance. Donley et al. (2012) found that temperature affects the power output in common thresher sharks *Alopias vulpinus* (Bonnaterre, 1788), showing muscle power increasing with temperature. There is also evidence to support behavioural thermoregulation in sharks. Speed et al. (2012) observed blacktip reef shark *Carcharhinus melanopterus* (Quoy & Gaimard, 1824) movements in relation to water temperatures finding their presence at an aggregation site to be linked with daily peaks in water temperatures. Tidal flow also plays a major role for several species, sometimes changing areas available at certain tides. Habitat use of leopard sharks *Triakis semifasciata* (Girard, 1855) in a coastal bay are directly influenced by tidal flow (Ackerman, 2000). Lemon sharks use a shallow mangrove fringed lagoon habitat near to shore at high tide but move to more open areas at low tide. This behaviour is thought to be a means by which juvenile sharks avoid deeper, high tide waters to avoid larger predatory sharks (Guttridge et al., 2012). For species such as the bull shark *Carcharhinus leucas* (Muller & Henle, 1839), salinity can play an important role in behavior, with juveniles of this species exhibiting a preference for lower salinity areas (Compagno, 1984).

Biological factors also play a central role in behaviour and habitat use of sharks. Food acquisition, predator avoidance and breeding are distinct components of most shark's behavioural routines at different life stages. A major factor affecting an animal's fitness is its ability to find and exploit food resources. Foraging plays an important role in an animal's capacity to survive and reproduce. Sharks can optimize their food intake through appropriate habitat use, search behaviours, activity levels and capture techniques (Heithaus, 2004). There are many studies of shark feeding ecology, including diet (Medved et al., 1985; Cortes & Gruber, 1990; Wetherbee et al., 1990; Newman et al., 2010) and mechanics of feeding (Motta et al., 1997; Wilga & Motta, 1998; Motta & Wilga, 2001). Stable isotope analysis has been used to estimate trophic position in sharks (Estrada et al., 2003; Speed et al., 2012), and observations of Levy flight foraging (optimal search patterns used when prey is sparse) have investigated search tactics used by sharks to maximise prey encounters (Sims et al., 2012). An example of shark movement patterns being driven by prey acquisition is that of the basking sharks *Cetorhinus maximus* (Gunnerus, 1765), which coordinate time spent in surface waters with abundance of zooplankton (Sims et al., 2003). Diet of school sharks *Galeorhinus galeus* (Linneaus, 1758) was found to vary both seasonally and ontogenetically (Lucifora et al., 2006). Investigating wild predatory behaviours, however, is challenging, largely due to inherent difficulties in observing free-ranging sharks. In some cases, observation of prey capture behaviour is feasible (Ebert, 1991; Martin et al., 2005; Martin et al., 2009), but these cases are often confined to species that display more inherently observable prey capture behaviour. Much of what is known of predatory behaviour in more difficult to observe sharks is the result of anecdotal observations (Pratt et al., 1982) or inferred from morphology (Motta & Wilga, 2001; Motta, 2004).

The effects of predation risk and predator avoidance are poorly understood in sharks, but anti-predator behaviours affect habitat use, activity patterns and decision-making (Heithaus, 2004; Guttridge et al., 2012). Although sharks are prey of cetaceans and teleosts (Randall, 1977; Fertl et al., 1996), by far the most common predators of sharks are other sharks. Elasmobranchs have been found in the diet of tiger sharks *Galeocerdo cuvier* (Peron & Lessueur, 1822), bull sharks, galapagos sharks *Carcharhinus galapogensis* (Snodgrass & Heller, 1905) and sevengill sharks *Notorhynchus cepedianus* (Peron, 1807) (Randall, 1977; Snelson et al., 1984; Wetherbee et al., 1996; Barnett et al., 2010) among many others. Lemon sharks are cannibalistic with larger conspecifics being the most significant predator of juveniles (Wetherbee et al., 1990).

Predator avoidance strategies in sharks are expressed through a range of tactics. One of the most obvious and long-term tactics is habitat selection (Lima & Dill, 1990). Sharks can significantly reduce the risk of predation by occupying low-risk habitats. However, in many cases low-risk habitat also offers low-yield in terms of food, with higher risk habitats offering greater foraging rewards. How a shark plays off these factors affects how often it will expose itself to higher predation risk (Heithaus, 2004). Many shark species use shallow water nursery habitats in the early stages of their lives to avoid predators (Morrisey & Gruber, 1993a; Simpfendorfer & Milward, 1993). As previously referenced, juvenile lemon sharks potentially use tidal fluctuations to trigger changes in areas of use, to avoid predators in deeper water (Guttridge et al., 2012). Heupel & Heuter (2002) found no relationship between nursery habitat use by juvenile blacktip sharks *Carcharhinus limbatus* (Muller & Henle, 1839) and prey availability, suggesting that preferences for zones away from high prey abundances could be driven by more important factors, such as predator avoidance.

Metabolic activity and energetic management are important factors to a sharks overall fitness and capacity to succeed. Many studies have investigated bioenergetics and metabolism in sharks (e.g. Gruber, 1984; Scharold & Gruber, 1991; Sundstrom et al., 1998; Lowe et al., 2001; Chen et al., 2008) The basic bioenergetics model, ‘Consumption = Production + Metabolism + Excretion’ (Sundstrom et al., 2002) indicates that energy consumed that is not used up in metabolism, or disposed of through excretion, is incorporated into tissue. Effective use of energy should then increase rates of growth and thus survival. Balancing bioenergetics costs and benefits are important drivers for behaviour and habitat use. Sharks must therefore make a decision on when and how intensively they hunt. Increased activity, likely increases chances of prey encounter but also comes at a metabolic cost (Papastamatiou et al., 2015).

1.2 Sharks in coastal ecosystems

Coastal areas have been negatively pervaded by human activity throughout history (Lotze et al., 2006). Several studies have described the effects of human presence on shark ecology and abundance. For example, in the protected, lightly fished North-western Hawaiian Islands (NWHI), sharks comprised 13% of total fish biomass, whereas in the neighbouring but densely populated and heavily fished Main Hawaiian Islands (MHI), there was almost no sharks compared to fish biomass (Friedlander & DeMartini, 2002). In the Northern Line Islands, shark biomass was far greater on an uninhabited island, decreasing concurrently with increases in human presence on other islands (DeMartini et al., 2008). In the Mediterranean Sea, records show many large predatory shark species to have undergone considerable (between 96-99.9%) declines, relative to their historical abundances (Ferretti et al., 2008). On Australia’s Great Barrier Reef, sharks and rays are considered extremely vulnerable to habitat loss from climate change (Chin et al., 2010).

Understanding how elasmobranchs use nearshore environments is important as different species will have differing requirements from and influences on community dynamics (Bethea et al., 2004). Nearshore areas are used as nursery sites for juveniles by many large bodied shark species (e.g. *Carcharhinus limbatus*, *Negaprion brevirostris*, *Carcharhinus plumbeus*, *Sphyrna lewini*) (Castro, 1993; Morrissey & Gruber, 1993a; Simpfendorfer & Milward, 1993) providing plentiful foraging resources whilst reducing exposure to predation, thus increasing recruitment to adult populations (Beck et al., 2001; Knip et al., 2010). Due to difficulties observing, and thus conserving, often highly migratory adults, emphasis is placed upon young age classes using discrete nursery habitats and the identification and protection of nursery sites as essential fish habitat (Heithaus, 2007; Kinney & Simpfendorfer, 2009).

Due to their productivity, protection afforded from predators and provisioning of food resources, mangroves act as an extremely important habitat, not just for sharks but for many fishes and crustaceans. Mangroves provide an important nursery habitat for many juvenile fish species (e.g. Roberton & Duke, 1987; Laegdsgaard & Johnson, 1995; Nagelkerken et al., 2000; Mumby et al., 2004; Sheaves, 2016). Juvenile lemon sharks in Bimini, Bahamas, routinely use mangrove habitat, remaining within well-defined areas close to the mangrove shoreline and often traversing shallow channels, deep into the mangrove thicket (Morrisey & Gruber, 1993a). Unfortunately, mangroves are being removed from coastal environments at an alarming rate and, in instances of mangrove destruction, significant reductions in species abundance and richness have been found (Huxham et al., 2004, Shinnaka et al., 2007). By understanding how juvenile sharks use important habitats, it is possible to develop a theoretical understanding of the behavioural responses to the existing and potential environmental perturbations associated with coastal development. From this, it is possible to theorise the consequences that such deviations in natural behaviour have to the fitness and survival of the individual. Lemon sharks in Bimini act as concentrated predators (Heupel et al., 2014) and it is possible that disturbances to the environment will influence fish abundance and therefore impact predator health and the ecosystem's stability. Consequently, highlighting the interactions and connectivity that key predators have with critical nursery habitat can help in defining appropriate policy and legislation to prevent habitat loss.

Many studies have investigated habitat use of young sharks at nursery sites. Simpfendorfer and Milward (1993) assessed the nursery habitat use of eight species of shark via various fishing and netting methods. Duncan and Holland (2006) measured nursery habitat use and growth rates of scalloped hammerhead sharks in a tag-and-recapture study. Heupel and Heuter (2002) used acoustic telemetry and faunal sampling techniques to investigate the importance of prey density in relation to blacktip shark movements in a nursery site. Many other studies have also examined aspects of nursery site use in different species (e.g. Morrissey & Gruber, 1993b; Heithaus et al., 2002; Heupel & Simpfendorfer, 2005; Heithaus, 2007; Heupel et al., 2007; Weng et al., 2007; Cartamil et al., 2010). Most of these studies consider the movements, habitat use and home range of species, often with the use of various capture techniques or ultrasonic telemetry, and some consider diet of different species within these habitats, often via stomach content analyses or faunal sampling. Even recent studies continue to resolve habitat use in the spatial context (Munroe et al., 2016; Oh et al., 2017) but do not offer more fine-scale understanding. This knowledge provides information on where sharks are and what resources may be important, however, in the context of understanding how sharks interact with these key environments, further information on what sharks are doing during spatial migrations is vital. Constraints in the ability to observe wild sharks for prolonged periods has meant that better quantifying sharks use of these habitats has been far more limited. To date, there have been no observations on the activity and range of specific behaviours that sharks display during spatial movements. It is therefore difficult to accurately assign causality to observed movement patterns (Papastomatiaou et al., 2012). Individual factors such as habitat structure, food availability and water temperature are all likely important elements defining shark behaviour and habitat use. Understanding how these factors link and how different ecological pressures and motivators are traded off against one another help to build a clearer picture of how sharks use nursery sites on a daily basis and what they require from them. For species reliant on such nurseries, the fitness and survival of entire populations is dependent on the fitness and survival of young sharks using these systems.

1.3 Aim, Objectives and Hypotheses

The aim of this research is to further the understanding of inshore nursery habitat use by young sharks by quantifying activity and specific behaviours in space and time. This is expected to elucidate their daily routine in this habitat at the fine-scale and define the biological and environmental factors that impact their fitness and survival. To do this the following objectives were defined:

O1: Develop a methodological approach for observing specific behaviour and activity, at fine-scale in space and time, for sharks using a nursery site.
O2: Produce scientifically rigorous data that are reliably representative of natural behaviour.
O3: Understand the natural patterns in habitat use, activity and behaviour of young, free ranging sharks using a nursery habitat.
O4: Elucidate foraging ecology in young nursery-bound sharks and understand the influence of food acquisition and prey abundance on routine patterns in behaviour.
O5: Understand changes in routine behavioural habits across ontogenetic phases and consider shifting drivers and pressures as sharks grow.
O6: Define the importance of the nursery habitat to young sharks in the context of fitness and survival, qualifying key requirements from the habitat in the conditions and resources it provides.

These objectives then lead to a set of testable hypotheses and research questions:

H1: A combination of technologies and techniques are able to resolve fine-scale spatio-temporal behaviour and activity in young nursery-bound sharks. With the development of technologies for studying specific animal movement patterns in the wild, by combining direct observations with accelerometer data in captive trials, and combining traditional telemetry and accelerometer logged data in wild trials, we can synonymously measure specific behaviour alongside habitat use and movements. This is addressed in chapters 3, 4 and 5.

H2: The methods used do not influence behaviour in the long-term and they produce reliable data representative of natural behaviour. A major consideration of animal attached logging devices is the potential impact they have on natural behaviour. With the advancement in technology, smaller, lighter, externally mounted devices now allow for less invasive tagging and handling procedures and less impact to physical movement. This thesis will address this in chapter 3.

H3: Sharks display distinct patterns in habitat use, activity and behaviour across differing temporal scales. A combination of biological and environmental factors determines these patterns. Combined tagging methods used here mean we can record specific behaviours and activity, and place these temporal components within a spatial context. Onboard recording of bio-physical and environmental data by accelerometers and habitat sampling allow for association of biological and environmental factors with patterns in spatio-temporal behaviour. This is addressed in chapter 4.

H4: Foraging and prey acquisition play an important role in defining habitat use, activity and behavioural patterns in nursery-bound sharks. High frequency accelerometer recording can distinguish even small postural changes and can identify the distinct acceleration outputs of foraging, prey capture and feeding behaviour in sharks. When ground-truthed with observed captive trials, these behaviours can be identified in unobserved wild shark data. This research addresses this in chapter 5.

H5: As sharks grow, ecological pressures and priorities shift, affecting changes in how sharks use the nursery habitat. By studying animals using a specific site, it is possible to study sharks across a size range and thus compare a suite of different data across ontogeny. As sharks grow, they will be differently impacted by various pressures and this will impact their patterns of behaviour. This is addressed in chapter 6.

H6: The nursery habitat provides specific conditions and resources necessary for the fitness and survival of young sharks. The structure and function of the nursery ecosystem is in a fine balance and even small changes can cause severe knock-on effects to the fitness and survival of sharks using it. Compiling the findings from chapters 4, 5 and 6 allows for the production of a complete spatio-temporal behavioural routine for young lemon sharks. From this a theoretical model for nursery habitat use can be developed and predictions made on the theoretical impacts of deleterious human activities within this nursery habitat. This is addressed in chapter 7.

1.4 Selection of study species and site

To achieve the above objectives, this research required the use of modern technologies and techniques to resolve fine-scale behaviour in sharks. Conducting the study at a known shark nursery site with reliable access to sharks at a range of sizes was essential to achieving the aims and objectives.

The lemon shark *Negaprion brevirostris* belongs to the family Carcharhinidae (requiem sharks), of the order Carcharhiniformes (ground sharks). The lemon shark is distinguished from other carcharhinid species by the second dorsal fin, which is almost as large as the first and its blunt snout, shorter than the width of its mouth. It is one of the larger coastal carcharhinid species with a max total length (TL) of ~ 3.4 m. It is an inshore species found in coastal waters of the Atlantic Ocean, ranging from the US to southern Brazil and in the Pacific Ocean from Baja California to Ecuador. It is currently also known from some areas off the West African coast, though it is not known if these two populations are in fact different species (Campagno, 1984; Sundstrom, 2015; Fig. 1.1). Feldheim et al. (2002) undertook a study into the population genetic structure in the western Atlantic, using DNA microsatellite markers, and found that there is extensive gene flow between sites. Size and growth rates vary greatly with location, with some sites, such as Bimini, favouring comparatively smaller, slower-growing individuals (Barker et al., 2005; DiBattista et al., 2007). The lemon shark is a placentally viviparous species, reaching sexual maturity between 12 - 16 years old, with a gestation period of ~ 10 - 12 months. Litter sizes range from four to 18 pups with multiple paternities. Females reproduce biennially, often returning to their own birth site for parturition (Compagno, 1984; Feldheim et al., 2001; Feldheim et al., 2002). Adults, however, provide no parental care after birth and juveniles are reliant on the sheltered nursery areas to maximize chances of survival in the early, and vulnerable life stages. Their diet consists mainly of teleost fishes but also includes molluscs, crustaceans and other elasmobranchs. Juvenile lemon sharks of Bimini are selective in their diet, preferring yellowfin mojarra *Gerres cinnereus* (Walbaum, 1792) over other species, despite their comparatively low abundance in the environment (Cortes & Gruber, 1990; Newman et al., 2010).

Fig 1.1 Map of the known global range of the lemon shark. Accessed via iucnredlist.org on 16/01/2019 (International Union for Conservation of Nature (IUCN). ***Negaprion brevirostris*****. The IUCN Red List of Threatened Species. IUCN 2015-3)**

The lemon shark is classified as ‘Near Threatened’ by the IUCN (IUCN, 2018) and as such is protected by law throughout parts of its range. In the coastal waters of Florida, Louisiana and Texas, commercial fishing for lemon sharks is prohibited (NOAA, 2009). In 2011, the waters of the Bahamas were designated as a shark sanctuary, prohibiting the targeting and retaining of caught sharks. Due to their size, survivability in captivity and resistance to hook and line capture, lemon sharks make an excellent study species (Gruber et al., 1988). Much research has been dedicated to examining the movement patterns and home ranges for these sharks. Juvenile sharks are highly site-attached to restricted home ranges for the first three years of their lives (Morrissey & Gruber, 1993a; Morrissey & Gruber, 1993b; Franks, 2007). Predator avoidance is likely the motivation behind such restricted patterns of movement. Larger, cannibalistic lemon sharks occupy the deeper waters of the open lagoon areas. It is likely that smaller juvenile sharks remain close to shore as a means to avoid encounters with larger conspecifics (Morrisey & Gruber, 1993a; Guttridge et al., 2012). Estimated first year survival varies annually between 38% and 65% (Gruber et al., 2001). Sharks select warmer shallower waters, on a sandy or rocky bottom type. Juveniles experimentally displaced from their home ranges by up to 16 km were found to return to these, demonstrating a keen homing ability (Edren & Gruber, 2005). Sub-adult sharks occupy larger, secondary home ranges, and demonstrate diel patterns in activity (Gruber et al., 1988; Sundstrom et al., 2001).

This study focused on juvenile and sub-adult lemon sharks between 0.7 - 1.8 m. Juvenile sharks smaller than 0.7 m have smaller dorsal fin surface area, increasing the chances of fin tearing and tag package rejection. This would likely cause stress to small, vulnerable sharks and risk loss of expensive tagging equipment. Larger sub-adult and adult sharks over 1.8 m are known to make extended excursions from the shallow waters surrounding Bimini, eventually migrating away from Bimini altogether. Thus, sharks of these sizes were rejected from this study as they had greatly reduced chances of recapture and tag recovery.

The populations of lemon shark occupying the Bimini Islands in the Bahamas are some of the most intensively studied shark populations in the world. Many years of research has provided detailed information on habitat use, home ranging, temperature preferences, bioenergetics and diet (Gruber et al., 1988; Morrissey & Gruber, 1993a; Morrissey & Gruber, 1993b; Sundstrom & Gruber, 1998; Newman et al., 2010; DiGirolamo et al., 2012). The information available for this species shows that lemon sharks have the potential to act as a key indicator species for sharks using coastal nursery habitats, providing a template for the ecological role of large coastal shark species at early life stages. Mathematical modelling approaches can be useful in determining potential drivers of population dynamics and can inform management actions (White et al., 2014). White et al. (2014) modelled Bimini lemon shark population dynamics using 17 year longitudinal data (1996-2012). The stochastic model used in this study accounted for 33-49% of the variation in the population size but the authors suggest that more accurate assessments of population viability require the incorporation of a suite of environmental effects and must account for predator-prey dynamics. The acquisition of these data are suggested by the authors as next steps to informing this model and better applying these methods.

Despite the breadth of available information, no study has delineated the daily routine behaviour and activity of this species whilst using a nursery site. To meet O1 (section 1.3), this study aimed to develop a methodological approach for this species at the Bimini site, to then achieve subsequent study objectives.

1.5 Methods to observe and study behaviour, activity and habitat use

In the study of sharks in the wild, constraints in scientific technology and the expense associated with the use of satellite and archival tagging have contributed to a comparatively heavy dependence on acoustic telemetry (Sims, 2010). Telemetric tools are, however, growing ever-more sophisticated and today allow for the close study of bioenergetics, spatial ecology and behaviour in aquatic animals' natural environments (Cooke et al., 2004;

Donaldson et al., 2014). In practice, acoustic telemetry involves either attachment or surgical implantation of an ultrasonic transmitter to an individual. This transmitter emits a continuous coded pulse on a set frequency. In many cases a directional hydrophone and portable acoustic receiver are used to enable a vessel to locate and follow the individual and track its movements (Sims, 2010), this is known as active telemetry. Many studies over the past 40 years have made use of this technology. (e.g. Klimley & Nelson, 1984; Holts & Bedford, 1993; Grubbs et al., 2007; Speed et al., 2011; Munroe et al., 2014; Cagua et al., 2015; Legare et al., 2015). Active telemetry provides fine-scale location data but is limited to the time during which the shark can be tracked.

Passive telemetry or acoustic monitoring employs stationary receiver arrays, moored at specific sites or on a buoyed line. These units can be deployed for months or even years, so this type of telemetry allows for long-term movement and behaviour to be recorded (Sims, 2010). Another advantage of passive telemetry is the ability of the receivers to collect a large volume of data from many different transmitters allowing for simultaneous observation of movement patterns of multiple animals. Passive receivers, however, only log the presence of a shark within their range and give no fine-scale data regarding location or movements between or within that range. Individuals must also remain within the detection range of the acoustic array as a whole. Such instruments are poorly adapted to observations of large-scale habitat movements or migratory behaviour. This technology is also limited as it does not allow for a more fine-scale understanding of the behavioural ecology of sharks. Basically, telemetry defines spatial elements well but temporal elements poorly, providing insight into the locations of sharks at varying spatial scales but information on specific quantitative behavior is deficient (Whitney et al., 2012).

Satellite tagging is commonly used to address research questions for marine animals moving over larger spatial scales. A tag, externally mounted on the animal, transmits a signal to a satellite system known as the ARGOS system. PAT, P-SAT and SPOT tags, though varying in function, offer scientists data on large-scale movement patterns (Hammerschlag et al., 2011). Domier and Nasby-Lucas (2008) used P-SAT tags to study migration patterns and habitat preferences of white sharks *Carcharadon carcharias* (Linnaeus, 1758) at Guadalupe Island. Findings highlighted patterns in migrations, use of migrations corridors and gender variances in migration timings as well as a shared foraging site. Satellite tags are also very useful in studying species of conservation concern. The porbeagle shark *Lamna nasus* is listed as Critically Endangered on the International Union for Conservation of Nature (IUCN) Red

List (IUCN, 2018). Pade et al. (2009) used pop-up satellite linked tags to monitor coastal area fidelity for these sharks, relating to coastal fishing pressures and found vulnerability to fishing and movements across international boundaries. This technology is unparalleled in its capacity to monitor broad scale movement for marine animals, but tags are very expensive and position estimates are not particularly accurate, with many position data being only within 10 km (Hays et al., 2001).

Archival data logging tags include depth, temperature and light-sensing units. These tags are usually small, externally mounted devices recording data over long periods. An accelerometer is an example of an archival data logging tag that is now being extensively used in marine and shark research. An accelerometer is a device that records a change in velocity (acceleration) when a small piezo-electrical sensor is deformed by gravitational or inertial acceleration (Mathie et al., 2004; Wilson et al., 2008). Measurements are recorded in one, two or three planes (surge, heave and sway) of acceleration and accurately represent true movement (Dow et al., 2009). Measurements of gravitational acceleration represent the orientation of the device, commonly referred to as static acceleration. Measurements of inertial acceleration represent the true movements of the device, referred to as dynamic acceleration. Accelerometers typically archive data, onto a digital memory chip. Sub-second recording regimes prohibit the transfer of such large data packages and require the recovery of the device to access the data (Whitney et al., 2012). Some telemetric devices are now being developed that are able to send data to a receiver (Brown et al., 2013). Within the realm of behavioural research, accelerometers are animal attached devices that measure animal orientation as well as dynamic movement. Tri-axial accelerometers (such as those used in this study) measure acceleration in all the axes of movement and thus can be used to identify a range of behaviours in free-ranging animals (Yoda et al., 1999; Shepard et al., 2008a).

The first study to use accelerometer technology to assess animal behaviour was Yoda et al. (1999). The study used accelerometers to monitor porpoising behaviour of Adelie penguins *Pygoscelis adeliae* (Hombron & Jacquinot, 1841). Accelerometers have since been used to infer many types of behavioural activities across a variety of aquatic vertebrates. Studies have investigated swimming kinematics (Tanaka et al., 2001; Kawabe et al., 2003), spawning behaviour (Tsuda et al., 2006) behavioural modes (Nathan et al., 2011; Brewster et al., 2018) energetic and metabolic rates (Gleiss et al., 2009; Murchie et al., 2011; Wright et al., 2014) and foraging ecology (Naito et al., 2010; Iwata et al., 2011; Kokubun et al., 2011; Makiguchi et al., 2012; Friedlaender et al., 2015).

Accelerometers have, for the last decade, been used in the study of elasmobranch behaviour. Whitney et al. (2007) used accelerometers to elucidate diel activity patterns of whitetip reef sharks *Triaenodon obesus* (Ruppell, 1837). Whitney et al. (2009) identified mating events in nurse sharks *Ginglymostoma cirratum* (Bonnaterre, 1788), Nakamura et al. (2011) investigated yo-yo dive behaviour in tiger sharks *Galeocerdo cuvier*, likely representing ambush predatory strategy and Watanabe et al. (2012) monitored the swim speed and tailbeat frequency of Greenland sharks *Somniosus microcephalus* (Bloch & Schneider, 1801). Accelerometers have proven an extremely effective tool in the study of animal behaviour, especially in the marine environment. The accelerometer has transcended the traditional limitations of biotelemetry and provides small, lightweight, tagging devices that record data at sub-second frequencies (0.5-1000 Hz) (Brown et al., 2013). Devices can record continuously or activate under specific conditions. The sensitivity of the tags means even the smallest of movements or postural changes are recorded.

An animal’s movements are the fundamental expression of its responses to both internal biological drivers and its external environment (Shepard et al., 2010). Accelerometers provide a means to measure the movement of wild animals and to quantitatively record changes in movement behaviour over spatio-temporal scales. When combined with other sensors or devices, they offer a powerful tool to assist in our understanding of animal behaviour.

The tagging of animals with various telemetric and archival devices provides a plethora of data on ranging aspects of animal biology. Studies that use these methods in marine animals depend on neither the attachment of the device nor the device itself affecting the natural behavior and physiology of tagged individuals (Jadot et al., 2005). It is important when conducting such research to measure the effects of tags on an animal’s natural behaviour to ensure data gathered and inferences drawn on natural behaviour are as accurate as possible. It will also be important in providing ethical committees with information on acceptable tagging practices (Wilson & McMahon, 2006). Various studies have been conducted to assess the effects of tags and their attachment on behaviour and performance of teleost fishes and marine mammals (Mellas & Haynes, 1985; Wilson et al., 1986; Ballard et al., 2001; Jadot et al., 2005). Few have endeavored to assess the impacts of externally attached data logging devices to the natural behaviour of sharks or the recovery of these animals from tag-attachment procedures.

1.6 Methodological design and thesis structure

This study used captive observation trials, small accelerometer tags, small acoustic transmitters (accelerometer and acoustic transmitters fitted together as fin-mounted tag packages), telemetric tracking tools, baited remote underwater video surveys (BRUVs) and environmental sampling methods to achieve its objectives. This thesis is structured as follows and cross references to the Objectives and Hypotheses set in section 1.3:

- Chapter 2 outlines the general methods applied to data collection for this research, developing the methodological approaches for observing behaviour (O1) and validating the use of technology to resolve fine-scale patterns in behaviour (H1).
- Chapter 3 uses captive and wild trials to observe the behaviour of tagged individuals(O2) and demonstrate the minimal impacts of small, fin mounted tag packages on natural behaviour as well as measure the impacts of capture and tagging on sharks, post release into the wild (H2).
- Chapter 4 uses captive behavioural ethogram and tagging and tracking of wild sharks to investigate the spatial and temporal patterns in habitat use, activity and behaviour in free-ranging lemon sharks (O3) and determines the biological and environmental drivers that determine these patterns (H3).
- Chapter 5 uses captive trials to delineate specific body movements and postures that produce acceleration signals unique to foraging and prey capture (O4). This chapter then applies these findings to uncover foraging behaviour in space and time for wild sharks, considering location, frequency, periodicity and success of foraging behaviour (H4). Baited remote underwater video survey is used to identify and correlate prey distributions and abundances with shark foraging behaviour.
- Chapter 6 investigates data gathered in Chapters 4 and 5 to consider shifts in habitat use, activity and behaviour of sharks across ontogeny pressures (O5). This chapter uses data across size range of sampled animals to show how changes in ecological pressures determine ontogenetic shifts in behaviour and habitat use (H5).
- Chapter 7 summarized findings and compared them to existing literature (O6). This chapter then applies study findings and theoretical modeling to define the importance of this nursery habitat to this species and to model the potential impacts of habitat disturbance and loss to these sharks (H6).

Chapter 2 – General Methods

2.1 Study Site

The Bimini Islands (25.44'N and 79.16'W) are a cluster of sub-tropical islands located to the western edge of the Andros platform of the Great Bahama Bank, approximately 86 km east of Miami, Florida. The waters to the east and south of the islands extend across the Great Bahama Bank, rarely exceeding 3 m in depth. The north and south islands (10 km^2 and 8 km^2 respectively) enclose shallow, mangrove fringed lagoons that act as a nursery site for many species including lemon sharks (Morrissey & Gruber, 1993a; Jennings et al., 2012). This study was conducted at two primary sites. Captive trials were held in semi-enclosed pens constructed in the shallow water flats behind the research facility (highlighted in Fig. 2.1).

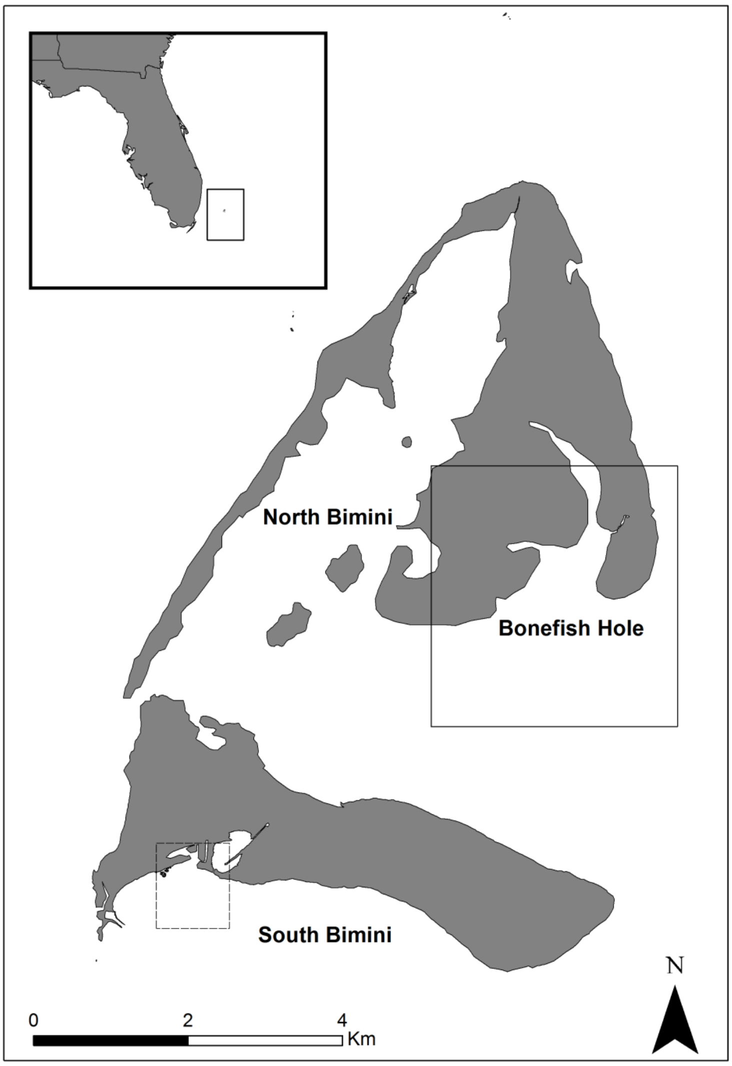

Fig. 2.1 Map of Bimini, Bahamas (25°44' N, 79° 16' W). The dashed line box represents the site at which captive trails were conducted and the solid line box represents the site at which free-ranging trials were conducted.

Wild trials were conducted in the south-eastern area of the north island, known as ‘Bonefish Hole’, and across the open lagoon flats between the two islands (see Fig. 2.1).

The Bonefish Hole site has many narrow inlets that retreat back into the body of the mangroves and these areas are subject to considerable tidal flow. This site was selected because of its lack of boat traffic and human interference in the area. Other lagoon areas around Bimini have been affected by extensive habitat degradation through dredging and mangrove clearing (Jennings et al., 2012). Such factors are likely affecting the natural patterns of behaviour of many of the species occupying those areas.

2.2 Tag Package Construction and Attachment

The accelerometer tag packages used in this study comprised a G6A tri-axial accelerometer (35 x 25 x 12 mm, 30Hz, 40MB, CEFAS Technologies Ltd, UK) and a PT-04 acoustic transmitter (9 x 25 mm, 134-136 dB, battery life: 3 months, Sonotronics Inc., Tucson AZ., Fig. 2.2). PT-04 transmitters were epoxy fastened to the top side of the accelerometer in such a manner as to minimise drag. Tag packages were fitted to the first dorsal fin of sharks with monofilament line. The monofilament line was passed through holes drilled in the accelerometer and then fastened to the shark through two small holes pierced in the shark’s fin using a hollow hypodermic needle, through which the monofilament line was fed. Small aluminium crimps held the tag flush to the reverse side of the fin. Insole material was used to pad the back of tag package and the fin surface side of the crimps to minimise abrasion (as per Whitney et al., 2009). Devices were oriented so that the horizontal acceleration plane of the accelerometer was in line with the horizontal posture of the animal. The smallest shark studied weighed 4.3 kg, meaning the tag package, which weighed 23 g in air, was only 0.53% of this animal’s body weight. It was thus assumed that tag weight, which was less than the 2% rule set by Winter (1983), did not adversely affect the behaviour of tagged individuals.

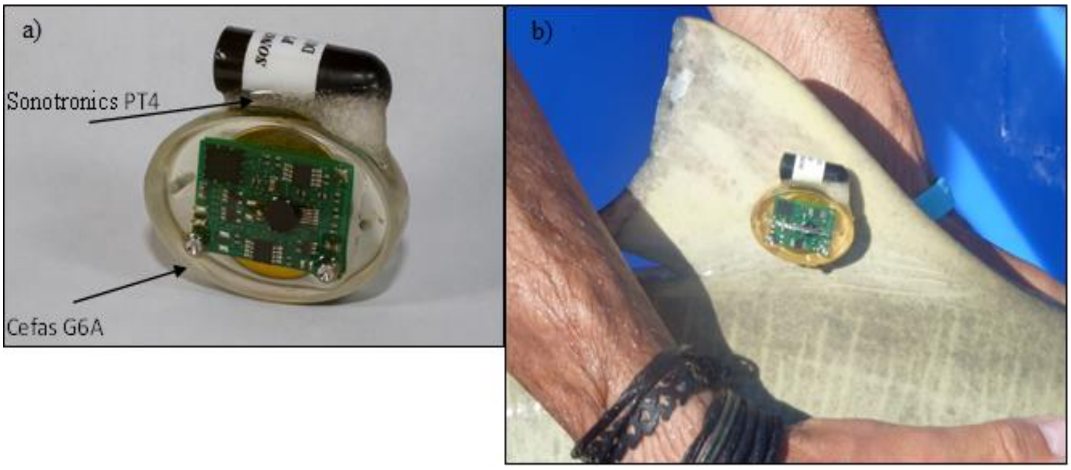

Fig. 2.2 a) Image of an accelerometer/acoustic transmitter tag package with PT-04 transmitter epoxy fastened to the G6A accelerometer b) Tag package attached to the first dorsal fin of a shark, oriented to align the horizontal acceleration plane with the posture of the animal.

CEFAS G6A tags recorded acceleration data (measured in units of standard gravity; 1 *g* = 9.8 m s^{-2}) in three axes of motion (surge, heave and sway; Fig. 2.3), as well as recording temperature (^{o}C) and depth (m). Acceleration recording was set to 30Hz, logging a value in each axis 30 times per second. Both temperature and depth data were logged once per second. The orientation of the tag, when attached to the fin of an animal, was such that dynamic acceleration was measured with surge representing the forward and backwards motion, heave representing the up and down motion and sway representing the sideways motion (Fig. 2.3).

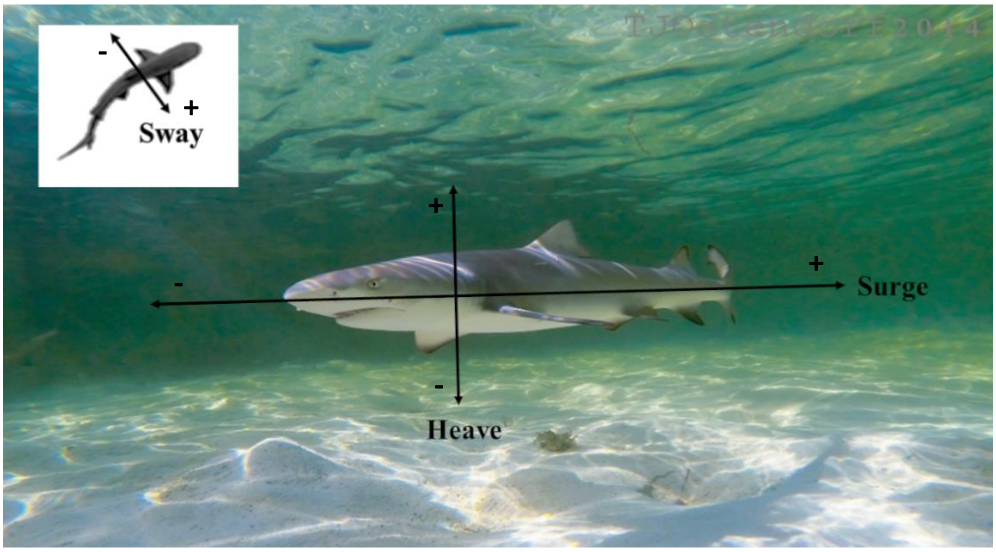

Fig. 2.3 Axial orientation of dynamic acceleration for fin-attached tags.

In sharks, swimming kinematics are altered through the differing power of undulating tailbeats. Such movements are recorded as sway acceleration in the G6A tags, and as such this axis of acceleration was used in several analyses of data and assignment of behaviours.

2.3 Shark Capture

For captive trials, sharks were caught in nearshore areas off South Bimini as this reduced travel time and stress on the sharks when moving them to the semi-enclosed pens behind the facility. For wild shark accelerometer deployments, sharks were caught in the shallow waters of the Bonefish Hole area and the adjacent lagoon to the south of this area (see Fig. 2.1).

Various capture methods were employed to capture sharks, all of which were well-tested and standardised methods used by the Bimini Biological Field Station (BBFS) team for many years. Traditional rod and reel fishing was used from shallow water vessels, usually no more than 1 km from the shore. Baited circle hooks on a steel leader wire were fastened to standard fishing rods with 50 kg breaking strain monofilament line. Menhaden chum blocks were used to generate a scent trail. Gill nets (100 x 1 m) set in shallow water areas adjacent to the shoreline were used to capture smaller sharks (< 1 m). Nets were checked regularly, and sharks were removed and handled by trained team members. In some instances, larger sharks (usually > 1.2 m) were caught using a specialised technique whereby large rope-mesh nets (75 x 1 m) with one weighted edge and one floating edge (as with standard gill nets) were deployed from the side of a moving skiff, with an anchor, in the path of a shark. This allowed the team to actively find and intercept suitably sized sharks which would become ensnared in the netting.

Upon capture, sharks were firstly secured. Sharks small enough, were transferred to an on-board circular tub (diameter 1.5 m) filled with seawater. Larger sharks were secured underwater to the side of the vessel. Both of these techniques were used as they were deemed the least invasive for the animal, as well as being the safest setup for the teams to work in. In all instances, unsuitably sized sharks (< 70 cm TL or > 180 cm TL) were quickly released. Sharks were also assessed for signs of elevated stress (blotchy appearance to the skin, lack of movement). Any shark expressing these signs was immediately released back into the wild and excluded from the study. For sharks displaying no signs of elevated stress, a series of standardised information was first gathered, and in the case of rod and reel capture, the hook removed. All lemon sharks caught in this study were tagged with a passive integrated transponder (PIT) coded identity tag (Destron Fearing Inc., Eagan MN, USA), injected just under the skin near the first dorsal fin. PIT tag readers were used to identify individuals by their PIT tag code. All sharks in this study are identified, from this point forth, by the last three digits of this PIT code. Sharks were first scanned for an existing PIT tag, as many sharks using the Bimini ecosystem are tagged every year as part of an ongoing survey at the site. For sharks with no existing tag, one was inserted under the skin and a note of the code was made. As well as PIT tagging, a series of standard data were taken for all caught sharks. Three measurements of animal length (pre-caudal length (PCL), fork length (FL) and total length (TL)) were taken in cm, measuring from the tip of the snout with a tape measure along the topside of the body. Girth measurements were taken by wrapping a tape measure around the body of the animal just anterior to the first dorsal fin. A small tissue sample was taken from its dorsal fin for genetic analysis, the animal sexed (presence of claspers) and the date and location of its capture recorded.

In the case of captive trials, individuals were then transferred to the circular tub within the vessel and immediately transported to a holding pen behind the research facility. Here sharks were given time to recover from capture and handling before being transferred to an observation pen at a later date. All sharks caught for captive trials fed to satiation 24 h after capture, indicating a full recovery. For wild shark deployment trials, once the shark had been secured and the standard capture information gathered, the tag package was attached as quickly as possible before releasing the shark back into the wild (described in section 2.2). Capture techniques, animal handling and tag package attachment techniques for both captive and wild trials were employed in chapters 3, 4 and 5.

2.4 Captive Trials

Essential to understanding animal behaviour is the development of an ethogram of discrete behaviours typically displayed by an animal under natural conditions (Sakamoto et al., 2009). It is important to construct this ethogram based on direct observations of behaviour (Martin & Bateson, 2007). Captive trials were conducted throughout the study in two main capacities. Firstly, to observe individual responses to the presence of the tag package, behaviour was observed prior to tagging, followed by trials observing behaviour with fin-attached devices (Chapter 3). Secondly, captive trials were used to develop an ethogram of behaviours of sharks with active accelerometer tags attached. This allowed direct and precise observation of different specific behaviours, which could then be used to distinguish acceleration outputs and provide accurate assumptions of behaviour in wild tagged sharks, for which direct observation is impossible (Chapters 4, 5 and 6).

A holding pen (12 x 6 m) was constructed in the shallow waters behind the research station (see Guttridge et al., 2009 for more information on housing environment details) and a 4 m high tower was erected on the south side of the pen to observe the sharks unobtrusively and minimise potential responses by sharks to observer presence (Fig. 2.4). All sharks held in the captive pens were observed daily and fed frequently. Sharks were held in the pens for as long as necessary to complete the trials (no longer than one week) before being released back into the wild at site of capture. Observed captive trials were used to measure tag effects in chapter 3, ethogram a series of behaviours in chapter 4 and characterise specific foraging and prey handling behaviours in chapter 5.

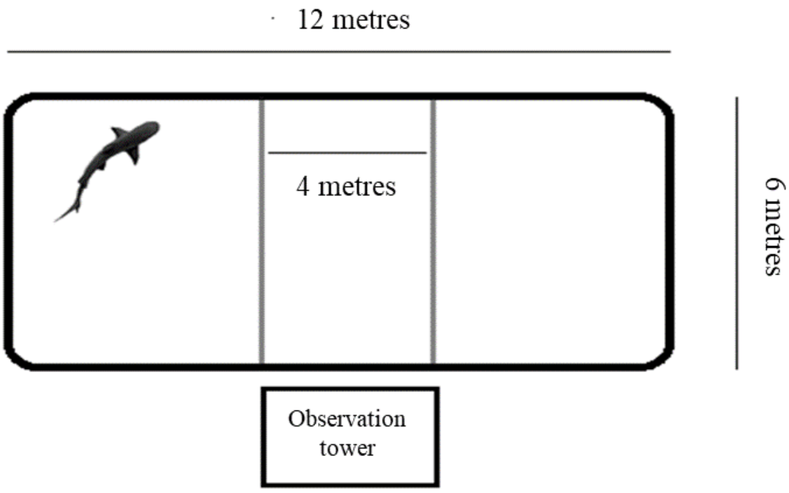

Fig. 2.4 Experimental setup for semi-captive trials.

2.5 Telemetry

2.5.1 Passive receiver array

A network of stationary acoustic receivers was deployed within the study site throughout wild deployments (Fig. 2.5). Submersible ultrasonic receivers (SUR, Sonotronics Inc., Tucson AZ, USA) detected passage of sharks with attached PT-04 acoustic transmitters. The devices logged the date and time of reception of individual tags within their range. Detection range has been thoroughly tested in these units, considering various environmental factors and their effect on detection range (van Zinnicq Bergmann, unpublished 2017).

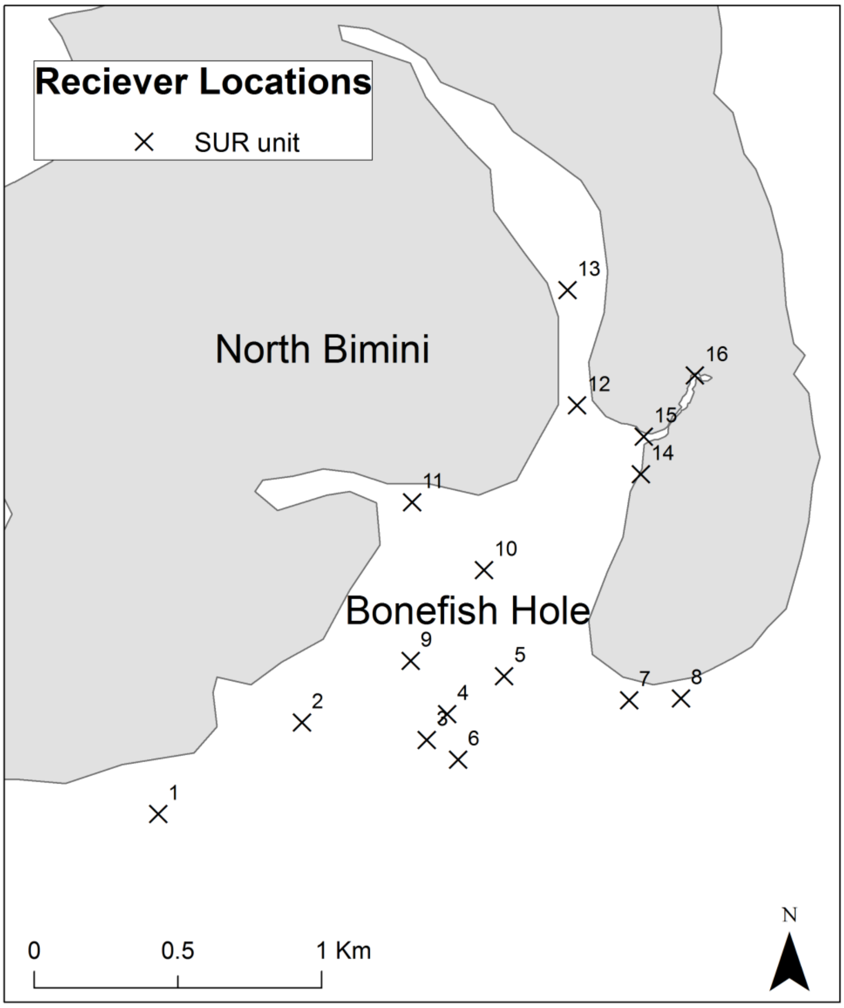

Fig. 2.5 Positions of SUR units within the study site. Each unit with a designated ID number. Units are set up to overlap, any sharks undetected by the array can be assumed to be occupying waters outside of the Bonefish Hole area.

The array was set up to cover the mangrove edged area of Bonefish Hole and to have receiver detection ranges overlap even at lower tides when range was reduced. This allowed for connectivity between receivers and a better understanding of shark movements between areas. SURs were housed in a PVC pipe moulded to a concrete base, buried into the substrate so that the receiver node itself was 30 cm above the seabed. The devices were left in the same position for the course of the study and data were downloaded at the end of a deployment period, after all sharks were recaptured. Passive telemetric tools were used to track shark movements and determine space use and range size for chapters 3, 4, 5 and 6.

2.5.2 Active tracking

Throughout free-ranging trials, sharks were also actively tracked in shallow water vessels using portable ultrasonic telemetric equipment (model USR-08, Sonotronics Incorporated, Tucson AZ USA). Tracking crews consisted of a boat driver, a tracker and a data recorder. Sharks were tracked over a 24-h-cycle, but logistical constraints meant that continuous tracking was impossible. Instead, a tracking schedule was set up whereby, typically, daytime crews tracked for 8 h before returning to the research station. After a break of several hours, night time crews drove out to the site and tracked for another 8 h. Start and end times depended on search locations and tide times. During periods of heavy rain and storms tracking was halted. To find sharks, random tracks were driven throughout the study site, originating at the initial site of capture. Once an acoustic signal was picked up by the tracker, teams began recording data. Estimates of shark location were recorded every 5 min with a handheld Global Positioning System (GPS) (Garmin Ltd, GPS 72H) along with a compass bearing and estimated distance (to nearest 5 m, based on audible signal intensity) from the animal. All crew members were given training in active tracking prior to fieldwork and inexperienced crew members were always teamed with more experienced ones. A distance of > 15 m was maintained between vessel and animal to minimize disturbance of the sharks' natural movements. In instances where sharks were tracked into areas too shallow for the vessel, two team members walked with the equipment, where feasible, continuing to record data. Active telemetric tools were used to track shark movements and determine space use and range size for chapters 3, 4, 5 and 6.

2.6 Recapture

Sharks were recaptured at the end of the deployment period using the same methods outlined for capture in section 2.3. In many instances active tracking was used to assist in the location and capture of tagged sharks. Once an individual was secured, the tag was quickly removed. The same series of data were taken as on capture. Once complete, the shark was released and visually tracked for five min to monitor its condition, post release. Recapture techniques were employed for all wild trials in chapters 3, 4 and 5.

2.7 Baited Remote Underwater Video

Baited remote underwater videos (BRUVs) were used to sample the local faunal assemblages (see Chapter 5). Two BRUV rigs were constructed from reinforced steel bars (Fig. 2.6). GoPro Hero 2 cameras were used in underwater housings, attached to the frame as shown in Fig. 2.6. A mass of 800 g (frozen weight) of Atlantic menhaden *Brevoortia tyranus* (Latrobe, 1802) chum was placed in mesh bags inside the chum cage (20 x 14 x 16 cm) at the end of the frame arm. This bait is used in fishing as well as many studies using BRUVs (Harvey et al., 2007). The chum was always fully defrosted before each deployment and the use of the mesh bag regulated flow of the chum. Small floats were attached to the top of the frames for identification at the sea surface. All deployment locations were randomly selected using a random points generator in ArcMap v 10.0 Maximum deployment depth was 4 m and minimum was 0.6 m. Garmin 72H GPS units and shallow water skiffs were used to navigate to the deployment site. All deployments were classified as either low tide or high tide (duration of deployment within 2 h of peak high/low tide time) and either as a mangrove edge site (ME: < 50 m from mangrove shoreline) or non-mangrove edge site (NME: > 50 m form mangrove shoreline). At low tide, many areas of the study site became inaccessible and so a preliminary mapping of accessible low tide depths was conducted. Low tide BRUVs were deployed within this range. All high tide deployments were conducted within the overall range for the whole study (Fig. 2.7). Upon arrival at a deployment site, a set of environmental data was taken. Temperature, salinity and dissolved oxygen were recorded using a YSI Pro 2030. Flow direction was determined using a water-soluble dye and a compass. Flow velocity was measured using a General Oceanics flow meter with a low velocity paddle wheel (General Oceanics, FL, USA). Visibility was measured using a small Secchi disk and a 20 m tape measure. Standard vertical measurements were not useful as all deployments were in shallow water, so measurements were taken horizontally in the water column. One crew member held the disk facing horizontally in the water and another used a mask and snorkel to swim away from the disk whilst holding on to the tape measure. At the point the disc markings could no longer be differentiated the measurement was taken. Depth measurements were taken using a marked depth stick (to nearest cm).

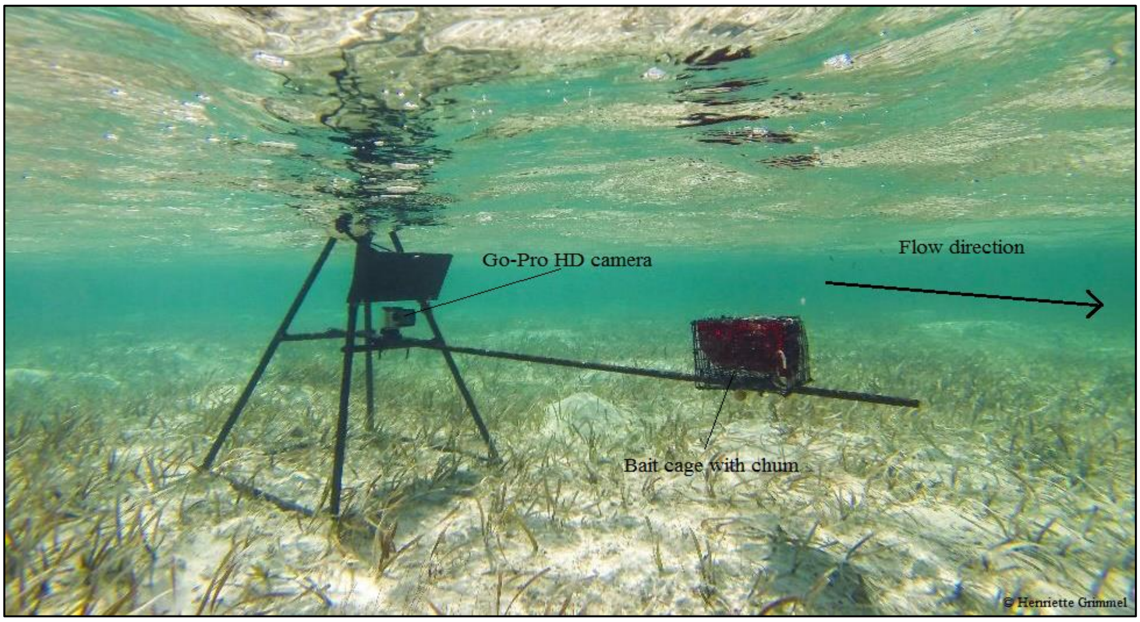

Fig. 2.6 BRUV rig setup. Rebar steel frame with attached Go-Pro Hero 2 in underwater housing, with 800g of chum in a mesh chum bag, inside a steel cage.

After collection of environmental data, rigs were positioned underwater at the site. The frame arm and view of the camera were faced down current to maximise observation of fish drawn to the chum scent. Deployment start time was recorded at the point the chum bag was inserted into the cage. The team and skiff then immediately left the site. Each deployment had a duration of 70 min. The first 10 min would be left unused in analysis to allow for a settling period after the disruption caused in the setup. No two deployments were simultaneously conducted within 1 km of one another so as to eliminate bias from potential chum scent crossover. After 70 min the team returned to the site, collected a second series of environmental data in the same manner as before and finally, estimated bottom type using a quadrat (105 x 105 cm containing 20 x 20 cm sections) to estimate sea-grass coverage in the immediate area. Quadrats were placed along alternating sides of a 20 m transect line, moving out from the rig and in the direction of the water flow. For each quadrat an estimate of seagrass cover was taken (to nearest 5%). A mean value was then derived from the collective samples. The site was then either categorised as sandy (< 20% seagrass cover), mixed (20% - 80%) or seagrass bed (> 80%). BRUVs were used to map faunal community abundance and distributions in chapters 5 and 6.

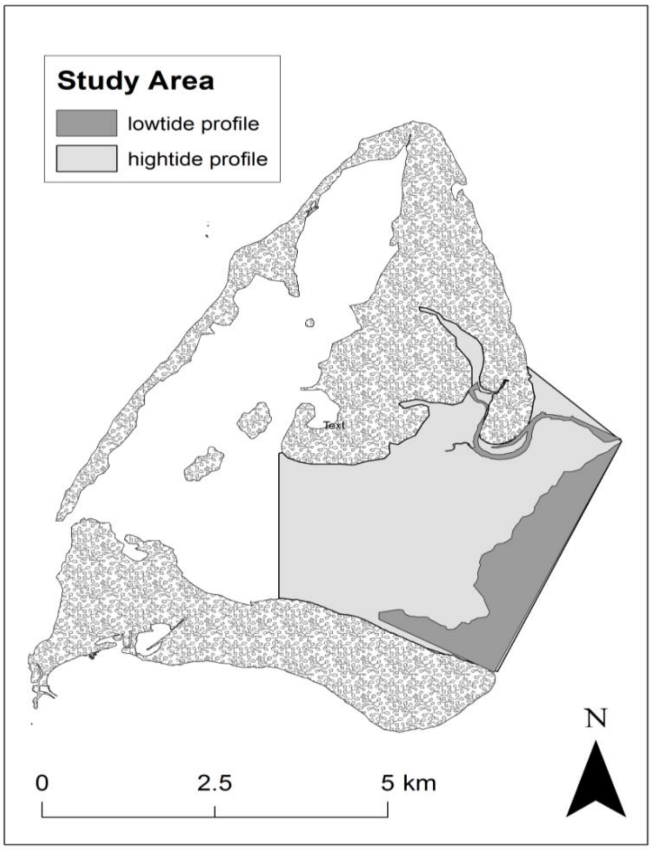

Fig. 2.7 Map of the Bimini Islands with high and low tide sampling areas for the BRUVs field work (low tide profile overlaid on high tide). Random points were selected within these profiles for BRUV deployments.

2.8 Data analysis

All statistical procedures, analyses and models were conducted and generated within R (v 3.2.3, R Core Development Team 2012) unless stated otherwise. All data were explored, models and tests selected, using the protocol outlined by Zuur et al. (2010).

2.8.1 Tracking data analysis

SUR data were downloaded from units to a computer and imported into an Excel file. Downloaded data were always filtered for spurious detections. Each tag had an individual pulse interval (measured in µs) and detections recorded with a pulse interval greater than 3 µs either side of the tags designated interval value were considered spurious and eliminated from analysis (as advised by Sonotronics team). For each detection, SURs recorded the acoustic tag frequency, the date and time of the detection and the ping interval. Detection data were sorted into Excel files for each shark.

Active tracking data were manually transferred into Excel files for each track. Active tracking data included Shark ID, GPS coordinates, compass bearing, estimated distance from vessel as well as environmental data (water temperature and depth, salinity and bottom type (sand, seagrass or mixed). Water temperature and salinity measurements were made using a YSI Pro 2030 water meter (YSI Inc., OH, USA).

Both passive and active tracking data were analysed using ArcGIS (v 10.0 software, ESRI Corp.). Data files were converted to CSV files, loaded into ArcMap v 10.0 and GPS coordinates used to display data. Bimini island shapefiles were used to provide accurate geographic context. Tracking maps for each shark were constructed using a ‘point to line’ function. Distance-to-shore data were acquired by applying a ‘NEAR table’ function to tracking data and using the Bimini shoreline as the feature to which distances were measured. This function automatically provided the distance to the nearest possible point of land. Distances were converted from decimal degrees to m.

2.8.2 Accelerometer data analysis

All accelerometer data were downloaded using a CEFAS host translation program and converted into IGOR packed experiment files. Acceleration data were explored and analysed using Igor Pro v 6.37 (WaveMetrics Inc., Lake Oswego, OR, USA) and Ethographer, a user-friendly application running within the Igor platform (Sakamoto et al., 2009). Raw acceleration data were first subjected to a 2 sec box smoothing function, to separate static acceleration (the orientation of the tag and thus the animal) from dynamic acceleration (true movements of the animal). The chosen smoothing interval was determined using the protocol of Shepard et al. (2008a) and has been used in similar studies (Brownscombe et al., 2013; Wilson et al., 2015).

2.8.2.1 Measuring activity

Overall dynamic body acceleration (ODBA) has been identified as a sound proxy for activity and energy expenditure in sharks as acceleration is intuitively a measure of locomotory activity (Halsey et al., 2011; Whitney et al., 2012). ODBA has been shown to have a linear relationship with energy expenditure in vertebrates and deemed a superior movement-related proxy of activity and energy expenditure to tailbeat frequency (Whitney et al., 2012). For this research, values of ODBA were generated by summing the absolute values for dynamic acceleration in each axis (surge, heave and sway). Mean values for each minute were obtained with a decimate function within Igor Pro.

2.8.2.2 Behaviour discrimination by acceleration ethogram

To characterise distinct behaviours, functions within Ethographer were used alongside visual observation data from accelerometer tagged sharks in captive trials (see section 2.5). The methods used in this study are based on the ‘automated procedure to categorise behaviour from body acceleration’ presented by Sakamoto et al. (2009). Firstly, a spectrogram, presenting a spectrum of time-series data was created from dynamic sway acceleration data (for each shark), by continuous wavelet transformation, using the Morlet mother wavelet function (Sakamoto et al., 2009) (Fig. 2.8). Continuous wavelet transformation decomposes the time-series of acceleration signals into time and periodicity domains. This allows for the observation of different parts of the signal by adjusting the focus (Torrence & Compo, 1998; Karlsson et al., 2000; Sakamoto et al., 2009). Continuous wavelet transformation is similar to fast Fourier transformation, however deemed to be more appropriate for use with time-series data as it can apply to non-static oscillation signals (Sakamoto et al., 2009). A *K*-means clustering algorithm was used to cluster the data into similar spectra, based on the characteristics of the frequency and amplitude of wave oscillations. *K*-means clustering is a popular method for interrogating large data sets and runs an unsupervised, interactive algorithm, using the number of clusters as a parameter (Jain et al., 1999; Sakamoto et al. 2009). This clustering process allowed for the identification of distinct behaviours from an acceleration ethogram.

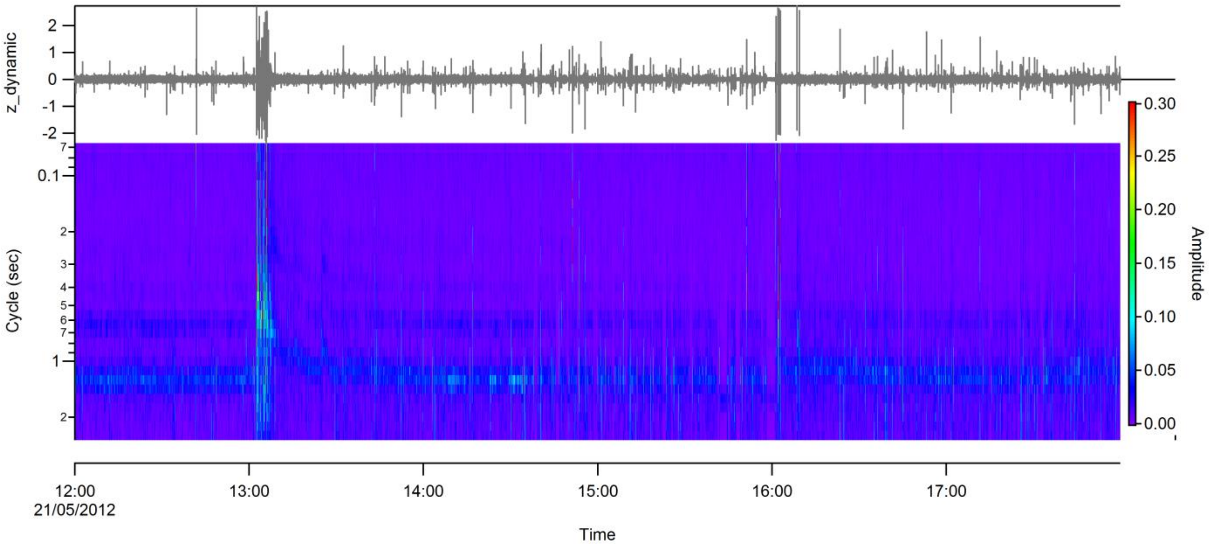

Fig. 2.8 Spectrogram of z axis dynamic acceleration for a full 6 h captive observation trial (for trial shark 15C), generated from continuous wavelet transformation in Ethographer.

This captive trailing process, corroborating the accurate assignment of clusters with observation data, meant that using the same clustering methods for the analysis of wild shark acceleration data would reliably classify behaviour in sharks where observation was not possible. All data packages recovered from wild shark acceleration loggers were therefore processed using the same methods as for captive trials. This procedure has been frequently used with the translation of acceleration data into behaviour for a range of animals (e.g. seabirds, fish and sharks) (Sakamoto et al., 2009; Whitney et al., 2009; Brownscombe et al., 2013).

Chapter 3 – Measuring the Behavioural Impacts of Externally Mounted Tag Packages and Capture Techniques on Young Lemon Sharks*

Quantifying the effects of capture, handling and device attachment on behaviour is important in elasmobranch tagging research, allowing methods and data interpretation to be refined. Behavioural responses of lemon sharks *Negaprion brevirostris* to a fin mounted tag package (CEFAS G6A tri-axial accelerometer with epoxied Sonotronics PT4 acoustic transmitter) were measured in a controlled captive environment (n = 10, size range - 80 to 140 cm) and in free-ranging sharks upon release (n = 7, size range - 100 to 160 cm). No changes were detected in behaviour (i.e. swimming speed, tailbeat frequency, time spent resting and frequency of chafing) between control and tagged captive shark trials, suggesting that the tag package itself does not alter behaviour. In the free-ranging trials an initial period of elevated swimming activity was found in all individuals (represented by overall dynamic body acceleration). However, sharks appeared to recover quickly, returning to a steady swimming state between 2 and 35 min after release. Post-release tracking found all sharks swim immediately for the shoreline and remain within 100 m of shore for prolonged periods. Hence, although lemon sharks are capable of quick adaptation to stressors and demonstrate rapid recovery in terms of activity, tracking data suggest that they may modify their spatial use patterns post release. This research is important in separating deviation in behaviour due to environmental stressors from artefacts caused by experimental techniques.

3.1 Introduction

In recent years, researchers have become increasingly reliant on remote devices to address a wide range of science and management questions, in a variety of species, including; marine mammals, turtles, teleosts, chondrichthyans and cephalopods (Stark et al., 2005; Shepard et al., 2008a; Grothues, 2009; Donaldson et al., 2014). Tools such as biotelemetry (radio and acoustic telemetry) and biologging (archival logger) devices offer a sophisticated means of evaluating the behaviour, spatial ecology, energetics and physiology of free-living animals in their natural environment.

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*Published as 'The behaviour and recovery of juvenile lemon sharks *Negaprion brevirostris* in response to external accelerometer attachment' – Journal of Fish Biology. See Appendix 1.

Implicit in studies that use these methods is the assumption that neither the attachment of the device nor the device itself affect the natural behaviour and physiology of tagged individuals (Mellas & Haynes, 1985; Jadot et al., 2005) and that observations should be comparable with those of untagged animals (Jepsen et al., 2001). For research findings to be viable, it is critical that tag-attached animals display normal behaviour to allow indicative data collection and extrapolation to the whole population (Bridger & Booth, 2003). As a result, studies have focused on measuring the effects of tagging devices on marine animals, but they mostly concentrate on teleost fishes (Bridger & Booth, 2003; Cooke et al., 2011).

Elasmobranchs occupy important roles in marine food webs whilst often being subjected to intense anthropogenic pressures (Dulvy et al., 2014). Tagging research is contributing greatly towards a better understanding and thus management for sharks, skates and rays, but without acknowledging and controlling for deviation in behaviour due to experimental techniques, researchers are liable to draw erroneous conclusions, hindering effective management and conservation (Sundstrom & Gruber, 2002; Bridger & Booth, 2003). Thus, evaluating the effects of tag package presence and measuring expected rates of recovery will lead to a more accurate interpretation of data.

Studies investigating anthropogenic stressors on elasmobranchs are few and most relate to physiological and biochemical effects of capture stresses (Skomal & Bernal, 2010; Skomal & Mandelman, 2012). How such physiological stresses manifest themselves in terms of behaviour is poorly understood. Some studies now use various archival tags to measure post-release mortality, behaviour and stress in sharks (Skomal et al., 2007; Campana et al., 2009; Gallagher et al., 2014; Hutchinson et al., 2015), but are limited in their capacity to measure sub-lethal behavioural responses, usually drawing conclusions from post-release depth profiles. For coastal species that occupy shallow waters this indicator is considered inadequate.

Accelerometers are becoming ever more advanced and thus more popular in marine research. Many studies of birds, marine mammals, fish and sharks now use accelerometers to elucidate aspects of behaviour, physiology and energetics (Gleiss et al., 2010; Kokobun et al., 2011; Whitney et al., 2012; Wright et al., 2014; Friedlaender et al., 2015). This technology offers a means to quantify sub-lethal, post-release behaviour in elasmobranchs, assessing the severity of changes in behavioural responses, as well as the rate at which individuals recover.

The objectives of this research were to: 1) identify potential behavioural compensation mechanisms that indicate stress in elasmobranchs, using a small accelerometer/acoustic transmitter tag package; 2) measure the expression of these behavioural responses; and 3) monitor the time taken for animals to return to a state of behavioural homeostasis. Trials were conducted on both captive and free-ranging sharks to evaluate the effects of tag presence, capture and attachment processes on the behaviour and activity of lemon sharks.

3.2 Materials and Methods

All sharks (n = 16) were caught for this research using standardised research methods, including traditional rod and reel fishing and gillnet capture within 1 km of the shoreline. Suitably sized sharks (70-180 cm TL) were secured upon capture. Small sharks (< 1 m) were transferred to an on-board circular tub (diameter 1.5 m) filled with seawater. Larger sharks were secured underwater to the side of the vessel using ropes wrapped around the nearside pectoral and caudal fin. Unsuitably sized sharks were immediately released. For captive trials (see section 2.4), sharks were transported to and held in a semi-captive mesh pen with an adjacent observation tower. Sharks were held in pens for no longer than 1 week and released back into the wild at site of capture. Accelerometer/acoustic transmitter tag packages (see section 2.2) were attached to the first dorsal fin of sharks with monofilament line and padded crimps. Both passive and active acoustic tracking techniques were used in wild trials to monitor movement and habitat use of sharks. Submersible ultrasonic receivers (SURs), set up across the wild habitat, logged presence-absence records for different sharks moving within their range and directional ultrasonic hydrophones, manually operated from small skiffs, were used to actively track sharks in the wild habitat. Overall dynamic body acceleration (ODBA) was used as a proxy for total activity to test for recovery rate in wild trials. See Chapter 2 for more detailed information on all capture (section 2.3), captive housing (section 2.4), tagging (section 2.2), acoustic tracking (section 2.5) and equipment used for this chapter.

All statistical procedures, analyses and models were conducted and generated within R (v 3.2.3, R Core Development Team 2012) unless stated otherwise. All data were explored, models and tests selected, using the protocol outlined by Zuur et al. (2010).

3.2.1 Tag weight

As stated in section 2.2, the smallest shark tagged in this study weighed 4.3 kg, the tag package being 0.53% of this animal’s body weight. Tag weight in all individuals tested was less than the 2% rule set by Winter (1983) and considered not to affect adversely the behaviour of tagged individuals.

3.2.2 Captive trials

In order to test for potential negative effects of tag presence and associated behavioural compensation mechanisms, ten lemon sharks, ranging from 97 - 135 cm in TL (Table 3.1), were caught and tested in captive trials (see section 2.4 for captive housing details). Sharks were tested in a standardised environment for 12 h both before and after tag attachment. All sharks were given 24 h post capture to become acclimatised to the pen environment before beginning trials. Only one shark was present in the pen at any one time to avoid interference behaviour. Sharks were then observed for two 6 h periods over two consecutive days during daylight hours to establish ‘control’ conditions, i.e. without tag attached. Two observers simultaneously recorded the type and time of all behavioural indicators. After the two days of control testing the shark was caught using dip-nets, transferred to a 1.5 m holding tub and the accelerometer tag package was attached. Once the tag package was in place the shark was released back into the pen and given another 24 h to recover, then two days of 6 h trials were repeated in the same manner as before the tag attachment.

Table 3.1 Summary data for sharks caught and used in captive trials. Shark denoted by PIT (passive integrated transponder) tag number. TL = total length of the shark, M = male, F = female.

Shark	Sex	TL (cm)
15C	F	104
C62	M	97
505	M	102
04E	M	116
000	M	105
36A	F	122
511	F	106
219	F	109
411	M	135

One of the most important concerns for externally attached devices is how it affects swimming behaviour and kinematics. Chafing behaviour, a means to remove unwanted parasites and foreign bodies (Myrberg & Gruber, 1974), was considered a reasonable indication that sharks with fin-attached data loggers were attempting to dislodge or remove a foreign object such as the tag. Accurate time recordings were taken of every chafing event. In addition, the weight, position and physical drag of the tag package could reduce hydrodynamics of sharks, especially with fin attached tags (Lowe et al., 1998; Thorstad et al., 2000). Changes to swimming behaviour were, therefore, examined by measuring each sharks swimming speed and tailbeat frequency between two marker poles set 4 m apart in the observation pen (see Fig. 2.4). As a shark's snout passed the first marker a timer was started and stopped again once its snout reached the second marker. At the same time a second observer counted the number of tailbeats whilst moving between the poles. One tailbeat was defined as the tail moving from a straightened position along the midline of the animal to a full extent, in either direction, and then back to the midline. This definition represents half of the commonly applied definition for a tailbeat (a complete to and fro tail movement in both directions). The reason for this is that, over a short distance, this allows for more accurate measurement of kinematics and differences in mechanical effort. Twenty simultaneous recordings of swim speed and tailbeat frequency were made at the beginning of control trials and 20 at the beginning of tagged trials. If a shark turned or was disturbed, the observation was rejected. Finally, tags affecting swimming efficiency, may influence time spent resting for energy recuperation, therefore the time sharks spent resting throughout trials was also recorded.

To control for the effects of hunger and feeding behaviour, sharks were fed 12 h before starting trials. In the control trials this was around 12 h after capture and for the tagging trials around 12 h after attachment. This method also served to set the level of satiation and eliminate the issue of sharks potentially changing behaviour over the observation period as they grew hungrier.

3.2.3 Free-range shark tagging

To measure impacts of capture, handling and tag attachment, a series of wild tagging trials were conducted. These trials aimed to record activity and track the movements of sharks immediately upon release from a tagging vessel. Seven sharks, ranging from 119 - 169 cm TL, were caught, tagged and released for free-range trials (Table 3.2). Mean handling duration was 15 min ± 2.6 SE (Standard Error); all sharks caught were cheek hooked and swam away from the vessel quickly upon release. Sharks were caught from the Bonefish Hole area of Bimini, south of the north island. This area was selected as it is a known nursery ground for young lemon sharks (Guttridge et al., 2012). Accelerometer tags were set to begin recording data immediately upon release of the shark. Once released, the sharks were actively tracked using a shallow water vessel using portable ultrasonic telemetric equipment (model USR-08, Sonotronics). Sharks were tracked continuously for as long as possible during daylight hours.

Shark locations were recorded every 5 min with a handheld (Garmin 72H) Global Positioning System (GPS) together with a compass bearing and estimated distance (to nearest 5 m) from the animal. Visual contact was maintained when possible, but maximum detection range for the PT4 transmitters was 80 m, thus allowing crews to maintain accurate distance estimates without visual confirmation. All sharks were tracked intermittently for five days after tagging. Samples of these data, between 24 h and 36 h after release, were used as control tracking data, representing the movement habits of the sharks after a significant period of recovery.

3.2.4 Data analysis

All data recorded for this research were interpreted relative to the research objectives set out in section 3.1. Interrogation of the captive data showed these data were non-normally distributed and as such were tested using non-parametric Wilcoxon signed rank tests to assess variance between control and tagged samples. This test was selected as it is commonly used to test for differences between individuals subjected to different conditions and does not assume normality in the distribution of the data. Acceleration data were explored and analysed using Igor Pro v 6.37 and Ethographer, as described in section 2.8.2. ODBA was used to quantify fine-scale swimming behaviour, post release (Whitney et al., 2010). ODBA data were analysed to determine the rate at which sharks recovered after release. Accelerometers produced 1800 data points every minute and a decimate function within Igor Pro was used to yield mean values for every minute. Data from 0 - 10 min after release were compared with the subsequent 11 - 20 min data using an unpaired *t*-test. Sequential testing of samples was carried out along the time-series (incrementally by 1 min) until no significant difference was generated between samples, indicating a levelling in swimming activity. This method proved to be the most intuitive approach to defining inter-sample variability. Tracking maps for each shark were constructed using ArcGIS version 10.0. Distance-to-shore data were acquired by applying a ‘NEAR table’ function to tracking data within ArcMap v 10.0. A Spearman rank correlation coefficient was used to determine the strength of associations between distance data and time for 1 h samples of both release tracking data and the subsequent control tracking data.

3.3 Results

No significant differences were found between behavioural indicators before and after tag attachment for any shark in the captive trails (Wilcoxon signed ranks tests, all $P > 0.05$). A comparison of mean data across all sharks also found no significant difference between control and tagged tests (Wilcoxon signed rank tests, all $P > 0.05$; Fig. 3.1). All sharks displayed consistent behaviour throughout trials and all fed readily post-capture and post-tag attachment.

In the free-ranging experiment, an initial period of elevated ODBA, and thus swimming activity, was observed immediately after release from the tagging vessel (Fig. 3.2). However, all seven individuals returned to a steady swimming state relatively quickly (mean = 12 min ± 4.4 SE; Table 3.2).

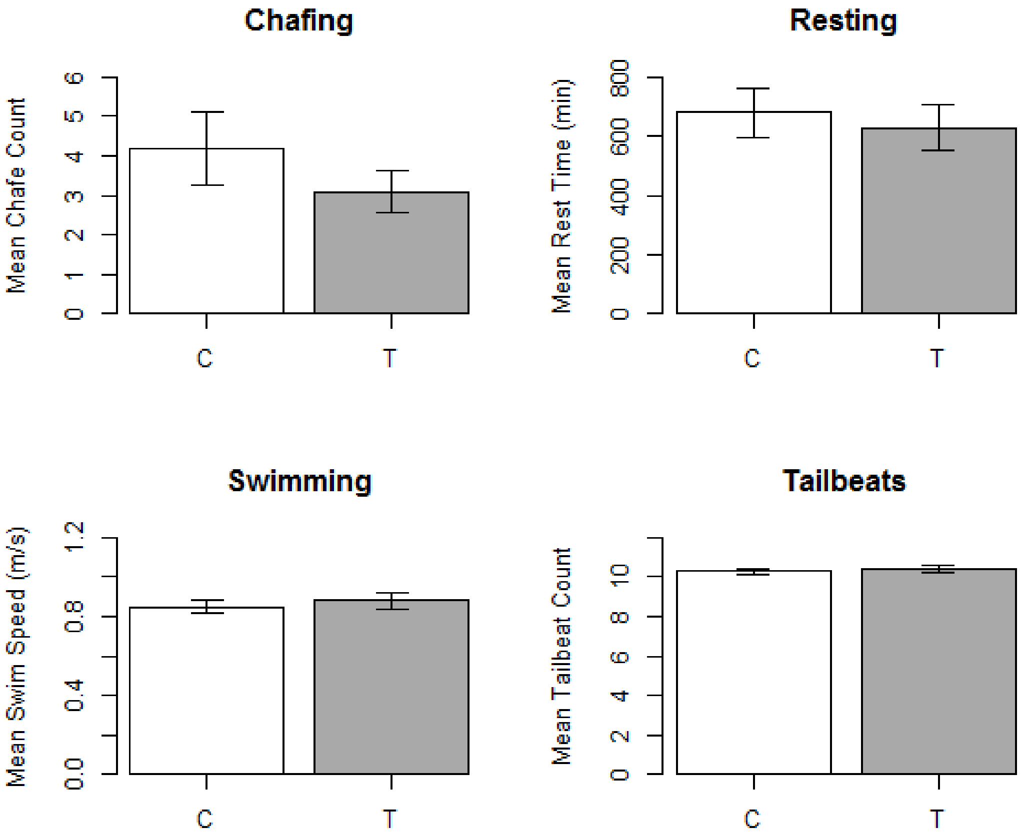


Fig. 3.1 Comparisons of mean totals ± SE for chafing and resting (n = 10) and swimming and tailbeats (n = 7), C = control, T= tagged. There were no significant differences found between control and tagged sharks for any indicator (Wilcoxon signed rank test, *P* > 0.05).

Table 3.2 Summary data for sharks used in wild deployments. Shark denoted by PIT (passive integrated transponder) tag number. Handling duration derived from the time a shark became hooked to the time of its release from the boat. M = male, F = female.

Shark	TL (cm)	Deployment Date	Sex	Handling Duration (min)	Time to recovery (min)
082	119	19/03/2014	M	14	6
C69	148	19/03/2014	F	30	3
773	169	16/07/2014	F	13	15
901	122	03/09/2014	F	11	10
E07	160	03/09/2014	M	11	11
345	129	29/09/2014	M	16	37
F7F	132	29/09/2014	F	10	4

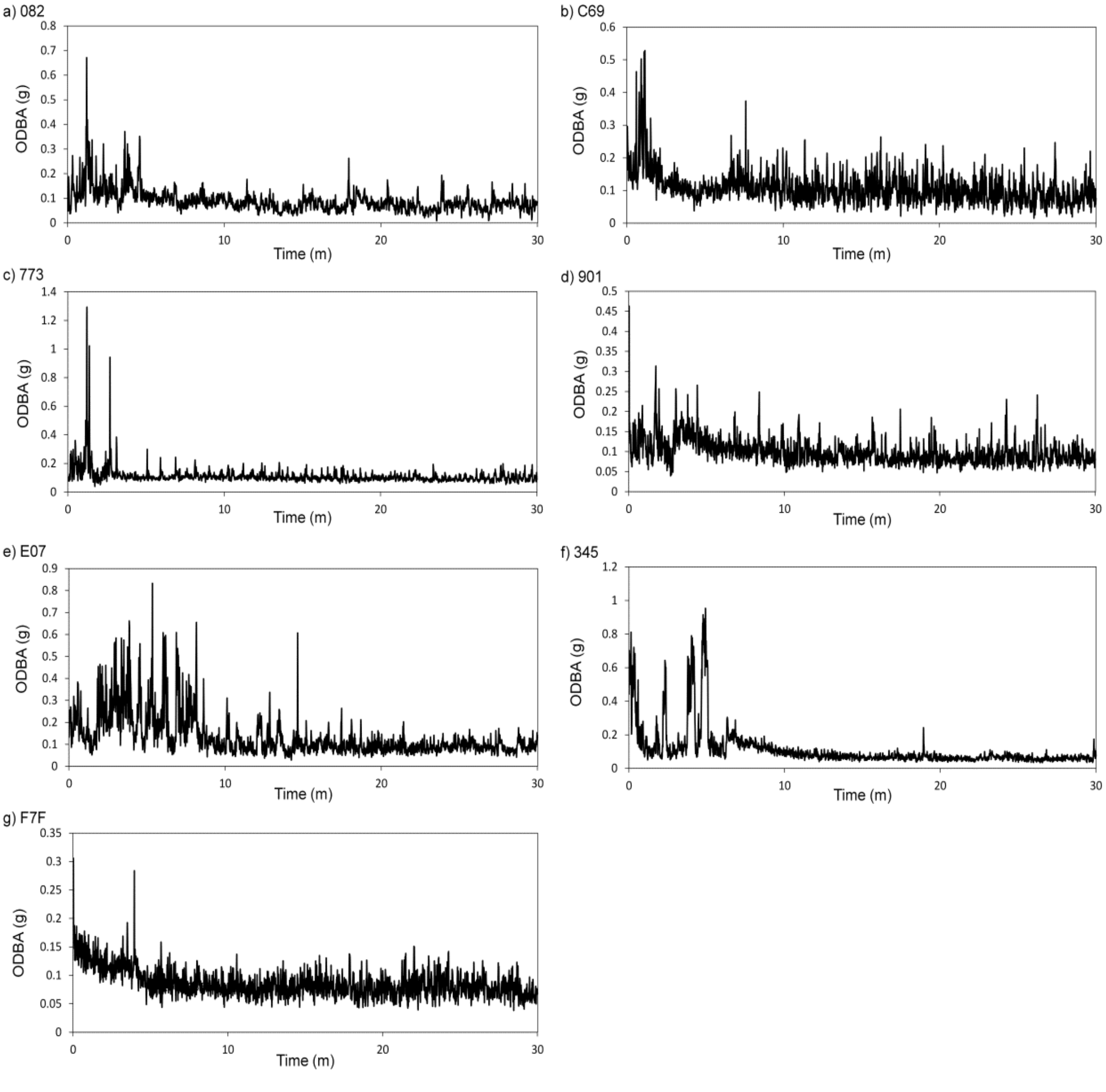


Fig. 3.2 Acceleration profiles for all free-ranging sharks, displaying overall dynamic body acceleration (ODBA) over the first 30 min of data logging after release from the tagging vessel.

Five sharks were successfully tracked for periods between 1-4 h post-release. Tracks of two sharks (901 and 345) were halted due to boat and equipment failure, respectively. All successfully tracked sharks moved towards the shoreline of the north island (Fig. 3.3). Once within 100 m of shore, all sharks remained there for prolonged periods. This behaviour persisted for a mean observed 90 min and continued until termination of tracks for all sharks except C69. Although this individual displayed the same pattern as other sharks after release, C69 began moving further than 100 m from shore 140 min after initial tagging whilst still being tracked. Spearman rank correlation revealed significant covariance between distance-to-shore and time during the release tracking period for all sharks except C69, for which no significant correlation was identified. Tests of control tracking periods indicated no significant covariance for any shark with exception of E07, for which a significant positive correlation was found (Table 3.3, Fig. 3.4).

Table 3.3 Spearman rank correlation between distance-to-shore and time. *P* = result from test over 1 h release period and *P* (control) = result from test over 1 h control period.

Shark ID	Track date	*P*	*P* (control)
82	19/03/2014	-	ns
C69	19/03/2014	ns	ns
773	16/07/2014	- - -	ns
E07	03/09/2014	- -	+ + +
F7F	29/09/2014	- -	ns

ns, P>0·05; + or -, 0·05≥P>0·01; + + or - -, 0·01≥P>0·001; + + + or - - -, P≤0·001

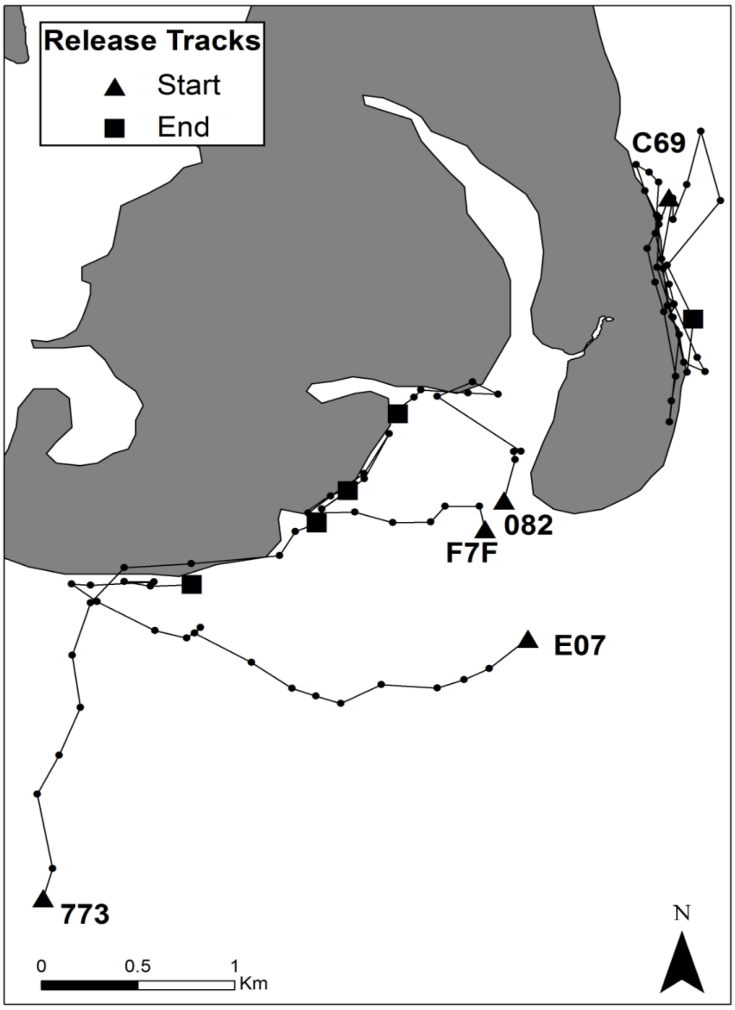


Fig. 3.3 Map displaying tracking coordinates for each shark upon release. Start = location of capture and tagging. End = location at which track was terminated.

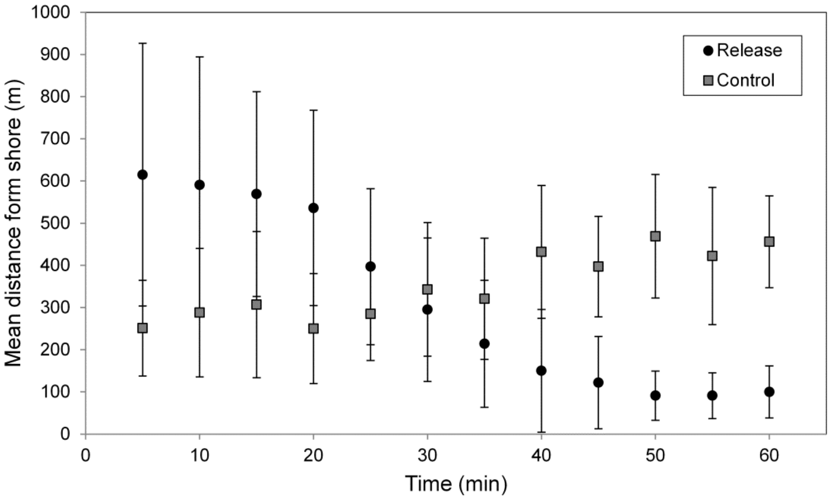


Fig. 3.4 Scatter plot displaying mean distance-to-shore (± SE), per 5 min interval. Release = mean total distance from shore for all sharks, over a 1 h period, immediately after release. Control = mean total distance from shore for all sharks, over a 1 h period, between 24 h and 36 h after release.

3.4 Discussion

3.4.1 Effects of tag presence

In terms of instrument-induced effects to behaviour in sharks, this study demonstrated that the presence of the accelerometer tag package itself had no significant influence on lemon shark behaviour. All sharks tested in the captive trials showed no variation from their routine behaviour, post attachment and shark behaviour did not change after recovery from handling. In teleost fishes, the presence of tagging devices shows conflicting effects on behaviour. For example, significantly slower swimming speeds were recorded in juvenile white sturgeon *Acipencer transmontanus* Richardson 1836 with externally mounted transmitters (Counihan & Frost, 1999), but in adult Atlantic salmon *Salmo salar* Linnaeus, 1758 swimming performance was not affected by external tags (Thorstad et al., 2000).

In sharks, Lowe et al. (1998) found that juvenile scalloped hammerhead sharks in captivity equipped with tailbeat transmitters worked harder and swam slower than those without. Bouyoucos et al. (2017) tested the effects of externally fin-mounted tagging devices on lemon sharks, finding the tag packages to alter the kinematics, activity levels and swimming performance of small sharks. These sharks were smaller than those tested in this study and tag packages were larger, which may explain the contrasting findings. The low tag to body weight ratio and small physical size of the packages used in this study could explain the apparent lack of any deleterious influence.

3.4.2 Rate of recovery from tagging

It is known that capture and handling procedures induce physiological and biochemical reactions that vary greatly in their nature and duration across species (Skomal & Mandelman, 2012). The way in which this is shown in post-release behaviour and energetics is poorly explained in the literature with very few studies describing responses and recovery (McKibben & Nelson, 1986; Lowe et al., 1998). Sundstrom & Gruber (2002) reported significantly higher swimming speeds in large juvenile lemon sharks, caught in Bimini, for the first 18 h after tagging with a speed-sensing transmitter, but the current findings, using ODBA as a measure of swimming activity, indicate a much quicker return to behavioural homeostasis, with elevated activity levels persisting for a maximum of only 35 min. The variation in these findings may be due to the methods of capture. Sundstrom & Gruber (2002) used baited long-lines and checked these lines every 4 h, whereas fishing methods used in this study ensured that the shark was always secured to the vessel within 5 min of hooking. The comparatively longer time spent on the long-lines before capture, tagging and release may induce a greater stress response in those animals and, subsequently, a longer time to recovery.

Gurshin & Szedlmayer (2004), using ultrasonic telemetry to measure post-release survival and movements of Atlantic sharpnose sharks *Rhizoprionodon terraenovae* (Richardson, 1836), found a higher net movement after initial release, but described a quick recovery from capture. Holts and Bedford (1993) found shortfin mako sharks *Isurus oxyrinchus* Rafinesque 1810 recovered from capture stress 30 - 90 min post-release. Rate of recovery is probably influenced by the method of capture, the duration and invasiveness of tagging procedures, and natural variations in animal condition prior to capture. The swift recovery times of sharks in this study could reflect the importance of brief and minimally invasive tagging processes. Further research should consider interspecific variations in behavioural stress responses and thus help to define best tagging practices (Wilson & McMahon, 2006).

3.4.3 Post-release behaviour

Beyond the assessment of post-release survivorship, studies investigating elasmobranch behaviour after capture, handling and tagging are rare. This is probably due to the logistical constraints associated with monitoring such behaviour, although new technologies enable valuable new developments. For example, Heithaus et al. (2001) used Crittercam technology to record video footage of tiger shark predation attempts and Skomal et al. (2007) used animal-borne video to assess post-release stress in grey reef sharks. Findings here demonstrated consistent patterns in post-release movement for all sharks. Such persistent proximity to shore, as observed in these sharks, is uncharacteristic for this population. Several studies investigating the movement habits of Bimini lemon sharks showed individuals to use open shallow lagoon areas at low tide, moving closer to the shoreline at high tide (Morrissey & Gruber, 1993a; Guttridge et al., 2012). The use of chum (800 g (frozen weight) mass of Atlantic menhaden *Brevoortia tyranus*) and baited hooks to capture sharks means that sharks may have moved large distances, potentially from near shore areas when attracted by the bait. It is therefore possible that sharks simply returned to this habitat once released. However, during different tidal phases chum slicks were, in some instances, running away from the shore.

The control tracking data aimed to represent movement behaviour of sharks, once given a significant period of recovery. These data showed no patterns similar to those found after release. It is then considered valid to contend that findings could represent a modification to space use patterns in response to the capture and tagging procedures. Such adaptive behaviour has been noted in other studies. For example, neonatal nurse sharks when occupying a habitat with available shelter, will hide under holes and rock crevices when disturbed by a potential predator (Garla et al., 2014) and grey reef sharks *Carcharhinus amblyrhynchos* (Bleeker,

1856) quickly vacate the area of a stressful encounter, i.e capture and tagging (McKibben & Nelson, 1986). Tiger sharks, equipped with satellite transmitters were found to move into deeper waters further offshore, upon release (Afonzo & Hazin, 2014).

Finding refuge is a common behavioural phenomenon found in many elasmobranch species (Holland et al., 1993; Heupel & Heuter, 2002; Knip et al., 2011). In many instances, finding refuge is observed as a predetermined behaviour, whereby sharks minimise risk, using certain habitat types at certain times and even congregating within these areas (Heupel & Simpfendorfer, 2005). It is possible that the findings here could represent an adaptive behaviour to seek refuge, in response to the stresses of capture and handling. However, further research with a greater sample size, longer track durations and consideration of other abiotic factors would give more firm conclusions relating to drivers of these observations.

3.5 Conclusions

This study demonstrates the applicability of combined accelerometer/acoustic transmitter tags to elucidate sub-lethal post tagging behaviour in a coastal shark species. Findings suggest no response to presence of the fin-mounted device and rapid recovery in terms of activity, post-release, but suggest potential alterations to space use patterns. It is likely that both responses to tag presence as well as capture and handling will vary from species to species. As such, it is important for tagging studies to incorporate behavioural testing of tagging into study design, thus improving the understanding of these aspects of behaviour and producing more reliable data.

Chapter 4 – A Quantitative Understanding of Habitat Use, Activity and Behaviour in a Site-attached, Coastal Shark Species (*Negaprion brevirostris*)

Observing fine-scale activity and behaviour of animals in their natural environment is necessary to better quantify habitat use and define the importance of key sites such as nurseries. Accelerometry and acoustic tracking techniques were used here to observe young lemon sharks using a coastal mangrove nursery site. Prior to observation of wild animals, sharks (n=7) were caught and observed in semi-captive conditions to calibrate tags and ensure accurate translation of acceleration data into specific behaviours. Tagging and subsequent tracking of wild sharks (n=23) revealed tidally associated patterns in movement. Sharks used areas closest to shore over high tide periods and moved into areas further from shore as tides fell. Accelerometer data collected from these sharks quantified these habitat use patterns, showing that sharks are generally less active when using nearshore areas, displaying increases in resting behaviour and increase their activity in areas further from shore, expressing bursting behaviour far more frequently. Accelerometer-logged temperature data suggest thermal associations in activity and behaviour, potentially representing a ‘hunt warm rest cool’ bio-energetic strategy. These findings represent a novel understanding of the activity and behaviour of this species in the wild and uncover potential environmental determinants of habitat use.

4.1 Introduction

The conservation and management of sharks has become a major priority of scientific research due to concerns over the declines of many elasmobranch populations (Stevens et al., 2000; Dulvy et al., 2014). Nursery sites are commonly deemed as critical habitats for sharks, providing food availability and protection from predation for juvenile sharks, thereby increasing recruitment to adult populations (Springer, 1967; Beck, 2001; Heupel et al., 2007). Effective management of such sites requires an understanding of how sharks use this habitat and what their requirements are from it. Much research has been dedicated to identifying nursery grounds and better understanding nursery habitat use in sharks. For example, Simpfendorfer & Millward (1993) investigated the use of a tropical bay as a nursery area by shark species of the families Carcharhinidae and Sphyrnidae. Different species were found to use the habitat for different durations and over different life stages and although diets between species were similar, little evidence for direct competition was found, emphasising the productivity of the site.. Parsons & Hoffmayer (2007) identified shark nursery grounds along the Mississippi and Alabama Gulf coasts in the US and found that differences in temperature, salinity and dissolved oxygen impacted the habitat use patterns of different species. DeAngelis et al. (2008) characterised nursery habitat partitioning between two species in the US Virgin islands and Conrath & Musick (2012) reviewed nursery use in sharks from the perspective of reproductive biology (see Heithaus, 2007 for theoretical review of nursery habitat for sharks). Some studies have used acoustic tracking techniques to better understand the habitat selectivity and home range of sharks using nursery areas (e.g. Holland et al., 1992, scalloped hammerhead sharks; Recchisky & Wetherbee, 2003, sandbar sharks; Heupel et al., 2004, blacktip sharks; Wetherbee et al., 2007, lemon sharks). The juvenile and sub-adult lemon shark populations of Bimini are some of the most thoroughly studied of any elasmobranch population. Research dedicated to the Bimini populations initially focused on understanding of habitat use in the spatial context. Gruber et al. (1988), and Morrisey & Gruber (1993a) focused research into acoustic telemetry and investigating movement patterns and habitat use, demonstrating distinct patterns of movement within the habitat, associated with physical characteristics. Morrissey and Gruber (1993b) showed that young sharks use well-defined home ranges and core areas of use. Further studies measured bioenergetics for this species, again using telemetric tools (Sundstrom et al., 1998). More recently Gruber et al. (2001) used mark-recapture techniques to estimate the survival rate of a cohort of sharks, finding evidence for strong density dependant survival. DiGirolamo et al. (2012) found temperature prefrerences in young sharks using an enclosed lagoon habitat and Feldheim et al. (2001) modelled population genetic structure, indicating gene flow throughout the western Atlantic stocks. Despite the breadth of existing literature, little is still known about the fine-scale behaviour and activity of lemon sharks. Scientists are often limited in their ability to observe specific behaviour. Acoustic telemetry defines spatial elements well, but information on specific, quantitative behaviour is still lacking (Whitney et al., 2013). Little is also known about how the physical features of the environment influence behaviour in nursery-bound sharks (Heithaus, 2007). Bio-logging devices are now advancing understanding of behaviour at a higher resolution. Acceleration data loggers measure both orientation and true movements of animals at a sub-second scale. Even the most subtle or rapid postural changes and movements are recorded, providing an accurate interpretation of behaviour.

As part of a large-scale resort development in the western areas of North Bimini (see Fig. 2.1), large segments of mangrove shoreline have been removed, significant dredging undertaken as well as other other activities. Jennings et al. (2008) found that, in areas affected by dredging for the development, first year survival of juvenile lemon sharks declined by 23.5% and that the habitat itself was significantly degraded. Development plans continue to extend into the natural environment of the Bimini Islands, highlighting a pressing need to better understand the value of this environment to a species with such sensitivity to habitat degradation.

This research employed both captive and wild trials, and used a combination of acoustic telemetry and acceleration data loggers to: 1) develop an ethogram of behaviour for lemon sharks and accurately categorise behaviour from acceleration data; 2) examine fine-scale patterns in behaviour and activity for young, free-ranging lemon sharks using the Bimini nursery site; and 3) consider the biological and environmental factors driving the observed patterns. By developing a quantified map of space use, this research aimed to better understand the interactions of lemon sharks with the nursery habitat and determine their requirements from this environment. Given the large-scale changes to vast areas of lagoon habitat in Bimini and the apparent deleterious impacts, this information is timely and important.

4.2 Methods

As described in chapter 2, all sharks (n = 30) were caught for this research using standardised gillnetting, and traditional rod and reel fishing methods (further details in section 2.3). Sharks tested in captive trials (n = 7) were caught on the south side of the island to minimise transport time and stress for the animals. Over the course of the trials sharks were observed for signs of stress (skin blotching) and were always released back into the wild at site of capture. Sharks tagged for wild trials (n = 23) were tracked using passive and active acoustic tracking techniques. Range tested, submersible receivers were set up strategically across the habitat (see section 2.5 for more information) and logged presence-absence records for sharks within their range over the deployment period. Active tracking telemetry equipment was used to find and track sharks at liberty. Active tracking was scheduled for small teams to track for 8 h shifts over day and night, throughout the deployment period. Tag package attachment for all sharks in captive and wild trials was accomplished swiftly under controlled circumstances, as described in chapter 2. Sharks were recaptured using the same techniques as outlined for capture and active tracking tools were used to assist in this process (further details in section 2.6).

Figures and statistical tests were produced using R (v 3.2.3, R Core Development Team 2012) unless stated otherwise. Decisions regarding assumptions of normality and statistical approach were made following protocols outlined by Zuur et al. (2010).

4.2.1 Captive Ethogram trials

Prior to tagging and tracking of wild sharks, a series of captive trials were conducted with the aim of developing an ethogram of behaviours that would then be used to translate wild shark acceleration data into different specific behaviours. Several key modes of behaviour, listed below, have been regularly recorded in wild sharks using the Bimini nursery site. Therefore captive trials focussed on isolating these behaviours from acceleration data.

1) Resting: A shark laying, unmoving on the seabed. Lemon sharks in Bimini commonly rest, both in captivity and in the wild.
2) Steady swimming: A shark swimming at a steady rate with no discernible stress or excitement stimulus. Different individual sharks expressed distinct swimming kinematics and varied swimming speeds at different times.
3) Bursting: A high speed swimming state only expressed in sharks responding to a distinct stimulus. Bursting was intermittently observed during times of foraging and feeding when sharks became more active overall. Sharks also expressed bursting behaviour when frightened by a sudden splash or movement.

Sharks were caught and held in semi-captive pens and, after a 24 h period of recovery and feeding, had a tag package attached. The accelerometer was set to begin recording at a specific time the following day. A team, positioned on the observation tower, equipped with a data sheet and watch (synchronised exactly with the time setting of the accelerometer) recorded all behaviours observed as well as precise (to the second) time changes between distinct behaviours.

4.2.2 Wild shark tagging

To examine fine-scale behaviour and activity of lemon sharks in the wild a series of wild shark tagging and tracking deployments were conducted. Some accelerometer tags (all tags deployed up to and including July 2013) were set with a 24 h delay to begin recording data after a significant period of recovery time, thus effectively excluding effects from capture and handling in activity and behavioural data. Other tags (tags deployed from March 2014 to

October 2014) were intentionally set to begin recording upon release, to address objectives set for Chapter 3 of this thesis. Findings relating to recovery periods for these sharks showed no shark taking longer than 35 min to recover, returning to normal activity and behaviour. A conservative first one hour of recording was excluded from data analyses.

4.2.3 Data analysis

Acceleration data were explored and analysed using Igor Pro version 6.37 and Ethographer, as described in section 2.8.2 (see Appendix 4 for sway acceleration profiles for all sharks across the deployment period). Using the procedures described in section 2.8.2 relating to behaviour discrimination by acceleration ethogram, behavioural elements were classified by a *K*-means clustering algorithm for each 6 h captive trial, for each shark. A Mask analysis function within Ethographer grouped these clusters into the distinct behaviours for all sharks.

To analyse patterns in diel and tidal cycles, time bins were allocated. Sunrise and sunset times as well as all tide times were acquired from the NOAA tides and currents database (North Bimini Station ID TEC4617: https://tidesandcurrents.noaa.gov/noaatidepredictions). Data were grouped into dawn, day, dusk and night using sunrise and sunset times rounded to the nearest 5 min. Dawn and dusk periods were set as one h either side of the respective event time (e.g. dawn time = 06:00, dawn period = 05:00 - 07:00). Day and night groups contributed the hours in between these periods. Data were assigned to high, mid or low tide in the same way. All data recorded two h and ten min either side of the high tide time were defined as high tide data. The same grouping was made for low tide data. All data recorded outside of either of these boundaries were labelled as mid tide data. These time groupings were all equal (4 h and 20 min for each tidal bracket) and summed to 13 h total, allowing for the shifting tide times over successive days. The data within these time-binned groups are from this point referred to as high tide phase, mid tide phase or low tide phase.

Acoustic tracking data were plotted and examined using ArcMap v 10.0. All SUR data were filtered for spurious detections, as described in section 2.8.1. Detection plots for SUR data were created in ArcMap using categorical display of total detections for each unit. Near distance tables, generated within ArcMap, were used to determine distance to the shoreline for all active tracking data points, for all sharks. After testing the data for assumptions, ANOVA was used to test for differences in distance-to-shore data at different tidal phases. Individual tests were conducted for sharks with at least ten data points recorded in each tidal phase and a singular test was conducted on all data grouped as a whole.

Plymouth Routines in Multivariate Ecological Research (PRIMER v 6, PRIMER-E, Plymouth, UK) with the PERMANOVA + add-on package was used for multivariate analysis of accelerometer data (ODBA, temperature (C°) and depth (m)) (Anderson et al., 2008). Time-series accelerometer data are recorded as acceleration in a positive or negative direction away from a null *g* value, representing a waveform. As such these data are non-normal and are not transformable to meet assumptions of normality. PRIMER uses permutation tests that make very few assumptions about the form of data and thus make these tests extremely robust, transparent, easily interpretable and widely applicable (Anderson et al., 2008). The PRIMER package has been applied to a variety of data structures including remote sensing, wavelength data. ODBA data for all sharks were tested using PRIMER, with each individual shark as a separate variable. Both ODBA and environmental data were tested against tidal (high, mid, low) and diel (dawn, day, dusk, night) conditions. Accelerometers record at sub-second frequency producing vast amounts of data. For each shark, one min mean ODBA values were used in analysis. ODBA data were square-root transformed and a Bray-Curtis resemblance matrix was applied, as per Anderson et al. (2008). Non-metric multi-dimensional scaling (nMDS) plots were used to present patterns in the data relative to tidal and diel conditions. Environmental data (temperature and depth) were first normalised, and a Euclidian distance-based resemblance matrix was created, as per Anderson et al. (2008). Principal coordinate ordinations (PCO) were used to analyse these data in relation to tidal and diel phase conditions. Two-way permutational multivariate analysis of variance (PERMANOVA), with 9999 permutations, was used to statistically describe any differences in data between these conditions. To assess strength of association between biological and environmental data, Spearman's rank correlation coefficient was calculated via seriation with 9999 permutations of the data using the RELATE and BEST routines within PRIMER.

Behavioural data were analysed as percentage of time spent in each behavioural group, either steady swimming, resting or bursting. Resting and bursting behaviours are associated with energy management and foraging, and thus data for each shark were individually compared against tidal and diel conditions using Kruskal-Wallis tests.

Temperature sampling was conducted at random locations within the study site (Fig. 4.1) (for information on how random sites were selected see section 5.2). A shallow water skiff drove to the site using a hand-held GPS unit. At each site a series of environmental data, including temperature, were taken using a YSI water meter to meet objectives set for other components of this research (for details see section 5.2). These temperature data were used to generate a map of temperature ranges across the study site at both high and low tide phases. Distance to shore was calculated for each sampling site (Fig. 4.1) and a simple linear regression model was used to determine any relationship between distance to shore and water temperature. A model was generated for all data collected from high tide sampling locations and another for all those collected from low tide locations. Student's t-tests, for high and low tide samples, tested differences between mangrove edge and non-mangrove edge samples (mangrove edge < 50 m from mangroves, non-mangrove edge ≥ 50 m from mangroves).

4.3 Results

4.3.1 Captive trials

Seven sharks, ranging from 97 - 122 cm were caught and tested in captive trials (Table 4.1). All sharks recovered well from capture, tagging and pen holding, and all were released back into the wild at site of capture.

As described in section 2.8.2, *K*-means clustering was used to discern behaviours from acceleration data. The clustering process works by breaking down the time series into a number of clusters defined by the user. For acceleration data representing behaviour we can define each cluster as representing a distinct behavioural element. The program groups every second of the time series data into a cluster based on wave characteristics (amplitude and cycle length). Increasing the number of clusters increases the sensitivity of the automated routines in clustering the time series data, separating the data into more groups. If too many clusters are applied, however, the process over separates the data (Sakamoto et al., 2009). By running the *K*-means clustering process with varying numbers of clusters, one can determine the optimum number of behavioural elements to distinguish between changes in actual behaviour without over-clustering. Here, grouped acceleration data were corroborated with timed observations from captive trials, concluding that twelve behavioural elements proved to be adequate to cover all behaviour expressed by sharks (Fig. 4.2). For this research (and other studies using this approach, see Sakamoto et al., 2009) it was necessary to cluster more behavioural elements than distinct behaviours. These behavioural elements represented the same pattern of behaviour with different intensities of movement and periodicities. A Mask analysis function within Ethographer was then used to group the 12 behavioural elements into the three described behaviours of resting, steady swimming and bursting (Fig. 4.2). The accuracy of this procedure was validated with direct observation data for each captive, tagged shark, showing in each instance that observations of behaviour and transitions between behavioural states matched closely with clustering results in the acceleration data.

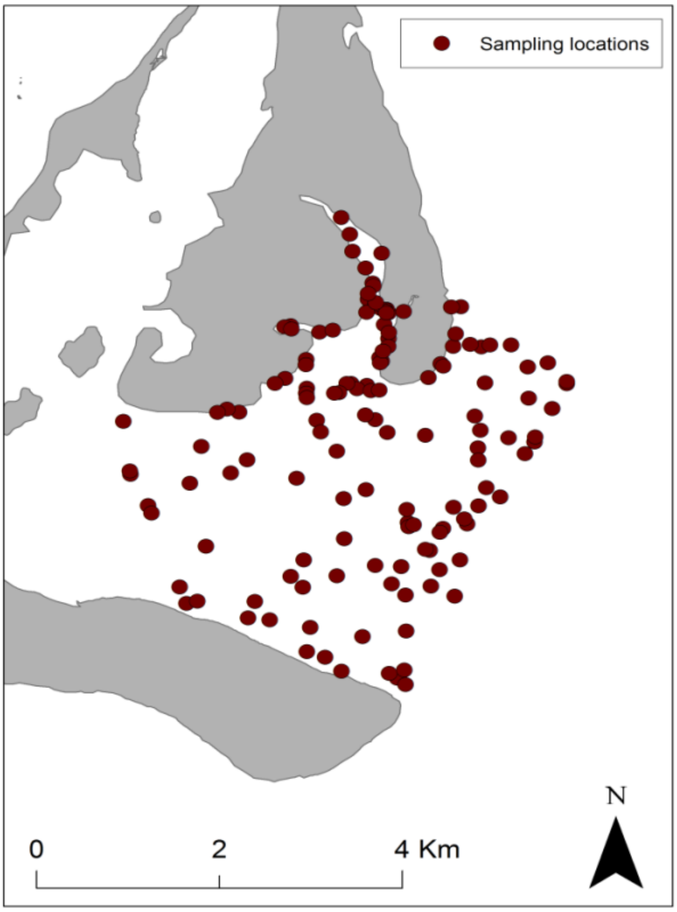


Fig. 4.1 Map displaying locations of environmental sampling across the study site. Water temperature was recorded at each location. Locations were selected randomly using a random-points generator in ArcMap v 10.0.

Table 4.1 Summary information for all sharks caught and tested in captive ethogram trials over the course of the study. TL = total length. M = male, F = female

Shark ID	TL (cm)	Sex	Trial Date
15C	104	F	May-12
C62	97	M	Nov-12
505	102	M	Feb-13
04E	116	M	Jun-13
36A	122	F	Apr-14
511	106	F	Aug-14
219	109	F	Sep-14

4.3.2 Wild trials

A total of 23 sharks, ranging from 78 to 169 cm (TL), were captured and tagged for wild trials. Of those sharks, three were lost at some point throughout the trial period and one accelerometer tag failed, leaving 19 complete five day deployments (Table 4.2). In translating acceleration data into specific behaviours, wild deployment data were subjected to the same procedures as described for captive trials.

4.3.2.1 Patterns of space use

Data collected from the SUR array show that, in general, sharks used areas closest to the shoreline over the high tide phase and areas furthest from shore during low tide phase (Fig. 4.3). 64.7% of all high tide detections were logged between three receivers (7,10 and 16) with the greatest number of all detections (39%) at receiver 16. 68.5% of all low tide detections were logged between four receivers (3,4,5 and 7) with 26.2% of all detections at receiver 7 (Fig. 4.3). For all sharks, there were significant differences for total detections between high, mid and low tide phases (ANOVA P <0.05). A mean total of 1240.4 ± 24.1 SE detections were logged throughout the high tide phase and only 138.2 ± 48.0 SE during low tide phase. No observable association was found between habitat use patterns and the diel cycle with tidal patterns persisting across the diel phase. Distance-to-shore data derived from active tracking GPS data support these findings (see 2.8.1 for explanation of distance-to-shore calculations). Sharks were found to occupy areas furthest from shore at low tides (ANOVA, $P < 0.001$, Fig. 4.4). Patterns of movement and proximity to the shoreline varied across ontogeny with larger sharks showing less distinct tidal patterns (expanded upon in Chapter 6).

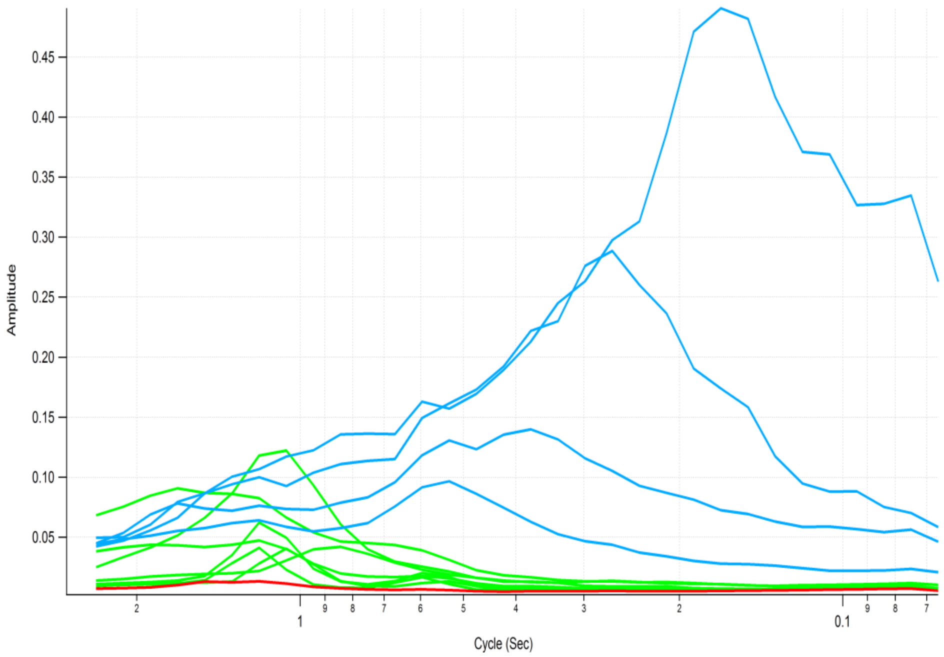


Fig. 4.2 *K*-means clustering results for shark 15C, representing amplitude and cycle length of acceleration for each of twelve behavioural elements. Each behavioural element is defined by the spectrum of an acceleration pattern. Behavioural elements are grouped by colour to represent distinct behaviours (red = resting, green = steady swimming, blue = bursting)

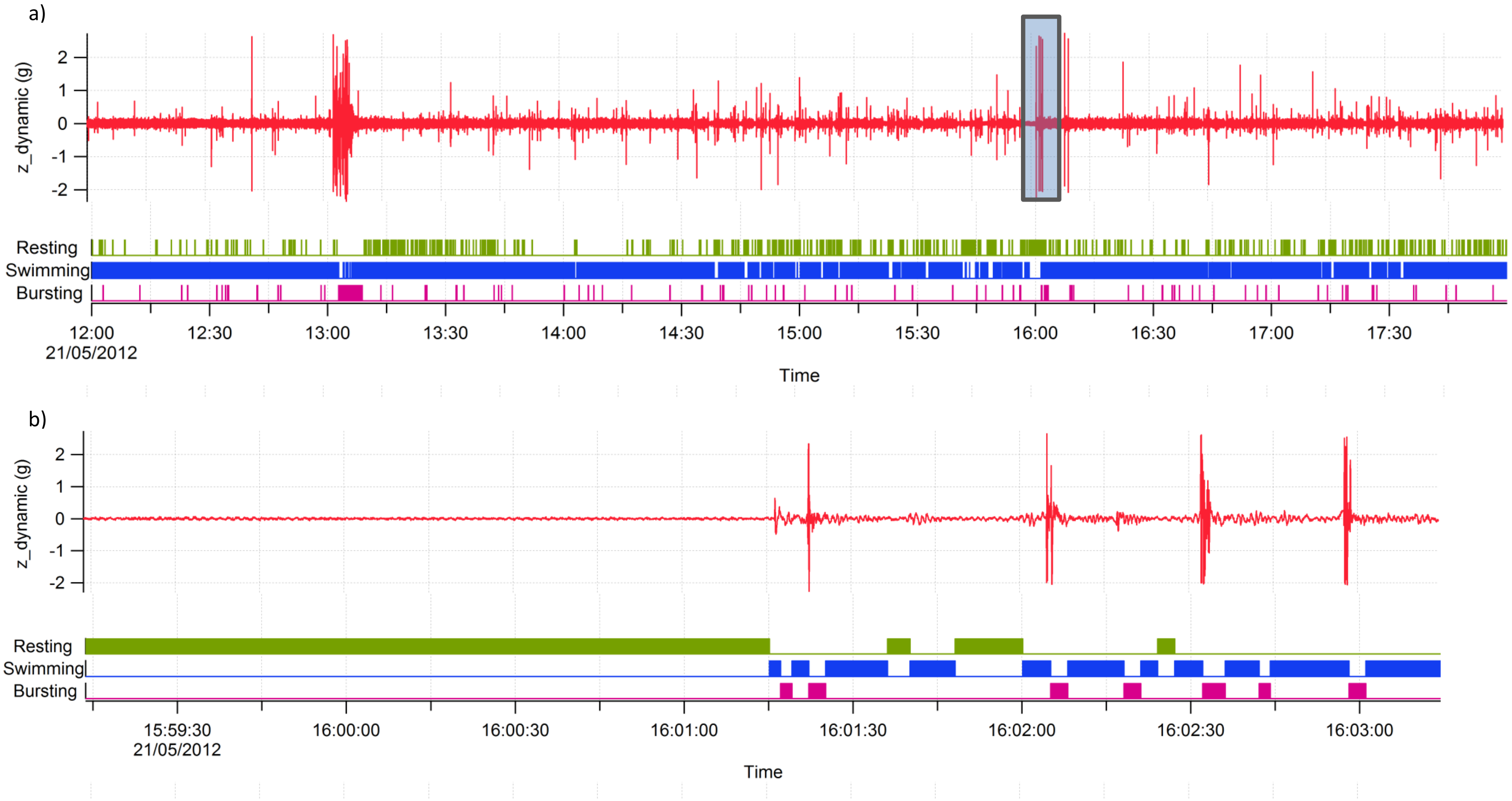


Fig. 4.3 Acceleration profile and associated masked clusters for specific behaviours, a) time-series of 6 h captive observation trial for shark 15C, the grey box identifies a section of the time-series zoomed and presented in b) showing transition between behavioural states, confirmed as accurate by direct observation records.

Table 4.2 Summary information for all sharks caught and tracked for wild trials. Greyed individuals were not used in data analysis due to either loss or failure of tag package. Acoustic tag and frequency relate to the ping codes of acoustic transmitters. TL = total length. M = male, F = female.

Shark ID	Deployment date	Recapture date	Acoustic tag	Frequency	Accelerometer tag	TL (cm)	Sex
4A73536511	29/07/2012	03/08/2012	688	80	A09317	84	F
4A0A043D40	29/07/2012	03/08/2012	3356	81	A09319	78	M
4A66401437	29/07/2012	03/08/2012	3357	82	A09318	78	F
4A75116708 (tag failed)	30/08/2012	05/09/2012	557	79	A08842	99	M
4B02774E50	30/09/2012	08/10/2012	3357	82	A09318	105	F
4A73233C69	30/09/2012	08/10/2012	688	80	A09317	101	M
4A67382C25	30/09/2012	06/10/2012	3356	81	A09319	103	M
4B1A556224	30/09/2012	09/10/2012	687	79	A09316	117	M
C25 second deployment	09/02/2013	22/02/2013	456	76	A09593	104	M
4A75116708	09/02/2013	27/02/2013	3356	81	A09590	101	M
4A676E2B4E (lost)	09/02/2013	-	488	75	A09594	103	M
C69 second deployment	21/07/2013	10/08/2013	3458	83	A09202	110	M
4B7B49385F	21/07/2013	29/07/2013	3464	69	A09591	120	F
4C3B001267	21/07/2013	30/07/2013	3674	76	A09593	135	F
C69 third deployment	19/03/2014	24/03/2014	3464	69	A09535	119	F
985121031817082	19/03/2014	24/03/2014	456	79	A09588	148	F
45486F6E6C (lost)	15/07/2014	-	444	70	A09208	187	F
4B026F7773	16/07/2014	21/07/2014	3656	71	A09843	169	F
4C4A492901	03/09/2014	26/09/2014	3656	71	A09843	122	M
48586F1E07	03/09/2014	08/09/2014	5557	78	A11530	160	F
4A65676345	29/09/2014	08/09/2014	5557	78	A11530	129	F
4C4A417F7F	29/09/2014	06/09/2014	6768	76	A11529	132	M
4A321E687D (lost)	29/09/2014	-	336	72	A09843	101	M

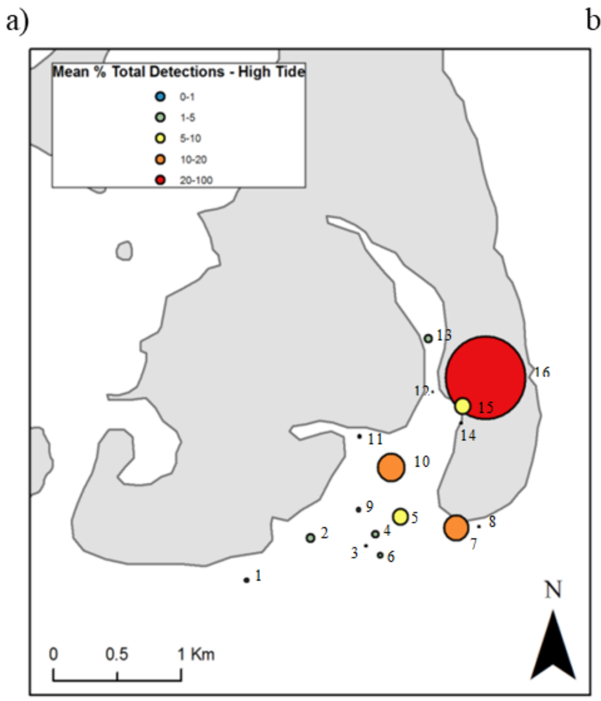


Fig. 4.4 Mean percentage total detections among SUR units, for all sharks, a) representing detections during high tide phase and b) representing detections during low tide phase. SURs with no detections recorded are not displayed.

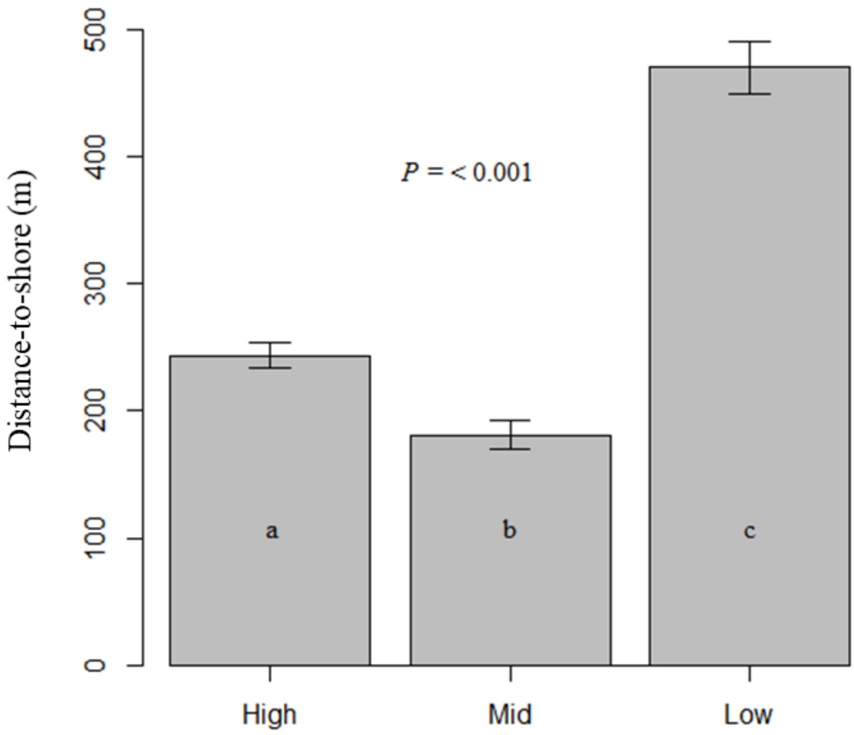


Fig. 4.5 Bar-plot showing mean distance-to-shore values between tides for all sharks. *P* value represents ANOVA result and letters denote groups based on Tukey's pairwise post hoc tests.

4.3.2.2 Activity patterns and associated environmental data

Non-metric MDS and a 2-way PERMANOVA of ODBA data revealed significant differences between tidal and diel phases (Table 4.3, Fig. 4.5). Most of the tagged sharks, in most instances, displayed greatest activity during the low tide phase (mean = 0.084 g ± 0.0007 SE) and were least active during the high tide phase (mean = 0.066 *g* ± 0.0004 SE). Sharks were also significantly more active during dusk periods (mean = 0.083 *g* ± 0.0021 SE) and least active at dawn and night (mean = 0.073 *g* ± 0.0004 SE, 0.074 *g* ± 0.0006 SE respectively). ANOVA for each individual shark found 13 of 19 sharks were significantly more active during either low or both low and mid tide phase. Two sharks showed no activity differences between tidal phase and four sharks were significantly more active during the high tide phase. These were four larger sharks no longer using near-shore areas as frequently. Diel patterns were more variable between individuals.

Table 4.3 Results for PERMANOVA tests of square root transformed ODBA data, based on Bray-Curtis similarities and normalised environmental (temperature and depth) data based on Euclidean distance. Two-way tests of two conditions (tidal and diel phase) were conducted in each instance.

Condition	df	pseudo-F p	MS	*P* (perm)
ODBA				
Tidal Phase	2	79.697	1500.5	<0.001
Diel Phase	3	55.655	1047.8	<0.001
Tide x Diel	6	55.355	1042.9	<0.001
Environmental				
Tidal Phase	2	105.71	28.622	<0.001
Diel Phase	3	625.64	169.64	<0.001
Tide x Diel	6	98.46	26.697	<0.001

Ten of 19 sharks were significantly more active at dusk than at other diel periods, but amongst others, activity patterns varied greatly across different diel periods. PCO and 2-way PERMANOVA of environmental data found significant differences in ambient temperatures and depths occupied by sharks at different tidal and diel phases (Table 4.3, Fig. 4.6). During the low tide phase, surrounding water temperatures were highest (mean = 28.61 ^{o}C ± 0.067 SE) and became lowest during high tide (mean = 27.66 ^{o} C ± 0.075 SE). Temperature variances between diel phase showed the greatest differences between groups. Highest temperatures recorded at dusk (mean = 29.81 ^{o} C ± 0.0328 SE) and lowest temperatures were recorded at dawn (mean = 27.09 ^{o} C ± 0.0161 SE). Concurrent with analysis of environmental data, shark activity positively correlated with temperature, with tags recording highest activity during periods of highest temperatures. The RELATE test indicated a significant relationship between ODBA and environmental resemblance matrices and the BEST function showed that the strongest correlation between ODBA and environmental data was achieved using only temperature and omitting depth (No. of variables = 2, $R^2 = 0.671$, $P < 0.05$).

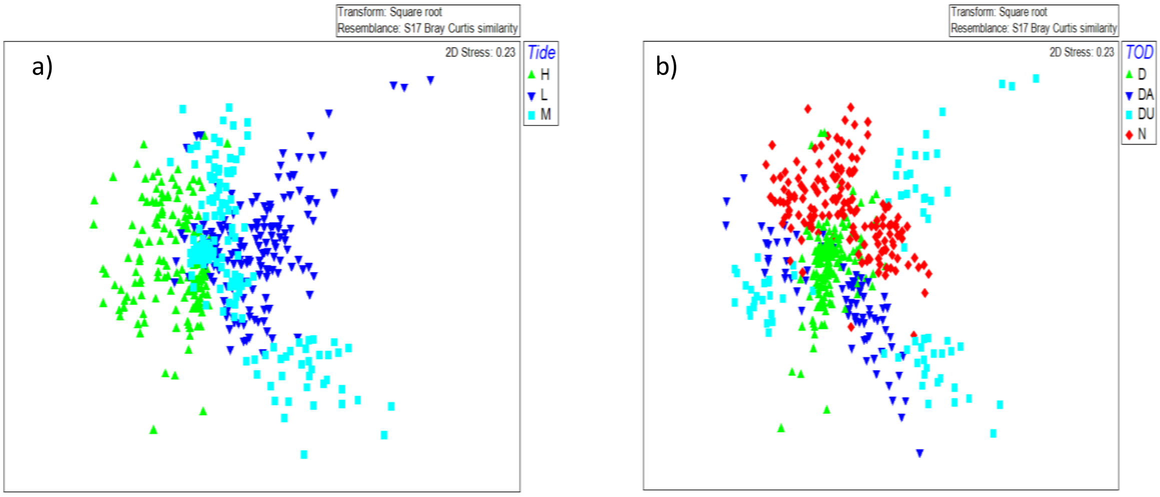


Fig. 4.6 non-metric MDS plots for ODBA across all sharks. a) shows colour designations of tidal phase for each sample (H=high, L=low, M=mid), b) shows colour designations for diel phase for each sample (D=day, DA=dawn, DU=dusk, N=night).

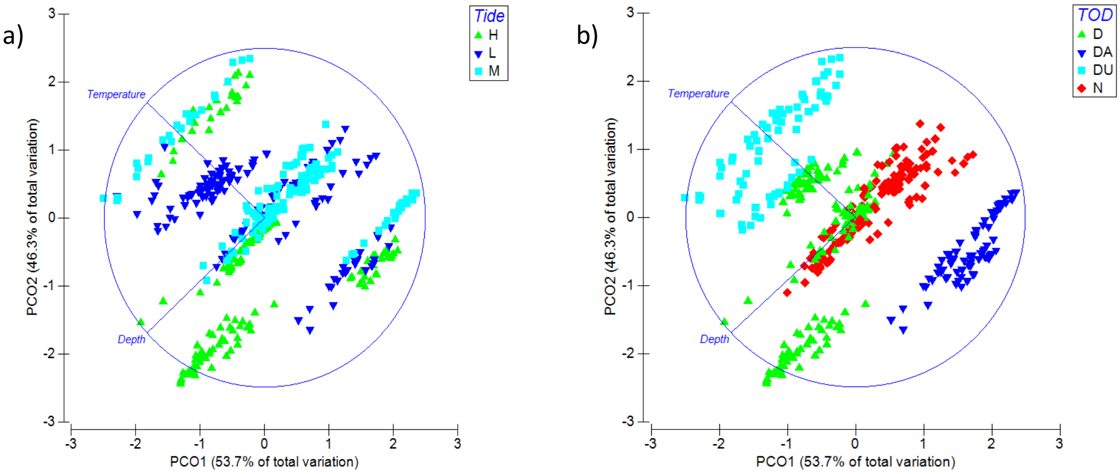


Fig. 4.7 PCO plots for all temperature and depth data for all sharks. a) shows colour designations of tidal phase for each sample (H=high, L=low, M=mid), b) shows colour designations for diel phase for each sample (D=day, DA=dawn, DU=dusk, N=night). Vectors show trends in data relative to individual variables.

4.3.2.3 Fine-scale behaviour

Analysis of behavioural clustering also found tide-related changes in key behaviours, resting and bursting (PERMANOVA $P < 0.001$). Individual tests found that percentage of overall time spent resting between tidal phases was significantly different in all but two sharks (E07 and 345), and of these, all but two sharks (267 and 082) showed higher percentage time spent resting during the high tide phase. When tested overall, a greater proportion of time was spent resting at high tide phase (mean = 12.24% ± 2.47 SE, Fig. 4.7), compared to mid and low tide phase (7.86% ± 1.27 SE and 3.56% ± 1.02 SE respectively). Conversely, bursting behaviour showed inverse patterns in occurrence. All but one shark showed significant differences in overall time spent bursting (F7F), and of these, 12 sharks displayed bursting behaviour more frequently at low and/or mid tide times. When tested overall, sharks showed significantly higher percentage time spent bursting at low tide phase (mean = 10.86% ± 0.61 SE) than mid and high tide phases (4.72% ± 0.56 SE and 3.96% ± 0.66 SE) (PERMANOVA $P < 0.001$, Fig. 4.7). Both resting, and bursting behaviour also varied significantly over the diel phase (PERMANOVA $P < 0.001$, Fig. 4.8). Although PERMANOVA indicated significant differences in resting between diel phases, individual Kruskal-Wallis tests found that no patterns emerge in particular diel periods selected for resting, with different sharks resting at different diel phases. Individual tests of bursting, however, showed distinct patterns at the diel scale between individuals, with the greatest mean percentage of time spent bursting recorded at dusk (mean = 7.43% ± 1.12 SE) and lowest at dawn (3.46% ± 0.65 SE). There were no significant differences between bursting behaviour at day and night phases.

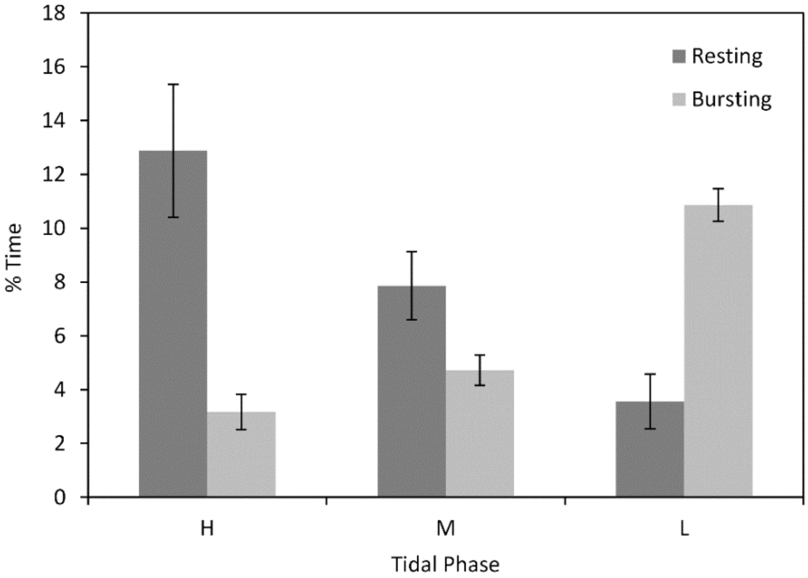


Fig. 4.8 Bar-plot displaying mean percentage time spent resting and bursting, for all sharks, across tidal phase. H = high, M = mid and L = low tide phase.

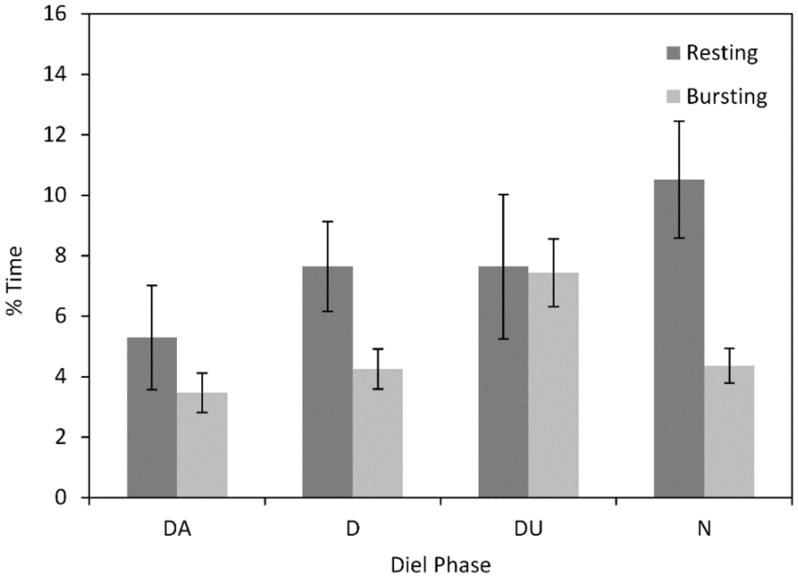


Fig. 4.9 Bar-plot displaying mean percentage time spent resting and bursting, for all wild sharks, across the diel phase. DA =dawn, D = day, DU = dusk and N = night.

4.3.2.4 Environmental testing of the study site

Linear regressions of distance-to-shore and temperatures at sample sites showed no relationship between these variables at either tidal period (High – d.f = 79, F = 1.427, r^2 = 0.004, *P* = 0.3801; Low –d.f = 57, F = 6.439, r^2 = 0.004, *P* = 0.257, Fig. 4.9) and student's t-tests found that temperature does not vary significantly between mangrove edge and non-mangrove edge habitat types at either tidal period (High tide – t = 0.42, *P* = 0.67, Low tide – t = 1.42, *P* = 0.15). These findings indicate that the temperature profile across the study site is spatially indifferent.

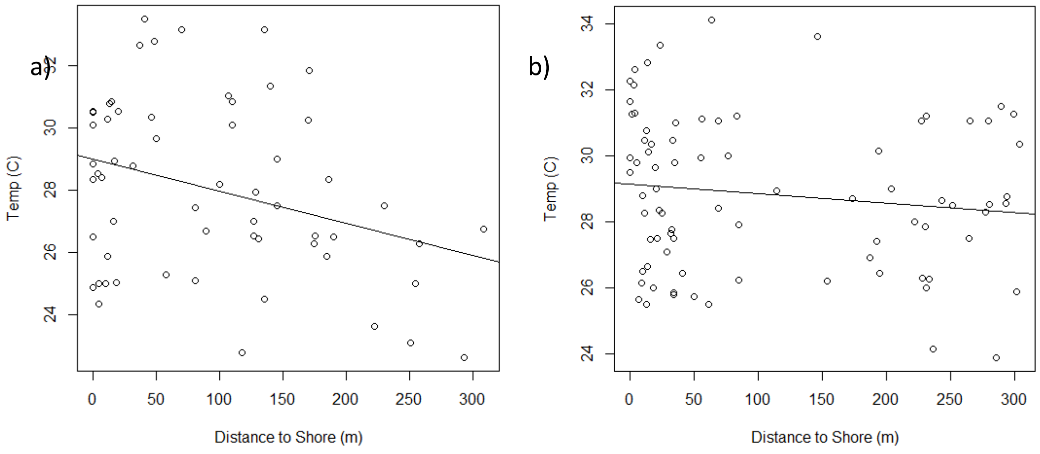


Fig. 4.10 Scatter plots displaying temperature data at each sampling location within the study site, at differing distances from the shoreline. a) High tide sample locations, b) low tide locations. Solid lines represent the regression line for the associated linear regression model. Data show no association between water temperature and distance-to-shore.

4.4 Discussion

4.4.1 Patterns of space use

Tidally-mediated patterns in movement were found for most sharks, based on active and passive telemetry data. In general, sharks used nearshore areas and shallow, mangrove-fringed inlets during high tide periods. At low tides, sharks used open areas further from shore. Tidal patterns in movement behaviour have been observed in a number of species. In leopard sharks, tidally driven movements were considered to be a means of accessing food resources (Ackerman, 2000). Shepard et al. (2006) also reported tidally linked patterns in diving behaviour of basking sharks that were considered to relate to movements of prey. Movements of juvenile sandbar sharks, using a nearshore area, were also found to relate to tidal patterns, with sharks movements being driven by the direction of tidal current flow (Medved & Marshall, 1983). The movements of the sandbar sharks in this study appeared to be driven by the acquisition of food resources but it is difficult to conclude through observations of movement alone. Studies of lemon sharks, at various sites, have found tidally-mediated patterns in movement for sharks of different age ranges (Wetherbee et al., 2007; Guttridge et al., 2012). These studies suggest that tidally-driven movements in lemon sharks are a means by which sharks avoid predators, using shallow near-shore areas at high tides, to avoid larger bodied predatory sharks able to access open lagoon areas at these times. Although there are few data to support this conclusively in these sharks, it is a valid hypothesis, given the theory that fish will use physically complex habitats to avoid predators (Laegdsgaard & Johnson, 2001) and captive, experimental trials on Bimini sharks have demonstrated refuging response in small sharks that aligns with findings from the wild (Stump et al., 2017). However, if sharks are using these areas only during higher tide times as a means of predator avoidance, it is important to consider why sharks then move into open lagoon areas instead of remaining in safer near shore areas at all times. It is likely that there is a fundamental ecological advantage to the animal in using these alternate areas. In this study, larger sharks became less bound to nearshore areas at high tides and spent time more consistently in open areas. As sharks grow their diet shifts and they become less vulnerable to predation. It is hypothesised that ontogenetic shifts in diet and higher energy requirements, combined with decreased predation threat, influence these shifts in movements (addressed in Chapter 6).

4.4.2 Activity and specific behaviour

Accelerometers recorded quantitative fine-scale data on activity and specific behaviour for all sharks. During high tide periods, sharks exhibited reduced overall activity and spent significantly more time resting. At low tides sharks became more active overall and displayed increased bursting behaviour. As well as the tidal phase, the diel cycle significantly affected activity and behaviour of sharks in this study. Trends in data showed that sharks were commonly most active at dusk and least active at dawn. Patterns in behaviour and activity of elasmobranchs have more commonly been associated with diel cycles than with tidal phase. Many studies have found that the diel cycle influences activity and behaviour for a range of species. For example, horn sharks and swell sharks displayed nocturnal patterns in activity when observed in a natural environment (Nelson & Johnson, 1970). Sixgill sharks *Hexanchus griseus* (Bonnaterre, 1788) make vertical migrations at dawn and dusk (Andrews et al., 2009) and locomotor activity of whale sharks, measured using acceleration data loggers, showed crepuscular patterns in activity, with sharks being most active at dusk, corresponding with periodic abundance of their prey (Gleiss et al., 2013). Diel patterns of activity in lemon sharks have been reasonably well documented; for example, Gruber (1982) initially reported crepuscular patterns in activity for lemon sharks, but subsequent lab and field studies showed sharks to be more active at night (Nixon & Gruber, 1988; Morrissey & Gruber, 1993a). More recent tracking studies concluded that large juvenile sharks are indeed crepuscular, more active at twilight times, and suggest that these times of lower light levels increase predatory efficiency in these sharks, potentially to make the most of vision, that in this species is adapted for low light levels (Sundstrom & Gruber, 1998). Other recent research, using accelerometer-derived estimates of lemon shark activity and behaviour, report a combined influence of tidal and diel phase, supporting findings from this study (Bouyoucos et al., 2018).

4.4.3 Environmental drivers

Data presented significant associations in both the tidal and diel phases of activity with temperature, as sharks were more active in warmer waters and less active in cooler waters. Increased ODBA and bursting behaviour associated with low tides and dusk periods were also times at which water temperatures were highest. At high tides and during dawn and night periods, when waters were coolest, sharks displayed lowest ODBA and increased expression of resting behaviour. It is therefore possible that these patterns are being driven by coinciding temperature shifts. Most biological processes are temperature-dependant and many ectothermic predators modify behaviour in response to temperatures (Magnuson et al., 1979; Papastamatiou et al., 2015). In sharks, temperature can influence metabolic efficiency as well as muscle and power output, in turn influencing foraging patterns and habitat selection (Miklos et al., 2003; Sims et al., 2006; Donley et al., 2012; Speed et al., 2012). By selecting for differing available temperatures animals can optimise physiological efficiency. This is known as behavioural thermotaxis and has been observed in several elasmobranch species

(Casterlin & Reynolds, 1979; Sims et al., 2006; DiSanto & Bennett, 2011). DiGirolamo et al. (2012) studied diel temperature patterns of lemon sharks in the neighbouring north sound nursery site in Bimini. This study found temperature differences within the site, and recorded sharks selecting warmer waters throughout the day until their peak at early evening, then moving to cooler waters during late evening and early morning hours. Morrissey & Gruber (1993a) found that lemon sharks, occupying the neighbouring North Sound habitat, used areas with higher temperature (> 30 °C) disproportionate to their availability. This shows a selection for warmer sub-habitat zones, although findings here indicate a lack of variance between areas at this study site.

Sims et al. (2006) proposed the 'hunt warm, rest cool' bioenergetic strategy for a benthic shark species, suggesting that foraging occurs at locations and/or times at which temperatures are elevated. Increased temperatures should improve metabolic rate and thus foraging performance. Resting at cooler temperatures will lower rates of metabolism and digestion, increasing time food is exposed to digestive enzymes and thus increasing digestive efficiency (Sims et al., 2006). Findings here, relating to activity as well as occurrence of specific behaviour, support this hunt warm, rest cool strategy for this population. Findings relating to environmental testing of temperature showed spatial thermal homogeneity across the study site, meaning that although temperature may influence activity and behaviour at a temporal scale, it is highly unlikely to be a factor driving habitat use for these sharks. In understanding the complete spatio-temporal routine of these sharks it can be hypothesised that predator avoidance is the primary motivator for sharks using nearshore areas over times of increased threat and that temperature plays an important role in optimising overall activity, foraging and metabolic efficiency, wherever sharks are within the habitat. Why sharks use areas further from shore at times of decreased threat, instead of remaining in safer nearshore areas, must then be quantified. Findings here relating to increased activity and bursting behaviour suggest increased foraging effort in these areas. Open lagoon areas may offer advantages to foraging efficiency or availability of prey. Accelerometer/acoustic transmitter tag packages proved an effective tool in uncovering movement and space use, activity and specific behaviour for these sharks, at liberty. Captive ethogram trials allowed for direct observation of different behaviours and validation of automated clustering techniques to then be used for wild shark acceleration data, and thus better discern behavioural states in wild sharks. Foraging is broadly considered an important factor in defining the behaviour of animals in the wild and identifying specific periods of foraging is particularly important for understanding the biological drivers of behavioural ecology in sharks. Bursting behaviour offers a good proxy for foraging, but sharks will also express this behaviour when frightened or pursued by predators. Some studies using accelerometers have been able to quantify more accurately the acceleration outputs that indicate foraging behaviour (Kokobun et al., 2011; Brownscombe et al., 2014). Being able to resolve specific foraging events in these sharks would improve understanding and development of theory in this regard. Alongside this, investigation into the distribution and abundance of prey communities will help to further elucidate their foraging ecology in this habitat. This topic is addressed in Chapter 5.

4.5 Conclusions

This research showed that a compilation of variables significantly influence the movement, activity and specific behaviour of young lemon sharks using the Bimini nursery site. Tidally mediated patterns in movement are likely to represent efforts to avoid exposure to predation, as concluded from similar studies. Both tidal and diel patterns in activity and behaviour correlate with changes in water temperatures and this could be a primary driver for observations made. The use of certain habitats at certain times, as well as periodic increases in activity whilst using those habitats could relate to attempts by sharks to increase prey acquisition success. The use of warmer waters, at lower tides and at the warmest points of the diel cycle, could be a means by which sharks optimise their metabolic output and thus their foraging efficiency. Although previous research has determined crepuscular activity in these sharks, this study showed that sharks were highly active at dusk but not so over the dawn period.

The strong relationship between activity and behaviour with temperature suggests that, rather than sharks using vision systems and light levels to increase predatory success (as suggested in previous literature, Sundstrom & Gruber, 1998), it is more likely diel patterns in activity and behaviour are influenced by surrounding water temperatures. Findings here uncover a complete spatio-temporal routine for these sharks and provide novel understanding of habitat use, activity and behaviour as well as the factors that are likely drivers of these observations.

Chapter 5 – Understanding the Foraging Ecology of Nursery Bound Lemon Sharks (*Negaprion brevirostris*): Assessing the Effects of Prey Availability and Environmental Variability on Habitat Use Patterns and Foraging Effort

Foraging and feeding plays an important role in how animals behave within their environment. Here, foraging behaviour was investigated in tandem with environmental conditions and distributions of prey species. Semi-captive experimental trials of sharks (n=5) equipped with tri-axial accelerometers recorded distinct acceleration outputs, validated by observation, that provided a novel description of foraging specific movements and prey handling behaviours. These acceleration outputs were used to estimate foraging behaviour in tagged wild sharks (n=23). Findings indicate tidally mediated patterns, as both foraging and prey handling behaviours were observed significantly more over the low tide or low and mid tide phase. In terms of success rates, on average 1 in 5 foraging events ended in successful prey capture. Baited remote underwater videos (BRUVs), used to survey the distributions and abundances of faunal communities within the habitat, uncovered a potential driver for observed foraging patterns. BRUVs data showed that foraging effort in sharks increased in areas overlapping with high abundances of their preferred prey species. Environmental sampling of the habitat also showed that areas in which foraging effort increased (offshore) were characterised by increased visibility and reduced seagrass abundance. This research evidences strategy by young sharks to optimise their foraging effort and maximise energy intake. These findings present a more comprehensive understanding of foraging in this species than has previously been available.

5.1 Introduction

How an animal chooses and uses its environment is driven, at least in part, by the acquisition of food. Feeding directly affects growth, survival and reproductive success (Stephens et al., 2007). Most animals, for some part of their lives, must balance their foraging efforts with the avoidance of predators and risk of death (Lima & Dill, 1990; Lima, 1998). This foraging versus predation risk trade-off is a concept well studied in both terrestrial and marine animals. Werner & Hall (1988) and Gotceitas & Colgan (1990) investigated this trade-off and its impacts to habitat shifts in Bluegill *Lepomis macrochirus*. Werner & Hall (1988) found size-specific habitat shifts strongly correlated with predation risk by largemouth bass. Gotceitas & Colgan (1990) supported these findings and also showed adaptive foraging effort at times of lower predation risk. Houston et al. (1993) produced general models analysing optimal trade-off between energy gain and predator avoidance, considering both short and long-term decisions by animals. The study produces a series of potential explanations for animals reduce energy intake over time. Cowlishaw (1997) showed how foraging and predator avoidance determine habitat use in a desert Baboon population. Lima (1998a) investigated non-lethal effects in the ecology of predator-prey interactions, highlighting the population level effects of anti-predator decision making, and Thaler et al. (2012) used a series of field and greenhouse experiments to understand the compensatory mechanisms tobacco hornworm caterpillar *Manduca sexta* use to balance growth with predator avoidance. For young sharks, predator avoidance plays an important role in how a habitat is used and how intensively a shark forages at different locations and times (Lima & Dill, 1990). The metabolic costs associated with foraging also require sharks to manage their energetic output as food resources are often not evenly distributed (Houston & McNamara, 2013). This means that sharks must make decisions on where, when and how intensively they forage (Lima & Dill, 1990; Sydeman et al., 2006; Papastamatiou et al., 2015). Elucidating spatial and temporal patterns in foraging ecology, as well as the factors that drive them, improve understanding of the processes that define habitat use (Pirotta et al., 2013).

Studies investigating predator-prey interactions and foraging in sharks are numerous and provide insights into various aspects of foraging ecology for different species. For example, Heithaus et al. (2002) found evidence for a stealth foraging tactic in tiger sharks and showed them to prefer areas of habitat with higher prey abundance. Bethea et al. (2004) compared the foraging ecology of four sympatric sharks species (Atlantic sharpnose, spinner, finetooth and blacktip sharks) showing that, though diet overlap between species was found to be high, habitat overlap was low, suggesting spatial resource partitioning.. Sims et al. (2006) modelled encounter success of basking sharks and showed high levels of foraging success when compared to ‘model’ sharks. Lucifora et al. (2006) showed ontogenetic and seasonal variation in the diet of school sharks and showed ontogenetic changes in the mean size of prey consumed.. Andrews et al. (2009) found consistent patterns of diel activity in sixgill sharks associated with responses to food stimuli. Newman et al. (2010) found that young lemon sharks preferentially prey on Yellowfin Mojarra regardless of their relative abundance in the habitat. Many studies suggest that habitat use in sharks is in part defined by the distribution of their prey. For example, studies of white shark habitat use showed them to select areas of higher prey abundance (Bruce, 1992; Martin et al., 2009) and sandbar sharks have been shown to exhibit site fidelity to ocean fish farms (Papastamatiou et al., 2011). Some studies have assessed the role of prey abundance gradients to foraging behaviour, suggesting that some species actively seek areas of high prey abundance at certain times (Sims & Quayle, 1998; Heithaus et al., 2002; Sims et al., 2008). However, other studies suggest that for some species, prey abundance does not drive patterns in foraging behaviour and movement, and that factors such as predator avoidance are more important (Heupel & Heuter, 2002; Duncan & Holland, 2006; Gutteridge et al., 2012). Studies have also considered the importance of environmental conditions on foraging (Hammerschlag et al., 2006) and the potential for cooperative foraging in some species (Ebert, 1991).

Despite the breadth of research and advances in technology allowing for more in-depth study, understanding of fine-scale foraging behaviour in the wild is still lacking for most species. Foraging strategies and tactics employed by sharks are of particular importance in determining the effects the marine environment has on behaviour at upper trophic levels and yet remains one of the least understood aspects of their ecology (Sims, 2010). Lemon sharks are one of the more intensively studied shark species, but most of what is known of their foraging ecology is indirectly implied or gleaned from diet studies (Cortes & Gruber, 1990; Wetherbee et al., 1990; Morrissey & Gruber, 1993a; Newman et al., 2010; Newman et al, 2012). No study, to date, has directly measured the fine-scale patterns of foraging effort in this species, nor considered the relationship between spatio-temporal foraging patterns and the distribution and abundance of prey species. The abundance of food resources is deemed to be an important factor determining nursery site use in these sharks (Heithaus, 2002), and yet little is known about how sharks using these sites interact with food resources. The use of acceleration data loggers to observe specific behaviour in wild animals has provided novel insights into otherwise cryptic behaviour in many species. In sharks, accelerometers have been used to identify various distinct behaviours (see section 2.1) but have not yet been used to identify foraging behaviour in particular.

The aim of this study was to record foraging behaviour in wild lemon sharks, to assess patterns in when, where and how frequently these sharks forage and consider what factors drive these patterns. In doing so, this research aimed to provide a clearer understanding of the importance of foraging ecology and predator-prey interactions to daily routine, fitness and survival in sharks using nursery sites.

The objectives of this research were to: 1) use fin-mounted accelerometer/acoustic transmitter tag packages to characterise distinct foraging behaviours in observable captive trials; 2) use fin-mounted accelerometer/acoustic transmitter tag packages to record foraging behaviours in sharks in the wild, investigating spatial and temporal patterns; 3) use baited remote underwater video surveys to map faunal species abundance and distribution within the study habitat; and 4) consider shark foraging patterns in relation to prey species abundance and distribution as well as other environmental factors.

5.2 Materials and Methods

This chapter uses the same capture, tagging and tracking procedures described in section 4.2. All wild sharks deployments (n = 23) were passively and actively tracked for a deployment period of five days. Additionally, trials specifically designed to characterise foraging behaviours were conducted for several sharks (n = 5) in the captive pen environment. All of these sharks were caught in waters off South Bimini, close to the captive pen site. Baited remote underwater videos (BRUVs) were used to sample the distribution and abundance of faunal communities within the habitat. Survey sites were selected at random, BRUVs were baited with chopped Menhaden and deployed for 70 minutes. A series of data were taken at deployment and upon collection describing the environmental conditions of the immediate site (water temperature, salinity, flow velocity, flow direction, water depth, visibility, bottom type and tidal state). Videos were analysed using the MaxN counting method to define relative abundance of different species groups. All BRUVs were categorised as either Mangrove Edge or Non-mangrove Edge based on their proximity to the mangrove shoreline (ME= ⩽ 50 m, NME = > 50 m). See section 2.7 for more information on BRUVs.

All data were explored using guidelines presented by Zuur et al. (2010) for statistical analysis of biological data. Figures, statistical tests and models were produced using R (v 3.2.3, R Core Development Team 2012) unless stated otherwise.

5.2.1 Captive trials - delineation of burst swimming behaviour

To estimate foraging behaviour in wild tagged sharks accurately, meeting the first objective of this research, a preliminary series of captive trials was conducted to observe and categorise foraging behaviour, prey handling behaviour and predator avoidance behaviour. A semi-captive pen with observation tower was set up as described in section 2.4. Sharks were fished for and caught as per section 2.3 and held in the pen for 24 h prior to any further interaction. All sharks were tagged with active accelerometer tag packages to observe foraging behaviour. After attachment, sharks were given another 24 h before observations began. At trial commencement a team of two observers introduced fish bait into the pen. Yellowfin mojarra were used as this species is considered a preferred food for Bimini lemon sharks (Newman et al., 2010). The size of fish used was based on findings from stomach eversion analyses in Newman et al. (2010) so as to match the size of prey fish selected in the wild. Accurate time recordings were made by observers on occurrence of and changes between specific behaviours. The aim of these trails was to use observation corroborated acceleration data to characterise different elements of foraging behaviour in these sharks. Fright responses were also induced in sharks to elicit bursting behaviour and compare acceleration outputs to foraging and prey handling behaviour. This was achieved by dropping a heavy weight into the pen, close to the shark, from the tower. An accurate time record was taken of the sharks' behavioural response.

5.2.2 Captive trials - identification of feeding events from acceleration data

All captive trail acceleration data were visually inspected after trials to distinguish different observed behavioural elements and matching timed observations to acceleration data. In Chapter 4 of this thesis, bursting behaviour was identified in acceleration data using *K*-means clustering analysis. Active foraging in lemon sharks likely involves the chase and capture of prey, during which times they will invariably exhibit periods of increased activity and bursting behaviour. Sharks that have been startled or are being chased by a predator of their own, however, will also express bursting behaviour. In defining foraging events and prey capture, bursting behaviour may not discriminate between foraging and predator avoidance responses. It was therefore an important objective of these trials to observe and categorise foraging-specific behaviours that could be accurately identified in acceleration data. Feeding and fright response trials were brief and in no instance caused undue stress in a shark. At the termination of trials, sharks were caught and the tag package removed. Sharks were held in the pen for another 48 h after trials to monitor behaviour, feed the shark and ensure healing of the fin without infection. Sharks were then released at site of capture. All sharks used in this study were in excellent condition upon release and swam away strongly.

5.2.3 Data Analysis

5.2.3.1 Analysis of Acceleration Data

Acceleration data were explored and analysed using Igor Pro v 6.37 and Ethographer, as described in section 2.8.2. Data collected from wild shark accelerometers were subjected to the same *K*-means clustering algorithms and mask analysis techniques. Findings from captive trials would determine the approaches used to categorise estimated foraging behaviour in wild shark data, upon which all estimated foraging events were recorded for all sharks. These data were then grouped as either dawn, day, dusk or night and high, mid or low tide phase (as per section 4.2.3). After testing for assumptions, hi Square tests for goodness of fit were used to examine whether data fitted the theoretical expectation that all data were distributed at a null hypothesis ratio among groups (diel = 10/24, 2/24, 2/24, 10/24 [day, dawn, dusk, night], tidal = 1/3, 1/3, 1/3 [high, mid, low]).

5.2.3.2 BRUV analysis

All BRUV videos were analysed by a team of two observers watching video playback in real time using VLC Media player (v 2.1.3). MaxN (= Maximum Number of Individuals) is a commonly used relative abundance index for analysis of fish assemblages (Brooks et al., 2011). It uses a count of the maximum number of individuals of a species in a single video frame at any one time. This represents a deliberately conservative estimate of abundance when individuals cannot be recognised. Fauna were consistently identified to the family level and, where possible, to species level, recording MaxN in each instance.

PRIMER software was used to analyse the BRUVs data for patterns in biological and environmental data. Non-metric multi-dimensional scaling (nMDS) plots and principal component analysis (PCA) were used to graphically represent patterns in data. One-way and two-way Permutational Multivariate Analysis of Variance (PERMANOVA) was used to test for significant variances in the data, with each BRUV representing a single variable. Similarity percentages analysis (SIMPER) provided a breakdown of contributions by different species to any dissimilarity between conditions. Wilcoxon-Mann-Whitney tests were used to identify differences in environmental variables between conditions. ArcMap v 10.0 was used to analyse acoustic tracking data and to generate all maps.

5.2.3.3 Analysis of predator-prey distribution overlap

For all active tracking data, each track point was grouped as ME or NME (same criteria as for BRUVs). Based on findings in Chapter 4 relating to tidal activity, sharks with adequate tracking data across each tidal category were compared in relation to overall faunal abundance and abundance of Gerreidae fishes. For each shark, ten sample location points recorded at each tidal phase were randomly selected. Each point was given a score based on the mean total relative abundance estimates from BRUV deployments within a 500 m radius of the track point. For total MaxN of all fishes, this was referred to as a ‘prey availability score’ or PAS.

Gerreidae data were denoted as PAS-G. For each shark, PAS and PAS-G data were compared for differences between tidal phase using a Kruskal-Wallis test.

5.3 Results

5.3.1 Captive trials

Five sharks were tested in captive foraging trials and all sharks recovered well and were released back into the environment (Table 5.1).

Table 5.1 Summary data for sharks caught and tested in captive foraging trials. A range of sizes were tested to account for changes in swimming kinematics. TL = total length. M = male, F = female.

Shark	TL (cm)	Sex
15C	104	F
C62	97	M
45E	102	F
505	102	M
411	135	M

At the introduction of prey items, all sharks responded similarly, increasing swimming activity and speed, intermittently exhibiting bursting behaviour and making sharp turns, representing apparent searching behaviour.

5.3.2 Captive trials – characterisation of feeding behaviour from acceleration data

Throughout captive feeding trials sharks were found to manipulate prey items, thrashing their head rapidly from side to side. This broke the prey item into smaller pieces that were then swallowed by the shark. This behaviour will from this point onwards be referred to as ‘prey handling behaviour’ and acceleration data associated with this behaviour were investigated alongside foraging and fright behaviours. Four of five captive sharks displayed prey handling behaviour with a total of 12 events observed and recorded. Further investigation of the data showed that prey handling behaviour produced different acceleration waveforms than foraging or fright responses, generating a rapid, high-amplitude oscillation in the acceleration waveform. During prey handling events all sharks remained almost stationary whilst rapidly and briefly thrashing their head from side to side. This behaviour produced mean sway axis, dynamic acceleration values over 1 sec that reliably exceeded those presented by bursting behaviour (Fig. 5.1).

All sharks responded to fright response trials with immediate bursting behaviour for very short periods before quickly returning to a steady swimming state. Investigation of the data showed that the swimming kinematics associated with foraging behaviour produced sustained bursting acceleration for at least two sec and frequently much longer, once food stimulus was introduced. Fright responses produced short and uneven, high amplitude spikes in acceleration for less than two sec in all instances and very often less than one sec. Sharks would sometimes maintain faster swimming behaviour for short periods after the introduction of the fright stimulus but did not express bursting behaviour again after the initial response at the moment the fright stimulus was detected (Fig. 5.2).

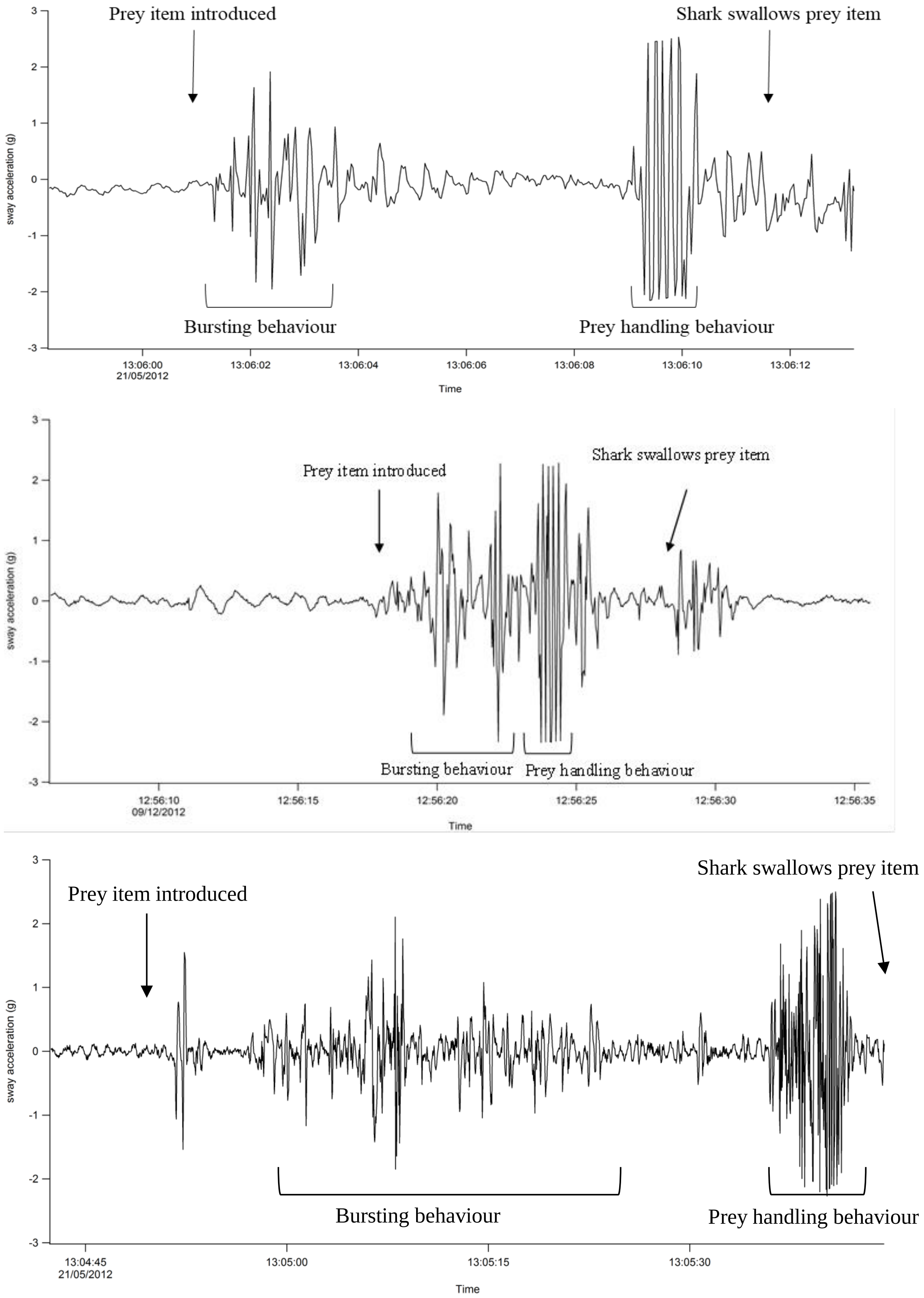


Fig. 5.1 Sample segments of the acceleration time-series for captive trials (Sharks 15C and 45E) at the introduction of a prey food item, showing differences in acceleration signatures of bursting behaviour and prey handling behaviour.

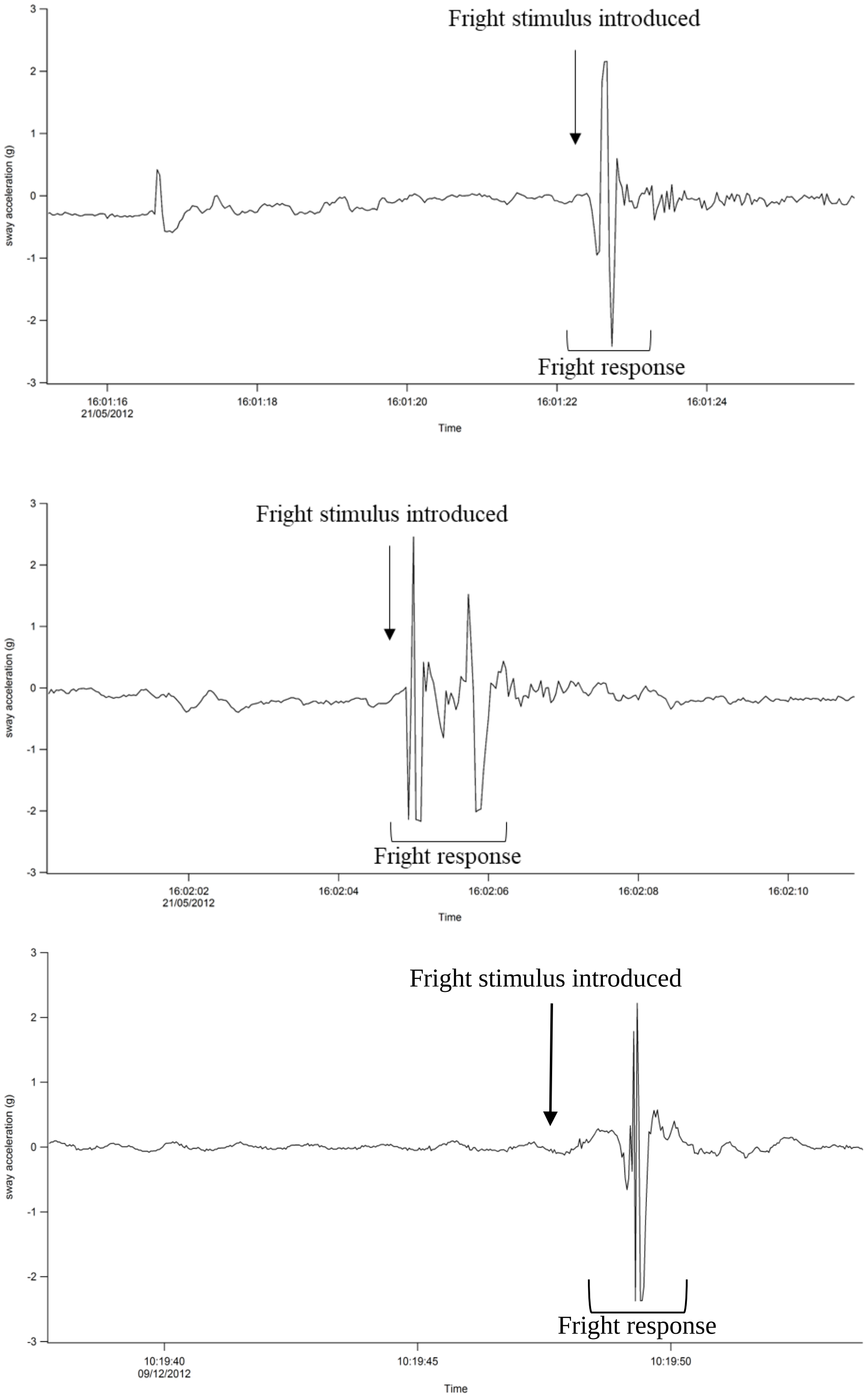


Fig. 5.2 Example segments of the acceleration time-series for captive trials (Sharks 15C and 45E) at the introduction of fright stimuli.

5.3.2 Wild shark foraging behaviour

Accelerometer data from 19 wild shark deployments were used to identify bursting behaviour, estimated foraging events and estimated prey handling events. *K*-means clustering and mask analyses were used to separate periods of bursting behaviour from all other data (as in 4.3.1). Based on captive trial findings, all bursting events in wild shark data could be separated by event duration. Events at < 2 sec were categorised as fright responses by the sharks and events ≥ 2 sec were categorised as likely foraging events. At periods in the time-series where a sequence of bursting events occurred together (within 10 sec of one another) they were grouped together as one foraging event. To further distinguish potentially successful foraging events and prey handling behaviour, sway axis, dynamic acceleration data were decimated to provide a mean value for every second over the duration of the foraging event. Where mean sway acceleration exceeded 1 g over one sec, this was estimated to represent prey handling behaviour and the successful capture of a food item. In each instance, data were visually inspected to ensure acceleration signatures matched with those representative of this behaviour (as per captive trials, rapid oscillating acceleration waveform, see Fig. 5.1). When acceleration exceeded 1 g over 1 sec but this was the result of a singular spike in acceleration, and not displaying the rapid oscillating acceleration waveform associated with prey handling behaviour, these data were not included as prey handling events.

All wild shark data were processed in the above described manner and produced an estimated total of 479 foraging events over a course of 83 active deployment days for all sharks, resulting in a mean 5.77 foraging events ± 4.36 SE per shark, per day. Total estimated foraging events contributed 46% of all burst swimming events. Data were compared at diel and tidal phase and Chi ^{2} tests for goodness of fit were used to assign statistical significance to these comparisons (Table 5.2). In terms of diel phase, associations with foraging were not consistent. Six of 19 sharks displayed significant differences to the null hypothesis ratio (see 5.2.4.1) and of these, five sharks displayed the most foraging events during the night (Fig. 5.3a). In terms of tidal phase, most sharks (14 of 19) recorded foraging behaviour in a pattern significantly different from the expected null hypothesis ratio. Of these sharks, the overall trend was towards greatest foraging effort at low tide (Fig. 5.3b) times with ten sharks displaying more foraging events at either low tide or both mid and low tide phase, with significantly less foraging at high tide. This was particularly prominent in smaller sharks. In some sharks, mostly larger individuals, this distribution of foraging effort was not present and patterns in foraging behaviour varied. The durations of foraging events ranged from 2-154 sec with mean event duration recorded as 6.37 sec ± 0.15 SE.

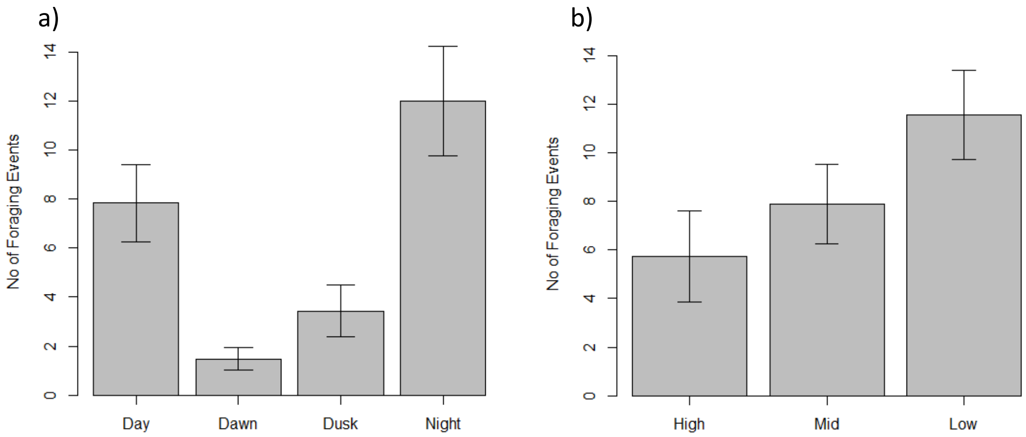


Fig. 5.3 Bar plots displaying mean foraging events for all sharks across entire deployment duration: a) showing differences between day, dawn, dusk and night, b) showing differences between high, mid and low tide.

A total of 74 estimated prey handling events were recorded for all sharks, resulting in a mean 0.91 successful predation attempts per shark, per day. Percentage foraging success ranged from 4.4% to 50% with a mean of 22.3% ± 3.1 SE. No patterns were found in prey handling behaviour at the diel phase. As with foraging behaviour, estimated prey handling events were found to be signficantly greater at the low tide phase (Fig. 5.4, Kruskal-Wallis test $p < 0.05$, Mann-Whitney pairwise tests identified low tide data as significnantly different from high and mid tides.

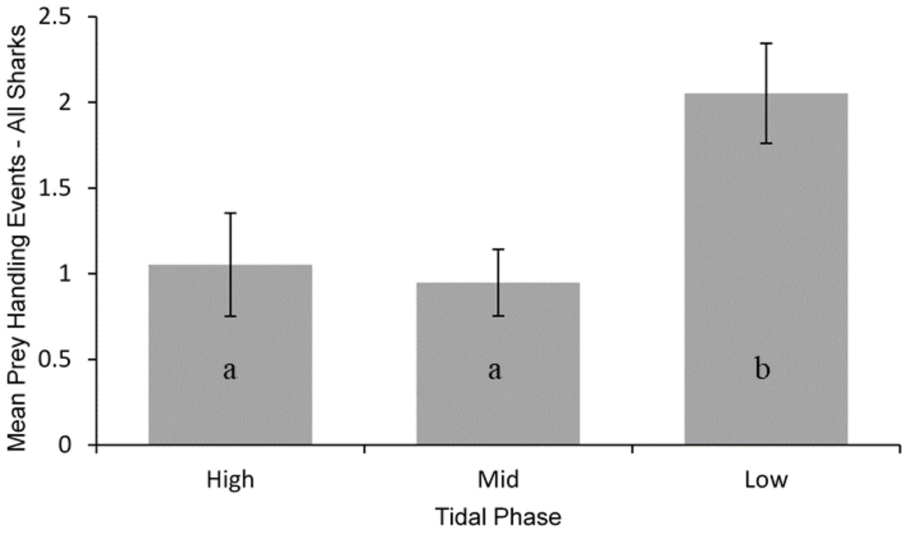


Fig. 5.4 Mean number of prey handling events over entire deployment duration for all sharks, tested across tidal phase. Letters denote groupings from Mann-Whitney post-hoc pairwise tests.

Table 5.2 Summary of estimated foraging events recorded by accelerometers. Chi^2 refers to the test statistic, *P* column refers to the p-value output from Chi^2 - tests. Values with *P* < 0.05 are highlighted grey, indicating a significant variance between data groups. Degrees of freedom = 3 for all diel tests and 2 for all tidal tests.

Shark	**Total Foraging Events**	**Diel**						**Tidal**				
		Day	Dawn	Dusk	Night	Chi2	*P*	High	Mid	Low	Chi2	*P*
D40	16	4	1	2	9	2.3	0.51	4	5	7	0.8	0.64
437	2	2	0	0	0	2.8	0.42	0	0	2	4	0.15
511	24	7	0	2	6	1.8	0.61	2	8	14	9	< 0.01
C69	19	10	4	0	5	6.9	0.07	2	6	11	6.4	< 0.05
708	14	1	0	1	12	11.7	< 0.01	2	2	10	5.8	< 0.05
C25	11	2	0	0	9	7.54	0.056	1	2	8	7.8	< 0.05
C25(2)	7	1	0	1	5	3.62	0.3	0	2	5	5.4	< 0.05
E50	18	1	0	7	10	28.1	< 0.01	0	4	14	17.3	< 0.01
C69(2)	24	12	0	5	7	7.8	0.06	2	18	4	19	< 0.01
224	11	5	0	1	5	1	0.8	0	4	7	6.7	< 0.05
C69(3)	31	6	4	2	19	7.5	0.058	4	12	15	6.7	< 0.05
85F	20	0	1	14	5	101.2	< 0.01	0	3	17	24.7	< 0.01
901	87	24	5	15	43	14.4	< 0.01	33	18	36	6.4	< 0.05
345	30	14	2	0	14	2.96	0.39	9	11	10	0.2	0.9
F7F	24	5	0	3	16	8.6	< 0.05	2	2	20	27	< 0.01
267	25	4	1	1	19	12.2	< 0.01	10	5	10	2	0.36
O82	21	7	1	1	12	12.2	0.53	7	7	7	0	1
E07	45	19	6	3	17	1.7	0.64	14	26	5	14.8	< 0.01
773	50	25	3	7	15	4.7	0.19	17	15	18	0.3	0.86

5.3.3 Distribution and Abundance of Faunal Species

One hundred and forty BRUV survey deployments were conducted over the course of 13 months (January April 2013 to May 2014). A total of 5786 individuals from 62 species, belonging to 27 family groups were identified through video analysis (Table 5.3). Distinct differences were found in the biota between habitat type (mangrove edge - ME and non-mangrove edge – NME, Fig. 5.5, Fig. 5.6) but not between tidal phase (High and Low) (2-Way PERMANOVA: Habitat type $P < 0.01$, Tide $P = 0.195$). SIMPER analysis showed that different faunal groups contribute to overall relative abundance in the different habitat types. In the mangrove edge zone, Lutjanids contributed over 56% of total abundance and in the non-mangrove edge zone Carangids and Hemiramphids combined to contribute over 56% of total abundance (Table 5.4a, b and c). Carangidae, Lutjanidae, Gerreidae, Haemulidae, Hemiramphidae and Scaridae all contributed greater than 1% of overall mean relative abundance and as such were deemed to best represent the biota of the study site as a whole. These family groups were also identified as significant components of the known diet of juvenile lemon sharks (Newman et al., 2010). These faunal groups were tested for differences in abundance between habitat types using Wilcoxon Signed Rank tests. All groups tested had significantly different mean abundance values between habitat types with the exception of Gerreidae, which was found evenly between the two habitat types (Table 5.4, Fig. 5.5). Maps of distribution and abundance for each family group displayed the differences between groups (Fig. 5.7 a - f).

Analysis of environmental variables highlighted differences between habitat type (2-way PERMANOVA: Habitat type $P < 0.01$, Tide $P = 0.139$). PCA specifically highlighted mean visibility and seagrass coverage as important variables (Fig. 5.8). Visibility was significantly reduced in the mangrove edge environment (Table 5.5, Wilcoxon- Mann-Whitney test $P < 0.01$). In the mangrove edge zone, mean visibility was 7.84 m ± 0.30 SE and in the non-mangrove edge zone it was 9.81 m ± 0.24 SE. Percentage seagrass coverage was expectedly significantly greater in the mangrove edge zone (Table 5.5, Wilcoxon-Mann-Whitney test $P < 0.01$).

Table 5.3 Summary of all identified teleost fishes from 140 BRUV deployments, organised alphabetically by family. All species names taken from the World Register of Marine Species (WoRMS Editorial Board, 2016).

Family	Binomial	Common Name	Total Individuals	Percentage
Acanthuridae	*Acanthurus bahianus*	Ocean surgeon	17	0.34
	Acanthurus chirurgus	Doctorfish	4	0.08
Atherinidae	*Atherinidae spp.*	Silversides	603	12.02
Belonidae	*Ablennes hians*	Flat needlefish	101	2.01
	Thilosurus crocodilus	Hound needlefish	4	0.08
Carangidae	*Carangoides bartholomaei*	Yellow jack	108	2.15
	Caranx ruber	Bar jack	273	5.44
	Caranc crysos	Blue runner	152	3.03
	Caranx latus	Horse-eye jack	8	0.16
	Trachinotus blochii	Permit	5	0.1
Chaetodontidae	*Elagatis bipinnulata*	Rainbow runner	1	0.02
Echeneiddae	*Chatodon ocellatus*	Spotfin butterflyfish	2	0.04
	Chatodon capistratus	Foureye butterflyfish	8	0.16
	Echeneis naucratoides	Whitefin sharksucker	30	0.6
Gerreidae	*Gerres cinereus*	Yellowfin mojarra	188	3.75
	Eucinostomus melanopterus	Flagfin mojarra	370	7.38
Haemulidae	*Haemulon aurolineatum*	Tomtate	47	0.94
	Haemulon flavolineatum	French grunt	5	0.1
	Pomadasys crocro	Burro grunt	4	0.08
	Haemulon carbonarium	Caesar grunt	40	0.8
	Haemulon plumierii	White grunt	11	0.22
	Haemulon sciurus	Bluestriped grunt	50	1
Hemiramphidae	*Hemiramphus brasiliensis*	Ballyhoo halfbeak	44	0.88
	Chriodorus atherinoides	Hardhead halfbeak	1005	20.04
Kyphosidae	*Kyphosus sectatrix*	Bermuda sea chub	12	0.24
Labridae	*Halichoeres bivittatus*	Slippery dick	150	2.99
	Xyrichtys novacula	Peraly razorfish	1	0.02
	Halichoeres maculipinna	Clown wrasse	13	0.26
	Halichoeres poeyi	Blackera wrasse	1	0.02
	Thalassoma bifasciatum	Bluehead wrasse	1	0.02
	Lachnolaimus maximus	Hogfish	3	0.06
Lutjanidae	*Lutjanus apodus*	Schoolmaster	116	2.31
	Lutjanus griseus	Grey/Mangrove snapper	843	16.81
	Lutjanus analis	Mutton snapper	9	0.18
	Lutjanus synagris	Lane snapper	11	0.22
	Ocyurus chrysurus	Yellowtail snapper	50	1
Mullidae	*Pseudupeneus maculatus*	Spotted goatfish	7	0.14

Ostraciidae	*Acanthostracion quadricornis*	Scrawled cowfish	5	0.1
	Acanthostracion polygonius	Honeycomb cowfish	3	0.06
Pomacentridae	*Stegastes variabilis*	Cocoa damselfish	8	0.16
	Microspathodon chrysurus	Yellowtail damselfish	2	0.04
	Abudefduf saxatilis	Sergeant-major	78	1.56
	Stegastes diencaeus	Longfin damselfish	2	0.04
Scaridae	*Scarus iseri*	Striped parrotfish	21	0.42
	Sparisoma rubripinne	Yellowtail parrotfish	6	0.12
	Scarus taeniopterus	Princess parrotfish	20	0.4
	Sparisoma viride	Stoplight parrotfish	11	0.22
Sparidae	*Calamus penna*	Sheepshead porgy	61	1.22
	Calamus arctifrons	Grass porgy	4	0.08
	Calamus pennatula	Pluma	8	0.16
Sphyraenidae	*Sphyraena barracuda*	Great barracuda	92	1.83
Tetradontidae	*Sphoeroides testudines*	Checkered puffer	43	0.86
	Sphoeroides spengleri	Bandtail puffer	3	0.06

Table 5.4a, b and c Teleost families identified by similarity percentages (SIMPER) for habitat type (a = NME, b = ME, c = Comparisons).

a) Non-Mangrove Edge

Faunal Groups	**Mean Abundance**	**% contribution**
Carangidae	1.64	32.69
Hemiramphidae	1.75	24.29
Gerreidae	1.13	13.08
Sphyraenidae	0.47	9.08
Labridae	0.6	8.12
Belonidae	0.35	3.45

b) Mangrove Edge

Faunal Groups	**Mean Abundance**	**% contribution**
Lutjanidae	4.65	56.04
Gerreidae	1.52	9.41
Haemulidae	1.37	7.4
Scaridae	1.4	5.57
Sphyraenidae	0.64	5.17
Carangidae	0.75	4.59
Sparidae	0.7	3.62

c) Comparisons	Group NME	Group ME	
Species	**Mean Abundance**	**Mean Abundance**	**% contribution**
Lutjanidae	0.52	4.65	24.28
Gerreidae	1.13	1.52	10.03
Hemiramphidae	1.75	0.24	9.67
Carangidae	1.64	0.75	8.87
Scaridae	0.11	1.4	6.8
Haemulidae	0.14	1.37	6.78
Labridae	0.6	0.65	5.08
Atherinidae	0.42	0.82	5.06
Belonidae	0.35	0.55	4.37
Sparidae	0.17	0.7	4.15
Pomacentridae	0.06	0.71	3.86
Sphyraenidae	0.47	0.64	3.7

Table 5.5 Wilcoxon-Mann-Whitney rank sum test statistic results and indicated significance values for comparisons of environmental data.

Environmental Variable	Habitat Type	Tidal Phase
Temperature	1725.5	1991.5
Visibility	1024***	1854*
Salinity	1638	2184.5
Flow Velocity	1937	2259.5
% Seagrass Coverage	585.5***	1865.5*

Note: * = $P < 0.05$, ** = $P < 0.01$, *** = $P < 0.001$

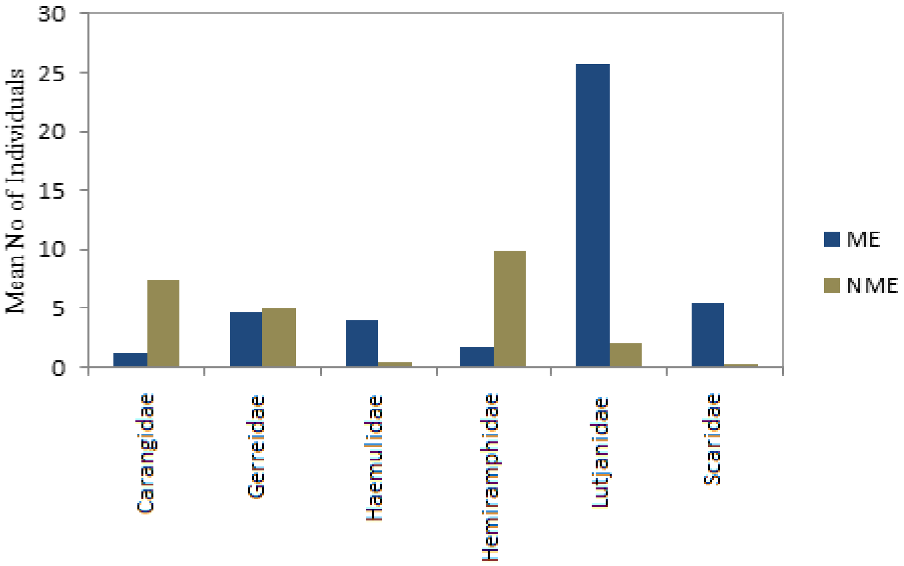


Fig. 5.5 Mean number of individuals recorded for each of the significantly contributing faunal groups. ME and NME show differences in abundances between habitat types. Only Gerreidae are distribution evenly among ME and NME zones.

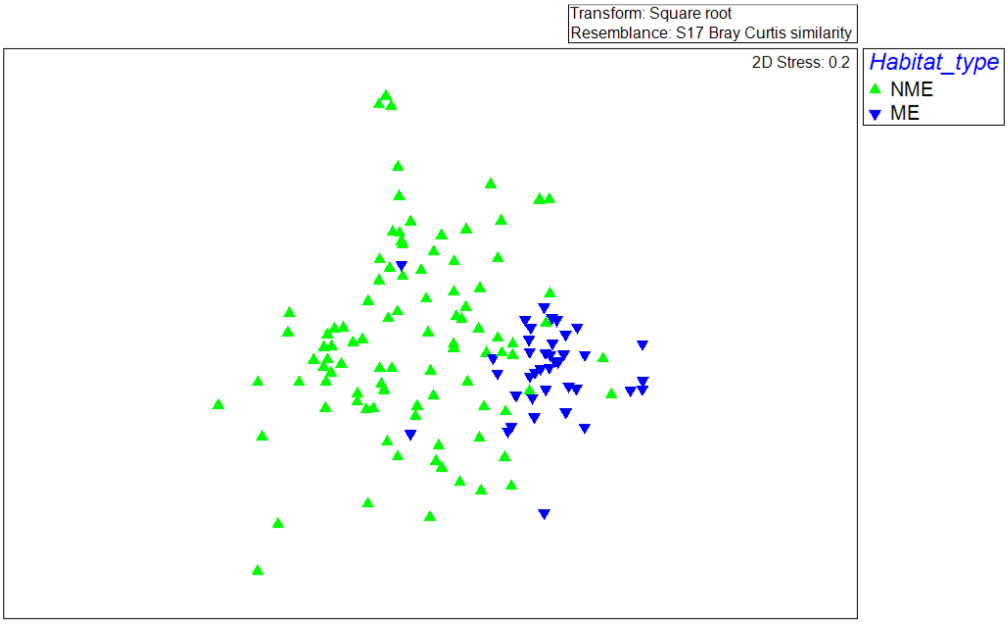


Fig. 5.6 Non-metric multi-dimensional scaling (MDS) plot of abundance data for all teleost family groups, sorted by habitat type (ME vs NME).

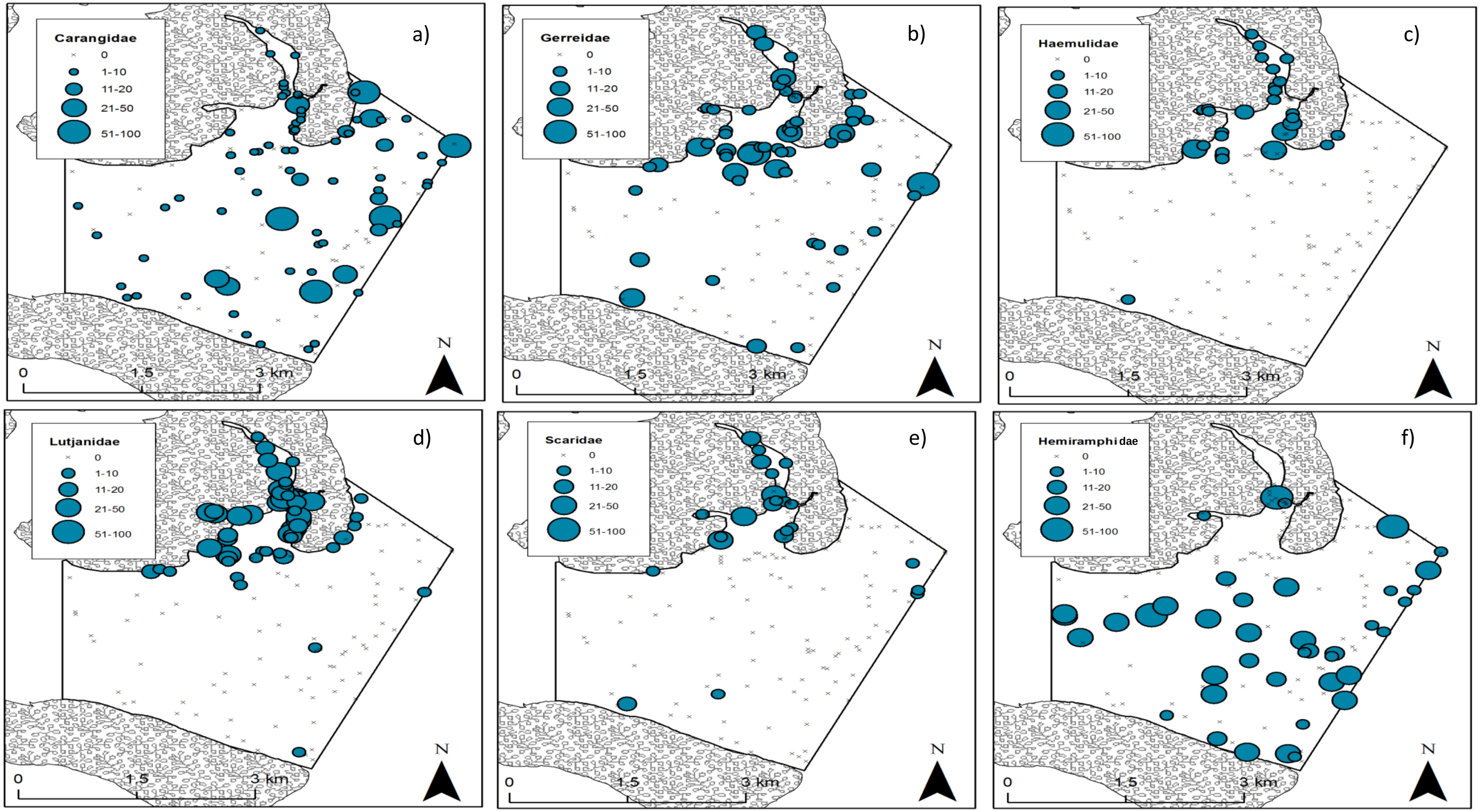


Fig. 5.7a - f Displays maps of abundance and distribution for the most prevalent and relevant teleost species (a = Carangidae, b = Gerreidae, c = Haemulidae, d = Lutjanidae, e = Scaridae, f = Hemiramphidae), as relates to lemon shark prey. All grey coloured 'x' marks represent BRUV deployment sites for which no individuals of that particular family group were recorded.

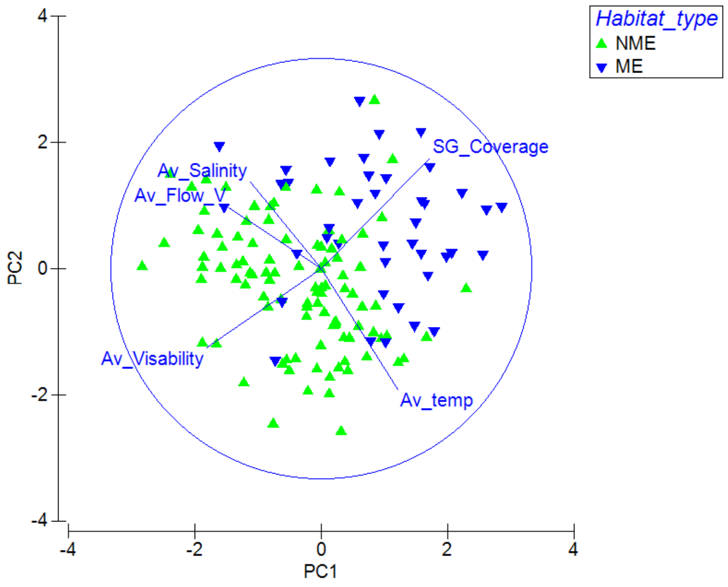


Fig. 5.8 Principal component analysis (PCA) of environmental data for all BRUVs, sorted by habitat type (ME vs NME). Vectors for each variable are labelled.

5.3.4 Shark-prey interactions

Acoustic tracking data for sharks, divided into ME and NME points, showed that only a small percentage (mean = 24.71% ± 5.83) of all track points were recorded in the ME zone. The ability to track sharks in lagoon areas at low tides was very limited as sharks often move into extremely shallow areas inaccessible by boat. Therefore, there were only limited data available for analysis of spatial overlap with faunal communities using PAS metrics. Six of the 19 wild sharks were tracked for long enough periods over all tidal phases to be tested in this way (Table 5.6). Tidal analysis of PAS and PAS-G for active tracking data showed that at low tides these sharks used areas of poorer overall faunal abundance, but that five of six sharks occupied areas with significantly higher abundances of Gerreidae fishes at low tides (Table 5.6, Fig. 5.9).

Table 5.6 Kruskal-Wallis tests for PAS and PAS-G of tracking points at different tidal phases. Coloured blocks represent groupings from post-hoc Mann-Whitney pairwise tests.

Shark	**PAS - High**	**PAS - Mid**	**PAS - Low**	***P***	**PAS-G - High**	**PAS-G - Mid**	**PAS-G - Low**	***P***
511	55.6	55.25	46.951	***	1.44	1.55	11.312	***
D40	54.688	58.115	50.512	n.s	6.18	4.944	9.948	**
C25	44.399	41.003	40.727	n.s	2.86	2.09	12.424	***
85F	38.737	34.55	16.24	**	17.226	8.542	12.529	**
267	25.815	51.168	42.566	***	9.854	11.308	15.309	**
E07	39.656	54.243	18.89	***	4	5.53	7.875	**
Mean	42.8992	50.6652	35.0318		7.74	6.3748	11.3946	

Note: n.s = $P > 0.05$, * = $P < 0.05$, ** = $P < 0.01$, *** = $P < 0.001$

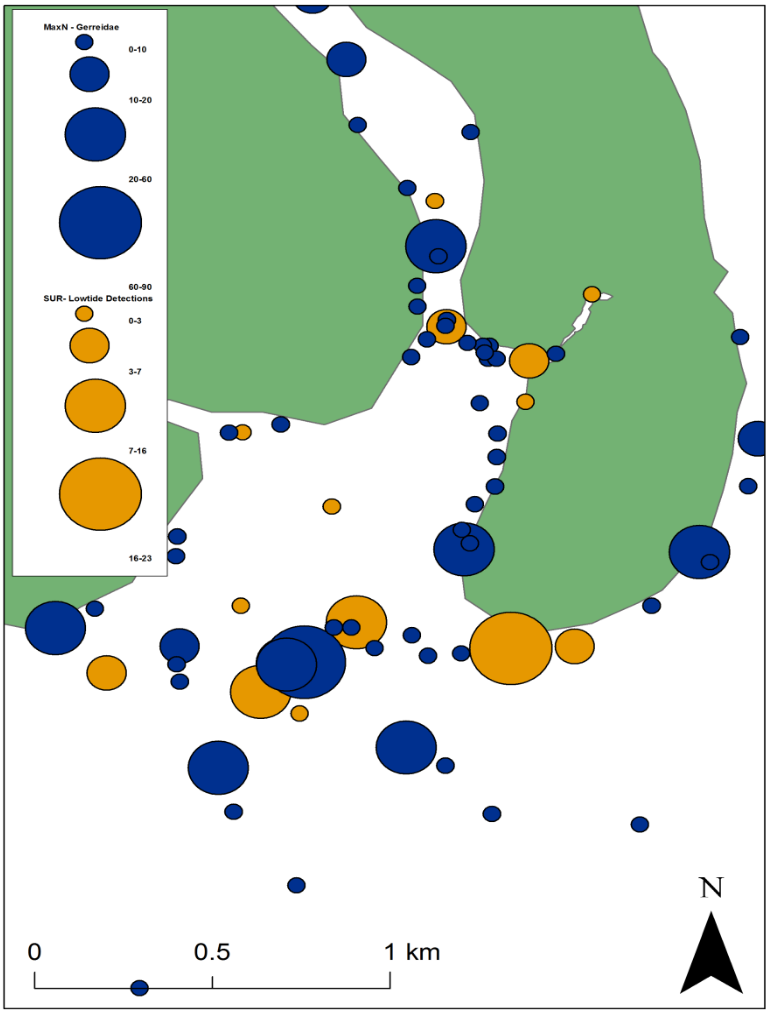


Fig. 5.9 Map of prey distributions relative to shark locations. Blue markers represent BRUV deployment locations, size of marker relative to MaxN Gerreidae. Orange markers represent SUR sites, size of marker relative to total detections of all sharks throughout the low tide period.

5.4 Discussion

5.4.1 Foraging and prey handling

Periodicity and frequency of foraging effort in free-ranging lemon sharks was investigated using direct measurements of swimming behaviour. Estimation of prey handling events provided a proxy for predatory success in these sharks and is supported by similar observations made of prey manipulating behaviour in other shark species, including the school shark (Lucifora et al., 2006). Findings relating to this suggest that around one in five foraging events involved a successful prey capture and that sharks captured prey a little under once per day. Foraging events in all sharks were mostly brief, rarely exceeding several seconds. This may represent a stalking tactic employed by the sharks to optimise predatory success. Stalking predators will approach their prey whilst concealed before making a rapid strike (Motta, 2004). Seven-gill sharks capture prey by approaching it with deliberately subtle body movements before making a quick dash at the prey (Ebert, 1991). Stalking prey reduces the energy expended in chasing fish for prolonged periods. Prey handling events were often evenly spread across the deployment time-series (at similar times on subsequent days) which may indicate a cessation of foraging for several hours after successful prey capture, potentially to facilitate digestive processes.

Lemon sharks do not appear to be random in their foraging habits. Most sharks increased foraging effort and prey capture at low and/or mid tide periods. These data are congruent with findings from Chapter 4 relating to increases in ODBA activity and bursting behaviour at lower tides (see section 4.3). Active and passive tracking data showed that most sharks use areas further from shore during these periods of increased foraging and return closer to shore at high tides. Few studies have identified tidally related patterns in foraging in elasmobranchs. Shepard et al. (2006) reported tidal patterns in vertical movements of basking sharks that are thought to relate to tidally-induced aggregations of zooplankton. Strong tidally associated patterns in movements of leopard sharks in a bay area are thought to be used as a means to exploit tidally available food resources (Ackerman et al., 2000). In white sharks, predatory success has been linked with the diel phase, with sharks' success rate greatest in the early morning, within one h of sunrise (55%) and diminishing rapidly thereafter. White sharks were shown to cease active predation attempts when success rate dropped below a certain threshold (40%) (Martin et al., 2005). It is possible that lemon sharks in this study could reduce foraging effort at unproductive times, suggesting that foraging success is potentially lower at higher tides in the Bimini environment. It is well established that juvenile and sub-adult sharks occupy a meso-predatory role and must balance the acquisition of food with avoidance of their own predators. Tidal patterns in foraging effort at this site likely represent management of such priorities in tandem with adequate foraging effort. Studies into movement and behavioural habits of young lemon sharks using nursery sites have commonly concluded that use of nearshore areas and movements within these areas are primarily driven by predator avoidance (Morrissey & Gruber, 1993a; Wetherbee et al., 2007). If this is the case, a reduction in foraging effort at times of increased threat (when sharks are driven to use nearshore areas) suggests that areas further from shore offer a foraging advantage and/or nearshore areas are of lower foraging value.

5.4.2 Relationships with faunal communities

Despite observed decreases in foraging close to shore, findings from BRUV deployment data show that areas closer to shore, throughout the study site, are home to a greater abundance and diversity of marine fauna and thus potential prey for lemon sharks. Some taxonomic groups of fishes were far more abundant in mangrove-fringed near-shore areas (i.e Haemulidae, Lutjanidae, Scaridae) whereas some were more abundant in open lagoon areas away from shore (i.e. Carangidae, Hemiramphidae). Distribution and relative abundances of fishes were found to be static and did not vary at tidal or diel scales. Thus, it is unlikely that tidal patterns in foraging effort relate to shifting distribution and movements of prey. Newman et al. (2010) studied the diet and prey preference of juvenile lemon sharks at two neighbouring sites in Bimini, finding young lemon sharks to be selective for certain fishes regardless of their abundance within their habitat. Yellowfin mojarra comprised over 50% of the diet of young lemon sharks. In this study, Mojarras as a group (Gerreidae) were found to be major components of both the mangrove edge and non-mangrove edge environments but in neither habitat type were they the most abundant fish group. Some of the largest relative abundance values for Gerreidae were found in an area on the southern edge of the Bonefish Hole inlet, an area that active and passive tracking data showed lemon sharks to be commonly found at low tide periods (Fig. 5.9). PAS and PAS-G data showed that several sharks move away from areas of higher overall fish abundance at low tides and into areas of higher Gerreidae abundance. This could mean that sharks are selecting particular areas within their range in which to forage and are selecting these areas based on presence of preferred prey items. Mojarra may offer a greater reward than other available prey, in terms of calorific value or could be easier prey to catch (Wetherbee et al., 1990). Mojarra are bottom-feeders often pecking at the sand as they forage for invertebrates (Sazima, 2002), and this behaviour could render them more susceptible to predators. Many shark species are known to be selective feeders, for example, basking sharks select the most profitable plankton patches, school sharks select specific prey types and avoid others, and silky sharks target squid and crab species (Sims & Quayle, 1998; Lucifora et al., 2006; Cabrera-Chavez-Costa et al., 2010). Sharks are often considered to be selective feeders when food is abundant, thus maximising energy intake, and are inclined towards opportunism when food is limited (Wetherbee et al., 1990). This theory suggests that the Bimini nursery habitat offers plentiful food resources that allow for sharks using the site to employ selective feeding habits.

5.4.3 Relationships with environmental conditions

It is also possible that sharks optimise their foraging efforts, not by selecting a specific prey type but rather selecting parts of the habitat that offer an environmental advantage. Mangroves act as a refuge for many fish species (Laegdsgaard & Johnson, 2001) and may well reduce foraging efficiency as sharks will be less able to successfully pursue and catch fish that can quickly access the protection of the mangrove root system. Gruber and Morissey (1993a) suggested that lemon sharks will avoid foraging in areas of high seagrass cover as this too may reduce foraging efficiency. In this study, seagrass coverage was found to be significantly greater in the mangrove-edge zone whereas visibility was conversely significantly greater in the non-mangrove-edge zone. Many sharks are considered to be highly visual predators (Gruber & Cohen, 1978) and it may be that the increased visibility and reduced cover for prey in open areas away from the mangrove shore increases foraging efficiency. Changes in visibility have been found to influence the attack frequency of white sharks (Hammerschlag et al., 2006) and slit-eye sharks have been shown to have an affinity for specific sites with highest water clarity (Gutteridge et al., 2011).

Whether observed patterns are driven by a selection for a specific type of prey or by the use of beneficial characteristics of specific areas of habitat, these findings show that foraging and prey acquisition have a significant influence on habitat use and behaviour in these sharks. For many animals, areas of profitable resource acquisition are also areas of increased risk of mortality (Werner & Anholt, 1993) and tidal regimes may frame this dynamic in this nursery system. Given that sharks should optimise their foraging strategies within the constraints of other important factors such as predator avoidance, it is noteworthy that their success rate is apparently so low. Several sharks (i.e. C25, C69) were caught several times over subsequent years in this study and so demonstrate that the observed patterns in foraging effort are sufficient to promote growth and survival. However, lemon sharks in Bimini have been shown to be slow growing when compared to other regions (DiBattista et al., 2007). It can be hypothesised that these sharks function at a fine energetic balance, meaning that changes to their habitat could have severe consequences to their fitness and survival.

5.5 Conclusions

This research represents valuable insight into foraging behaviour in elasmobranchs, providing a fine-scale spatio-temporal understanding that remains unavailable for most species. Accelerometry proved an effective tool in determining specific foraging behaviours at a fine scale. Nursery-bound lemon sharks displayed tidally mediated patterns in foraging effort, increasing overall foraging effort at lower tide periods. The study provides evidence that suggests that sharks hunt more frequently in sites with higher abundances of preferred prey species, but that significant differences in the environment between sub-habitat types could also motivate habitat selection for foraging. This study provides evidence to support the theory that young lemon sharks occupying nursery habitats optimise their foraging efforts to maximise energetic intake, which in turn affects their growth and survival. These findings contribute to a better understanding of the importance of mangrove ecosystems to the fitness of sharks at key life stages.

Chapter 6 – Ontogenetic Shifts in Habitat Use, Activity and Behaviour of Lemon Sharks *Negaprion brevirostris* at a Sub-Tropical Nursery Site

Understanding the influence of ontogenetic change is essential to developing a full understanding of how and why animals behave as they do in the wild. Ontogenetic change represents shifting biological and ecological requirements by an animal as it grows. This research provided quantitative understanding of ontogenetic changes in habitat use by young nursery-bound lemon sharks across a range of sizes (78-169 cm TL) and thus ages. Findings demonstrated ontogenetic shifts in both overall ranges of sharks, with larger sharks using larger areas overall, and in amount of time sharks spent further from the shoreline. Across ontogeny, sharks spent increasing amounts of time in areas further from the mangrove shoreline, eventually not using these areas at all. Findings also showed that tidal patterns in movement and habitat use, apparent in smaller, younger sharks, diminish as sharks grow larger. Relating to activity and specific behaviour, findings demonstrated a decreasing trend in overall activity and resting behaviour as sharks grew. Data showed ontogenetic shifts in foraging events and effort but no corresponding changes in successful predation events. Further, results relating to mean relative fish abundance showed that, over ontogeny, sharks habitat use patterns and ranges did not overlap with greater fish abundances overall, but did increasingly overlap with areas of higher Carangidae fish abundance specifically. The findings of this research provide new information relating to observed ontogenetic changes in how sharks use nursery sites as well as the potential drivers for these observations. Such observed changes in habitat use represent shifting needs from the habitat as a whole. These needs must be considered fully if conservation of these sites is to be effective.

6.1 Introduction

Ontogenetic shifts in movement, habitat use and behaviour are common across many, if not all, marine species (Grubbs, 2010). For young sharks, habitat selection and behaviour are the functions of a trade-off between maximising energy intake and avoiding predation (Lima, 1998; Heithaus, 2007). Nearshore coastal areas are used as nursery sites by many shark species. These habitats are commonly characterised by considerable changes in physical conditions such as temperature, depth, salinity and dissolved oxygen. Such extremes can make these coastal environments challenging, but they offer increased foraging potential whilst predation risk is often considerably diminished (Knip et al., 2011). As sharks grow, risk of predation decreases, whilst at the same time energetic requirements and feeding capability increase. These shifts in energetic and metabolic priorities influence changes in habitat use and behaviour. Different life stages of a species will often occupy changing ecological roles within an ecosystem (Grubbs, 2010), and, with such changes, exert shifting pressures on other organisms whilst having different requirements from that system. Understanding the dynamics of different life stages of a species using a nursery habitat is important to facilitate more effective management for these habitats and to understand better how sharks at different ontogenetic stages, with changing biological requirements, share an important habitat.

Ontogenetic shifts in diet have been documented for many species of elasmobranchs. For example, Lowe et al. (1996) and Ebert (2002) recorded ontogenetic dietary shifts in tiger sharks and sevengill sharks, respectively, coinciding with expanding ranges. Wetherbee and Cortes (2004) reviewed food consumption and feeding habits across different species of elasmobranch. Lucifora et al. (2009) considered the ecological and applied implications of ontogenetic diet shifts in the copper shark, suggesting alterations to current fishing practice and Newman et al. (2012) found dietary shifts and ontogenetic changes to prey selection in nursery bound lemon sharks. Such changes in diet are often linked to variations in habitat use, prey selectivity, enhanced foraging ability and metabolism (Jackson et al., 2004; Grubbs, 2010). Due to the difficulties associated with observation of foraging behaviour in wild sharks, no study has considered the potential changes in periodicity and frequency of foraging behaviour in sharks as they grow. It is well established that for most vertebrates, home range increases as an animal grows (McNab, 1963; Börger et al., 2008). Increases in home range and area of occupancy across ontogeny have been documented for several shark species (Chapman et al., 2005; Dicken et al., 2007; Weng et al., 2007). The lemon shark is a comparatively well documented species in this regard. Research at the Bimini Islands in the Bahamas has been dedicated to understanding home range and habitat use for this species (Morrisey & Gruber, 1993a; Morrissey & Gruber, 1993b). Franks (2007) studied ontogenetic shift in space use for juvenile lemon sharks using distinct nursery sites at the Bimini Islands finding a significant positive relationship for both distance to shoreline and space use when tested against age/body size. Sharks consistently showed a high degree of site fidelity to their natal nursery and there was no movement between nurseries. Young sharks had small home ranges, often spending most of their time in certain core areas, closer to shore. Larger individuals had larger home ranges and spent more time in areas further from the shoreline (Franks, 2007).

However, such studies have been limited to the use of acoustic telemetry and have, to date, been unable to determine ontogenetic changes at a quantitative behavioural scale. Franks was unable to determine which environmental or biological factors influence changes in home range and space use nor how activity and behaviour of animals changed as their space use expanded. Adapting behaviour to match ontogenetic changes in energetic and dietary requirements profoundly affects the ability of an organism to obtain food resources and thus survive (Lowry & Motta, 2007). Measuring behaviour and activity of sharks at different ages will improve understanding of the important factors associated with ontogenetic shifts and thus, survival. Several studies have shown that more than one shark species can often occupy the same nearshore area, either using the same resources or partitioning the habitat at different scales. Simfendorfer & Milward (1993) compared use of a tropical bay, with some species using the site only seasonally (whitecheek, creek whaler, blacktip and Australian blacktip shark) and others using it year round (spot-tail, milk and Australian sharpnose shark). Pikitch et al. (2005) assessed demographic population structure of lemon, nurse and Caribbean reef sharks, and southern stingrays at a Caribbean atoll. DeAngelis et al. (2008) characterized a nursery habitat in the UC Virgin islands and found evidence for habitat partitioning by two shark species (blacktip and lemon sharks) and Knip et al. (2010) reviewed the multi-species use of nearhshore environments. Although these studies represent an understanding of differences in interspecific use of nearshore habitat, intraspecific differences in behaviour and habitat use have received little attention (Knip et al., 2011). Understanding ontogenetic shifts in patterns of behaviour and habitat use will help to understanding further intraspecific competition as well as shifts in habitat requirements and ecosystem role in a shared environment.

The aim of this study was to build upon existing knowledge by using novel tag packages to investigate ontogenetic shifts in spatio-temporal patterns of behaviour and activity for young lemon sharks, using a shallow-water coastal nursery habitat at Bimini, Bahamas. Further, the study aimed to consider the environmental and biological drivers for hypothesised ontogenetic shifts in habitat use, activity and behavioural patterns.

Specifically, the objectives of this research were: 1) to measure habitat use in a spatial context, overall activity and specific behaviour of sharks during a range of ontogenetic stages (based on total length of animals); 2) to delineate ontogenetic changes in habitat use, activity and specific behaviour, if there are any; and 3) to determine which factors, if any, affect observed ontogenetic shifts.

6.2 Materials and Methods

This chapter` explores data collected using methods described in chapters 2, 4 and 5 of this thesis. Data from captive trials were not used for data analysis in this chapter. Capture, tagging and recapture of wild sharks, summarised in section 4.2, yielded high frequency acceleration data across a size range of sharks (78 – 169 cm TL). Comparisons of overall dynamic body acceleration (ODBA; as a measure of overall activity) and specific behaviours, including resting, foraging and prey handling, were explored using this data. Acoustic telemetry techniques described 4.2 provided data on movement and habitat use. Data collected from baited remote underwater videos (BRUVs) deployments, described in section 2.7, were used in this chapter to consider ontogenetic changes in habitat use and prey choice. All data were explored and analysed relative to the research objectives outlined in section 6.1. The R software package (v 3.2.3, R Core Development Team 2012) was used to present figures and findings from statistical testing, unless stated otherwise. All data were tested for assumptions of normality and statistical procedures were selected as per Zuur et al. (2010).

6.2.1 Data analysis

Comparisons of habitat use across different sized sharks were conducted by generating a minimum convex polygon (MCP) for each shark, using a series of active tracking data. MCPs were only generated for sharks with at least 12 data points summing to a minimum of one h of tracking. Geospatial modelling environment (GME), software that operates within R, produces shapefiles to be visualised in ArcGIS. A ‘genmcp’ function was used within GME to convert tracking point data into minimum convex polygons, which were then uploaded into ArcGIS and presented as maps. For each MCP, a value of overall area was calculated in km^2. This area value was used to test for differences in overall activity space in different sized sharks. Distance-to-shore data were generated for each shark as described in 2.8.1 and a mean overall distance-to-shore value for each shark was compared across individuals. Six sharks of different sizes were tracked for over one h in each tidal phase and mean distance-to-shore, calculated for each tidal phase, were compared between sharks. A one-way ANOVA tested for significant differences between these data for each individual and Tukey’s pairwise comparisons identified differences between tidal groups.

Acceleration data were explored and analysed using Igor Pro v 6.37 and Ethographer, as described in section 2.8.2. A mean ODBA value across the entire deployment period was calculated for each shark, representing its overall mean activity. Mean tidal ODBA values were generated for each shark for the entire deployment. Data describing percentage time spent resting in each shark were produced using *K*-means clustering and mask analysis procedures described in section 4.3.1. Foraging and prey handling data, described in section 5.3.1 were also compared between sharks. Total foraging events, total prey handling events and % foraging success were analysed between individuals. Per-second temperature data from accelerometers were also analysed. Mean temperature and depth values for the whole deployment period were calculated for every shark and tested across ontogeny. BRUV data (see Chapter 5 for more information on BRUV surveys and their methodological use in this research) were investigated alongside space use data to assess potential size-related changes in prey availability and selection. A total fish abundance value was derived for each shark by summing the total fish MaxN found within the MCP of that shark (sum of MaxN values at all deployment sites inside the MCP). Larger MCP ranges, however, are likely to encompass more BRUV deployment sites and a greater overall value for fish abundance. Therefore, relative available fish abundance was derived by dividing total fish abundance by the MCP area. Abundances of key faunal groups (classified in Chapter 5) were also compared between shark MCPs. All of the above described variables were individually compared against shark size using simple linear regression models. Using individual tests eliminated issues with overfitting models and multicollinearity. The aim of data analysis, in this instance, was to explore the relationships of individual variables with shark size and, as such, more complex model fitting was unnecessary. A Welch's t-test, used to test unequal variances in samples of unequal sizes, was selected to test for differences in faunal group abundance between Mangrove Edge and Non Mangrove Edge (described in section 2.7) sub-habitat types.

6.3 Results

Nineteen wild shark deployments were conducted for this research (Table 6.1). Minimum convex polygons were generated for 14 of these 19 deployments (all sharks with ≥ 1 h tracking time) (Table 6.1, Fig. 6.1). Small sharks were found to use smaller overall area than larger sharks; the smallest shark (D40, 76 cm) had an MCP of 0.43 km^2 and the largest shark (773, 169 cm) had an MCP of 4.75 km^2. A positive linear relationship was found between MCP and size for all sharks ($r^2 = 0.466$, $P < 0.01$) (Table 6.2, Fig. 6.2a). Mean distance-to-shore data, gathered from all tracked sharks, also showed smaller sharks to occupy areas generally closer to the shoreline than larger individuals, with linear regression again indicating a positive relationship with size ($r^2 = 0.577$, $P < 0.01$) (Table 6.1, Table 6.2, Fig. 6.2b). Findings from Chapter 4 showed strong tidal patterns in movement, whereby sharks occupied areas furthest from shore at low tides and areas closest to shore at high tides. As sharks grow and use areas further from shore in general, this tidal pattern in proximity to shore appears to persist (Table 6.3).

Interrogation of ODBA data showed that smaller sharks were more active overall than larger sharks. The smallest shark tested in this study had a mean ODBA of 0.079 *g* ± 0.0004 across the entire deployment period and the largest shark had a mean ODBA of 0.040 *g* ± 0.0002. A significant negative relationship was found between mean ODBA and increasing size of sharks ($r^2 = 0.653$, $P < 0.01$) (Table 6.2, Fig. 6.2c). Assessment of temporal variables at an individual level showed that tidal patterns in activity varied across ontogeny. Smaller sharks displayed more distinct differences in activity between tidal phases. These sharks were consistently most active at low tide phase and least active at high tide. However, these tidal patterns became generally less distinct in larger sharks with much smaller differences in activity between high, mid and low tide phases (Table 6.2, Fig. 6.3a). Diel patterns in activity also varied over ontogeny, smaller sharks showed greater differences in mean ODBA between diel phases whereas larger sharks mean activity was more even over different diel phases (Table 6.2, Fig. 6.3b). Mean percentage resting behaviour displayed by sharks was much higher in smaller individuals than in larger ones with a significant negative relationship with size ($r^2 = 0.588$, $P < 0.01$) (Table 6.2, Fig. 6.2d). Analysis of foraging behaviour across ontogeny showed that total foraging events tended to be greater in larger sharks ($r^2 = 0.561$, $P < 0.01$, Table 6.2, Fig. 6.2e) Shark 901 was eliminated from this model as an outlier, producing 87 recorded foraging events. Despite data showing an increase in foraging events in larger individuals, a model for prey handling events showed no such pattern across ontogeny and percentage foraging success calculated from these data had no significant relationship with shark size (Table 6.2, Fig. 6.2f).

Analysis of temperature data from accelerometers showed that mean water temperature in areas occupied over the deployment period did not vary across ontogeny and the only distinct temperature differences between individuals were those accounted for by seasonal fluctuations (Table 6.2, Fig. 6.2g). The four sharks deployed in the cooler dry season had markedly lower mean water temperatures of areas occupied as would be expected in a sub-tropical system.

Data from all 140 BRUV deployments were used to investigate faunal distributions relating to shark activity space. Mean relative fish abundance did not have a discernible relationship with size of shark (Table 6.2, Fig. 6.2h). However, as sharks grew, their area of occupancy overlapped with areas of increased abundance of Carangid fishes. Using mangrove-edge and non-mangrove-edge parameters, mean Carangidae abundance was much greater in non-mangrove edge areas (mean NME abundance = 7.44 ± 1.76 SE, mean ME abundance = 1.19 ± 0.26 SE). Gerreidae fishes were identified in Chapter 5 and previous research (Newman et al., 2010) as potentially valuable prey items for these sharks. Abundance was not different between mangrove-edge and non-mangrove-edge zones (mean NME abundance = 4.92 ± 1.31 SE, mean ME abundance = 4.70 ± 1.20 SE) (Table 6.4).

Table 6.1 Summary information for all sharks tested in this study. Distance-to-shore, ODBA, % resting, % bursting, temperature and depth data area all mean values calculated for each individual. TL = total length. Shark ID codes d2* and d3* refer to second and third deployments of the same shark.

Shark ID	TL (cm)	MCP (km^2)	Distance-to-shore (m)	ODBA (*g*)	% Resting	Foraging Events	Prey Handling	% success	Temp (ºC)	Relative Faunal Abundance
D40	76	0.43	42.82	0.079	14.26	16	3	18.8	31.52	753.23
437	78	0.65	142.11	0.086	17.71	2	1	50.0	31.28	564.01
511	84	n/a	205.24	0.101	4.47	24	5	20.8	31.48	n/a
C69	101	n/a	n/a	0.092	8.95	19	2	10.5	28.81	n/a
C25	101	1.26	162.5	0.075	11.91	14	4	28.6	24.06	888.93
708	101	0.89	158.87	0.082	5.35	11	3	27.3	28.75	290.38
C25(d2*)	104	1.89	176.53	0.073	9.8	7	3	42.9	23.91	462.86
E50	105	0.65	180.57	0.082	8.21	18	3	16.7	29.04	823.95
C69(d2*)	110	5.48	158.87	0.086	12.03	24	2	8.3	30.76	440.4
224	117	n/a	n/a	0.082	11.09	11	2	18.2	28.71	n/a
C69(d3*)	119	3.72	82.32	0.075	3.93	31	4	12.9	25.12	1153.79
85F	120	n/a	442.75	0.071	12	20	5	25.0	30.59	n/a
901	122	n/a	n/a	0.07	2.01	87	9	10.3	29.91	n/a
345	129	4.02	341.67	0.054	0.54	30	6	20.0	30.68	283.28
F7F	132	1.85	150.15	0.063	0.44	24	3	12.5	30.66	831.38
267	135	1.8	322.04	0.074	6.09	25	6	24.0	30.5	431.03
O82	148	3.22	383.68	0.069	2.6	21	4	19.0	25.32	695.58
E07	160	9.15	477.49	0.06	0.78	45	2	4.4	30.22	268.8
773	169	4.75	397.44	0.041	2.6	50	3	6.0	30.33	837.48

Table 6.2 Results from linear regression models for each variable tested against size of shark.

Variable	r^2	*P*	d.f	F Statistic
MCP	0.466	**< 0.01**	12	10.49
Distance-to-shore	0.577	**< 0.01**	14	19.06
Mean ODBA	0.653	**< 0.01**	17	31.92
Resting	0.588	**< 0.01**	17	25.20
Foraging events	0.561	**< 0.01**	16	22.74
Prey handling events	0.022	0.555	16	0.36
Mean temperature	0.002	0.850	17	0.03
Relative overall fish abundance	0.017	0.660	12	0.20

Table 6.3 Summary of tidal differences in mean distance-to-shore values for different sharks. Different colours represent statistical differences between groups, identified by post hoc tests. H = high tide phase, M = mid tide phase, L = low tide phase.

Shark	Size	H	M	L	*P*
D40	76	12.12	79.64	334.55	***
437	78	5.87	46.74	75.86	***
511	84	39.34	193.76	382.61	***
C69(2)	110	281.34	134.45	200.92	**
267	135	148.40	246.15	571.57	***
E07	160	257.41	250.58	824.46	***

Table 6.4 t-test results comparing relative abundance data for fish groups between habitat-type. ME = Mangrove-edge, NME = Non-mangrove-edge.

	Mean Relative Abundance				
	ME	NME	d.f	t	*P*
Total fish Abundance	57.17	33.07	69	3.28	**< 0.01**
Carangidae fishes	1.19	7.44	120	-3.53	**< 0.01**
Gerreidae fishes	4.7	4.92	102	-0.12	0.90

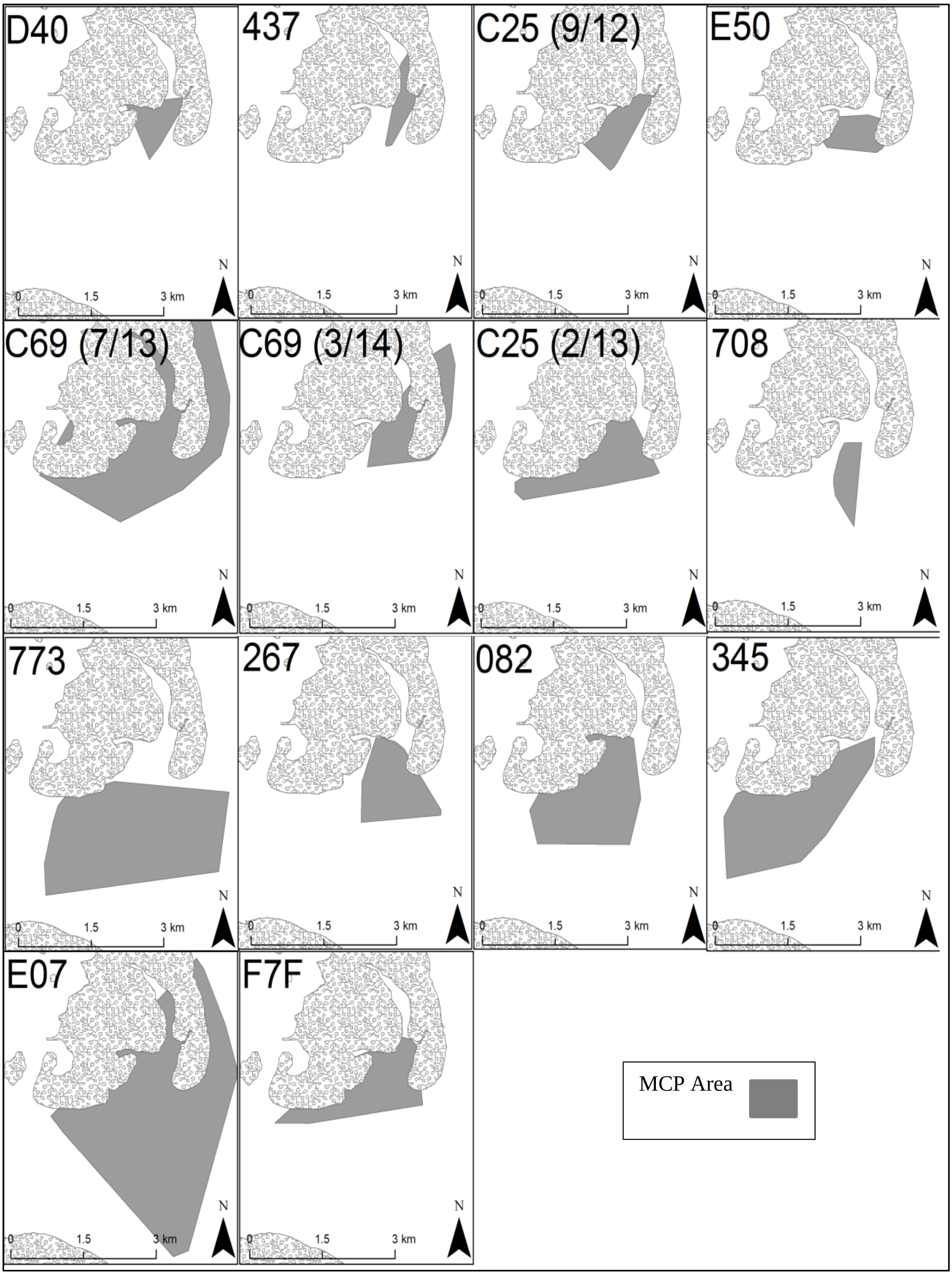
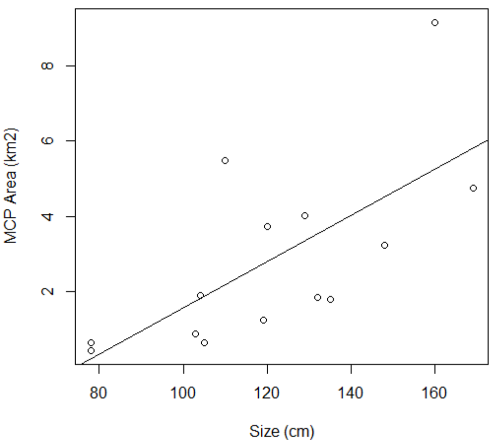
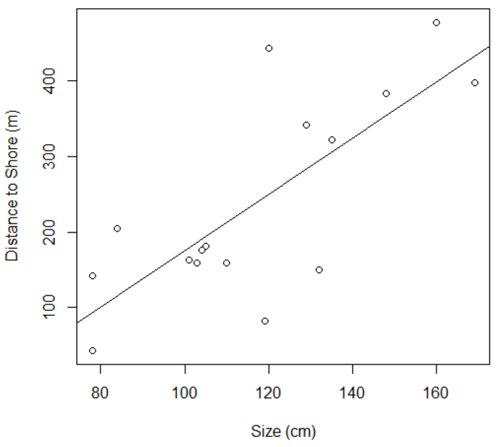


Fig. 6.1 Minimum convex polygons (MCPs) representing area of use for each actively tracked shark. MCP was calculated for 14 of 19 total wild deployments, each with at least one h of tracking data. Date of deployment is included for each shark with multiple deployments over the study.

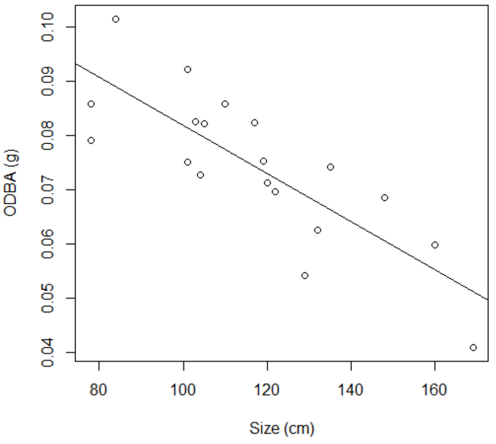
a)


MCP Area (km2)
2
4
6
8
80
100
120
140
160
Size (cm)

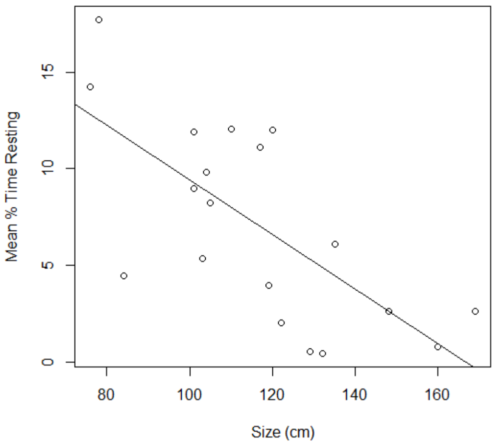
b)


Distance to Shore (m)
100
200
300
400
80
100
120
140
160
Size (cm)

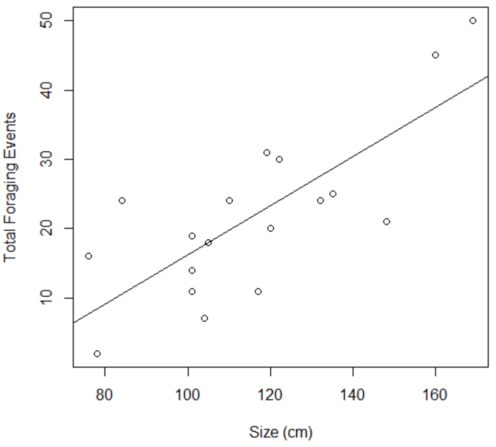
c)


ODBA (g)
0.04
0.05
0.06
0.07
0.08
0.09
0.10
80
100
120
140
160
Size (cm)

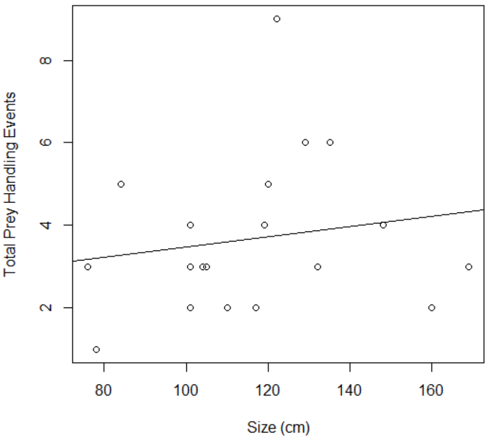
d)


Mean % Time Resting
0
5
10
15
80
100
120
140
160
Size (cm)

e)


Total Foraging Events
10
20
30
40
50
80
100
120
140
160
Size (cm)

f)


Total Prey Handling Events
2
4
6
8
80
100
120
140
160
Size (cm)

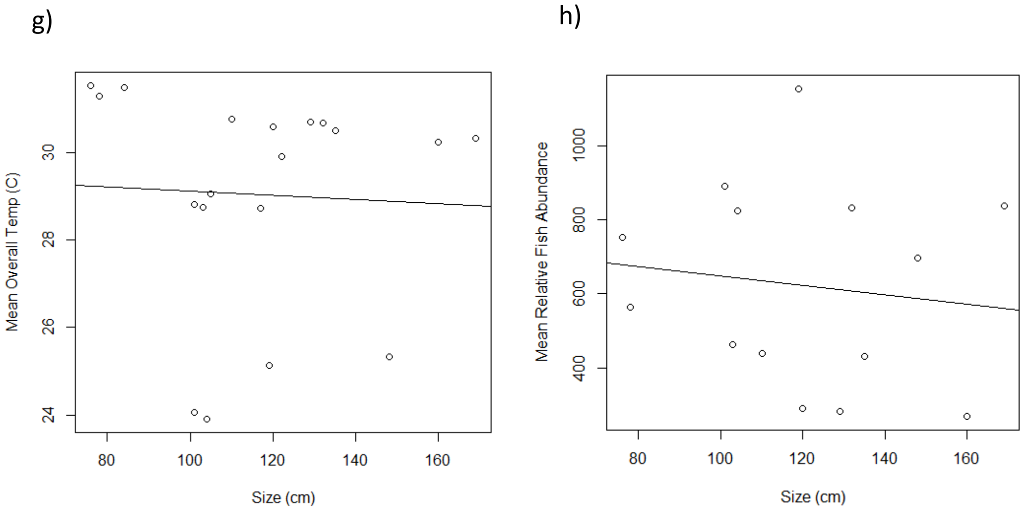


Fig. 6.2a-h Simple linear regression models for all variables tested against size of shark. Each circle marker represents a value for each shark and solid lines represent linear regression lines (summary in table 6.2). Size (x-axis label) = total length of sharks.

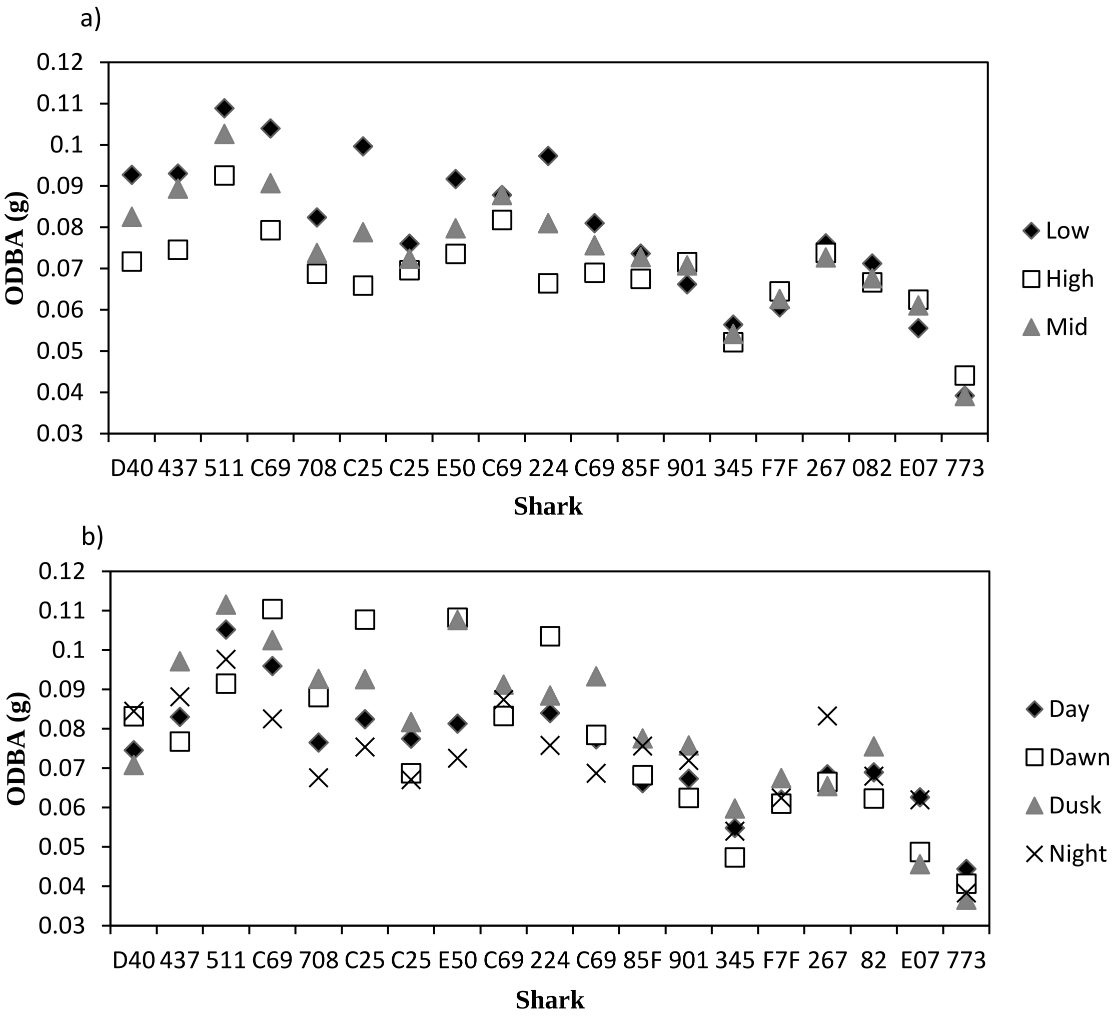


Fig. 6.3 a) Mean ODBA values at different tidal phases (high, mid and low) for each shark; b) Mean ODBA values at different diel phases (dawn, day, dusk and night) for each shark. Sharks displayed in increasing size order from left to right.

6.4 Discussion

6.4.1 Ontogenetic changes in habitat use

This research provides quantitative understanding of ontogenetic changes in habitat use by sharks. Findings relating to MCP and area occupied are congruent with findings for other species as well as previous research on juvenile populations of this species at the Bimini site. Juvenile pigeye sharks *Carcharhinus amboinensis* (Muller & Henle, 1839), for example, showed ontogenetic shifts in habitat use with older juveniles using larger areas. Movements of all sharks were tidally related, but tide had the strongest effect on the movements of the youngest sharks (Knip et al., 2011). Activity space for larger juvenile bull sharks is an order of magnitude larger than for smaller juveniles (Yeiser et al., 2008) and juvenile lemon sharks in Bimini increase activity space as they grow. Smaller sharks use more restricted areas, closer to shore but expand their range and time spent in areas further from shore as they grow (Morrissey & Gruber, 1993b; Franks, 2007). Small juvenile lemon sharks using a nursery site at a tropical atoll in Brazil restricted their activity space to near shore, reef flat areas, whereas larger sharks roamed more freely in open waters (Wetherbee et al., 2007).

Findings here, considering a broader size range than previously studied for this species, showed general increases in MCP over ontogeny and distance-to-shore data showed larger sharks to consistently use areas further from shore (Fig. 6.1, 6.2a). Previous research as well as findings discussed in Chapters 4 and 5 show tidal phase to be a primary driver of movement patterns in site-attached lemon sharks. These tidal patterns are primarily considered to be predator avoidance behaviour by smaller sharks, using shallow, near-shore refuge areas at high tides when most vulnerable to predation (Morrissey & Gruber, 1993a; Guttridge et al., 2012). Larger sharks were here found to consistently use areas further from shore, rarely using areas as close to the shoreline as smaller individuals. However, movement patterns in terms of distance to the shoreline did remain tidally mediated across ontogeny. These findings support existing theory in that larger sharks, with a reduced threat of predation, have a lesser need to use nearshore areas for the purpose of refuge. Experimental studies of predation risk effects on semi-captive lemon sharks showed a negative relationship between shark size and the use of an artificial refuge area, in the presence of a predator (Stump et al., 2017). This study concluded that size is an important factor in determining antipredator behaviour of lemon sharks.

6.4.2 Ontogenetic changes in activity and behaviour

Although some research measuring ontogenetic changes to swimming performance and energetics in fish exists (e.g. Fuiman & Webb, 1988), prior to this study, few have provided direct data on changes in physical activity of sharks over an ontogenetic timescale. Findings here identified a significant negative correlation between overall activity and increasing shark size. Sundstrom and Gruber (1998) used speed sensing transmitters to construct a bioenergetics model for lemon sharks and found smaller juvenile sharks (0 - 2 years) spent 22% of their consumed energy on production and 50% on metabolism, whereas larger sub-adult sharks spent only 7% on production and 66% on metabolism. This suggests larger-bodied sharks must commit more consumed energy to greater metabolic costs than smaller individuals. The observed reduction in overall swimming activity as sharks grow could represent a means to reduce metabolic costs and maintain a bioenergetic balance. As discussed in section 4.4, it is whilst occupying near-shore areas at high tides that smaller sharks exhibit most resting behaviour. Increases in resting behaviour over the high tide phase could represent a means to conserve energy in areas of low productivity, until valuable foraging areas, further from shore, become safer to use. This theory could explain why, as sharks grow, they reduce their overall expression of resting behaviour, as reduced time spent in near-shore, low productivity areas also reduces the need to rest.

The management of bioenergetics and energetic output are deemed to be extremely important in determining activity space (Shank, 1986). Therefore, an animal will occupy the smallest area that satisfies all of its energetic requirements (Abramsky & Tracy, 1980; Morrissey & Gruber, 1993b). Larger lemon sharks with greater energetic requirements meet these needs by expanding their range southward into open lagoon areas. These areas must therefore offer greater reward in either abundance of prey, type and quality of prey or environmental conditions facilitating metabolic performance. Temperature determines the rate of key physiological processes such as digestion and growth (Grubbs, 2010) and water temperature influences movements and habitat use for several shark species (e.g. Morrissey & Gruber, 1993a; Grubbs et al., 2007). However, water temperatures did not vary significantly across this study site at a spatial scale (see section 4.3). It is therefore reasonable to assume that ontogenetic expansion of area use, in this nursery, relates to the acquisition of food. Data showed that total foraging events recorded by accelerometer increased as sharks grew larger. These findings are supported by those of Newman et al. (2012), in which stomach content analyses of Bimini lemon sharks suggested an ontogenetic shift in number of prey consumed. As discussed in section 6.4.1, tracking data showed that larger sharks spend less time in nearshore areas and expand their range further into open lagoon areas and it is likely that recorded increases in foraging effort coincide with a decrease in predation risk and associated use of nearshore areas. As predation risk decreases, sharks are less constricted in the time available for foraging in productive areas further from shore. Sharks may also increase their foraging efforts overall to meet increasing energetic requirements associated with growing body size (Sundstrom & Gruber, 1998; Carlson et al., 2004). Data, however, also showed that prey handling events did not increase over ontogeny with foraging effort and that larger sharks displayed similar numbers of prey handling events to smaller ones. Percentage foraging success of sharks varied between individual but there was a decreasing trend in % foraging success with size, and the two lowest success rates (4.4, 6%) were calculated for the two largest sharks. This suggests that larger sharks may need to invest a greater amount of time and energy into foraging to achieve the same rate of capture success. Lucifora et al. (2009) referred to a potential senescence related decline in performance and changes in sensory capabilities as sharks age, influencing ontogenetic changes in diet. Larger sharks may be less able to employ effective stealth approach and ambush tactics that smaller sharks use to capture their prey. Larger sharks observed in this study may adjust their diet to meet greater energetic demands but this may be driven by a reduced foraging success. More profitable prey captures (i.e. larger prey or prey with greater calorific value) would be required to account for this reduced success. It may also be, however, that larger sharks, with a larger gape, swallow more prey items whole and make use of prey handling behaviours less often. As discussed in section 6.1, ontogenetic shifts in diet are common in almost all shark species (e.g. Bethea et al., 2006; Estrada et al., 2006; McElroy et al., 2006).

Results relating to mean relative fish abundance showed there were no ontogenetic differences found in availability of fish taxa overall. Areas further from shore did not have greater relative fish abundances and, when compared using mangrove-edge and non-mangrove-edge parameters, were found to be poorer in overall fish abundance. In terms of key prey groups, previous studies in Bimini (Newman et al., 2010) and findings from section 5.3 have shown a preference for Gerreidae fishes in juvenile lemon sharks. There was no correlation found between abundance of Gerreidae fishes within MCP ranges of different sized sharks. Both smaller and larger sharks used areas with similar Gerreidae abundance. Carangidae fishes, however, were found in greater abundances overlapping MCPs of larger sharks.

In general, these fishes were found in greater abundances in areas further from shore, where larger sharks spend more time (see section 5.3). Carangids are the most abundant and largest fish food source regularly inhabiting the Bimini site. Larger sharks may make changes to areas of the habitat they use to accommodate an ontogenetic shift in diet to exploit a more profitable food source. Such dietary shifts to larger food items across ontogeny is very common among most marine species, especially sharks (e.g. Cocheret de la Morinière et al., 2003; Estrada et al., 2006; Ellis & Musick, 2007; Graham et al., 2007). Tiger sharks, sevengill sharks and silky sharks *Carcharhinus falciformis* (J.P. Muller & Henle, 1839), for example, display ontogenetic shifts in their diet, feeding on larger prey items and different prey types as they grow (Lowe et al., 1996; Ebert, 2002; Duffy et al., 2015). These findings provide a more complete understanding of the changes in habitat use, activity and specific behaviour that are associated with shifts in diet.

6.5 Conclusions

Lemon sharks occupy the Bimini island nursery site for the first 3 - 5 years of their lives. Findings here represent a novel understanding of how their use of this habitat and their behaviour within it changes over this time, as they grow. Sharks change the areas they use, spending more time in open waters, further from shore as they grow larger. Overall activity reduces as does the frequency in expression of resting behaviour. Foraging events increase across ontogeny, but foraging success does not. As is well established, predator avoidance drives young sharks to use refuge habitats near shore and influences other important drivers such as prey acquisition. Findings from this research are likely demonstrating a shift in behavioural priority. As sharks grow and predation risk decreases they are driven by a combination of increased metabolic cost, optimal energy intake and decreased foraging efficiency to expand their area of use and to increase their foraging efforts towards larger, more profitable prey items.

Sharks, like most animals, must balance this optimal intake of food with the avoidance of predation. Shifts in this balance across ontogeny influence their bioenergetics, fitness and survival through the early stages of their lives. Understanding these processes helps understand the sensitivity of these animals to human-induced habitat alterations that can ultimately affect recruitment to adult populations.

Chapter 7 – General Discussion

7.1 Summary of research findings

As coastal ecosystems face increasing pressure from human interference, understanding how species that rely on these systems use them and how they respond to changes within their environments is becoming increasingly important (Borja et al., 2016; Borja et al., 2017). This research used a combination of technologies and techniques to provide a fine-scale understanding of how lemon sharks use a shallow-water, tropical nursery habitat. This study investigated patterns in space use, activity and specific behaviour to better understand the interactions these sharks have with the biological and environmental components of the nursery system and to identify key requirements from that system. In doing so, this study aimed to produce a complete spatio-temporal map of activity and behaviour, thus better describing the use of nursery habitat in the context of fitness and survival.

Findings from the research have contributed new understanding of this species and how it uses its nursery environment. Chapter 3 shows that sharks are resilient to capture, handling and tagging processes but that they do modify their natural habitat use patterns after capture and handling. In chapter 4, tidal patterns in movement show that sharks use nearshore areas over the high tide and move away from the shoreline into open areas at low tide. Coinciding with these movements, sharks tend to be more active over the low tide in open areas further from shore. Measurements of specific behaviour in wild animals show that sharks mostly rest over the high tide, near shore, but express most bursting behaviour when using open, offshore areas. Patterns in activity and behaviour correlate with temperature, as sharks are generally less active and rest more frequently in cooler temperatures and increase activity and bursting behaviour as temperatures increase. Chapter 5 shows that most foraging events and prey handling events occur over the low tide period, in areas further from shore. Increased foraging effort occurs in areas with lower overall faunal abundance but in areas coinciding with high abundance of preferred prey species and increased visibility. Chapter 6 shows that as they grow, sharks occupy a larger home range and use nearshore areas less frequently. As they grow sharks continue to move into and use areas further from shore and some larger sharks tidal patterns in movement, activity and behaviour become far less distinct. Sharks become generally less active overall as they grow larger and greatly reduce the expression of resting behaviour. Foraging rate increases as sharks grow, though frequency of successful prey capture does not. Overall, sharks display behaviour representative of an adaptive optimal mode, responsive to short term environmental changes, biological cues and relative changes over ontogeny,

This research contributed to scientific understanding of:

1) the response of young sharks to capture and tagging processes;
2) the requirement for minimally invasive externally mounted devices;
3) the value of captive trials and ethograms for accelerometer-derived behaviour studies;
4) quantitative habitat use, activity and specific behaviour of young sharks in a nursery system.
5) the biological and environmental variables that influence habitat use, activity and behaviour in young sharks using a nursery system;.
6) the bioenergetics and metabolic performance in young sharks;
7) foraging ecology in wild sharks, including periodicity and frequency of foraging behaviour as well as potential foraging tactics and success rates;
8) biological and environmental variables influence foraging and food acquisition behaviours in young sharks;
9) ontogenetic shifts in nursery habitat use of young sharks, including changes to spatio-temporal patterns of activity and behaviour across the ontogenetic scale representing responses to shifting pressures;
10) ontogenetic shifts in the ecological role and/or trophic position of the lemon shark;
11) the critical importance of shallow-water nursery sites to the survival of young sharks.

To meet O2 of this research, to produce scientifically rigorous data that are reliably representative of natural behaviour, the study firstly investigated the effects of external fin-mounted tag packages and the responses of sharks to capture and tagging processes. The presence of fin-attached tag packages did not affect swimming kinematics and behaviour in tested sharks. Capture and tagging processes caused elevated activity and swimming kinematics upon release, but this period of increased activity was brief, and all sharks resumed 'normal' swimming behaviour quickly (no later than 35 min after release). Sharks demonstrated a perceived refuging response upon release from capture and tagging, with sharks heading towards the shoreline in all cases for up to an hour. H2 of this thesis stated that the methods used do not influence behaviour in the long-term and they produce reliable data representative of natural behaviour. Findings support this hypothesis in that tags do not cause deviations in natural behaviour and that data collected reliably reflects natural behaviour of wild sharks. Findings from existing literature, relating to tagging effects of sharks, is varied, evidencing the need to incorporate testing into experimental design of studies using different tag packages and animal handling techniques. The small and light tag package used for this research and the brief handling times likely played an important role in ensuring minimal effects on the animals.

Behaviour represents an animal's response to its environment and the other organisms with which it interacts. In order to survive, all animals must alter their behaviour over space and time. For immature animals, not yet influenced by factors associated with reproduction, daily routine behaviour must represent a balance between maximising foraging success and minimising risk of predation whilst optimising energetic and metabolic performance (Papastamatiou et al., 2015). To meet O3, O4 and O5 of this research (see section 1.3), captive ethogram trials, tagging and tracking of wild sharks, analysis of faunal communities and environmental sampling were used to elucidate the complete behavioural routine of young lemon sharks using the nursery habitat.

Predation is well established as a factor defining the morphological and behavioural attributes of animals across an evolutionary scale but more recently evidence points to predation as a driver for behavioural changes within an animal's lifetime. Studies have investigated the implications of predation risk to animal behaviour and how predation risk is perceived by animals (Lima & Dill, 1990). Much research has been dedicated to understanding the concept of decision-making under the risk of predation. Non-lethal predator-prey interactions and behavioural modifications, made to reduce predation risk, have been documented for a range of species (Lima, 1998a; Lima, 2002). Anti-predator behaviours can influence reproductive behaviour, overall fitness and even influence the function of ecological systems at a fundamental level (Lima, 1998b).

At the Bimini site, predation risk for young lemon sharks is considered to increase greatly at higher tides and in associated deeper waters (Guttridge et al., 2012; Stump et al., 2017). During these times, many sharks in this study moved into mangrove-fringed nearshore areas, likely using these areas as a refuge from predators. Various studies have investigated habitat use patterns of this species and the investment in predator avoidance behaviour as part of daily routine. Guttridge et al. (2012) described similar tidally influenced movement patterns and noted intra-specific predation threat as a major motivator for these patterns. Size-dependant anti-predator investment was suggested as a factor in how long sharks spent nearshore, with smaller individuals staying near the shoreline for longer periods and moving away from these areas latest. Other studies, observing lemon sharks at different sites (Wetherbee et al., 2007), also reported tidally influenced movement patterns and suggest predator avoidance as the primary driver. In semi-captive, experimental conditions, predator presence (larger conspecific) proved a significant driver of artificial refuge use by Bimini lemon sharks (Stump et al., 2017). This research supports the conclusions drawn by these studies.

In terms of impact to fitness and survival, the consequences of failure to avoid predation are severe. Anti-predator behaviour, therefore, is often the primary determining factor influencing overall behaviour in most meso-predatory animals. Predation risk and predator avoidance, however, only explain part of the behavioural routine of the sharks in this study. The conceptual trade-off between predation risk and foraging is well explored and, for many taxa, areas of increased foraging profit are also riskier in terms of predation (Lima & Dill, 1990; Heithaus, 2007). Findings relating to O3, O4 and O5 of this research further the current understanding of coastal sharks using nursery sites, demonstrating a behavioural strategy to trade-off predator avoidance with foraging and energy intake, exploring how this changes over ontogeny as well as how the nursery habitat provides the conditions necessary for this trade-off.

The relationship between energy acquisition and energy expenditure is an important one that defines the success of any animal (Brown et al., 2004, Gleiss et al., 2011). Quantifying how energy is used and gained in the daily routine of sharks is critical in better understanding how they manage their daily energy budget against competing drivers such as predator avoidance. Food acquisition is the result of successful foraging. Sharks that gain more energy (by successfully capturing prey) than they lose during attempts to acquire it are able to incorporate more of that energy into growth. Foraging behaviour is, however, one of the most difficult to study and least understood aspects of shark ecology (Sims, 2010). Understanding foraging habits in lemon sharks has been, to date, largely derived from diet studies. For example, Wetherbee and Cortes (2004) described feeding in lemon sharks as asynchronous and intermittent, showing no pattern in its periodicity. Though this may be the case for larger, adult sharks, that may have more limited access to food resources, this research, using accelerometry, has shown distinct and repeatable patterns in feeding periodicity among most sharks studied, although these patterns are absent in the largest sharks. Foraging tactics displayed by different shark species have been studied and many different species utilise various behavioural means of hunting for and capturing prey (see section 5.1). In this study, findings showed that many sharks, influenced by predator avoidance, selectively increased overall activity and foraged in lagoon areas away from the shoreline at times of lower predation threat. These areas were defined by lower percentage seagrass coverage, increased visibility and higher abundances of known preferred prey. This suggests an effort by these sharks to maximise their foraging efficiency, potentially either through increased capture success or higher energetic reward. Furthermore, few foraging events lasted for prolonged periods and most less than 10 sec. Many short bursts ended in prey capture, as estimated by accelerometry. Stealth approach and ambush predatory tactics may increase success in this environment, minimising energetic costs associated with foraging behaviour. Tiger sharks observed using a seagrass ecosystem did not attack vigilant prey that were aware of their presence and neither did they engage in prolonged pursuits of prey. It is suggested that tiger sharks employ stealth tactics and rely on close undetected approaches for successful prey capture (Heithaus et al., 2002). Observation of young lemon sharks using an archipelago in Brazil found sharks to commonly perform “substrate inspection” of crevices and holes in rocks and reef (Garla et al., 2017). The sand bottom of the Bimini lagoon is characterised by uneven mounds and gulley’s that sharks could be patrolling and inspecting in similar ways. Although sharks must commit energy through increased costs of more strenuous and fast muscle movements when foraging, they must also minimise metabolic costs associated with foraging, and energy reducing tactics such as those highlighted above are one way to reduce the cost of foraging behaviour in this regard. Findings also showed that foraging behaviour became less tidally driven in larger sharks, however, these sharks still foraged in open lagoon areas with the same environmental characteristics (lower seagrass cover, higher visibility than nearshore areas) and foraging events remained brief.

Selecting suitable areas in which to forage and using foraging tactics to minimise energy costs is important but when foraging must be traded off against predator avoidance, time spent in suitable foraging habitat can be restricted which can impact overall energetics. Sharks that are constrained to using the apparently unproductive nearshore areas during high tides may increase activity and foraging effort in productive areas further from shore out of necessity to acquire energy within a restricted timeframe. Swimming faster allows for more area to be covered and increases chance of prey encounter but costs more metabolic energy and could explain why over high tides, in nearshore areas, these smaller sharks significantly reduce activity and rest. Larger sharks, with less predation pressure, have a broader timeframe over which they can potentially forage, and data show that despite an increase in recorded foraging events in larger sharks, overall activity decreased with increasing size of shark. Differences in activity over ontogeny could be explained by energy costs associated with swimming.

Studies have shown that larger sharks have a lower cost of transport than smaller sharks (Carlson et al., 2004) and may be able to maintain the same swimming speeds with less physical activity. Accelerometers measured mechanical movements of the animals to which they were attached but not the speed. The observed reduction in ODBA in larger sharks may evidence a reduced cost to maintaining swimming speeds.

Previous research describes a “bigger is better” theory and a selection towards feeding efficiently under predation pressure in order to grow faster (Milinski, 1986, DiBattista et al., 2007). Large size confers benefits in a reduced susceptibility to predators, access to more food types and earlier maturation (Urban 2007). Findings here, however, suggest that predation pressure limits the ability of young sharks to pursue a strategy towards faster growth and larger size. Most sharks in this study routinely altered their behaviour to account for elevated predation risk, at the cost of foraging rate. This suggests that in nursery habitats, smaller size and slower growth are more effective strategies towards overall fitness, under the threat of predation. DiBattista et al. (2007) investigated the size and growth of several populations of Bimini lemon sharks, observing small size and low growth rates at this site, suggested that lemon sharks in Bimini select against large size and fast growth. Various other studies have also produced results that conflict with the ‘bigger is better’ theory (Quinn et al., 2001; Carlson et al., 2004b). DiBattista et al. (2007) offered several hypotheses for their findings, identifying the most likely cause as predation pressure. It is presumed that faster growing individuals forage more frequently at times of higher predation risk and that, in the Bimini environment, the risk of predation outweighs the benefits of further foraging. The study conducted by DiBattista et al. (2007) focussed on small juvenile sharks for which predation pressure is a significant limitor to foraging and energy gain.

Employing tactics to be efficient when actively foraging is key, but moderating activity at times of lower forage potential is also important to conserve energy. In this study, many sharks spent periods of time resting on the seabed, unmoving. Resting behaviour has been recorded in various shark species. In Caribbean reef sharks, resting is attributed to facilitation of cleaning interactions with a goby species (Sazima & Moura, 2000) but for many species resting is linked to metabolic processes and energy management (Whitney et al., 2012). When using areas of lower foraging profit, sharks are able to modify their energetic output to minimise wasted energy. Resting behaviour can facilitate digestion and minimise unnecessary energy wasted in swimming. Resting behaviour could be a key factor in determining the growth rates of lemon sharks, especially younger, smaller sharks that are restricted to less productive habitats for longer periods and with a likely higher cost of transport than larger individuals. Indeed, smaller sharks spent much more time resting than larger ones. Foraging behaviour and prey handling events in larger sharks were more evenly spread across the tidal cycle. If larger sharks are not expending large amounts of energy in time-constrained foraging periods, they may have less need to reduce output and recuperate that energy loss at other times, hence the reduction in expression of resting behaviour. A lower cost of transport could also explain the reduced resting seen in larger sharks, as lower energetic costs associated with swimming reduce the relative benefits or necessity for this behaviour.

Temperature is a key variable in controlling metabolic rate and has been shown to impact activity and behaviour in many elasmobranch species (Carlson et al., 2004, Sims et al., 2006). Findings from this research found associations between activity and foraging behaviour with temperature. Most sharks were least active in cooler water, increasing their activity as water temperatures increased. Various studies have provided evidence for a “hunt warm, rest cool” bioenergetics strategy for several elasmobranch species (Matern et al., 2000, Sims et al., 2006). Findings from this research could indicate a similar bioenergetic strategy for these sharks, maximising foraging efficiency in warm waters and facilitating digestion in cooler waters. Other research, however, indicates that digestion rates are not shown to be largely impacted by water temperature (e.g. Carlson, et al., 2004, Heithaus, 2007) and ontogenetic shifts found in activity and foraging periodicity of sharks in this study show that larger sharks showed no relationship between activity and foraging behaviour with water temperature. Larger sharks, with a reduced predation threat influencing their daily routine may adopt an alternative bioenergetics strategy or the observed patterns in activity and foraging behaviour of smaller sharks may be connected to the above described trade-off between predator avoidance and energy acquisition. Temperature regimes coincide with rising and falling tides, but so does predation risk in this habitat. Associations with temperature may simply be an artefact of the sharks responding to a different cue.

Ecosystem dynamics can be complex for many meso-predators. This research has shown that non-consumptive predation risk effects are of high importance in driving overall behaviour of lemon sharks in this study. Findings have also uncovered other important factors that mediate anti-predator behaviour. As previously stated, many organisms must maximise energy intake through food acquisition at times or in areas of lower predation pressure. At optimal fitness, an individual will manage this energy acquisition versus predator avoidance trade-off well, feeding optimally (within the limits of the trade-off) and avoiding death. However, for most animals, it is not possible to constantly achieve this balance. The condition of an animal can determine its thresholds for risk. Green sea turtles *Chelonia mydas* (Linnaeus, 1758), for example, demonstrate condition-dependant risk taking in seagrass communities. Turtles in poor physical condition select profitable but high-risk (in terms of predation) micro-habitats, whereas turtles in good condition select safer but less profitable micro-habitats (Heithaus et al., 2007). Threespine stickelbacks *Gasterosteus aculeatus* Linnaeus, 1758 that are parasitized and have a greater need for energy than uninfected individuals have been shown to compromise safety from predation for foraging and energetic gain. Parasitized fish remained longer in areas near a predation hazard and when experimentally frightened (by a model heron), fled shorter distances, remained hidden for shorter times and returned to forage sooner (Godin & Sproul, 1988). In crucian carp *Carassius carassius*, under experimental conditions, level of hunger, as well as predation risk significantly affected habitat used. Well-fed carp stayed in safe zones away from higher risk habitat, but hungry individuals used open areas, despite predation risk (Pettersson & Bronmark, 1993).

For predatory animals, such as sharks, success, in terms of energy gain, is dependent on foraging success. It is a fundamental understanding of foraging ecology and behaviour that success varies and consequent impacts on animal condition from failure to acquire energy can impact the overall behaviour of the animal. It is likely that a shark that has been successful in hunting will be responsive to threat cues and move to safer areas. A shark that has not been successful may choose to alter its behaviour and remain in more exposed or higher risk areas for longer, increasing its chances of successful prey capture at the cost of potential mortality. Such condition-dependant risk taking is a likely a function of adaptive decision making towards overall fitness. In young lemon sharks, failure to catch prey will likely impact animal condition and thus incite changes to risk taking from an increased hunger and need for food and energy. Sharks may continue to use open areas for foraging over increasingly risky periods and water depths. Previous research of lemon sharks in Bimini has provided no evidence for starvation in these sharks, suggesting that predation is the major cause of mortality in young lemon sharks and thus signifying that these sharks will tolerate greater risk of mortality to forage and avoid starvation (Gruber pers. comm. *fide* Heupel et al., 2007).

The most prevalent occurrence of state-dependent risk taking is that of size-related differences in risk taking. For many species, increasing body size correlates with decreasing predation risk, especially in the marine world due to gape limitation whereby prey can outgrow a predator’s capacity to hunt them (Urban, 2007). Lemon sharks in this study showed shifts in habitat use, activity and foraging periodicity and frequency that indicate a shift in how they use the habitat as predation risk decreases over ontogeny. Many species, faced with a foraging rate versus predation risk trade-off, have also been reported to make changes to behaviour, or to habitat occupied, based on changes in body size (e.g. Werner & Hall, 1988; Magnhagen & Borcherding, 2008). Lima and Dill (1990) referred to a hierarchy of decision making in feeding animals under the threat of predation. This starts with when to feed, then where to feed, then what to eat and how to consume it. Predation threat in this system most directly influences when and where these sharks feed. The use of terms such as 'choice' and 'decision making' can imply selection and adaptive reasoning and should not be ruled out in lemon sharks, given the research into their social structures and personalities (Guttridge et al., 2009; Guttridge et al., 2010; Finger et al., 2017). The routine nature of these animals, however, may represent less of an active decision-making process and more a response to cues in biological and environmental factors over ecological time.

The following theoretical model (Fig 7.1) projects the habitat use, activity and specific behaviour expressed by young lemon sharks under the threat of predation, framed as an individual's determination towards growth and survival. At times of low predation risk, sharks move into and use open lagoon areas. If sharks are hungry their drive to forage will be high and they will increase activity and foraging effort, being either successful or not. Over time, as long as predation risk remains low, a shark will behave in this way until it is no longer hungry, at which time it will reduce activity to a steady swimming state and may potentially forage opportunistically. For most sharks, predation threat will at some point increase and, when it does, a shark's decision-making processes change in response to it. If a shark is not hungry, having foraged successfully at times of lower risk, it will use mangrove-fringed, nearshore areas, reduce activity and foraging effort, and rest. It may forage opportunistically in these areas. As long as predation risk remains high, a shark will continue to behave in this way. If, having been unsuccessful in foraging at times of lower risk, elevated hunger may drive the shark to continue actively foraging in open lagoon areas, despite the increased threat of mortality. This risk could pay off in foraging success, reducing hunger and subsequent movement into safer nearshore areas to reduce activity and rest. It may also prove unsuccessful, leading to continued hunger and further deteriorated condition, exposure to risk or death. As sharks grow larger, theory described in this section suggests they experience a reduced predation risk, meaning, in this model, that larger sharks would more often perceive a lower threat, spend more time in open lagoon areas and thus less time in nearshore areas. This allows larger sharks to respond to hunger cues more readily, as they spend more of their time in suitable foraging areas. This, in turn, allows larger sharks to maintain a lower overall activity, throughout the day. Less time spent using nearshore areas also reduces expression of resting behaviour overall.

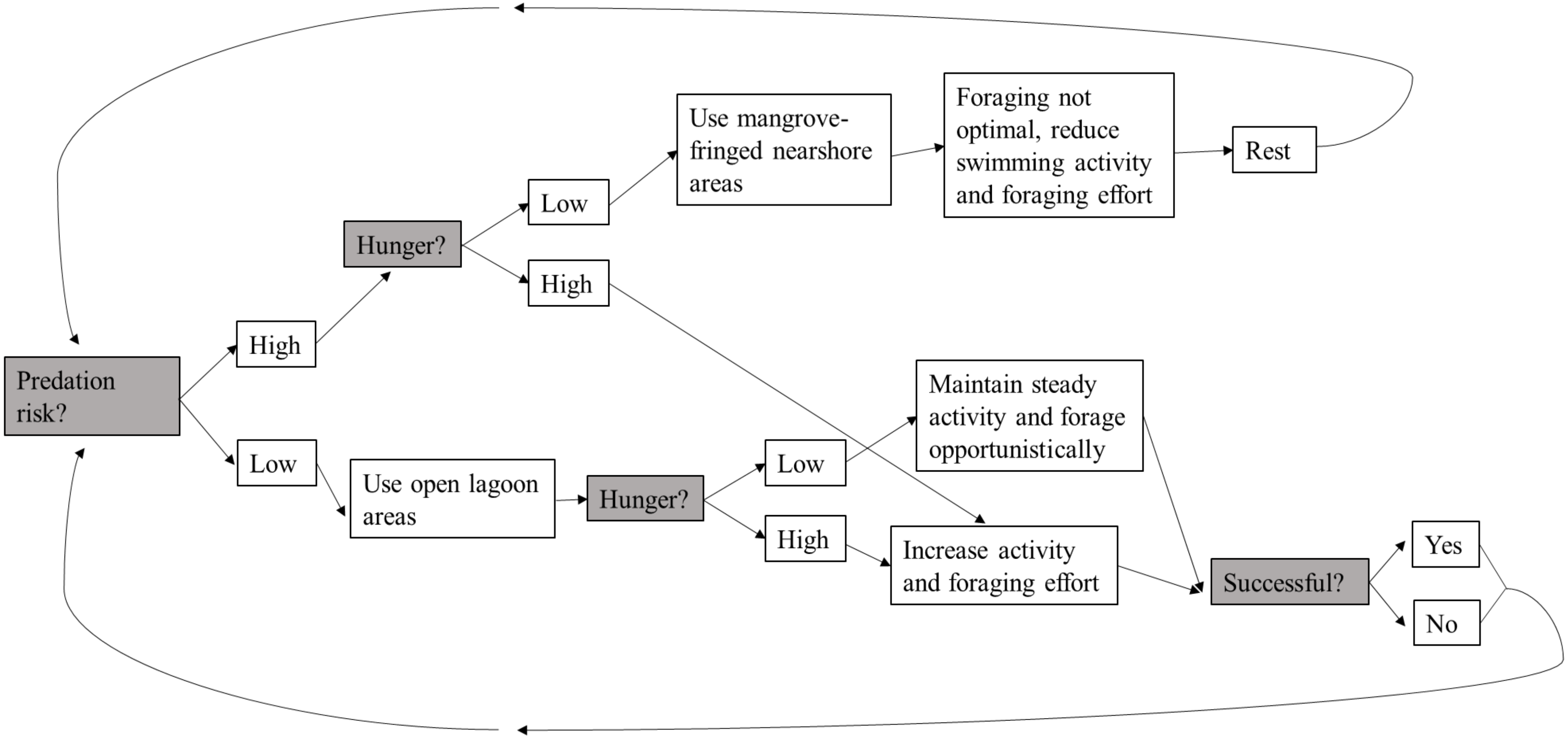


Fig 7.1 Theoretical model explaining spatio-temporal activity and behaviour relative to the threat of predation (by author, 2018).

Sharks use the Bimini site for the first 3-5 years of their lives before moving away into broader ranges and other habitats. This transition is not likely to be induced by reproductive motives as these sharks reach maturity at later ages (Feldheim et al., 2001; Chapman et al., 2009). It is more likely that the habitat no longer provides sufficient food resources to meet the energetic needs of larger individuals (Gruber et al., 1982). Larger sharks spent most time in open lagoon areas and ventured further from shore than other smaller sharks. BRUV survey data showed that larger carangid fishes used these areas more often. More time spent by larger sharks in these areas likely indicates an ontogenetic dietary shift from the preferred Mojarra prey of smaller sharks to larger prey items. This diet shift may only sustain the energetic needs of these sharks for a period of months before they move away from the islands in search of more profitable forage sites. DiBattista et al. (2007) noted that changes in selection relating to size and growth can occur at different life history stages and that, at a later life stage, lemon sharks may then shift to faster growth and larger size. Findings here relating to time spent foraging and increased time spent in more profitable areas may suggest that, as predation pressure diminishes, sharks may become more able to pursue a strategy towards faster growth and larger size. This may coincide with their departure from the nursery site and a potential shift in their trophic position. Further research into the habits of lemon sharks after they leave the nursery site will delineate this theory further.

7.2 Intrinsic Value of the Nursery Habitat and Impacts of Environmental Perturbation and Habitat Loss

The value of nursery sites to various shark species has been well explored (e.g. Heithaus, 2007; Heupel et al., 2007; Parsons & Hoffmayer, 2007; Oh et al., 2017). A suite of factors derive benefit to the fitness and survival of sharks using them. The sixth and final hypothesis of this research was that the nursery habitat provides conditions and resources necessary for the fitness and survival of sharks using that habitat. Study findings strongly support this hypothesis, providing a detailed understanding of how the nursery site provides key variables to facilitate survival in these sharks. Shoreline habitat features and tidal regimes offer protection, stable and plentiful prey resources are provided by the habitat and environmental conditions such as temperature and visibility likely play important roles in bioenergetic strategy, foraging and fitness. In Bimini, the natural survival rate of juvenile lemon sharks is estimated to be between 38 and 65% (Gruber et al. 2001). Given this, it is valid to assume that even with the series of physical components, resources and conditions of the habitat that support survival, the daily lives of these sharks and their survival is in a delicate balance. It is therefore also valid to assume that even relatively small changes in the habitat could have drastic impacts to the fitness and survival of these sharks. Freitas et al. (2009), studying the survivorship of juvenile lemon sharks in a Brazilian nursery, suggest that even modest threatening activities may dramatically affect the nursery-bound populations at this site.

As discussed in section 2.1, mangrove systems are important to the survival of many marine species. Jennings et al. (2008) described the impacts of the development of a large resort to the North Sound area of the Bimini Islands. They described dredging and sediment dumping as well as the removal of large areas of the mangrove forest shoreline. Their findings, when coupled with those of this study, can be used to infer the potential consequences to behaviour and survival of lemon sharks, when exposed to such environmental perturbation (Fig. 7.2).

Large-scale loss of mangrove habitat could reduce sharks access to refuge and supply of forage food, noise disturbance could elicit refuging behavioural responses similar to those found after capture and tagging, and changes to habitat and faunal community structure could negatively impact foraging success. Habitat degradation, loss of key habitat components and physical changes to the environment in the Bimini site would likely alter routine behaviour in these sharks, potentially increasing exposure to predation and decreasing foraging efficacy, thus negatively impacting their rate of survival. Jennings et al. (2008) found a significant reduction in survival rate of lemon sharks using the affected site compared with sharks using unperturbed areas of the islands. As the availability of suitable habitat is reduced, sharks may shift their areas of occupancy from degraded sites to the nearest suitable sites. More sharks using limited space will, however, inevitably lead to problems with resource competition and potential cascading effects of over-exploitation of prey species.

Coastal development around the Bimini Islands continues with little knowledge of the impacts from these activities. So far, the area of the islands used for this study remains unperturbed but with plans to remove large chunks of the eastern parts of the islands for a golf course, this likely will not remain the case for much longer. The findings from this research and the insights they provide are valuable to management and conservation bodies by demonstrating the minimum habitat requirements needed to maintain healthy populations of a species, and theorise the likely impacts to species conservation status, when these requirements are not met. This information can contribute to the mitigation of harmful anthropogenic interference in this ecosystem. Secondarily, this research acts as a blueprint for routine behaviour and habitat use in an unperturbed environment. Degrading impacts from human activities can then

be evaluated against potential changes in observed routine behaviour and used as an indicator of disturbance.
As mentioned already, lemon sharks are currently listed as Near Threatened on the IUCN Red List of threatened species with no current management plans in place (IUCN, 2018). Lemon sharks exhibit natal philopatry, returning to the exact same site to give birth, throughout their lives (Feldheim et al., 2014). There is no evidence to suggest that these sharks will change where they give birth if a site is degraded or even if that would be possible in some regions. The survival of young lemon sharks is requisite to the availability of suitable nursery habitat. If human activities continue to reduce the availability and quality of critical nursery habitat, this will have severe consequences for the conservation status of this species.

In building a thorough understanding of how these sharks behave within nursery sites, this study can act as a tool for informing conservation and management measures to ensure the stability of this key species, moving forward, both at the Bimini site and across its range. In 2000, Bimini was designated as the highest priority site in the Bahamas for the implementation of a Marine Protected Area (MPA) and despite this, this MPA has not been designated. Consideration of other management measures is needed to address the protection needs in Bimini. Marine environmental management is increasingly informed by problem-structuring frameworks (Elliott et al., 2017). These frameworks are informed by fine-scale data on the structures and interactions of the ecosystems for which they are applied. These research findings provide evidence for a state change in the natural ecosystem of Bimini, brought about by pressures from human activities. The use of problem-structuring frameworks, such as DAPSI(W)R(M) (Elliott et al., 2017), could mitigate the effects of human activity in the form of coastal development, mangrove loss, sediment dumping and dredging. Key Biodiversity Areas (KBAs) are sites that contribute significantly to the global persistence of biodiversity (IUCN, 2016). Sites can qualify as KBAs by meeting one or more of a series of criteria relating to threatened biodiversity; geographically restricted biodiversity; ecological integrity; biological processes; and irreplaceability (IUCN, 2016). The Bimini islands could be assessed under these criteria and may qualify as a KBA. This KBA listing then flags this site as a priority for conservation attention and catalyses conservation planning processes that can impact legislation, support the implementation of protected areas and inform the description of the site under international conventions. The greatest threat to the overall stability of the Bimini nursery system and to lemon shark survival rates is the loss of the complex shoreline habitat that the mangrove root thicket provides. Protection of mangroves at this site is of paramount importance in this regard.

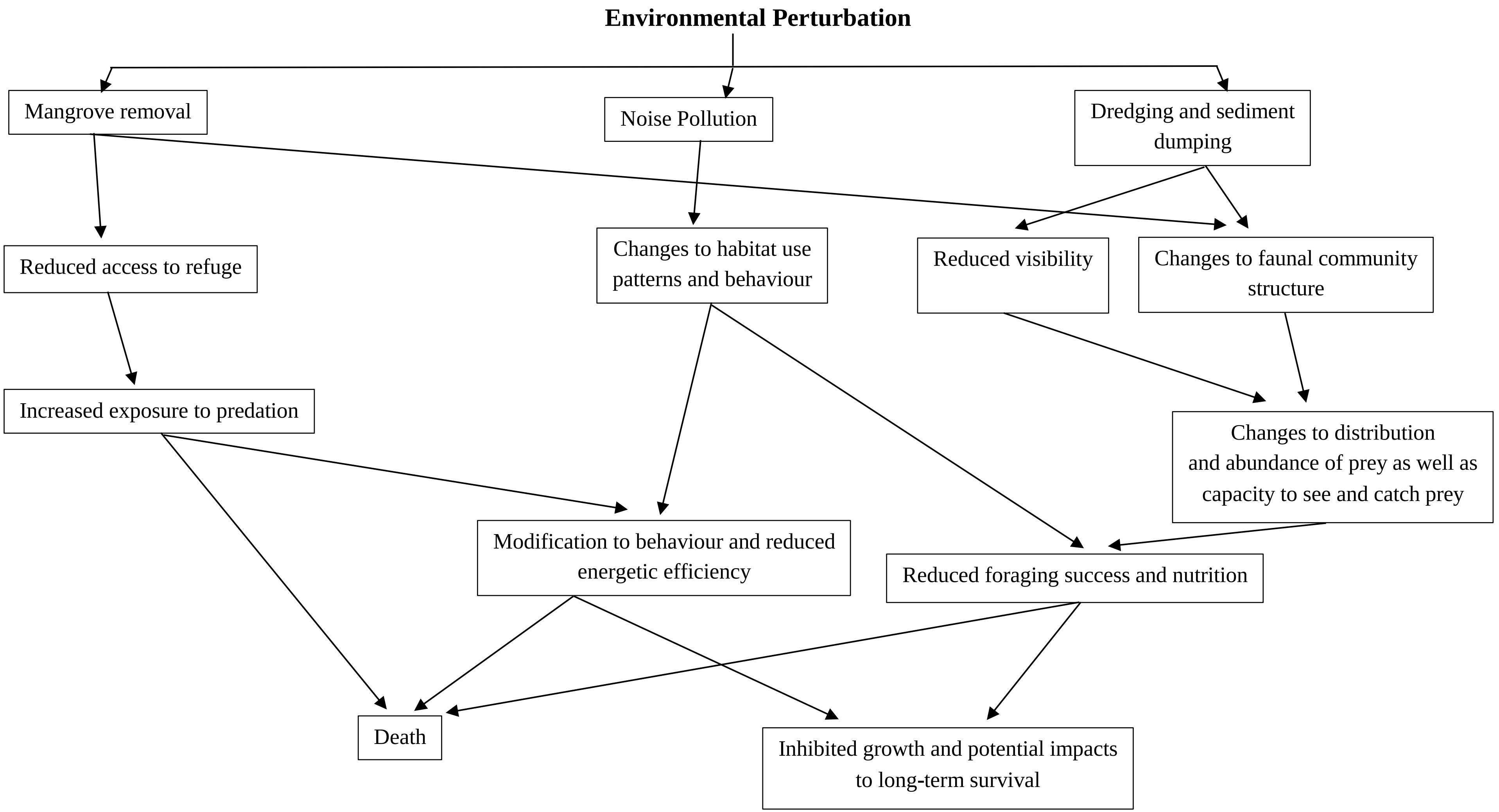


Fig. 7.2 Theoretical model exploring the consequences of environmental perturbation due to coastal development, based on the behavioural understanding gained from this research and the known perturbations experienced in neighbouring habitat (by author, 2018).

7.3 Limitations of this study and suggested directions for future work and research

7.3.1 Logistical constraints

Of the 19 wild shark deployments carried out in this study only four were conducted in the dry season. This was largely due to the nature of the research and education schedule of the field station. Busy periods of priority seasonal research, inclement weather, university courses and funding limited the capacity for large-scale field research commitments over these times. Future ecological studies are needed to incorporate a more even spread of deployments across different seasons, allowing for more in-depth investigation into the potential influences this factor may have in animal behaviour. Despite this, season had little observable influence to the outputs of this research and despite significant changes in seasonal water temperatures; routine spatio-temporal behavioural patterns were not shown to alter between seasons.

This study was limited to working with sharks > 70 cm and < 180 cm. Sharks smaller than 70 cm were deemed to be too small to be unaffected by the presence of the tag package and there were concerns relating to the rejection of the tag package by the thinner tissue of smaller sharks’ fins. Sharks larger than 180 cm rarely use the study site, venturing much further into open waters where tracking and recapture were near impossible. Although information on movements, activity and behaviour of larger sharks would have proven valuable, the risk of losing the shark and thus the expensive tag package was too great. Indeed, the largest shark tagged in this study (181 cm) was tracked for a short period upon release and never located again. The small PT4 transmitters used in this study proved invaluable in maintaining a light and small external tag package but to do so compromised signal strength and duration. These limitations may be what prohibited locating and recapturing several sharks with attached tags. The limited signal strength also meant tracking crews inevitably spent more time searching for sharks to track than if sharks were tagged with stronger, longer-range transmitters. Tracking was also limited in extreme low tides when large areas of the lagoon habitat would become extremely shallow, preventing trackers access to some shallow areas sometimes used by sharks. Logistical restrictions relating to fuel costs, inclement weather and operator fatigue also affected tracking durations.

As discussed in Chapter 5, stomach lavages were conducted on several sharks to provide sample diet data. These data would have been used to corroborate findings relating to distribution and abundance of prey species within the habitat. Lavages, however, proved ineffective, with all sharks having empty stomachs. Over the duration of this research the commitment of time and resources necessary to produce useable results from these methods were unavailable. Newman et al. (2010) conducted in-depth studies into the diet and prey preference of juvenile lemon sharks, and results from this research guided the conclusions drawn in the present study. Sharks were shown to more often occupy areas further from shore as they grew. Lemon sharks, like most shark species experience ontogenetic shifts in their diet over their lives. It is possible that sharks change their diet over their time occupying the nursery site, selecting different prey at different life stages and sizes. Studying potential dietary differences in sharks across the entire size range regularly using the nursery site could help to understand changes in habitat use and behaviour. It is also possible, however, that sharks opportunistically forage at all life stages, selecting habitat for other reasons and preying upon whatever species are present.

BRUV surveys were limited to daylight hours as recording at night requires specialist equipment that was not available for this research. Although faunal distributions remained largely static throughout different times of day and tidal phase, it is possible that at night distributions could alter significantly. Studies have shown distinct differences in mangrove fish assemblages across the diel, lunar and seasonal scale (Rooker & Dennis, 1991) and further research could consider the use of existing methods (Harvey et al., 2012) to operate BRUVs during night time hours.

7.3.2 Technology and methods

This study has shown that acceleration data loggers are capable of measuring specific behaviour in sharks over prolonged periods, whilst remaining minimally invasive and having only minor impacts to behaviour and activity post capture and tagging (Chapter 3). Although this is the case in this study, it is advised that similar research concepts and protocols should be incorporated into study design when using animal mounted tag packages.

The accelerometers used in this study were selected for their size, weight; sensitivity and capacity. CEFAS G6A accelerometers are most importantly small and light. In working with small, juvenile sharks, large and bulky tag packages would have been more likely to cause deviations in natural behaviour, caused stress in the animal or physical damage to it, or been too large to physically attach to the small dorsal fins of the sharks. G6A tags recorded at 30Hz frequency which was a fast enough logging rate to identify individual tailbeats and to separate all major behaviour states. As discussed in Chapter 3, the small size and light weight of the tag package was likely key in their remaining unobtrusive to tagged sharks and thus not affecting perceived natural behaviour. Tags recorded at the maximum log rate for a total of 5 days before memory was filled.

Another logistical limitation to this study was the cost of tags and their depletion after only two memory fills. Accelerometer tag technology continues to advance, offering smaller and lighter tags, capable of logging for longer periods, with longer lasting batteries. All of these advances translate to more sharks being tagged for longer periods, producing more data. The ability to study behaviour over longer periods allows investigation into longer-term changes to behaviour and activity over tidal and lunar phases, as well as potential associated shifts to seasonal changes in the physical environment. Larger data sets could also reveal patterns in foraging effort over broader time scales. Critter cams and other camera tags are also now being employed to better understand various aspects of marine animal behaviour. Skomal et al. (2015) used an automated underwater vehicle to track white and basking sharks and study their behaviour.

Being able to directly observe behaviour in sharks proved extremely useful in both defining the effects of the tags on the animals and in accurately categorising behaviours in acceleration data. Ethograms of different behaviours in a captive environment allowed for more reliable translation of wild shark acceleration data. It is therefore advised, where possible, to incorporate ethogram trials into studies using such tags for the delineation of shark behaviour in future research.

7.3.3 Foraging ecology

Accelerometers and the techniques used to analyse the data in this study were able to identify specific movements and behaviours associated with foraging. Throughout captive feeding trials, sharks were seen to manipulate prey items, tearing them into smaller pieces, which likely made prey easier to swallow and quicker to digest. This behaviour was unique to the handling of prey and allowed for estimation of these behaviours in wild, unobserved animals, expressing the same acceleration signatures. Although this was valuable for picking out foraging behaviour and even categorising successful events, it only provided an indication of this behaviour and was not collaborated by stomach content analysis or direct observation of foraging in the wild. As research moves forward and technologies advance, more in depth analysis of foraging and associated behaviours would provide a clearer picture of this critical aspect of ecology. Future studies could use alternative accelerometers, different accelerometer placement or different data analysis techniques to better identify foraging behaviour and specific foraging tactics or strategies. The use of technologies such as mandible accelerometers and gape sensors have become popular in other areas of marine research and would also allow for identification of prey capture and handling (Iwata et al., 2012; Ydesen et al., 2014). These methods would allow researchers to more reliably isolate and quantify foraging and prey handling in wild shark data. It would be possible to interpret encounter success in sharks across a range of factors such as habitat used, ontogenetic stage or individual skill. It would also be possible to investigate predatory tactics of different shark species. Ambush predatory species such as angel sharks (Squatinidae) and carpet sharks (Orectolobidae) capture prey from a static position and such rapid bursts in movement would be easily identifiable in acceleration data. Further to the study of physical behaviour, development of diet studies in different species would also produce a better understanding of foraging ecology as a whole. Advances in stable isotope analysis now means stable isotope profiles can provide information on trophic position, diet and movement habits in sharks (Hussey et al., 2011). Data on daily ration as well as preferred prey and prey size would be valuable in producing a complete understanding for different species with different environments, pressures and drivers.

As ability to observe the success rates of sharks develops, further research into condition-dependant risk taking could uncover adaptive decision-making processes and risk versus reward changes in habitat use and behaviour. Success rates could be compared to time spent in high risk habitat zones over a series of days, investigating the influence of hunger and/or energetic deficit on animal condition and survival.

7.3.4 Other concepts and directions

Personality and social behaviour in sharks is now receiving more scientific attention (Sims et al., 2000; Guttridge et al., 2010; Jacoby et al., 2012; Jacoby et al., 2014). Guttridge et al. (2009) studied Bimini lemon sharks, finding intra-specific size sorting with sharks spending more time with size-matched conspecifics. Testing repeatability in traits such as boldness and exploration is becoming more popular, as well as understanding the social structures and motives expressed by sharks. Interesting developments moving forward would involve the overlap between ecological behaviour and personality at an individual, intra-specific level. It may be that sharks defined as more ‘bold’ spend time foraging in higher reward, higher risk areas or more time foraging overall. Investigating the behavioural traits associated with sharks of different personalities could help to understand the costs and benefits of being more or less bold or explorative in different environments. Byrnes and Brown (2016) found individual personality differences in Port Jackson sharks *Heterodontus portusjacksoni* Blainville, 1816, with sharks showing predictably repeatable responses to stressors. Keller et al. (2017) found, in controlled trials, Bimini lemon sharks preferred to interact with familiar individuals, showing recognition potential in these sharks.

There is a growing need to link animal behaviour with conservation action and using changes in or altered states of behaviour as indicators of degradation or exploitation. However, with few empirical examples to support this, it has proven difficult (Caro, 2005). Some studies, however, have shown how behavioural theory can influence conservation management. For example, mammal species were tested for their wariness to observers in a national park in western Tanzania. Mammal species that were subject to covert human hunting pressure were significantly warier of observers than species exposed to lower exploitation levels. Such behavioural measurement can be used as a useful indicator of hunting pressure for different species and help to direct conservation management (Caro, 2005).

Heithaus et al. (2007) suggested that habitat selection behaviour has potential as a key indicator of anthropogenic impact in marine environments. In studies of a pristine seagrass ecosystem they found that habitat selection by prey species was largely determined by the edge micro-habitat used for predator avoidance and that the proportion of this edge micro-habitat is a better measure of overall habitat quality than size or availability in general. Habitat depletion already experienced in Bimini has involved large-scale removal of mangrove trees along the shoreline of the North Sound site. Such activities only minimally reduce the overall available habitat to sharks but do remove key habitat zones that sharks require for avoiding predators. Further research could investigate the spatio-temporal behaviour of sharks in such impacted areas and compare their habitat use patterns and specific foraging behaviour, for example, to that expressed in a similar un-impacted environment. Such research could help to demonstrate the potentially severe reduction in overall habitat quality by the removal of critical habitat components.

This study elucidates various aspects of ontogenetic shift in habitat use, activity and behaviour in young lemon sharks. This was, however, limited to the study of animals using the Bimini nursery site and did not investigate the behavioural ecology of adult sharks. Large sub-adult sharks eventually leave the Bimini site and move away from the shallow waters surrounding the islands. Although more research is being done to track and monitor larger sharks and uncover their patterns of movement at a broad scale, very little is understood of these animals, where they go and why. Although it is known that female sharks return biennially to deposit their pups in the safety of the mangroves, male sharks have rarely been recorded to return to the site once leaving. It is likely that sharks move away from the islands either to exploit more profitable food resources and sites, or to pursue reproductive imperatives. Genetic research into the population genetic structure found significant gene flow occurring in the western Atlantic and no evidence for distinct stocks (Feldheim et al., 2001). Uncovering the migration patterns, habitat use and foraging ecology of adult lemon sharks would help to create a complete picture of the life history of this species.

7.4 Conclusions

Upon conclusion of this study, the status of its hypotheses can be assessed.

H1: A combination of technologies and techniques can resolve fine-scale spatio-temporal behaviour and activity in young nursery-bound sharks.

H2: The methods used do not influence behaviour in the long-term and they produce reliable data representative of natural behaviour.

H3: Sharks display distinct patterns in habitat use, activity and behaviour across differing temporal scales. A combination of biological and environmental factors determines these patterns.

H4: Foraging and prey acquisition play an important role in defining habitat use, activity and behavioural patterns in nursery-bound sharks.

H5: As sharks grow, ecological pressures and priorities shift, affecting changes in how sharks use the nursery habitat.

H6: The nursery habitat provides specific conditions and resources necessary for the fitness and survival of young sharks. The structure and function of the nursery ecosystem is in a fine balance and even small changes can cause severe knock-on effects to the fitness and survival of sharks using it.

All hypotheses are supported by the findings of the research and can be accepted. Relating to H2; though the methodological approach did cause deviations in natural behaviour and space use in the short term, these effects were recorded and accounted for in subsequent analyses. All objectives of this research were met.

Many studies have investigated the importance and impact of particular factors on the overall behaviour and survival of animals in the wild. Far fewer, however, have considered how a suite of factors interplay in defining a complete behavioural routine. This research has quantitatively examined the habitat use, activity and behaviour of lemon sharks using a nursery environment in the Bahamas. Advances in remote technologies and associated techniques have allowed scientists to better understand where sharks are but have been far more restricted in resolving why they are there, from a behavioural perspective. By capturing high-resolution data on activity and specific behaviours, accurately in space and time, this research contributes novel understanding of why these animals behave in the way they do, how this behaviour represents fitness and survival, and consolidates the necessity of nursery habitats to the survival of young sharks, providing evidence to support the critical value of healthy marine ecosystems.

References

Abramsky, Z. and Tracy, C.R. (1980) Relation between home range size and regulation of population size in *Microtus ochrogaster*. *OIKOS* **34,** 347-355.

Ackerman, J.T., Kondratieff, M.C., Matern, S.A. and Cech, Jr. J.J. (2000) Tidal influence on spatial dynamics of leopard sharks, *Triakis semifasciata*, in Tomles Bay, California. *Environmental Biology of Fishes* **58**, 33-43.

Afonso, A.S. and Hazin, F.H.V. (2014). Post-release survival and behavior and exposure to fisheries in juvenile tiger sharks, Galeocerdo cuvier, from the South Atlantic. *Journal of Experimental Marine Biology and Ecology* **454**, 55-62.

Airoldi, L., Balata, D. and Beck, M.W. (2008) The Gray Zone: Relationships between habitat loss and marine diversity and their applications in conservation. *Experimental Marine Biology and Ecology* **366**, 8-15.

Anderson, M.J., Gorley, R.N. and Clarke, K.R. 2008. PERMANOVA+ for PRIMER: Guide to Software and Statistical Methods. PRIMER-E: Plymouth, UK. 1-214.

Andrews, K.S., Williams, G.D., Farrer, D., Tolimieri, N., Harvey, C.J., Bargmann, G. and Levin, P.S. (2009) Diel activity patterns of sixgill sharks, *Hexanchus griseus*: the ups and downs of an apex predator *Animal Behaviour* **78**(2), 525-536.

Ballard, G., Ainley, D.G., Ribic, C.A. and Barton, K.R. (2001) Effect of Instrument Attachment and Other Factors on Foraging Trip Duration and Nesting Success of Adelie Penguins. *The Condor* **103**(3), 481-490.

Barker, M.J., Gruber, S.H., Newman, S.P. and Schluessel V. (2005) Spatial and Temporal Variation in Growth of Nursery-Bound Juvenile Lemon Sharks (*Negaprion brevirostris*) A Comparison of Age Assigned Techniques. *Environmental Biology of Fish* **72**, 343-355.

Barnett, A., Abrantes, K.G., Stevens, J.D., Bruce, B.D. and Semmens, J.M. (2010) Fine-Scale Movements of the Broadnose Sevengill Shark and Its Main Prey, the Gummy Shark. *PlosOne* DOI:10.1371/journal.pone.0015464.

Martin, P. and Bateson, P.P.G. (2007) Measuring behaviour: an introductory guide. Cambridge, Cambridge University Press.

Baum, J.K. and Worm, B., (2009) Cascading top-down effects of changing oceanic predator abundances. *Journal of Animal Ecology* **78**(4), 699-714.

Beck, M.W., Heck Jr., K.L., Able, K.W., Childers, D.L., Eggleston, D.B., Gillanders, B.M., Halpern, B., Hays, C.G., Hoshino, K., Minello, T.J., Orth, R.J., Sheridan, P.F. and Weinstein, M.P. (2001) The identification, conservation and management of estuarine and marine nurseries for fish and invertebrates. *Bioscience* **51**(8), 633-641.

Bethea, D.M., Buckel, J.A. and Carlson, J.K. (2004) Foraging ecology of the early life stages of four sympatric shark species. *Marine Ecology Progress Series* **268**, 245-264.

Bethea, D.M., Carlson, J.K., Buckel, J.A. and Satterwhite, M. (2006) Ontogenetic and site-related trends in the diet of the Atlantic sharpnose shark *Rhizoprionodon terraenovae* from the northeast Gulf of Mexico. *Bulletin of Marine Science* **78**(2), 287-307.

Block, B.A., Jonsen, I.D., Jorgensen, S.J., Winship, A.J., Shaffer, S.A.,Bograd, S.J., Hazen, E.L., Foley, D.G., Breed, A.G., Harrison, A.L., Ganong, J.E., Swithenbank, A., Castleton, M., Dewar, H., Mate, B.R., Shillinger, G.L., Shaefer, K.M., Benson, S.R., Weise, M.J., Henry, R.W., Costa, D.P. (2011) Tracking apex marine predator movements in a dynamic ocean. *Nature* **475**, 86-90.

Börger, L., Dalziel, B.D. and Fryxell, J.M. (2008) Are there general mechanisms of animal home range behaviour? A review and prospects for future research. *Ecology Letters* **11**(6), 637-650.

Borja, A., Elliott, M., Andersen, J.H., Berg, T., Carstensen, J., Halpern, B.S., Heiskanen, A.S., Korpinen, S., Lowndes, J.S.S., Martin, G., Rodriguez-Ezpeleta, N. (2016) Overview of integrative assessment of marine systems: the ecosystem approach in practice. *Frontiers in Marine Science* **3**(20), doi:10.3389/fmars.2016.00020.

Borja, A., Elliott, M., Uyarra, M.C., Carstensen, J. and Mea, M. (2017) Bridging the gap between policy and science in assessing the health status of marine ecosystems, 2nd edition, doi: 10.3389/fmars.2017.00032, 548.

Bouyoucos, I.A., Suski, C.D., Mandelman, J.W. and Brooks, E.J. (2017) Effect of weight and frontal area of external tag packages on the kinematics, activity levels and swimming performance of small-bodied sharks. *Journal of Fish Biology* **90**, 2097-2110.

Bouyoucos, I.A., Suski, C.D., Mandelman, J.W. and Brooks, E.J. (2018) In situ swimming behaviours and oxygen consumption rates of juvenile lemon sharks (Negaprion brevirostris). *Environmental Biology of Fishes* **101**(5), 761-773.

Brewster, L.R., Dale, J.J., Guttridge, T.L., Gruber, S.H., Hansell, A.C., Elliott, M., Cowx, I.G., Whitney, N.M. and Gleiss, A.C. (2018) Development and application of a machine learning algorithm for classification of elasmobranch behaviour from accelerometry data. *Marine Biology* **165**: 62. https://doi.org/10.1007/s00227-018-3318-y.

Bridger, C.J. and Booth, R.K. (2003) The effects of biotelemetry transmitter presence and attachment procedures on fish physiology and behaviour. *Reviews in Fisheries Science* **11**, 13-34.

Brook, B.W., O'Grady, J.J., Chapman, A.P., burgman, M.A., Akcayaka, H.R. and Frankham, R. (2000) Predictive accuracy of Population Viability Analysis in Conservation Biology. *Letters to Nature* **404**, 385-387.

Brooks, E.J., Sloman, K.A., Sims, D.W. and Danylchuk, A.J. (2011) Validating the use of baited remote underwater video surveys for assessing the diversity, distribution and abundance of sharks in the Bahamas. *Endangered Species Research* **13**(3), 231-243.

Brown, L.R. and Moyle, P.B. (2011) Changes in Habitat and Microhabitat Partitioning within an Assemblage of Stream Fishes in Response to Predation by Sacramento Squawfish (*Ptychocheilus grandis*). *Canadian Journal of Fisheries and Aquatic Sciences* **48**(5), 849-856.

Brown, D.D., Kays, R., Wikelski, M., Wilson, R. and Klimley, A.P. (2013) Observing the unwatchable through acceleration logging of animal behaviour. *Animal Biotelemetry* **1**(20), 1-20.

Brownscombe, J.W., Thiem, J.D., Hatry, C., Cull, F., Haak, C.R., Danylchuk, A.J. and Cooke, S.J. (2013) Recovery bags reduce post-release impairments in locomotory activity and behavior of bonefish (Albula spp.) following exposure to angling-related stressors. *Journal of Experimental Marine Biology and Ecology* **440**, 207-215.

Brownscombe, J.W., Gutowsky, L.F.G., Danylchuk, A.J. and Cooke, S.J. (2014) Foraging behaviour and activity of a marine benthivorous fish estimated using tri-axial accelerometer biologgers. *Marine Ecology Progress Series* **505**, 241-251.

Bruce, B.D. (1992) Preliminary observations on the biology of the White Shark, *Carcharodon carcharias*, in South Australian waters. *Australian Journal of Marine and Freshwater Research* **43**(1), 1-11.

Buchholz, R. (2007) Behavioural biology: an effective and relevant conservation tool. *Trends in Ecology and Evolution* **22**, 401-407.

Byrnes, E.E. and Brown, C. Individual personality differences in Port Jackson sharks *Heterodontus portusjacksoni*. *Journal of Fish Biology* **89**(2), 1142-1157.

Cabrera-Chavez-Costa, A.A., Galvan-Magana, F. and Escobar-Sanchez, O. (2010) *Journal of Applied Ichthyology* **26**(4), 499-503.

Cagua, E.F., Cochran, J.E.M., Rohner, C.A., Prebble, C.E.M., Sinclair-Taylor, T.H., Pierce, S.J. and Berumen, M.L. (2015) Acoustic telemetry reveals cryptic residency of whale sharks. *Biology Letters* **11**(4), DOI: 10.1098/rsbl.2015.0092.

Campana, S.E., Joyce, W. and Manning, M.J. (2009) Bycatch and discard mortality in commercially caught blue sharks Prionace glauca assessed using archival satellite pop-up tags. *Marine Ecology Progress Series* **387**, 241-253.

Candolin, U. and Wong, B.B.M. (2012) Behavioural Responses to a Changing World; Mechanisms and Consequences. Oxford, Oxford University Press.

Carlson, J.K., Goldman, K.J. and Lowe, C.G. (2004a) Metabolism, energetic demand and endothermy. In Sharks and their relatives II: biodiversity, adaptive physiology and conservation. Carrier, J.C., Musick, J.A. and Heithaus, M.R. (eds) 203-224. Boca Raton, FL: CRC Press and Taylor and Francis Group.

Carlson, S.M., Hendry, A.P. and Letcher, B.H. (2004b) Natural selection acting on body size, growth rate and compensatory growth: an empirical test in a wild trout population. *Evoluionary Ecology Research* **6**, 955–973.

Caro, T.M. (2005) Behavioural indicators of exploitation. *Ethology*, *Ecology and Evolution* **17**(2), 189-194.

Caro, T. and Sherman, P.W. (2013) Eighteen reasons animal behaviourists avoid involvement in conservation. *Animal Behaviour* **85**, 305-312.

Cartamil, D., Wegner, N.C., Aalbers, S., Sepulveda, C.A., Baquero, A. and Graham, J.B. (2010) Diel movement patterns and habitat preferences of the common thresher shark *Alopias vulpinus* in the Southern California Bight. *Marine and Freshwater Research* **61**(5), 596-604.

Casterlin, M.E. and Reynolds, W.W. (1979) Diel Activity Patterns of the Smooth Dogfish Shark, *Mustelus Canis*. *Bulletin of Marine Science* **29**(3), 440-442.

Castro, J.I. (1993) The shark nursery of Bulls Bay, South Carolina, with a review of the shark nurseries of the south-eastern coast of the United States. *Environmental Biology of Fishes* **38**(1-3), 37-48.

Chapman, D.D., Pikitch, E.K., Babcock, E. and Shivji, M.S. (2005) Marine Reserve Design and Evaluation Using Automated Acoustic Telemetry: A Case-study Involving Coral Reef-associated Sharks in the Mesoamerican Caribbean. *Marine Technology Society Journal* **39**(1), 42-55.

Chapman, D.D., Babcock, E.A., Gruber, S.H., DiBattista, J.D., Franks, B.R., Kessel, S.A., Guttridge, T., Pikitch, E.K. and Feldheim, K.A. (2009) Long-term natal site-fidelity by immature lemon sharks (Negaprion brevirostris) at a subtropical island. *Molecular Ecology* **18**(16), 3500-3507.

Chen, W.K., Liu, K.M. and Liao, Y.Y., 2008: Bioenergetics of juvenile whitespotted bamboo sharks *Chiloscyllium plagiosum*. *Journal of Fish Biology* **72**, 1245-1258.

Chin, A., Kyne, P.M., Walker, T.I. and McAuley, R.B. (2010) An integrated risk assessment for climate change: analysing the vulnerability of sharks and rays on Australia's Great Barrier Reef. *Global Change Biology* **16**(7), 1936-1953.

Cocheret de la Morinière, E., Pollux, B.J.A., Nagelkerken, I., Hemminga, M.A., Huiskes, A.H.L. and van der Velde, G., 2003: Ontogenetic dietary changes of coral reef fishes in the mangrove-seagrass-reef continuum: stable isotopes and gut-content analysis. *Marine Ecology Progress Series* **246**, 279-289.

Compagno, L.J.V. (1984) FAO Species Catalogue Volume 4. Sharks of the World: An Annotated and Illustrated Catalogue of Shark Species Known to Date. Part 2. Carcharhiniformes. FAO Fisheries Synopsis No 125. FAO, Rome, Italy, 251-655.

Compagno, L.J.V. (1990) Alternative life-history styles of cartilaginous fishes in time and space. *Environmental Biology of Fishes* **28**(1-4), 33-75.

Conrath, C.L. and Musick, J.A. (2012) Reproductive Biology of Elasmobranchs. In Carrier, J.C., Musick, J.A. and Heithaus, M.R. (eds) Biology of Sharks and Their Relatives, 2nd edition. Boca Raton, FL: CRC Press and Taylor and Francis Group, 291-306.

Cooke, S.J., Bunt, C.M.and Schreer, J.F. (2004) Understanding Fish Behavior, Distribution, and Survival in Thermal Effluents Using Fixed Telemetry Arrays: A Case Study of Smallmouth Bass in a Discharge Canal During Winter. *Environmental Management* **33**(1), 140-150.

Cooke, S.J., Woodley, C.M., Eppard, M.B., Brown, R.S. and Nielsen, J.L. (2011). Advancing the surgical implantation of electronic tags in fish: a gap analysis and research agenda based on a review of trends in intracoelomic tagging effects studies. *Reviews in Fish Biology and Fisheries* **21**, 127-151.

Cortes, E. and Gruber, S.H. (1990) Diet, feeding habits and estimates of daily ration of young lemon sharks, *Negaprion brevirostris* (Poey). *Copeia* **1990**(1), 204-218.

Counihan, T.D. and Frost, C.N. (1999). Influence of externally attached transmitters on the swimming performance of juvenile white sturgeon. *Transactions of the American Fisheries Society* **128**, 965-970.

Cowlishaw, G. (1997) Trade-offs between foraging and predation risk determine habitat use in a desert Baboon population. *Animal Behaviour* **53**(4), 667-686.

DeAngelis, B.M., McCandless, C.T., Kohler, N.E., Recksiek, C.W. and Skomal, G.B. (2008) First characterization of shark nursery habitat in the United States Virgin Islands: evidence of habitat partitioning by two shark species. *Marine Ecology Progress Series* **358**, 257-271.

DeMartini, E., Friedlander, A., Sandin, S. and Sala, C. (2008) Differences in fish-assemblage structure between fished and unfished atolls in the northern Line Islands, central pacific. *Marine Ecology Progress Series* **365**, 199-215.

DeMatos Dias, D., Bueno de Campos, C. and Guimaraes Rodrigues, F.H. (2018) Behavioural ecology in a predator-prey system. *Mammalian Biology* **92**, 30-36.

DiBattista, J.D., Feldheim, K.A., Gruber, S.H. and Hendry, A.P. (2007) When bigger is not better: selection against large size, high condition and fast growth in juvenile lemon sharks. *Journal of Evolutionary Biology* **20**(1):201-212.

DiBattista, J.D., Feldheim, K.A., Garant, D., Gruber, S.H. and Hendry, A.P. (2011) Anthropogenic disturbance and evolutionary parameters: a lemon shark population experiencing habitat loss. *Evolutionary Applications* **4**(1), 1-17.

Dicken, M.L., Booth, A.J., Smale, M.J. and Cliff, G. (2007) Spatial and seasonal distribution patterns of juvenile and adult raggedtooth sharks (Carcharias taurus) tagged off the east coast of South Africa. *Marine and Freshwater Research* **58**(1), 127-134.

DiGirolamo, A.L., Gruber, S.H., Pomoroy, C. and Bennett, W.A. (2012) Diel temperature patterns of juvenile lemon sharks *Negaprion brevirostris*, in a shallow water nursery. *Journal of Fish Biology* **80**(5), 1436-1448.

DiSanto, V. and Bennet, W.A. (2011) Is post-feeding thermotaxis advantageous in elasmobranch fishes? *Journal of Fish Biology* **78**(1), 195-207.

Domier, M.L. and Nasby-Lucas, N. (2008) Migration patterns of white sharks *Carcharadon carcharias* tagged at Guadalupe Island, Mexico, and identification of an eastern Pacific shared offshore foraging area. *Marine Ecology Progress Series* **370**, 221-237.

Donaldson, M.R., Hinch, S.G., Suski, C.D., Fisk, A.T., Heupel, M.R. and Cooke, S.J. (2014) Making connections in aquatic ecosystems with acoustic telemetry monitoring. *Frontiers in Ecology and the Environment* **12**, 565-573.

Donley J.M., Sepulveda C.A., Aalbers S.A., McGillivray D.G., Syme D.A. and Bernal D. (2012) Effects of temperature on power output and contraction kinetics in the locomotor muscle of the regionally endothermic common thresher shark (*Alopias vulpinus*). *Fish Physiology and Biochemistry* **38**(5), 1507-1519.

Dow, C., Michel, K.E., Love, M. and Brown, D.C. (2009) A review of shark satellite tagging studies. American Journal of Veterinary Research **70**(4), 444-448.

Duffy, L.M., Olson, R.J., Lennert-Cody, C.E., Galvan-Magana, F. and Bocanegra-Castillo, N. (2015) Foraging ecology of silky sharks, *Carcharhinus falciformis,* captured by the tuna purse-seine fishery in the eastern Pacific Ocean. *Marine Biology* **162**(3), 571-593.

Dulvy, N.K., Fowler, S.L., Musick, J.A., Cavanagh, R.D., Kyne, P.M., Harrison, L.R., Carlson, J.K., Davidson, L.N.K., Fordham, S.V., Francis, M.P., Pollock, C.M., Simpfendorfer, C.A., Burgess, G.H., Carpenter, K.E., Compagno, L.J.V., Ebert, D.A., Gibson, C., Heupel, M.R., Livingstone, S.R., Sanciangco, J.C., Stevens, J.D.,Valenti, S. and White, W.T. (2014) Extinction risk and conservation of the world's sharks and rays. eLife 3, e00590.

Duncan, K.M. and Holland, K.N. (2006) Habitat use, growth rates and dispersal patterns of juvenile scalloped hammerhead sharks *Sphyrna lewini* in a nursery habitat. *Marine Ecology Progress Series* **312**, 211-221.

Ebert, D.A. (1991) Observations on the predatory behaviour of the sevengill shark *Notorynchus cepedianus*. *South African Journal of Marine Science* **11**(1), 455-465.

Ebert, D.A. (2002) Ontogenetic changes in the diet of the sevengill shark (*Notorynchus cepedianus*). *Marine and Freshwater Research* **53**(2), 517-523.

Edren, S.M.C. and Gruber, S.H. (2005) Homing ability of young lemon sharks, *Negaprion brevirostris*. *Environmental Biology of Fishes* **72**(3), 267-281.

Ellis, J.K. and Musick, J.A. (2007) Ontogenetic changes in the diet of the sandbar shark, *Carcharhinus plumbeus*, in lower Chesapeake Bay and Virginia (USA) coastal waters. *Environmental Biology of Fishes* **80**(1), 51-67.

Estes, J.A., Terborgh, J., Brashares, J.S., Power, M.E., Berger, J., Bond, W.J., Carpenter, S.R., Essington, T.E., Holt, R.D., Jackson, J.B.C., Marquis, R.J., Oksanen, L., Oksanen, T., Paine, R.T., Pikitch, E.K., Ripple, W.J., Sandin, S.A., Scheffer, M., Schoener, T.W., Shurin, J.B., Sinclair, A.R.E., Soule, M.E., Virtanen, R. and Wardle, D.A., 2011. Trophic Downgrading of Planet Earth. *Science* **333**(6040), 301-306.

Estrada, J.A., Rice, A.N., Lutcavage, M.E. and Skomal, G.B. (2003) Predicting trophic position in sharks of the north-west Atlantic Ocean using stable isotope analysis. *Journal of the Marine Biological Association of the United Kingdom* **83**(6), 1347-1350.

Estrada, J.A., Rice, A.N., Natanson, L.J. and Skomal, G.B. (2006) Use of isotopic analysis of vertebrae in reconstructing ontogenetic feeding ecology in white sharks. *Ecology* **87**(4), 829-834.

Feldheim, K.A., Gruber, S.H. and Ashley, M.V. (2001) Multiple Paternity of a Lemon Shark Litter (Chondrichthyes: Carcharhinidae). *Copeia* **3**, 781-786.

Feldheim, K.A., Gruber, S.H. and Ashley, M.V. (2002) The breeding biology of lemon sharks at a tropical nursery lagoon. *Prcoceedings of the Royal Society* **269** (1501), 1655–1661.

Feldheim, K.A., Gruber, S.H., DiBattista, J.D., Babcock, E.A., Kessel, S.T., Hendry, A.P., Pikitch, E.K., Ashley, M.V. and Chapman, D.D. (2014) Two decades of genetic profiling yields first evidence of natal philopatry and long-term fidelity to parturition sites in sharks. *Molecular Ecology* **23**(1), 110-117.

Ferretti, F., Myers, R.A., Serena, F. and Lotze, H.K. (2008) Loss of Large Predatory Sharks from the Mediterranean Sea. *Conservation Biology* **22**(4), 952-964.

Ferretti, F., Worm, B., Britten, G.L., Heithaus, M.R. and Lotze, H.K. (2010) Patterns and ecosystem consequences of shark declines in the ocean. *Ecology Letters*, **13**, 1055-1071.

Fertl, D., Acevedo-Gutierrez, A. and Darby, F.L. (1996) A report of killer whales (*Orcinus orca*) feeding on a carcharhinid shark in Costa Rica. *Marine Mammal Science* **12**(4), 606-611.

Finger, J.S., Dhellemes, F. and Guttridge, T.L. (2017) Personality in elasmobranchs with a focus on sharks: early evidence, challenges and future directions. In Vonk, J., Weiss, A. and Kuczaj, S.A. (eds) Personality in Non-human Animals. Cham, Switzerland, Springer International Publishing 129-152.

Franks B.R. (2007) The Spatial Ecology and Resource Selection of Juvenile Lemon Sharks (*Negaprion brevirostris*) in their Primary Nursery Areas. Ph.D. thesis. Drexel University.

Freitas, R.H.A., Rosa, R.S., Wetherbee, B.M. and Gruber, S.H. (2009) Population size and survivorship for juvenile lemon sharks (Negaprion brevirostris) on their nursery grounds at a marine protected area in Brazil. *Neotropical Ichthyology* **7**(2), 205-212.

Friedlander A.M. and DeMartini E.E. (2002) Contrasts in density, size and biomass of reef fishes between the northwestern and the main Hawaiian Islands: the effect of fishing down apex predators. *Marine Ecology Progress Series* **230**, 253-264.

Friedlaender, A.S., Goldbogen, J.A., Hazen, E.L., Calambokidis, J. and Southall, B.L. (2015) Feeding performance by sympatric blue and fin whales exploiting a common prey resource. *Marine Mammal Science* **31**, 345-354.

Fuiman, L.A. and Webb, P.W. (1988) Ontogeny of routine swimming activity and performance in zebra danios (Teleostei: Cyprinidae). *Animal Behaviour* **36**(1), 250-261.

Gallagher, A.J., Serafy, J.E., Cooke, S.J. and Hammerschlag, N. (2014). Physiological stress response, reflex impairment, and survival of five sympatric shark species following experimental capture and release. *Marine Ecology Progress Series* **496**, 207-218.

Garla, R.C., Garrone-Neto, D. and Gadig, O.B.F. (2014). Defensive strategies of neonate nurse sharks, *Ginglymostoma cirratum*, in an oceanic archipelago of the western central Atlantic. *Acta ethologica* DOI: 10.1007/s10211-014-0200-x

Garla, R.C., Gadig, O.B.F., Garica Jr., J., Veras, L.B. and Garrone-Neto, D. (2017) Hunting tactics of the lemon shark, Negaprion brevirostris, in shallow waters of an oceanic insular area in the western equatorial Atlantic. *Neotropical Ichthyology* **15**(1), e160119.

Gleiss A.C., Gruber S.H. and Wilson, R.P. (2009a) Multi-Channel Data-Logging: Towards Determination of Behaviour and Metabolic Rate in Free-Swimming Sharks. *Tagging and Tracking of Marine Animals with Electronic Devices* **9**, 211-228.

Gleiss A.C., Norman B., Liebsch N., Francis C. and Wilson R.P. (2009b) A new prospect for tagging large free-swimming sharks with motion-sensitive data-loggers. *Fisheries Research* **97**, 11-16.

Gleiss, A.C., Dale, J.J., Holland, K.N. and Wilson, R.P. (2010) Accelerating estimates of activity-specific metabolic rate in fishes: testing the applicability of acceleration data-loggers. *Journal of Experimental Marine Biology and Ecology* **385**, 85-91.

Gleiss, A.C., Wilson, R.P. and Shepard, E.L.C. (2011) Making overall dynamic body acceleration work: on the theory of acceleration as a proxy for energy expenditure. *Methods in Ecology and Evolution* **2**(1), 23-33.

Gleiss A.C., Wright S, Liebsch N., Wilson R.P. and Nelson B., 2013: Contrasting diel patterns in vertical movements and locomotor activity of whale sharks at Ningaloo Reef. *Marine Biology* **160**(11), 2981-2992.

Godin, J.J. and Sproul, C.D. (1988) Risk taking in parasitized sticklebacks under threat of predation: effects of energetic need and food availability. *Canadian Journal of Zoology* **66**(11), 2360-2367.

Gorini, L., Linnell, J.D.C., May, R., Panzacchi, M., Boitani, L., Odden, M., Nilsen, E.B., (2012) Habitat heterogeneity and mammalian predator-prey interactions. MammalRev 42 (1), 55–77.

Gotceitas, V. and Golgan, P. (1990) The effects of prey availability and predation risk on habitat selection by juvenile Bluegill sunfish. *Copeia* **1990**(2), 409-417.

Graham, B.S., Grubbs, R.D., Holland, K. and Popp, B.N. (2007) A rapid ontogenetic shift in the diet of juvenile yellowfin tuna from Hawaii. *Marine Biology* **150**(4), 647-658.

Grothues, T.M. (2009). A review of acoustic telemetry technology and a perspective on its diversification relative to coastal tracking arrays. *Tagging and Tracking of Marine Animals with Electronic Devices* **9**, 77-90.

Grubbs, R. D., Musick, J.A., Conrath, C.L. and Romine, J.G. (2007) Long-term movements, migration, and temporal delineation of summer nurseries for juvenile sandbar sharks in the Chesapeake Bay region. In McCandless, C.T., Kohler, N.E. and Pratt. H.L. Jr. (eds) Shark Nursery Grounds of the Gulf of Mexico and the East Coast Waters of the United States. American Fisheries Society Symposium 50, 87–108.

Grubbs, R.D. (2010) Ontogenetic shifts in movements and habitat use. In Carrier, J.C., Musick, J.A. and Heithaus, M.R. (eds) Biology of Sharks and Their Relatives II, Biodiversity, Adaptive Physiology and Conservation. Boca Raton, FL: CRC Press and Taylor and Francis Group, 319-350.

Gruber, S.H. and Cohen, J.L. (1978) Visual system of the elasmobranchs: state of the art 1960-1975. In Hodgson, E.S. and Matthewson, R.F. (eds) Sensory Biology of Sharks, Skates, and Rays. Washington DC, US Government Printing Office, 11–105.

Gruber, S.H. (1982) Role of the lemon shark, *Negaprion brevirostris* (Poey) as a predator in the tropical marine environment: A multidisciplinary study. *Florida Scientist* **45**(1), 46-75.

Gruber, S.H. (1984) Bioenergetics of the captive and free-ranging lemon shark. AAZPA Annual Conference Proceedings, 340-373.

Gruber, S.H., Nelson, D.R. and Morrissey, J.F. (1988) Patterns of Activity and Space Utilization of Lemon Sharks, *Negaprion brevirostris*, in a Shallow Bahamian Lagoon. *Bulletin of Marine Science* **43**(1), 61-76.

Gruber, S.H., de Marignac, J.R.C. and Honig, J.M. (2001) Survival of Juvenile Lemon Sharks at Bimini, Bahamas, Estimated by Mark–Depletion Experiments. *Transactions of the American Fisheries Society* **130**(3), 376-384.

Gurshin, C.W.D. and Szedlmayer, S.T. (2004). Short-term survival and movements of Atlantic sharpnose sharks captured by hook-and-line in the north-east Gulf of Mexico. *Journal of Fish Biology* **65**, 973-986.

Gutteridge A.N., Bennett M.B., Huveneers C. and Tibbetts I.R. (2011) Assessing the overlap between the diet of a coastal shark and the surrounding prey communities in a sub-tropical embayment. *Journal of Fish Biology* **78**, 1405-1422.

Guttridge, T.L., Gruber, S.H., Gledhill, K.S., Croft, D.P., Sims, D.W. and Krause, J. (2009) Social preferences of juvenile lemon sharks (*Negaprion brevirostris*). *Animal Behaviour* **78**, 543-548.

Guttridge, T.L., Gruber, S.H., Krause, J. and Sims, D.W. (2010) Novel acoustic technology for studying free-ranging shark social behaviour by recording individuals’ interactions. *Plos One* **5**(2), DOI:10.1371/journal.pone.0009324.

Guttridge, T.L., Gruber, S.H., Franks, B.R., Kessel, S.T., Gledhill, K.S., Uphill, J., Krause, J. and Sims, D.W. (2012) Deep danger: intra-specific predation risk influences habitat use and aggregation formation of juvenile lemon sharks *Negaprion brevirostris*. *Marine Ecology Progress Series* **445**, 279-291.

Halsey, L.G., White, C.R., Enstipp, M.R., Wilson, R.P., Butler, P.J., Martin, G.R., Gremillet, D. and Jones, D.R. (2011) Assessing the Validity of the Accelerometry Technique for

Estimating the Energy Expenditure of Diving Double-Crested Cormorants *Phalacrocorax auritus*. *Physiological and Biochemical Zoology* **84**(2), 230-237.

Hammerschlag N., Martin R.A. and Fallows C., 2006: Effects of environmental conditions on predator–prey interactions between white sharks (Carcharodon carcharias) and Cape fur seals (Arctocephalus pusillus pusillus) at Seal Island, South Africa. *Environmental Biology of Fishes*. **76**, 341-350.

Hammerschlag, N., Gallagher, A.J. and Lazarre, D.M. (2011) A review of shark satellite tagging studies. *Journal of Experimental Marine Biology and Ecology* **398**(1-2), 1-8.

Harvey, E.S., Newman, S.J., McLean, D.L., Cappo, M., Meeuwig, J.J. and Skepper, C.L. (2012) Comparison of the relative efficiencies of stereo-BRUVs and traps for sampling tropical continental shelf demersal fishes. *Fisheries Research* **125-126**, 108-120.

Hays G.C., Akesson S., Godley B.J., Luschi P, and Santidrian P. (2001) The implications of location accuracy for the interpretation of satellite-tracking data. *Animal Behaviour* **61,** 1035–1040.

Heithaus, M.R., Marshall, G.J., Buhleier, B.M. and Dill, L.M. (2001). Employing Crittercam to study habitat use and behaviour of large sharks. *Marine Ecology Progress Series* **209**, 307-310.

Heithaus M.R., Dill L., Marshall G. and Buhleier B. (2002) Habitat use and foraging behaviour of tiger sharks (*Galeocerdo cuvier)* in a seagrass ecosystem. *Marine Biology* **140**, 237-248.

Heithaus, M.R. (2004) Predator-prey interactions. In Carrier, J.C., Musick, J.A. and Heithaus, M.R. (eds) Biology of Sharks and Their Relatives. Boca Raton, FL: CRC Press and Taylor and Francis Group, 487-522.

Heithaus M.R. (2007) Nursery areas as essential shark habitats: A theoretical perspective. *American Fisheries Society Symposium* **50**, 3-13.

Heithaus, M.R., Wirsing, A.J., Burkholder, D., Thompson, J. and Dill, L.M. (2009) Towards a predictive framework for predator risk effects: the interaction of landscape features and prey escape tactics. *Animal Ecology* **78**(3), 556-562.

Heupel, M.R. and Heuter, R.E. (2001) Use of an Automated Acoustic Telemetry System to Passively Track Juvenile Blacktip Shark Movements. *Electronic Tagging and Tracking in Marine Fisheries* **1**, 217-236.

Heupel, M.R. and Heuter, R.E. (2002). Importance of prey density in relation to the movement patterns of juvenile blacktip sharks (Carcharhinus limbatus) within a coastal nursery area. *Marine and Freshwater Research* **53**, 543-550.

Heupel M.R., Simpfendorfer C.A. and Heuter R.E. (2004) Estimation of shark home ranges using passive monitoring techniques. *Environmental Biology of Fishes* **71**, 135-142.

Heupel, M.R. and Simpfendorfer, C.A. (2005). Quantitative analysis of aggregation behaviour in juvenile blacktip sharks. *Marine Biology* **147**, 1239-1249.

Heupel, M.R., Carlson, J.K. and Simpfendorfer, C.A. (2007) Shark nursery areas: concepts, definition, characterization and assumptions. *Marine Ecology Progress Series* **337**, 287-297.

Heupel, M.R. and Simpfendorfer, C.A. (2008) Movement and distribution of young bull sharks Carcharhinus leucas in a variable estuarine environment. *Aquatic Biology* **1**, 277-289.

Heupel, M.R., Knip, D.M. and Simpfendorfer, C.A. (2014) Sizing up the ecological role of sharks as predators. *Marine Ecology Progress Series* **495,** 291-298.

Holland K.N., Lowe C.G., Peterson J.D. and Gill A. (1992) Tracking coastal sharks with small boats: Hammerhead shark pups as a case study. *Journal of Marine and Freshwater Research* **43**(1), 61-66.

Holland, K.N., Wetherbee, B.M., Peterson, J.D. and Lowe, C.G. (1993). Movements and distribution of hammerhead shark pups on their natal grounds. *Copeia* **2**, 495-502.

Holts, D.B. and Bedford, D.W. (1993) Horizontal and Vertical Movements of the Shortfin Mako Shark, Isurus oxyrinchus, in the Southern California Bight. Australian *Journal of Marine and Freshwater Research* **44**, 901-909.

Hutchinson, M.R., Itano, D.G., Muir, J.A. and Holland, K.A. (2015). Post-release survival of juvenile silky sharks captured in a tropical tuna purse seine fishery. *Marine Ecology Progress Series* **521**, 143-154.

Houston A.I., McNamara J.M. and Hutchinson J.M.C., 1993: General results concerning the trade-off between gaining energy and avoiding predation. *Philospohical Transactions of the Royal Society B* **341**(1298), DOI: 10.1098/rstb.1993.0123.

Houston A.I. and McNamara J.M. (2014) Foraging currencies, metabolism and behavioural routines. *Journal of Animal Ecology* **83**(1), 30-40.

Hussey, N.E., Dudley, S.F.J., McCarthy, I.D., Cliff, G. and Fisk, A.T. (2011) Stable isotope profiles of large marine predators: viable indicators of trophic position, diet, and movement in sharks? *Canadian Journal of Fisheries and Aquatic Sciences* **68**(12), 2029-2045.

Huxham, M., Kimani, E. and Augley, J. (2004) Mangrove fish: a comparison of community structure between forested and cleared habitats. *Estuarine, Coastal and Shelf Science* **60**(4), 637-647.

Iwata T., Sakamoto K.Q., Takahashi A., Edwards E.W.J., Staniland I.J., Trathan P.N., Naito Y. (2012) Using a Mandible Accelerometer to Study Fine-Scale Foraging Behaviour of Free-Ranging Antarctic Fur Seals. *Marine Mammal Science* **28**(2), 345-357.

IUCN (2018) IUCN Red List of Threatened Species. Version 2018.1 http://www.iucnredlist.org/ (accessed on 10/01/2018).

Jacoby, D.M.P., Croft, D.P. and Sims, D.W. (2012) Social behaviour in sharks and rays: analysis, patterns and implications for conservation. *Fish and Fisheries* **13**(4), 399-417.

Jacoby, D.M.P., Fear, L.N., Sims, D.W. and Croft, D.P. (2014) Shark personalities? Repeatability of social network traits in a widely distributed predatory fish. *Behavioural Ecology and Sociobiology* **68**(12), 1995-2003.

Jackson, A.C., Rundle, S.D., Attrill, M.J. and Cotton, P.A. (2004) Ontogenetic changes in metabolism may determine diet shifts for a sit-and-wait predator. *Journal of Animal Ecology* **73**(3), 536-545.

Jadot, C., Donnay, A.,Ylieff, M. and Poncin, P. (2005). Impact implantation of a transmitter on Sarpa salpa behaviour: study with a computerized video tracking system. *Journal of Fish Biology* **67**, 589-595.

Jain, A.K., Murty, M.N. and Flynn, P.J. (1999) Data clustering: a review. *ACM Computing Surveys* **31**, 264–323.

Jennings D.E., Gruber S.H., Franks B.R., Kessel S.T. and Robertson A.L. (2008) Effects of large-scale anthropogenic development on juvenile lemon shark (*Negaprion brevirostris*) populations of Bimini, Bahamas. *Environmental Biology of Fishes* **83**(4), 369-377.

Jennings, D.E., DiBattista, J.D., Stump, K.L., Hussey, N.E., Franks, B.R., Grubbs, R.D. and Gruber, S.H. (2012) Assessment of the aquatic biodiversity of a threatened coastal lagoon at Bimini, Bahamas. *Journal of Coastal Conservation* **16**(3), 405-428.

Jepsen, N., Davis, L.E., Schreck, C.B. and Siddens, B. (2011). The physiological response of chinook salmon smolts to two methods of radio-tagging. *Transactions of the American Fisheries Society* **130**, 495-500.

Karlsson, S., Yu, J., Akay, M. (2000) Time-frequency analysis of myoelectric signals during dynamic contractions: a comparative study. *IEEE Transactions on Biomedical Engineering* **47**, 288–237.

Kawabe, R., Nashimoto, K., Hiraishi, T., Naito, Y. and Sato, K. (2003) A new device for monitoring the activity of freely swimming flatfish, Japanese flounder Paralichthys olivaceus. *Fisheries Science* **69**(1), 3-10.

Keller, B.A., Finger, J.S., Gruber, S.H., Abel, D.C. and Guttridge, T.L. (2017) The effects of familiarity on the social interactions of juvenile lemon sharks, *Negaprion brevirostris*. *Journal of Experimental Marine Biology and Ecology* **489**, 24-31.

Kinney, M.J. and Simpfendorfer, C.A. (2009) Reassessing the value of nursery areas to shark conservation and management. *Conservation Letters* **2**(2), 53-60.

Klimley, A.P. and Nelson, D.R. (1984) Diel movement patterns of the scalloped hammerhead shark (Sphyrna lewini) in relation to El Bajo Espiritu Santo: a refuging central-position social system. *Behavioural Ecology and Sociobiology* **15**(1), 45-54.

Kokobun, N., Kim, J., Shin, H., Naito, Y. and Takahashi, A. (2011) Penguin head movement detected using small accelerometers: a proxy of prey encounter rate. *The Journal of Experimental Biology* **214**, 3760-3767.

Knight, J. (2001) If they could talk to the animals. *Nature* **414**, 246-247.

Knip, D.M., Heupel, M.R. and Simpfendorfer, C.A. (2010) Sharks in nearshore environments: models, importance, and consequences. *Marine Ecology Progress Series* **402**, 1-11.

Knip, D.M., Heupel, M.R., Simpfendorfer, C.A., Tobin, A.J. and Moloney, J. (2011). Ontogenetic shifts in movement and habitat use of juvenile pigeye sharks Carcharhinus amboinensis in a tropical nearshore region. *Marine Ecology Progress Series* **425**, 233-246.

Laegdsgaard, P. and Johnson, C. (1995) Mangrove habitats as nurseries: unique assemblages of juvenile fish in subtropical mangroves in eastern Australia. *Marine Ecology Progress Series* **126**(1-3), 67-81.

Laegdsgaard P. and Johnson C. (2001) Why do juvenile fish utilise mangrove habitats? *Journal of Experimental Marine Biology and Ecology* **257**(2), 229-253.

Lechner, A.M., Doerr, V., Harris, R.M.B., Doerr, E. and Lefroy, E.C. (2015) A framework for incorporating fine-scale dispersal behaviour into biodiversity conservation planning. *Landscape and Urban Planning* **141**, 11-23.

Legare, B., Kneebone, J., DeAngelis, B. and Skomal, G. (2015) The spatiotemporal dynamics of habitat use by blacktip (Carcharhinus limbatus) and lemon (Negaprion brevirostris) sharks in nurseries of St. John, United States Virgin Islands. *Marine Biology* **162**(3), 699-716.

Lima S.L. and Dill L.M. (1990) Behavioural decisions made under the risk of predation: a review and prospectus. *Canadian Journal of Zoology* **68**(4), 619-640.

Lima S.L. (1998a) Nonlethal Effects in the Ecology of Predator-Prey Interactions. *Bioscience* **48**(1), 25-34.

Lima, S.L. (1998b) Stress and Decision Making under the Risk of Predation: Recent Developments from Behavioral, Reproductive, and Ecological Perspectives. *Advances in the Study of Behaviour* **27**, 215-290.

Lima, S.L. (2002) Putting predators back into behavioral predator–prey interactions. *Trends in Ecology and Evolution* **17**(2), 70-75.

Lotze H.K., Lenihan H.S., Bourque B.J., Bradbury R.H., Cooke R.G., Kay M.C., Kidwell S.M.., Kirby M.X., Peterson C.H. and Jackson J.B.C. (2006) Depletion, degradation and recovery potential of estuaries and coastal seas. *Science* **312**, 1806-1809.

Lowe, C.G., Wetherbee, B.M., Crow, G.L. and Tester, A.L. (1996) Ontogenetic dietary shifts and feeding behavior of the tiger shark, Galeocerdo cuvier, in Hawaiian waters. *Environmental Biology of Fishes* **47**(2), 203-211.

Lowe, C.G., Holland, K.M. and Wolcott, T.G. (1998). A new acoustic tailbeat transmitter for fishes. *Fisheries Research* **36**, 275-283.

Lowe, C.J. and Goldman, K.J. (2001) Thermal and bioenergetics of elasmobranchs: bridging the gap. *Environmental Biology of Fishes* **60**, 251-266.

Lowry, D. and Motta, P.J. (2007) Ontogeny of feeding behavior and cranial morphology in the whitespotted bambooshark *Chiloscyllium plagiosum*. *Marine Biology* **151**(6), 2013-2023.

Lucifora L.O., Garcia V.B., Menni R.C. and Escalante A.H., 2006: Food habits, selectivity and foraging modes of the school shark *Galeorhinus galeus*. *Marine Ecology Progress Series* **315**, 259-270.

Lucifora, L.O., Garcia, V.B., Menni, R.C., Escalante, A.H. and Hozbor, N.M. (2009) Effects of body size, age and maturity stage on diet in a large shark: ecological and applied implications. *Ecological Research* **24**(1), 109-118.

Magnhagen, C. and Bocherding, J. (2008) Risk-taking behaviour in foraging perch: does predation pressure influence age-specific boldness? *Animal Behaviour* **75**(2), 509-517.

Magnuson J.J., Crowder L.B. and Medvik P.A. (1979) Temperature as an ecological resource. *American Zoologist* **19**(1), 331-343.

Martin, R.A., Hammerschlag, N., Collier, R.S. and Fallows, C. (2005) Predatory behaviour of white sharks (*Carcharodon carcharias*) at seal island, South Africa. **85**(5), 1121-1135.

Martin, R.A., Rossmo, D.K. and Hammerschlag, N. (2009) Hunting patterns and geographic profiling of white shark predation. *Jounal of Zoology* **279**(2), 111-118.

Mathie, M.J., Coster, A.C.F., Lovell, N.H. and Celler, B.G. (2004) Accelerometry: providing an integrated, practical method for long-term, ambulatory monitoring of human movement. *Physiological Measurement* **25**, R1-R20.

Matich, P. and Heithaus, M.R. (2012) Effects of an extreme temperature event on the behavior and age structure of an estuarine top predator, *Carcharhinus leucas*. *Marine Ecology Progress Series* **447**, 165-178.

Makiguchi Y., Sugie Y., Kojima T. and Naito Y. (2012) Detection of Feeding Behaviour in Common Carp *Cyprinus carpio* by Using an Acceleration Data Logger to Identify Mandibular Movement. *Journal of Fish Biology* **80**(6), 2345-2356.

McElroy, W.D., Wetherbee, B.M., Mostello, C.S., Lowe, C.G., Crow, G.L. and Wass, R.C. (2006) Food habits and ontogenetic changes in the diet of the sandbar shark, Carcharhinus plumbeus, in Hawaii. *Environmental Biology of Fishes* **76**(1), 81-92.

McKibben, J.N. and Nelson, D.R. (1986). Patterns of movement and grouping of gray reef sharks, Carcharinus amblyrhynchos, at Enewetak, Marshall Islands. *Bulletin of Marine Science* **38**, 89-110.

McNab B.K. (1963) Bioenergetics and the determination of home range. *The American Naturalist* **97**(894), 133-140.

Medved R.J. and Marshall J.A. (1983) Short-term movements of young sandbar sharks, *Carcharhinus plumbeus* (Pisces, *Carcharhinidae*). *Bulletin of Marine Science* **33**(1), 87-93.

Medved, R.J. (1985) Gastric evacuation in the sandbar shark, *Carcharhinus plumbeus*. *Journal of Fish Biology* **26**(3), 239-253.

Mellas, E.J. and Haynes, J.M. (1985) Swimming performance and behavior of rainbow trout (*Salmo gairdneri*) and white perch (*Morone americana*): effects of attaching telemetry transmitters. *Canadian Journal of Fisheries and Aquatic Sciences* **42**, 488-493.

Miklos P., Katzman S.M. and Cech Jr., J.J. (2003) Effects of temperature on oxygen consumption of the leopard shark, *Triakis semifasciata*. *Environmental Biology of Fishes* **66**(15), 15-18.

Milinski, M. (1986) Constraints placed by predators on feeding behaviour. In Pitcher, T.J. (ed) The Behaviour of Teleost Fishes, Croom and Helm, 236-252.

Morrissey, J.F. and Gruber, S.H. (1993a). Habitat selection by juvenile lemon sharks, *Negaprion brevirostris*. *Environmental Biology of Fishes* **38**, 311-319.

Morrissey, J.F. and Gruber, S.H. (1993b) Home range of juvenile lemon sharks, *Negaprion brevirostris*. *Copeia* **1993**(2), 425-434.

Motta, P., Tricas, T. and Summers, R. (1997) Feeding mechanism and functional morphology of the jaws of the lemon shark Negaprion brevirostris (Chondrichthyes, Carcharhinidae). *Journal of Experimental Biology* **200**, 2765-2780.

Motta, P.J. and Wilga, C.D. (2001) Advances in the Study of Feeding Behaviors, Mechanisms, and Mechanics of Sharks. *Environmental Biology of Fishes* **60**(1-3), 131-156.

Motta, P.J. (2004) Prey capture behaviour and feeding mechanics of elasmobranchs. In Carrier, J.C., Musick, J.A. and Heithaus, M.R. (eds) Biology of Sharks and Their Relatives. Boca Raton, FL: CRC Press and Taylor and Francis Group, 165-203.

Mumby, P.J., Edwards, A.J., Ernesto Erias-Gonzalez, J., Lindeman, K.C., Blackwell, P.G., Gall, A., Gorczynska, M.I., Harborne, A.R., Pescod, C.L., Renken, H., Wabnitz, C.C.C. and Llewelyn, G. (2004) Mangroves enhance the biomass of coral reef fish communities in the Caribbean. *Nature* **427**, 533-536.

Munroe, S.E.M., Simpfendorfer, C.A. and Heupel, M.R. (2014) Habitat and space use of an abundant nearshore shark, *Rhizoprionodon taylori*. *Marine and Freshwater Research* **65**(11), 959-968.

Munroe, S.E.M., Simpfendorfer, C.A. and Heupel, M.R. (2016) Variation in blacktip shark movement patterns in a tropical coastal bay. *Environmental Biology of Fishes* **99**(4), 377-389.

Murchie K.J., Cooke S.J., Danylchuk A.J. and Suski C.D. (2011) Estimates of Field Activity and Metabolic Rates of Bonefish (*Albula vulpes*) in Coastal Marine Habitats Using Acoustic Tri-Axial Accelerometer Transmitters and Intermittent-Flow Respirometry. *Journal of Experimental Marine Biology and Ecology* **396**(2), 147-155.

Myrberg, A.A. and Gruber S.H. (1974). The behavior of the bonnethead shark, *Sphyrna tiburo*. *Copeia* **1974**, 358-374.

Nagelkerken, I., van der Velde, G., Gorissen, M.W., Meijer, G.J., Van't Hof, T. and den Hartog, C. (2000) Importance of Mangroves, Seagrass Beds and the Shallow Coral Reef as a Nursery for Important Coral Reef Fishes, Using a Visual Census Technique. *Estuarine, Coastal and Shelf Science* **51**(1), 31-44.

Nathan R., Spiegel O., Fortmann-Roe S., Harel R., Wikelski M. And Getz W.M., 2011: Using Tri-axial Acceleration Data to Identify Behavioural Modes of Free-Ranging Animals: General Concepts and Tools Illustrated for Griffon Vultures. *Journal of Experimental Biology* **215**, 986-996.

Nakamura I., Watanabe Y.Y., Papastamatiou Y.P., Sato K. and Meyer C.G. (2011) Yo-yo Vertical Movements Suggest a Foraging Strategy for Tiger Sharks *Galeocerdo cuvier*. *Marine Ecology Progress Series* **424**, 237-246.

Nelson, D.R. and Johnson, R.H., 1970. Diel Activity Rhythms in the Nocturnal, Bottom-Dwelling Sharks, *Heterodontus francisci* and *Cephaloscyllium ventriosum*. *Copeiai* **1970**(4), 732-739.

Newman, S.P., Handy, R.D. and Gruber, S.H. (2010) Diet and prey preference of juvenile lemon sharks *Negaprion brevirostris*. *Marine Ecology Progress Series* **398**, 221-234.

Newman, S.P., Handy, R.D. and Gruber, S.H. (2012) Ontogenetic diet shifts and prey selection in nursery bound lemon sharks, Negaprion brevirostris, indicate a flexible foraging tactic. *Environmental Biology of Fishes* **95**(1), 115-126.

Nixon, A.J. and Gruber, S.H. (1988) Diel metabolic and activity patterns of the lemon shark (*Negaprion brevirostris*). *Comparitive Physiology and Biochemistry* **248**(1), 1-6.

Oh, B.Z.L., Thums, M., Babcock, R.C., Meeuwig, J.J., Pillans, R.D., Speed, C. and Meekan, M.G. (2017) Contrasting patterns of residency and space use of coastal sharks within a communal shark nursery. *Marine and Freshwater Research* **68**(8), 1501-1517.

Pade N.G., Queiroz N., Humphries N.E., Witt M.J., Jones C.S., Noble L.R. and Sims D.W. (2009) First results from satellite-linked archival tagging of porbeagle shark, *Lamna nasus*. Area fidelity, wider-scale movements and plasticity in diel depth changes. *Journal of Experimental Marine Biology and Ecology* **370**(1-2), 64-74.

Papastamatiou, Y.P., Itano, D.G., Dale, J.J., Mayer, C.G. and Holland K.N. (2011) Site fidelity and movements of sharks associated with ocean-farming cages in Hawaii. *Marine and Freshwater Research* **61**(12), 1366-1375.

Papastamatiou, Y.P. and Lowe, C.G. (2012) An analytical and hypothesis-driven approach to elasmobranch movement studies. *Journal of Fish Biology* **80**(5), 1342-1360.

Papastamatiou Y.P., Watanabe Y.Y., Bradley D., Dee L.E., Weng K., Lowe C.G. and Caselle J.E., 2015: Drivers of daily routines in an ectothermic marine predator: Hunt warm, rest warmer? *PlosOne* **10**(6), e0127807.

Parsons, G.R. and Hoffmayer, E.R. (2007) Identification and Characterization of Shark Nursery Grounds along the Mississippi and Alabama Gulf Coasts. *American Fisheries Society Symposium* **50**, 301-316.

Pettersson, L.B. and Bronmark, C. (1993) Trading off safety against food: state dependant habitat choice and foraging in crucian carp. *Oceologia* **95**(3), 353-357.

Pikitch, E.K., Chapman, D.D., Babcock, E.A. and Shivji, M.S. (2005) Habitat use and demographic population structure of elasmobranchs at a Caribbean atoll (Glover's Reef, Belize). *Marine Ecology Progress Series* **302**, 187-197.

Pirotta E., Thompson P.M., Miller P.I., Brookes K.L., Cheney B., Barton T.R., Graham I.M. and Lusseau D., 2013: Scale-dependant foraging ecology of marine top predator modelled using passive acoustic data. *Functional Ecology* DOI: 10.1111/1365-2435.12146.

Pratt Jr. H.L., Casey J.G. and Conklin R.B. (1982) Observations on Large White Shark, Carcharadon carcharias, off Long Island, New York. *U.S. Fishery Bulletin*. **80**, 153-156.

Quinn, T.P., Hendry, A.P. and Buck, G.B. (2001) Balancing natural and sexual selection in sockeye salmon: interactions between body size, reproductive opportunity, and vulnerability to predation by bears. *Evolutionary Ecology Research* **3**, 917–937.

Randall, J.E. (1977) Contribution to the Biology of the Whitetip Reef Shark (*Triaenodon obesus*). *Pacific Science* **31**(2), 143-164.

Recchisky, E.L. and Wetherbee, B.M. (2003) Short-term movements of juvenile and neonate sandbar sharks, *Carcharhinus plumbeus*, on their nursery grounds in Delaware Bay. *Environmental Biology of Fishes* **68**, 113-128.

Robertson, A.I. and Duke, N.C. (1987) Mangroves as nursery sites: comparisons of the abundance and species composition of fish and crustaceans in mangroves and other nearshore habitats in tropical Australia. *Marine Biology* **96**(2), 193-205.

Rooker, J.R. and Dennis, G.D. (1991) Diel, lunar and seasonal changes in a mangrove fish assemblage off Southwestern Puerto Rico. *Bulletin of Marine Science* **49**(3), 684-698.

Sakamoto K.Q., Sato K., Ishizuka M., Watanuki Y., Takahashi A., Daunt F. and Wanless S. (2009) Can Ethograms be Automatically Generated Using Body Acceleration Data from Free-Ranging Birds? *Plos ONE* **4**, e5379.

Sazima, I. and Moura, R.L. (2000) Shark (*Carcharhinus perezi*), Cleaned by the goby (*Elacatinus randalli*), at Fernando de Noronha Archipelago, Western South Atlantic. *Copeia* **2000**(1), 297-299.

Sazima I., (2002) Juvenile Snooks (Centropomidae) as Mimics of Mojarras (Gerreidae), with a Review of Aggressive Mimicry in Fishes. *Environmental Biology of Fishes* **65**(1), 37-45.

Scharold, J. and Gruber, S.H. (1991) Telemetered Heart Rate as a Measure of Metabolic Rate in the Lemon Shark, *Negaprion brevirostris*. *Copeia* **1991**(4), 942-953.

Shank, C.C. (1986) Territory Size, Energetics, and Breeding Strategy in the Corvidae. *American Naturalist* **128**(5), 642-652.

Sheaves, M. (2016) Mangroves as fish habitat. In Murchie, K.J. and Daneshgar, P.P. (eds) Fisheries, Bethesda, MD, American Fisheries Society, 148.

Shepard E.L.C., Ahmed M.Z., Southall E.J., Witt M.J., Metcalfe J.D. and Sims D.W. (2006) Diel and tidal rhythms in diving behaviour of pelagic sharks identified by signal processing of archival tagging data. *Marine Ecology Progress Series* **328**, 205-213.

Shepard, E.L.C., Wilson, R.P., Quintana, F., Laich, A.G., Liebsch, N., Albareda, D.A., Halsey, L.G., Gleiss, A. Morgan, D.T., Myers, A.E., Newman, C., Macdonald, D.W. (2008a) Identification of animal movement patterns using tri-axial accelerometry. *Endangered Species Research* **10**, 47-60.

Shepard, E.L.C., Wilson, R.P., Halsey, L.G., Quintana, F., Leich, A.G., Gleiss, A.C., Liebsch, N., Myers, A.E. and Norman, B. (2008b). Derivation of body motion via appropriate smoothing of acceleration data. *Aquatic Biology* **4**, 235-241.

Shinnaka, T. Sano, M., Ikejima, K., Tongnunni, P., Horinouchi, M. and Kurokura, H. (2007) Effects of mangrove deforestation on fish assemblage at Pak Phanang Bay, southern Thailand. *Fisheries Science* **73**(4), 862-870.

Simpfendorfer C.A. and Millward N.E., 1993: Utilisation of a tropical bay as a nursery area by sharks of the families Carcharhinidae and Sphyrnidae. *Environmental Biology of Fishes*. **37**, 337-345.

Simpfendorfer, C.A. and Heupel, M.R. (2004) Assessing habitat use and movement. In Carrier, J.C., Musick, J.A. and Heithaus, M.R. (eds) Biology of Sharks and Their Relatives. Boca Raton, FL: CRC Press and Taylor and Francis Group, 553-572.

Simpfendorfer, C.A and Kyne, P.M. (2009) Limited potential to recover from overfishing raises concerns for deep-sea sharks, rays and chimaeras. *Environmental Conservation* **36**(2), 97-103.

Sims, D.W. and Quayle, V.A. (1998) Selective foraging behaviour of basking sharks on zooplankton in a small-scale front. *Nature* **393**, 460-464.

Sims, D.W., Southall, E.J., Quayle, V.A. and Fox, A.M. (2000) Annual social behaviour of basking sharks associated with coastal front areas. *Proceedings of the Royal Society B* **267**(1455), DOI: 10.1098/rspb.2000.1227.

Sims D.W., Southall E.J., Merrett D.A. and Sanders J. (2003) Effects of zooplankton density and diel period on surface swimming duration of basking sharks. *Journal of the Marine Biological Association of the UK* **83**(3), 643-646.

Sims D.W., Witt M.J., Richardson A.J., Southall E.J. and Metcalfe J.D. (2006) Encounter success of free-ranging marine predator movements across a dynamic prey landscape. *Proceedings of the Royal Society B* **273**(1591), DOI: 10.1098/rspb.2005.3444.

Sims, D.W. (2008) Sieving a Living: A Review of the Biology, Ecology and Conservation Status of the Plankton-Feeding Basking Shark *Cetorhinus Maximus*. *Advances in Marine Biology* **54**, 171-220.

Sims, D.W. (2010) Tracking and analysis techniques for understanding free-ranging shark movements and behaviour. In Carrier, J.C., Musick, J.A. and Heithaus, M.R. (eds) Biology of Sharks and Their Relatives II, Biodiversity, Adaptive Physiology and Conservation. Boca Raton, FL: CRC Press and Taylor and Francis Group, 351-392.

Sims, D.W., Humphries, N.E., Bradford, R.W. and Bruce, B.D. (2012) Lévy flight and Brownian search patterns of a free-ranging predator reflect different prey field characteristics. *Journal of Animal Ecology* **81**(2), 432-442.

Skomal, G.B., Lobel, P.S. and Marshall, G. (2007). The use of animal-borne imaging to assess post-release behavior as it relates to capture stress in grey reef sharks, Carcharhinus amblyrhynchos. *Marine Technology Society Journal* **41**, 44-48.

Skomal, G.B. and Bernal, D. (2010). Physiological responses to stress in sharks. In Carrier, J.C., Musick, J.A. and Heithaus, M.R. (eds) Biology of Sharks and Their Relatives II, Biodiversity, Adaptive Physiology and Conservation. Boca Raton, FL: CRC Press and Taylor and Francis Group, 459-490.

Skomal, G.B. and Mandelman, J.W. (2012) The physiological response to anthropogenic stressors in marine elasmobranch fishes: A review with a focus on the secondary response. *Comparitive Biochemistry and Physiology, Part A* **162**, 146-155.

Skomal, G.B., Hoyos-Padilla, E.M., Kukulya, A. and Stokey, R. (2015) Sub-surface observations of white shark Carcharodon carcharias predatory behaviour using an autonomous underwater vehicle. *Journal of Fish Biology* **87**(6), 1293-1312.

Snelson, F.F., Mulligan, T.J. and Williams, S.E. (1984) Food Habits, Occurrence, and Population Structure of the Bull Shark, *Carcharhinus leucas*, in Florida Coastal Lagoons. *Bulletin of Marine Science* **34**(1), 71-80.

Speed, C.W., Meekan, M.G., Field, I.C., McMahon, C.R., Stevens, J.D., McGregor, F., Huveneers, C., Berger, Y. and Bradshaw, C.J.A. (2011) Spatial and temporal movement patterns of a multi-species coastal reef shark aggregation. *Marine Ecology Progress Series* **429**, 261-275.

Speed CW., Meekan M.G., Field I.C., McMahon C.R. and Bradshaw C.J.A. (2012) Heat-seeking sharks: support for behavioural thermoregulation in reef sharks. *Marine Ecology Progress Series* **463**, 231-244.

Springer, S. (1967) Social organization of shark populations. In Gilbert, P.W., Mathewson, R.F. and Rail, D.P. (eds) Sharks, skates, and rays. Baltimore, Maryand, John Hopkins University Press, 149−174.

Stark, K.E., Jackson, G.D. and Lyle, J.M. (2005). Tracking arrow squid movements with an automated acoustic telemetry system. *Marine Ecology Progress Series* **299**, 167-177.

Stephens, D.W. (2007) Decision ecology: Foraging and the ecology of animal decision making. *Cognitive*, *Affective and Behavioural Neuroscience* **8**(4), 475-484.

Stevens, J.D., Bonfil, R., Dulkvy, N.K. and Walker, P.A. (2000) The effects of fishing on sharks, rays, and chimaeras (chondrichthyans), and the implications for marine ecosystems. *ICES Journal of Marine Science* **57**(3), 476-494.

Stuart-Smith, R.D., White, R.W.G. and Barmuta, L.A. (2008) A shift in the habitat use pattern of a lentic galaxiid fish: an acute behavioural response to an introduced predator. *Environmental Biology of Fishes* **82**(1), 93-100.

Stump, K.L., Crooks, C.J., Fitchett, M.D., Gruber, S.H. and Guttridge, T.L. (2017) Hunted hunters: an experimental test of the effects of predation risk on juvenile lemon shark habitat use. *Marine Ecology Progress Series* **574**, 85-95.

Sundstrom, L.F. and Gruber, S.H. (1998) Using speed-sensing transmitters to construct a bioenergetics model for subadult lemon sharks *Negaprion brevirostris* (Poey), in the field. *Hydrobiologia,* **371/372,** 241-247.

Sundstrom, L.F., Sterk, J. and Gruber, S.H. (1998) Effects of a speed-sensing transmitter on the swimming speed of lemon sharks. Bahamas Journal of Science **6**(1), 12-22.

Sundstrom, L.F., Gruber, S.H., Clermont, S.M., Correia, J.P.S., Marignac, J.R.C., Morrissey, J.F., Lowrance, C.R., Thomassen, L. and Oliveira, M.T. (2001) Review of elasmobranch behavioral studies using ultrasonic telemetry with special reference to the lemon shark, *Negaprion brevirostris*, around Bimini Islands, Bahamas. *Environmental Biology of Fishes* **60**, 225-250.

Sundstrom, L.F. and Gruber, S.H. (2002). Effects of capture and transmitter attachments on the swimming speed of large juvenile lemon sharks in the wild. *Journal of Fish Biology* **61**, 834-838.

Sundstrom, L.F. (2015) *Negaprion brevirostris. The IUCN Red List of Threatened Species* 2015:e.T39380A81769233. http://dx.doi.org/10.2305/IUCN.UK.2015.RLTS.T39380A81769233.en

Sydeman W.J., Brodeur R.D., Grimes C.B., Bychkov A.S. and McKinnell S. (2006) Marine habitat 'hotspots' and their use by migratory species and top predators in the Pacific

Ocean. Introduction. *Deep Sea Research Part II: Topcial Studies in Oceanography* **53**, 247-248.

Tanaka H., Takagi Y. And Naito Y. (2001) Swimming Speeds and Buoyancy Compensation of Migrating Adult Chum Salmon *Oncorhynchus keta* Revealed by Speed/Depth/Acceleration Data Logger. *The Journal of Experimental Biology* **204***,* 3895-3904.

Taylor S.M. and Bennett M.B. (2013) Size, sex and seasonal patterns in the assemblage of Carcharhiniformes in a sub-tropical bay. *Journal of Fish Biology* **82**(1), 228-241.

Thaler J.S., McArt S.H. and Kaplan I. (2012) Compensatory mechanisms for ameliorating the fundamental trade-off between predator avoidance and foraging. *Proceedings of the National Academy of Sciences of the United States of America*. **109**(30), 12057-12080.

Thorstad, E.B., ØKland, F. and Finstad, B. (2000). Effects of telemetry transmitters on swimming performance of adult atlantic salmon. *Journal of Fish Biology* **57**, 531-535.

Torrence, C. and Compo, G.P. (1998) A practical guide to wavelet analysis. *Bulletin of American Meteorological Society* **79**, 61–78.

Tsuda Y., Kawabe R., Tanaka H., Mitsunga Y., Hiraishi T., Yamamoto K. and Nashimoto K. (2006) Monitoring the Spawning Behaviour of Chum Salmon With an Acceleration Data Logger. *Ecology of Freshwater Fish* **15**(3), 264-274.

Urban, M.C. (2007) The growth-predation risk trade-off under a growing gape-limited predation threat. *Ecology* **88**(10), 2587-2597.

Watanabe, Y.Y., Lydersen, C., Fisk, A.T. and Kovacs, K.M. (2012) The slowest fish: Swim speed and tail-beat frequency of Greenland sharks. *Journal of Experimental Marine Biology and Ecology* **426-427**, 5-11.

Weng K.C., O'Sullivan J.B., Lowe C.G., Winkler C.E., Dewar H. and Block B.A. (2007) Movements, behaviour and habitat preferences of juvenile white sharks *Carcharadon carcharias* in the Eastern Pacific. *Marine Ecology Progress Series* **338**, 211-224.

Werner E.E. and Hall D.J. (1988) Ontogenetic habitat shifts in bluegill: The foraging rate-predation risk trade-off. *Ecology* **69**(5), 1352-1366.

Werner, E.E. and Anholt, B.R. (1993) Ecological consequences of the trade-off between growth and mortality rates mediated by foraging activity. *The American Naturalist* **142**(2), 242-272.

Wetherbee, B.M., Gruber, S.H. and Cortes, E. (1990) Diet, feeding habits, digestion, and consumption in sharks, with special reference to the lemon shark, *Negaprion brevirostris*. In Pratt, H.L., Gruber, S.H. and Taniuchi, T. (eds) Elasmobranchs as Living Resources:

Advances in the Biology, Ecology, Systematics and the Status of Fisheries. NOAA Technical Report 90, 29-47.

Wetherbee, B.M., Crow, G.L. and Lowe, C.G. (1996) Biology of the Galapagos shark, *Carcharhinus galapagensis*, in Hawai'i. *Environmental Biology of Fishes* **45**(3), 299-310.

Wetherbee, B.M. and Cortes, E. (2004) Food consumption and feeding habits. In Carrier, J.C., Musick, J.A. and Heithaus, M.R. (eds) Biology of Sharks and Their Relatives. Boca Raton, FL: CRC Press and Taylor and Francis Group, 225-246.

Wetherbee, B.M., Gruber, S.H. and Rosa, R.S. (2007) Movement patterns of juvenile lemon sharks *Negaprion brevirostris* within Atol das Rocas, Brazil: a nursery characterised by tidal extremes. *Marine Ecology Progress Series* **343**, 283-293.

Whitney N.M., Papastamatiou Y.P., Holland K.N and Lowe C.G. (2007) Use of an acceleration data logger to measure diel activity patterns in captive white tip reef sharks, *Triaenodon obesus*. *Aquatic Living Resources* **20**, 299-305.

Whitney, N.M., Pratt Jr., H.L., Pratt, T.C. and Carrier, J.C. (2010). Identifying shark mating behaviour using three-dimensional acceleration loggers. *Endangered Species Research* **10**, 71-82.

Whitney, N.M., Papastamatiou, Y.P. and Gleiss, A.C. (2012). Integrative multisensor tagging: emerging techniques to link elasmobranch behavior, physiology and ecology. In Carrier, J.C., Musick, J.A. and Heithaus, M.R. (eds) Biology of Sharks and Their Relatives, 2nd edition. Boca Raton, FL: CRC Press and Taylor and Francis Group, 265-290.

Wilga, C.J. and Motta, P.J. (1998) Feeding mechanism of the atlantic guitarfish rhinobatos lentiginosus: modulation of kinematic and motor activity. *Journal of Experimental Biology* **201**, 3167-3183.

Wilson R.P., Stewart Grant W. and Duffy D.C. (1986) Recording Devices on Free-Ranging Marine Animals: Does Measurement Affect Foraging Performance? *Ecology* **67**(4), 1091-1093.

Wilson, R.P. and McMahon, C.R. (2006) Measuring devices on wild animals: what constitutes acceptable practice? *Frontiers in Ecology and the Environment* **4**, 147-154.

Wilson, R.P., Shepard, E.L.C. and Liebsch, N. (2008) Prying into the intimate details of animal lives: use of a daily diary on animals. *Endangered Species Research* **4**, 123-137.

Wilson A.D.M., Brownscombe J.W., Krause J., Krause S., Gutowsky L.F.G., Brooks E.J. and Cooke S.J. (2015) Integrating network analysis, sensor tags, and observation to understand shark ecology and behaviour. *Behavioural Ecology* **1–10**, DOI:10.1093/beheco/arv115.

Winter, J. (1983) Underwater Biotelemetry. In Nielsen, L.A., Johnson, D.L. and Bethesda, M.D. (eds) *Fisheries Techniques*. American Fisheries Society 371–395.

Wirsing, A.J., Heithaus, M.R. and Dill, L.M. (2007) Living on the edge: dugongs prefer to forage in microhabitats that allow escape from rather than avoidance of predators. *Animal Behaviour* **74**(1), 93-101.

Wright, S., Metcalfe, J.D., Hetherington, S. and Wilson, R. (2014). Estimating activity-specific energy expenditure in a teleost fish, using accelerometer loggers. *Marine Ecology Progress Series* **496**, 19-32.

Ydesen, K.S., Wisneiwska, D.M., Hansen, J.D., Beedholm, K., Johnson, M. and Madsen, P.T. (2014) What a jerk: prey engulfment revealed by high-rate, super-cranial accelerometry on a harbour seal (*Phoca vitulina*). *Journal of Experimental Biology* **217**, 2239-2243.

Yeiser, B.G., Heupel, M.R. and Simpfendorfer, C.A. (2008) Occurrence, home range and movement patterns of juvenile bull (Carcharhinus leucas) and lemon (*Negaprion brevirostris*) sharks within a Florida estuary. *Marine and Freshwater Research* **59**(6), 489-501.

Yoda K., Sato K., Niizuma Y., Kurita M., Bost C.A., Le Maho Y. and Naito Y. (1999) Precise Monitoring of Porpoising Behaviour of Adelie Penguins Determined Using Acceleration Data Loggers. *Journal of Experimental Biology* **202**, 3121-3126.

Yoda, K., Naito, Y., Sato, K., Takahashi, A., Nishikawa, J., Ropert-Coudert, Y., Kurita, M. and Le Maho, Y. (2001) A new technique for monitoring the behaviour of free-ranging Adelie penguins. *Journal of Experimental Biology* **204**, 685-690.

Zuur A.F., Ieno E.N. and Elphick C.S. (2010) A protocol for data exploration to avoid common statistical problems. *Methods in Ecology and Evolution* **1**(1), 3-14.

Appendices

Appendix I

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The behaviour and recovery of juvenile lemon sharks *Negaprion brevirostris* in response to external accelerometer tag attachment

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Behavioural responses of lemon sharks *Negaprion brevirostris* to a fin-mounted tag package (CEFAS G6A tri-axial accelerometer with epoxied Sonotronics PT4 acoustic transmitter) were measured in a controlled captive environment (n = 10, total length, LT range 80 – 140 cm) and in free-ranging sharks upon release (n = 7, LT range 100 – 160 cm). No changes were detected in behaviour (i.e. swimming speed, tailbeat frequency, time spent resting and frequency of chafing) between control and tagged captive shark trials, suggesting that the tag package itself does not alter behaviour. In the free-ranging trials, an initial period of elevated swimming activity was found in all individuals (represented by overall dynamic body acceleration). *Negaprion brevirostris*, however, appeared to recover quickly, returning to a steady swimming state between 2 and 35 min after release. Post-release tracking found that all sharks swim immediately for the shoreline and remain within 100 m of shore for prolonged periods. Hence, although *N. brevirostris* are capable of quick adaptation to stressors and demonstrate rapid recovery in terms of activity, tracking data suggest that they may modify their spatial use patterns post release. This research is important in separating deviation in behaviour due to environmental stressors from artefacts caused by experimental techniques.

Key words: accelerometer; capture stress; elasmobranch; post-release behaviour; sharks.

INTRODUCTION

In recent years, researchers have become increasingly reliant on remote devices to address a wide range of science and management questions, in a variety of species, including marine mammals, turtles, teleosts, chondrichthyans and cephalopods (Stark et al., 2005; Shepard et al., 2008a; Grothues, 2009; Donaldson et al., 2014). Tools such as biotelemetry (radio and acoustic telemetry) and biologging (archival logger) devices offer a sophisticated means of evaluating the behaviour, spatial ecology, energetics and physiology of free-living animals in their natural environment.

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Implicit in studies that use these methods is the assumption that neither the attachment of the device nor the device itself affect the natural behaviour and physiology of tagged individuals (Mellas & Haynes, 1985; Jadot et al., 2005) and that observations should be comparable with those of untagged animals (Jepsen et al., 2001). For research findings to be viable, it is critical that tag-attached animals display normal behaviour to allow indicative data collection and extrapolation to the whole population (Bridger & Booth, 2003). As a result, studies have focused on measuring the effects of tagging devices on marine animals, but they mostly concentrate on teleosts (Bridger & Booth, 2003; Cooke et al., 2011).

Elasmobranchs occupy important roles in marine food webs whilst often being subjected to intense anthropogenic pressures (Dulvy et al., 2014). Tagging research is contributing greatly towards a better understanding and thus management for sharks, skates and rays, but without acknowledging and controlling for deviation in behaviour due to experimental techniques, researchers are liable to draw erroneous conclusions, hindering effective management and conservation (Sundstrom & Gruber, 2002; Bridger & Booth, 2003). Thus, evaluating the effects of tag package presence and measuring expected rates of recovery will lead to a more accurate interpretation of data.

Studies investigating anthropogenic stressors on elasmobranchs are few and mostly relate to the physiological and biochemical effects of capture stresses (Skomal & Bernal, 2010; Skomal & Mandelman, 2012). How such physiological stresses manifest themselves in terms of behaviour is poorly understood. Some studies now use various archival tags to measure post-release mortality, behaviour and stress in sharks (Skomal et al., 2007; Campana et al., 2009; Gallagher et al., 2014; Hutchinson et al., 2015) but are limited in their capacity to measure sublethal behavioural responses, usually drawing conclusions from post-release depth profiles. For coastal species that occupy shallow waters, this indicator is considered inadequate.

Accelerometers are miniaturized archival logging tags, becoming ever more advanced and thus more popular in marine research. Many studies of birds, marine mammals and fishes, including sharks, now use accelerometers to elucidate aspects of behaviour, physiology and energetics (Gleiss et al., 2010; Kokobun et al., 2011; Whitney et al., 2012; Wright et al., 2014; Friedlaender et al., 2015). This technology offers a means to quantify sublethal, post-release behaviour in elasmobranchs, assessing the severity of changes in behavioural responses, as well as the rate at which individuals recover.

The objectives of this study were (1) to identify potential behavioural compensation mechanisms that indicate stress in elasmobranchs using a small accelerometer and acoustic transmitter tag package, (2) to measure the expression of these behavioural responses and (3) to monitor the time taken for animals to return to a state of behavioural homeostasis. Trials were conducted on both captive and free-ranging lemon sharks *Negaprion brevirostris* (Poey 1868) to evaluate the effects of tag presence, capture and attachment processes on their behaviour and activity.

MATERIALS AND METHODS

STUDY SITE

This study was conducted at Bimini, Bahamas (25·44′ N; 79·16′ W; Fig. 1), a cluster of sub-tropical islands located to the western edge of the Andros platform of the Great Bahama

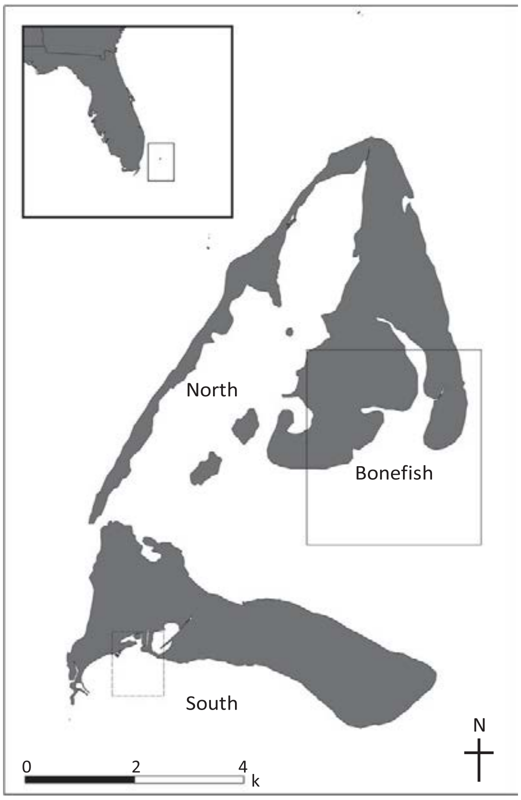

FIG. 1. Bimini, Bahamas (25◦ 44′ N; 79◦ 16′ W). The ⬚ represents the site at which captive trails were conducted and the □ represents the site at which free-ranging trials were conducted.

Bank, c. 86 km east of Miami, Florida. The two major islands, North and South Bimini, enclose a shallow mangrove-fringed lagoon that provides a nursery site for young *N. brevirostris*.

TAG PACKAGES AND ATTACHMENT

Accelerometer tag packages comprised a G6A tri-axial accelerometer (35 mm × 25 mm × 12 mm, 30 Hz, 40 MB, CEFAS Technologies Ltd; www.cefastechnology.co.uk) and a PT-04 acoustic transmitter (9 mm × 25 mm, 134 – 136 dB, battery life: 3 months, Sonotronics Inc.; www.sonotronics.com). PT-04 transmitters were epoxy fastened to the top side of the accelerometer. Tag packages were fitted to the first dorsal fin of *N. brevirostris* with 68 kg breaking-strain monofilament line. Two small holes were pierced in the shark's fin using a hollow hypodermic needle, through which the monofilament was fed. Small 1/16th aluminium crimps held the tag flush to the reverse side of the fin. Insole material was used to pad the back of tags to minimize abrasion. The smallest shark tagged weighed 4.3 kg, meaning the tag package, which weighed 23 g in air, was only 0.53% of this animal's body mass. Thus, the tag mass was less than the 2% rule set by Winter (1996) and considered not to affect adversely the behaviour of tagged individuals.

SHARK CAPTURE

Negaprion brevirostris were caught in the shallow waters surrounding Bimini using traditional rod and reel fishing from shallow water vessels, usually no more than 1 km from the shore. Sharks were then secured to the vessel, measured (total length, LT, cm) and the hook was removed. Unsuitable sharks (<80

TABLE I. Summary data for *Negaprion brevirostris* caught and used in captive trials. Shark denoted by passive integrated transponder tag number.

Shark	Sex	Trial start date	L_T (cm)
15C	F	16 May 2012	104
C62	M	30 June 2012	97
505	M	19 October 2012	102
04E	M	24 June 2013	116
000	M	3 November 2013	105
502	F	12 November 2013	135
36A	F	9 March 2014	122
511	F	10 August 2014	106
219	F	28 August 2014	109
411	M	20 September 2014	135
Mean ± s.e.			113 ±4

L_T, total length; M, male; F, female.

cm LT) were quickly released. Selected individuals for captive trials were first transferred to a circular tub (diameter, 1·5 m) on the vessel and then immediately transported to a temporary holding pen at the research facility. All sharks fed to satiation 24 h after capture, indicating full recovery. For free-ranging trials in which sharks were released back into the environment, they were secured to the side of the vessel, the tag package attached as quickly as possible and then released. All *N. brevirostris* were tagged with a passive integrated transponder (PIT) coded identity tag (Destron Fearing Inc.; www.destronfearing.com), injected just under the skin near the first dorsal fin. All sharks were identified by the last three digits of this PIT code.

CAPTIVE TRIALS

A total of 10 *N. brevirostris*, ranging from 97 to 135 cm LT (Table I), were caught and tested in captive trials. A holding pen (12 m × 6 m) was constructed in the shallow waters behind the research station (Guttridge et al., 2009) and a 4 m high tower was erected on the south side of the pen to observe the sharks' unobtrusively and minimize potential responses by sharks to observer presence. *Negaprion brevirostris* were tested in a standardized environment for 12 h both before and after tag attachment. All *N. brevirostris* were given 24 h postcapture to become acclimatized to the pen environment before beginning trials. Only one shark was present in the pen at any one time to avoid interference behaviour. *Negaprion brevirostris* were then observed for two 6 h periods over two consecutive days during daylight hours to establish control conditions, i.e. without tag attached. Two observers simultaneously recorded the type and time of all behavioural indicators. After 2 days of control testing, the shark was caught using dip-nets, transferred to a 1·5 m holding tub and the accelerometer tag package was attached. Once the tag package was in place, the shark was released back into the pen and given another 24 h to recover, then 2 days of 6 h trials were repeated in the same manner as before the tag attachment. One of the most important concerns for externally attached devices is how it affects swimming behaviour and kinematics. Chafing behaviour, a means to remove unwanted parasites and foreign bodies (Myrberg & Gruber, 1974), was considered to be a reasonable indication that *N. brevirostris* with fin-attached data loggers were attempting to dislodge or remove a foreign object such as the tag. Accurate time recordings were taken of every chafing event. In addition, the mass, position and physical drag of the tag package could reduce hydrodynamics of

TABLE II. Summary data for *Negaprion brevirostris* used in wild deployments. Shark denoted by passive integrated transponder tag number. Handling duration derived from the time a shark became hooked to the time of its release from the boat.

	Sex	Deployment date	L_t (cm)	Handling duration (min)	Time to recovery (min)	Track duration (min)
082	M	19 March 2014	119	14	6	60
C69	F	19 March 2014	148	30	3	170
773	F	16 July 2014	169	13	15	60
901	F	3 September 2014	122	11	10	n/a
E07	M	3 September 2014	160	11	11	65
345	M	29 September 2014	129	16	37	120
F7F	F	29 September 2014	132	10	4	n/a
Mean ± s.e.			140 ± 7	15 ± 3	12 ± 4	95 ± 22

L_T, total length; M, male; F, female.

sharks, especially with fin-attached tags (Lowe et al., 1998; Thorstad et al., 2000). Changes to swimming behaviour were, therefore, examined by measuring each *N. brevirostris*' swimming speed and tailbeat frequency between two marker poles set 4 m apart in the observation pen. As a shark's snout passed the first marker, a timer was started and stopped again once its snout reached the second marker. At the same time, a second observer counted the number of tailbeats whilst moving between the poles. One tailbeat was defined as the tail moving from a straightened position along the midline of the animal to a full extent, in either direction, and then back to the midline. Twenty simultaneous recordings of swim speed and tailbeat frequency were made at the beginning of control trials and 20 at the beginning of tagged trials. If a shark turned or was disturbed, the observation was rejected. Finally, tags affecting swimming efficiency may influence time spent resting for energy recuperation, therefore the time *N. brevirostris* spent resting throughout trials was also recorded. To control for the effects of hunger and feeding behaviour, N. brevirostris were fed 12 h before starting trials. In the control trials, this was around 12 h after capture and for the tagging trials around 12 h after attachment. This method also served to set the level of satiation and eliminate the issue of *N. brevirostris* potentially changing behaviour over the observation period as they grew more hungry.

FREE-RANGE SHARK TAGGING

Seven *N. brevirostris*, ranging from 119 to 169 cm LT, were caught, tagged and released for free-range trials (Table II). Mean ± s.e. handling duration was 15·0 ± 2·6 min; all *N. brevirostris* caught were cheek hooked and swam away from the vessel quickly upon release. *Negaprion brevirostris* were caught from the Bonefish Hole area of Bimini, south of the north island. This area was selected as it is a known nursery ground for young *N. brevirostris* (Guttridge et al., 2012). Accelerometer tags were set to begin recording data immediately upon release of the shark. Tags were set to record acceleration in three axes at 30 Hz and to record temperature and depth each at 1 Hz. Once released, the *N. brevirostris* were actively tracked using a shallow-water-vessel with portable ultrasonic telemetric equipment (model USR-08, Sonotronics). *Negaprion brevirostris* were tracked continuously for as long as possible during daylight hours. *Negaprion brevirostris* locations were recorded every 5 min with a handheld GPS (Garmin 72H; www.garmin.com) together with a compass bearing and estimated distance (to nearest 5 m) from the animal. A distance of >15 m was maintained between boat and animal to minimize disturbance of the shark's natural movements. Visual contact was maintained when possible, but maximum detection range for the PT4 transmitters was 80 m, thus allowing crews to maintain accurate distance estimates without visual confirmation. All *N. brevirostris* were tracked intermittently for 5 days after tagging. Samples of these data, between 24 and 36 h after release, were used as control tracking data, representing the movement habits of the *N. brevirostris* after a significant period of recovery.

DATA ANALYSIS

All captive data were tested using non-parametric Wilcoxon signed rank tests to assess variance between control and tagged samples. Accelerometer data for free-ranging trials were processed and analysed using Igor Pro 6.34 wave analysis software (WaveMetrics Inc.; www. wavemetrics.com). Static acceleration, representing the orientation of the tag and thus not the shark's movement, was separated from dynamic (true movement) acceleration data using a 2 s box-smoothing function within Igor Pro (Shepard et al., 2008b). Overall dynamic body acceleration (ODBA), the sum of absolute dynamic acceleration in all three axes, was used to quantify fine-scale swimming behaviour, post release (Whitney et al., 2010). ODBA data were analysed to determine the rate at which *N. brevirostris* recovered after release. Accelerometers produced 1800 data points every minute, and a decimate function within Igor Pro was used to yield mean values for every minute. Data from 0 to 10 min after release were compared with the subsequent 11 – 20 min data using an unpaired t-test. Sequential testing of samples was carried out along the time series (incrementally by 1 min) until no significant difference was generated between samples, indicating a levelling in swimming activity. Tracking maps for each *N. brevirostris* were constructed using ArcGIS 10.0. Distance-to-shore data were acquired by applying a NEAR table function to tracking data within ArcMap 10.0. A Spearman rank correlation coefficient was used to determine associations between distance data and time for 1 h samples of both release tracking data and the subsequent control tracking data. All statistical analyses were conducted using R software, version 3.0.0 (R Development Core Team; www.r-project.org).

RESULTS

No significant differences were found between behavioural indicators before and after tag attachment for any shark in the captive trails (Wilcoxon signed ranks tests, all $P > 0{\cdot}05$). A comparison of mean data across all *N. brevirostris* also found no significant difference between control and tagged tests (Wilcoxon signed rank tests, all $P > 0{\cdot}05$; Fig. 2). All *N. brevirostris* displayed consistent behaviour throughout trials and all fed readily postcapture and post-tag attachment. In the free-ranging experiment, an initial period of elevated ODBA, and thus swimming activity, was observed immediately after release from the tagging vessel (Fig. 3). All seven individuals, however, returned to a steady swimming state relatively quickly (mean ± s.e. = 12·0 ± 4·4 min; Table II). Five *N. brevirostris* were successfully tracked for periods between 1 and 4 h post release. Tracks of two *N. brevirostris* (901 and 345) were halted due to boat and equipment failure, respectively. All successfully tracked *N. brevirostris* were traced towards the shoreline of the north island (Fig. 4). Once within 100 m of shore, all *N. brevirostris* remained there for prolonged periods. This behaviour persisted for a mean observed 90 min and continued until termination of tracks for all *N.*

brevirostris except C69. Although this individual displayed the same pattern as other *N. brevirostris* after release, C69 began moving further than 100 m from shore 140 min after initial tagging whilst still being tracked. Tracking was terminated either due to loss of light, poor weather conditions or falling tides restricting access to the lagoon area. Spearman rank correlation revealed a significant negative relationship between distance to shore and time during the release tracking period for all *N. brevirostris* except C69, for which no significant correlation was identified. Tests of control tracking periods indicated no significant covariance for any *N. brevirostris* with exception of E07, for which a significant positive correlation was found (Table III).

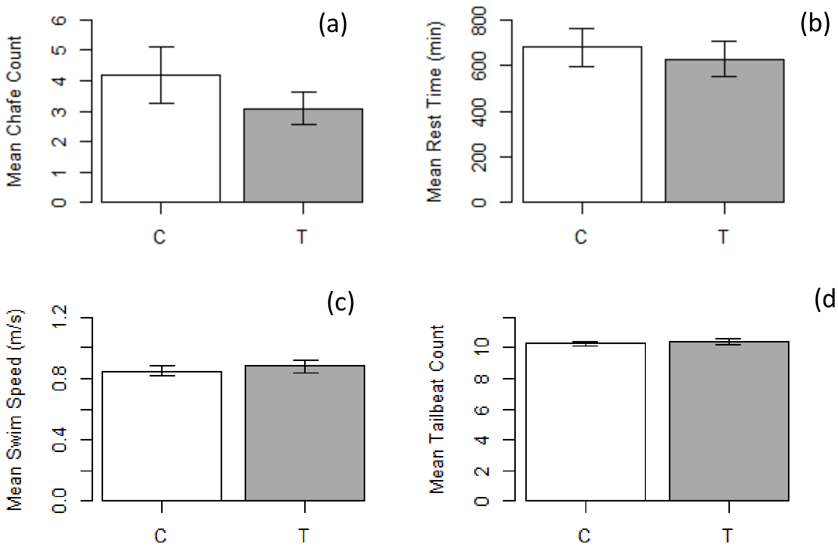

FIG. 2. Comparisons of mean±s.e. totals for (a) chafing, (b) resting (n=10), (c) swimming and (d) tailbeats (n=7) (C=control; T=tagged). There were no significant differences found between control and tagged *Negaprion brevirostris* for any indicator (Wilcoxon signed rank test, P>0·05).

DISCUSSION

EFFECTS OF TAG PRESENCE

In terms of instrument-induced effects to behaviour in sharks, this study demonstrated that the presence of accelerometer tag package itself had no significant influence on *N. brevirostris* behaviour. All *N. brevirostris* tested in the captive trials showed no variation from their routine behaviour, post attachment and behaviour did not change after recovery from handling. In teleosts, the presence of tagging devices shows conflicting effects on behaviour. For example, significantly slower swimming speeds were recorded in juvenile white sturgeon *Acipenser transmontanus* Richardson 1836 with externally mounted transmitters (Counihan & Frost, 1999), but in adult Atlantic salmon *Salmo salar* L. 1758, swimming performance was not affected by external tags (Thorstad et al., 2000). In sharks, Lowe et al. (1998) found that juvenile scalloped hammerhead sharks *Sphyrna lewini* (Griffith & Smith 1834) tagged in captivity with tailbeat transmitters worked harder and swam slower than those without tailbeat transmitters. The low tag to body mass ratio and small physical size of the packages used in this study could explain the apparent lack of any deleterious influence.

RATE OF RECOVERY FROM TAGGING

It is known that capture and handling procedures induce physiological and biochemical reactions that vary greatly in their nature and duration between species (Skomal & Mandelman, 2012). The way in which this is shown in post-release behaviour and energetics is poorly explained in the literature with very few studies describing responses and recovery (McKibben & Nelson, 1986; Lowe et al., 1998). Sundstrom & Gruber (2002) reported significantly higher swimming speeds in large juvenile *N. brevirostris*, caught in Bimini, for the first 18 h after tagging with a speed-sensing transmitter, but the current findings, using ODBA as a measure of swimming activity, indicate a much quicker return to behavioural homeostasis, with elevated activity levels persisting for a maximum of only 35 min. The variation in these findings may be due to the methods of capture. Sundstrom & Gruber (2002) used baited longlines and checked these lines every 4 h, whereas rod and reel fishing was used in this study, with the *N. brevirostris* always secured to the vessel within 5 min of hooking. The comparatively longer time spent on the longlines before capture, tagging and release may induce a greater stress response in those animals and, subsequently, a longer time to recovery. Gur- shin & Szedlmayer (2004), using ultrasonic telemetry to measure post-release survival and movements of Atlantic sharpnose sharks *Rhizoprionodon terraenovae* (Richardson 1836), found a higher net movement

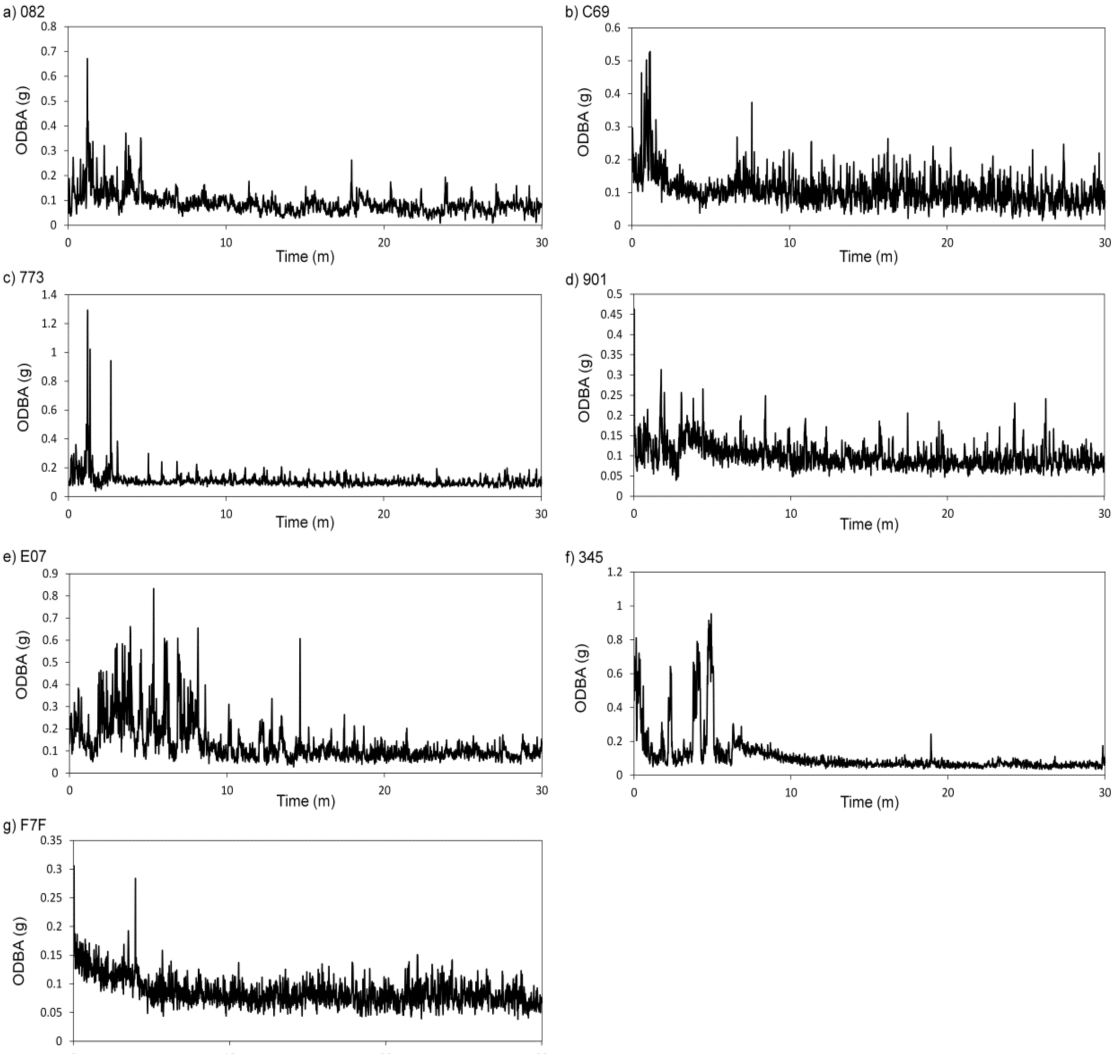

FIG. 3. (a–g) Acceleration profiles for all free-ranging *Negaprion brevirostris,* displaying overall dynamic body acceleration (ODBA) over the first 30 min of data logging after release from the tagging vessel. Individual fish identified by three-part alphanumeric code above each panel.

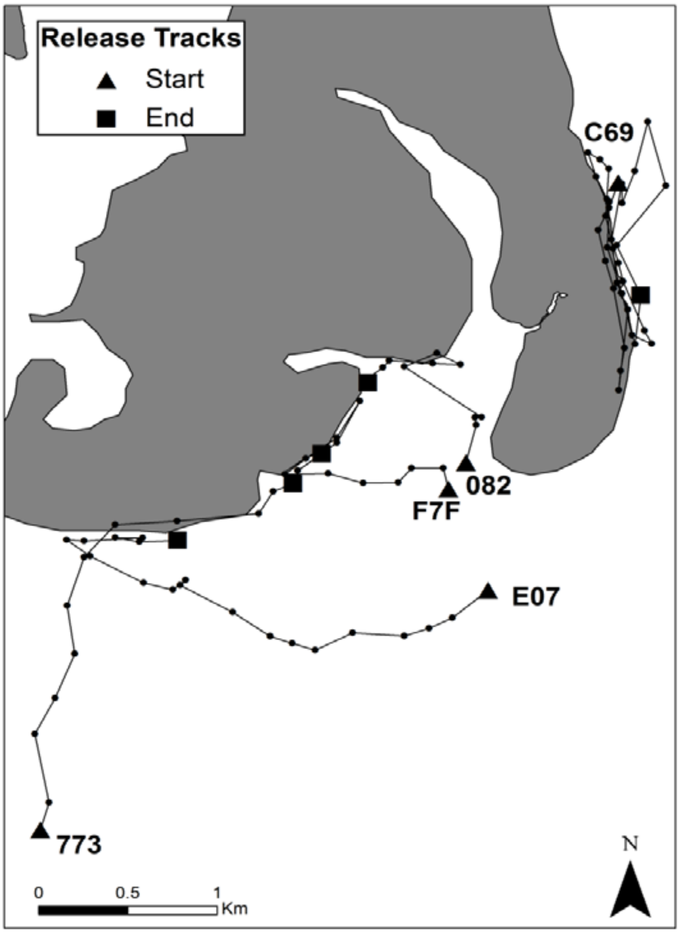

FIG. 4. Map displaying tracking co-ordinates for each Negaprion brevirostris (identified by three-part alphanumeric code) upon release. Start (▲), location of capture and tagging; end (■), location at which track was terminated; shark position at 5 min intervals (●).

after initial release but described a quick recovery from capture. Holts & Bedford (1993) found that shortfin mako sharks *Isurus oxyrinchus* Rafinesque 1810 recovered from capture stress 30 – 90 min post release. Rate of recovery is probably influenced by the method of capture, the duration and invasiveness of tagging procedures and natural variations in animal condition prior to capture. The swift recovery times of *N. brevirostris* in this study could reflect the importance of brief and minimally invasive tagging processes. Further research should consider interspecific variations in behavioural stress responses and thus help to define best tagging practices (Wilson & McMahon, 2006).

POST-RELEASE BEHAVIOUR

Beyond the assessment of post-release survivorship, studies investigating elasmobranch behaviour after capture, handling and tagging are rare. This is probably due to the logistical constraints associated with monitoring such behaviour, although new technologies enable valuable new developments (Heithaus et al., 2001; Skomal et al., 2007). Findings here demonstrated consistent patterns in post-release movement for all *N. brevirostri*s. Such persistent proximity to shore, as observed in these *N. brevirostris*, is uncharacteristic for this population. Several studies investigating the movement habits of Bimini *N. brevirostris* showed individuals to use open shallow lagoon areas at low tide, moving closer to the shoreline at high tide (Morrissey & Gruber, 1993; Guttridge et al., 2012). The use of chum and baited hooks to

TABLE III. Spearman rank correlation between distance to shore and time. P=result from test over 1 h release period and P (control)=result from test over 1 h control period.

Shark ID	Track date	*P*	*P* (control)
82	19 March 2014	*	NS
C69	19 March 2014	NS	NS
773	16 July 2014	***	NS
E07	3 September 2014	**	***
F7F	29 September 2014	**	NS

NS, $P > 0{\cdot}05$; * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

capture *N. brevirostris* means that sharks may have moved large distances, potentially from near-shore areas when attracted by the bait. It is therefore possible that *N. brevirostris* simply returned to this habitat once released. During different tidal phases, however, chum slicks were, in some instances, running away from the shore.

The control tracking data aimed to represent movement behaviour of sharks, once given a significant period of recovery. These data showed no patterns similar to those found after release (Table III and Fig. 5). It is then considered valid to contend that findings could represent a modification to space-use patterns in response to the capture and tagging procedures. Such adaptive behaviour has been noted in other studies. For example, neonatal nurse sharks *Ginglymostoma cirratum* (Bonnaterre 1788), when occupying a habitat with available shelter, will hide in holes and rock crevices when disturbed by a potential predator (Garla et al., 2014), and grey reef sharks *Carcharhinus amblyrhynchos* (Bleeker 1856) quickly vacate the area of a stressful encounter, i.e. capture and tagging (McKibben & Nelson, 1986). Tiger sharks *Galeocerdo cuvier* (Péron & LeSueur 1822), equipped with satellite transmitters, were found to move into deeper waters further offshore, upon release (Afonso & Hazin, 2014).

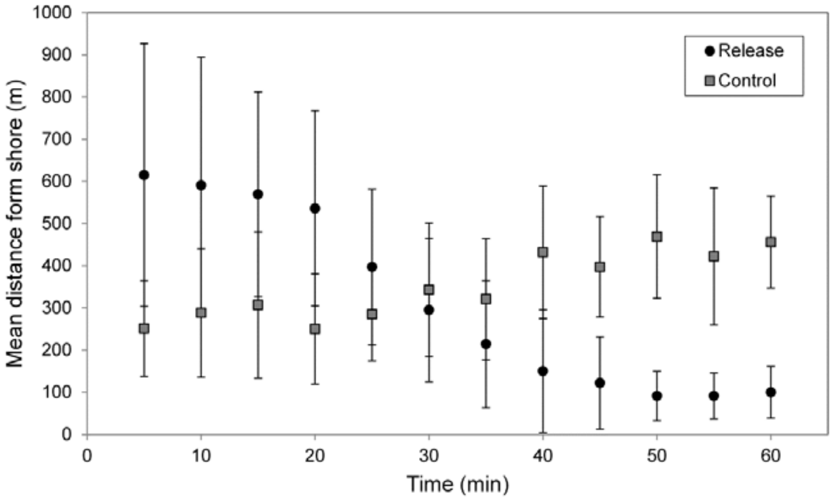

FIG. 5. Scatter plot displaying mean±s.e. distance to shore per 5min interval. Release (●, mean total distance from shore for all five tracked Negaprion brevirostris, over a 1 h period, immediately after release; control (■), mean total distance from shore for all sharks, over a 1 h period, between 24 and 36 h after release.

Finding refuge is a common behavioural phenomenon found in many elasmobranch species (Holland et al., 1993; Heupel & Heuter, 2002; Knip et al., 2011). In many instances, finding refuge is observed as a predetermined behaviour, whereby sharks minimize risk, using certain habitat types at certain times and even congregating within these areas (Heupel & Simpfendorfer, 2005). It is possible that the findings presented here could represent an adaptive behaviour to seek refuge, in response to the stresses of capture and handling. Further research with a greater sample size, longer track durations and consideration of other abiotic factors would give more firm conclusions relating to drivers of these observations.

This study demonstrates the applicability of combined accelerometer and acoustic transmitter tags as a means to elucidate sublethal post-tagging behaviour in a coastal shark species. Findings suggest no response to the presence of a fin-mounted device and rapid recovery in terms of activity, post release, but suggest potential alterations to space-use patterns. It is likely that both responses to tag presence as well as capture and handling will vary from species to species. As such, it is important for tagging studies to incorporate behavioural testing of tagging into study design, thus improving the understanding of these aspects of behaviour and producing more reliable data.

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REFERENCES

Afonso, A. S. & Hazin, F. H. V. (2014). Post-release survival and behavior and exposure to fisheries in juvenile tiger sharks, Galeocerdo cuvier, from the South Atlantic. Journal of Experimental Marine Biology and Ecology 454, 55 – 62.

Bridger, C. J. & Booth, R. K. (2003). The effects of biotelemetry transmitter presence and attachment procedures on fish physiology and behaviour. Reviews in Fisheries Science 11, 13 – 34.

Campana, S. E., Joyce, W. & Manning, M. J. (2009). Bycatch and discard mortality in commer- cially caught blue sharks Prionace glauca assessed using archival satellite pop-up tags. Marine Ecology Progress Series 387, 241 – 253.

Cooke, S. J., Woodley, C. M., Eppard, M. B., Brown, R. S. & Nielsen, J. L. (2011). Advancing the surgical implantation of electronic tags in fish: a gap analysis and research agenda based on a review of trends in intracoelomic tagging effects studies. Reviews in Fish Biology and Fisheries 21, 127 – 151.

Counihan, T. D. & Frost, C. N. (1999). Influence of externally attached transmitters on the swim- ming performance of juvenile white sturgeon. Transactions of the American Fisheries Society 128, 965 – 970.

Donaldson, M. R., Hinch, S. G., Suski, C. D., Fisk, A. T., Heupel, M. R. & Cooke, S. J. (2014). Making connections in aquatic ecosystems with acoustic telemetry monitoring. Frontiers in Ecology and the Environment 12, 565 – 573.

Dulvy, N. K., Fowler, S. L., Musick, J. A., Cavanagh, R. D., Kyne, P. M., Harrison, L. R., Carl- son, J. K., Davidson, L. N. K., Fordham, S. V., Francis, M. P., Pollock, C. M., Simpfendor- fer, C. A., Burgess, G. H., Carpenter, K. E., Compagno, L. J. V., Ebert, D. A., Gibson, C., Heupel, M. R., Livingstone, S. R., Sanciangco, J. C., Stevens, J. D., Valenti, S. & White, W. T. (2014). Extinction risk and conservation of the world's sharks and rays. eLife 3, e00590.

Friedlaender, A. S., Goldbogen, J. A., Hazen, E. L., Calambokidis, J. & Southall, B. L. (2015). Feeding performance by sympatric blue and fin whales exploiting a common prey resource. Marine Mammal Science 31, 345 – 354.

Gallagher, A. J., Serafy, J. E., Cooke, S. J. & Hammerschlag, N. (2014). Physiological stress response, reflex impairment, and survival of five sympatric shark species following exper- imental capture and release. Marine Ecology Progress Series 496, 207 – 218.

Garla, R. C., Garrone-Neto, D. & Gadig, O. B. F. (2014). Defensive strategies of neonate nurse sharks, Ginglymostoma cirratum, in an oceanic archipelago of the western central Atlantic. Acta Ethologica 18, 167 – 171. doi: 10.1007/s10211-014-0200-x

Gleiss, A. C., Dale, J. J., Holland, K. N. & Wilson, R. P. (2010). Accelerating estimates of activity-specific metabolic rate in fishes: testing the applicability of acceleration data-loggers. Journal of Experimental Marine Biology and Ecology 385, 85 – 91.

Grothues, T. M. (2009). A review of acoustic telemetry technology and a perspective on its diver- sification relative to coastal tracking arrays. Tagging and Tracking of Marine Animals with Electronic Devices 9, 77 – 90.

Gurshin, C. W. D. & Szedlmayer, S. T. (2004). Short-term survival and movements of Atlantic sharpnose sharks captured by hook-and-line in the north-east Gulf of Mexico. Journal of Fish Biology 65, 973 – 986.

Guttridge, T. L., Gruber, S. H., Gledhill, K. S., Croft, D. P., Sims, D. W. & Krause, J. (2009). Social preferences of juvenile lemon sharks (Negaprion brevirostris). Animal Behaviour 78, 543 – 548.

Guttridge, T. L., Gruber, S. H., Franks, B. R., Kessel, S. T., Gledhill, K. S., Uphill, J., Krause, J. & Sims, D. W. (2012). Deep danger: intra-specific predation risk influences habitat use and aggregation formation of juvenile lemon sharks Negaprion brevirostris. Marine Ecology Progress Series 445, 279 – 291.

Heithaus, M. R., Marshall, G. J., Buhleier, B. M. & Dill, L. M. (2001). Employing Crittercam to study habitat use and behaviour of large sharks. Marine Ecology Progress Series 209, 307 – 310.

Heupel, M. R. & Heuter, R. E. (2002). Importance of prey density in relation to the movement patterns of juvenile blacktip sharks (Carcharhinus limbatus) within a coastal nursery area. Marine and Freshwater Research 53, 543 – 550.

Heupel, M. R. & Simpfendorfer, C. A. (2005). Quantitative analysis of aggregation behaviour in juvenile blacktip sharks. Marine Biology 147, 1239 – 1249.

Holland, K. N., Wetherbee, B. M., Peterson, J. D. & Lowe, C. G. (1993). Movements and dis- tribution of hammerhead shark pups on their natal grounds. Copeia 1993, 495 – 502.

Holts, D. B. & Bedford, D. W. (1993). Horizontal and vertical movements of the shortfin mako shark, Isurus oxyrinchus, in the Southern California Bight. Australian Journal of Marine and Freshwater Research 44, 901 – 909.

Hutchinson, M. R., Itano, D. G., Muir, J. A. & Holland, K. A. (2015). Post-release survival of juvenile silky sharks captured in a tropical tuna purse seine fishery. Marine Ecology Progress Series 521, 143 – 154.

Jadot, C., Donnay, A., Ylieff, M. & Poncin, P. (2005). Impact implantation of a transmitter on Sarpa salpa behaviour: study with a computerized video tracking system. Journal of Fish Biology 67, 589 – 595.

Jepsen, N., Davis, L. E., Schreck, C. B. & Siddens, B. (2001). The physiological response of Chinook salmon smolts to two methods of radio-tagging. Transactions of the American Fisheries Society 130, 495 – 500.

Kokobun, N., Kim, J., Shin, H., Naito, Y. & Takahashi, A. (2011). Penguin head movement detected using small accelerometers: a proxy of prey encounter rate. Journal of Experi- mental Biology 214, 3760 – 3767.

Knip, D. M., Heupel, M. R., Simpfendorfer, C. A., Tobin, A. J. & Moloney, J. (2011). Onto- genetic shifts in movement and habitat use of juvenile pigeye sharks Carcharhinus amboinensis in a tropical nearshore region. Marine Ecology Progress Series 425, 233 – 246.

Lowe, C. G., Holland, K. M. & Wolcott, T. G. (1998). A new acoustic tailbeat transmitter for fishes. Fisheries Research 36, 275 – 283.

McKibben, J. N. & Nelson, D. R. (1986). Patterns of movement and grouping of gray reef sharks, Carcharhinus amblyrhynchos, at Enewetak, Marshall islands. Bulletin of Marine Science 38, 89 – 110.

Mellas, E. J. & Haynes, J. M. (1985). Swimming performance and behavior of rainbow trout (Salmo gairdneri) and white perch (Morone americana): effects of attaching telemetry transmitters. Canadian Journal of Fisheries and Aquatic Sciences 42, 488 – 493.

Morrissey, J. F. & Gruber, S. H. (1993). Habitat selection by juvenile lemon sharks, Negaprion brevirostris. Environmental Biology of Fishes 38, 311 – 319.

Myrberg, A. A. & Gruber, S. H. (1974). The behavior of the bonnethead shark, Sphyrna tiburo. Copeia 1974, 358 – 374.

Shepard, E. L. C., Wilson, R. P., Quintana, F., Laich, A. G., Liebsch, N., Albareda, D. A., Halsey, L. G., Gleiss, A., Morgan, D. T., Myers, A. E., Newman, C. & Macdonald, D. W. (2008a). Identification of animal movement patterns using tri-axial accelerometry. Endangered Species Research 10, 47 – 60.

Shepard, E. L. C., Wilson, R. P., Halsey, L. G., Quintana, F., Leich, A. G., Gleiss, A. C., Lieb- sch, N., Myers, A. E. & Norman, B. (2008b). Derivation of body motion via appropriate smoothing of acceleration data. Aquatic Biology 4, 235 – 241.

Skomal, G. B. & Bernal, D. (2010). Physiological responses to stress in sharks. In Sharks and Their Relatives, Vol. II (Carrier, J. C., Musick, J. A. & Heithaus, M. R., eds), pp. 203 – 224. Boca Raton, FL: CRC Press and Taylor & Francis Group.

Skomal, G. B. & Mandelman, J. W. (2012). The physiological response to anthropogenic stres- sors in marine elasmobranch fishes: a review with a focus on the secondary response. Comparative Biochemistry and Physiology Part A 162, 146 – 155.

Skomal, G. B., Lobel, P. S. & Marshall, G. (2007). The use of animal-borne imaging to assess post-release behavior as it relates to capture stress in grey reef sharks, Carcharhinus amblyrhynchos. Marine Technology Society Journal 41, 44 – 48.

Stark, K. E., Jackson, G. D. & Lyle, J. M. (2005). Tracking arrow squid movements with an automated acoustic telemetry system. Marine Ecology Progress Series 299, 167 – 177.

Sundstrom, L. F. & Gruber, S. H. (2002). Effects of capture and transmitter attachments on the swimming speed of large juvenile lemon sharks in the wild. Journal of Fish Biology 61, 834 – 838. doi: 10.1006/jfbi.2002.2058

Thorstad, E. B., Økland, F. & Finstad, B. (2000). Effects of telemetry transmitters on swimming performance of adult Atlantic salmon. Journal of Fish Biology 57, 531 – 535.

Whitney, N. M., Pratt, H. L. Jr., Pratt, T. C. & Carrier, J. C. (2010). Identifying shark mating behaviour using three-dimensional acceleration loggers. Endangered Species Research 10, 71 – 82.

Whitney, N. M., Papastamatiou, Y. P. & Gleiss, A. C. (2012). Integrative multisensor tagging: emerging techniques to link elasmobranch behavior, physiology and ecology. In Biology of Sharks and Their Relatives, 2nd edn (Carrier, J. C., Musick, J. A. & Heithaus, M. R., eds), pp. 265 – 290. Boca Raton, FL: CRC Press and Taylor & Francis Group.

Wilson, R. P. & McMahon, C. R. (2006). Measuring devices on wild animals: what constitutes acceptable practice? Frontiers in Ecology and the Environment 4, 147 – 154.

Winter, J. (1996). Advances in underwater biotelemetry. In Fisheries Techniques, 2nd edn (Murphy, B. R. & Willis, D. W., eds), pp. 555 – 590. Bethesda, MD: American Fisheries Society.

Wright, S., Metcalfe, J. D., Hetherington, S. & Wilson, R. (2014). Estimating activity-specific energy expenditure in a teleost fish, using accelerometer loggers. Marine Ecology Progress Series 496, 19 – 32.

Appendix II

Table summarising mean ODBA values over different tidal and diel phases and accelerometer-recorded mean temperature and depth for each shark, over the entire deployment period.

		ODBA (*g*)									
Shark	**Total Length (cm)**	**Low**	**Mid**	**High**	**Dawn**	**Day**	**Dusk**	**Night**	**Mean Overall**	**Mean Overall Temp (ºC)**	**Mean Overall Depth (m)**
D40	76	0.083	0.083	0.072	0.083	0.075	0.071	0.084	0.083	31.520	-1.244
437	78	0.093	0.089	0.075	0.077	0.083	0.097	0.088	0.091	31.282	-1.472
511	84	0.109	0.103	0.093	0.091	0.105	0.112	0.098	0.106	31.476	-1.285
C69	101	0.104	0.091	0.079	0.110	0.096	0.103	0.083	0.097	24.061	-0.532
708	101	0.082	0.074	0.069	0.088	0.077	0.093	0.068	0.078	30.764	-1.898
C25	103	0.100	0.079	0.066	0.108	0.082	0.093	0.075	0.089	28.812	-1.013
C25	104	0.076	0.072	0.070	0.069	0.078	0.082	0.067	0.074	29.044	-1.996
E50	105	0.092	0.080	0.074	0.108	0.081	0.108	0.073	0.086	23.913	-0.292
C69	110	0.088	0.088	0.082	0.083	0.083	0.091	0.087	0.088	28.713	-1.613
224	117	0.097	0.081	0.066	0.104	0.084	0.088	0.076	0.089	28.749	-1.562
C69	119	0.081	0.076	0.069	0.078	0.077	0.093	0.069	0.078	29.912	-0.531
85F	120	0.074	0.073	0.068	0.068	0.066	0.078	0.076	0.073	25.123	-1.880
901	122	0.066	0.071	0.072	0.062	0.067	0.076	0.072	0.069	30.498	-0.071
345	129	0.056	0.054	0.052	0.047	0.055	0.060	0.054	0.055	30.221	-0.166
F7F	132	0.061	0.063	0.065	0.061	0.062	0.067	0.062	0.062	30.329	-0.810
267	135	0.076	0.073	0.074	0.067	0.068	0.066	0.083	0.074	30.585	-2.012
82	148	0.071	0.068	0.067	0.062	0.069	0.076	0.068	0.069	30.682	-0.196
E07	160	0.056	0.061	0.062	0.049	0.063	0.046	0.062	0.058	25.320	-0.910
773	169	0.039	0.039	0.044	0.041	0.044	0.037	0.038	0.039	30.659	-0.250

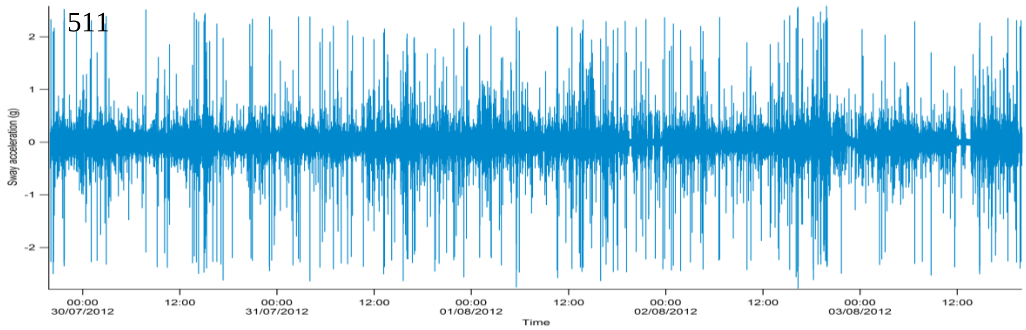
Appendix III

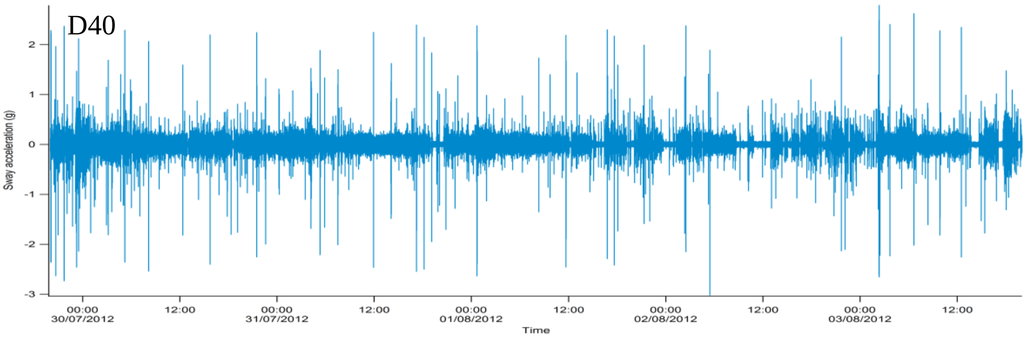
Summary of prey handling and foraging events for all sharks across entire deployment period.

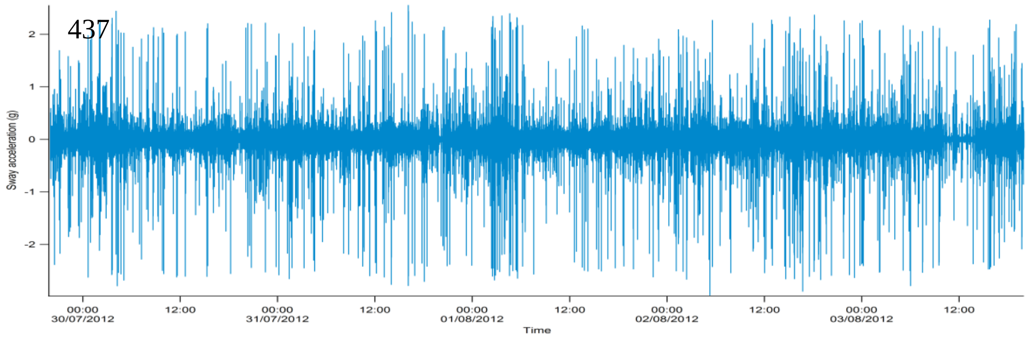
Shark	Prey Handling	Foraging	% success	High	Mid	Low
D40	3	16	18.8	1	1	1
437	1	2	50.0	0	0	1
511	5	24	20.8	0	2	3
C69	2	19	10.5	2	0	0
708	4	14	28.6	1	0	3
C25	3	11	27.3	0	2	1
C25(2)	3	7	42.9	0	1	2
E50	5	18	27.8	0	1	4
C69(2)	2	24	8.3	0	1	2
224	4	11	36.4	0	1	3
C69(3)	4	31	12.9	0	2	2
85F	5	20	25.0	0	2	3
901	9	87	10.3	4	1	4
345	6	30	20.0	4	2	0
F7F	3	24	12.5	2	0	1
267	6	25	24.0	2	2	2
O82	4	21	19.0	1	0	3
E07	2	45	4.4	2	0	0
773	3	50	6.0	1	0	2
Total	**74**	**479**	**22.3**	**1.05**	**0.95**	**1.95**

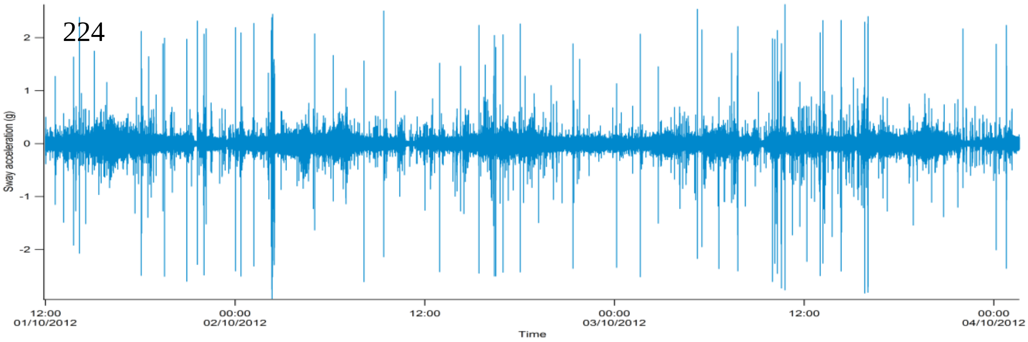
Appendix IV

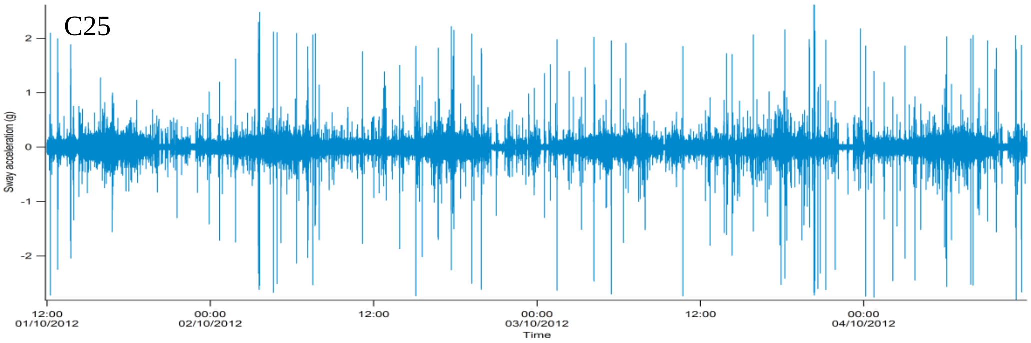
Summary sway acceleration profiles for all wild sharks (denoted by PIT code identifier in each figure) across entire deployment period. Routine swimming behaviour in sharks is characterised by oscillating tailbeats that are represented by dynamic sway acceleration, presented here. Data represent an oscillating waveform whereby greater degrees and speeds of tailbeat movements are recorded as higher values of acceleration.










C25
Sway acceleration (g)
2
1
0
-1
-2
12:00
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00:00
02/10/2012
12:00
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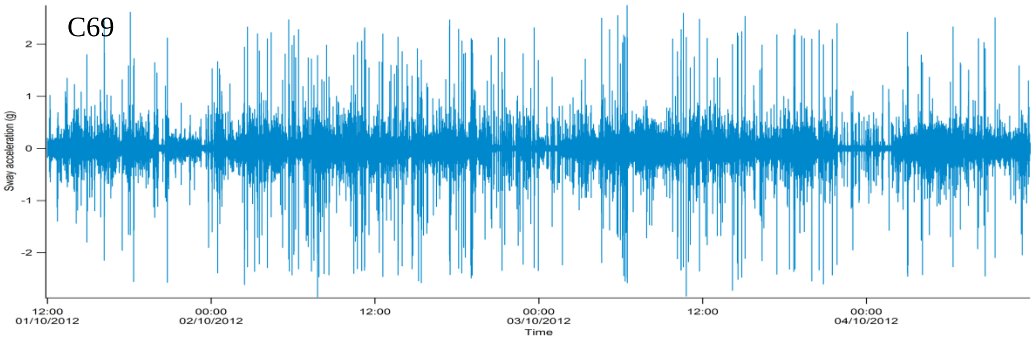
12:00
00:00
04/10/2012
Time


C69
Sway acceleration (g)
2
1
0
-1
-2
12:00
01/10/2012
00:00
02/10/2012
12:00
00:00
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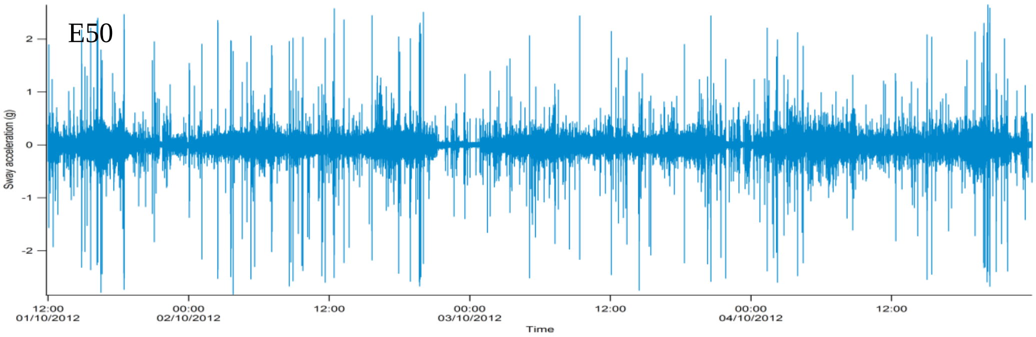
00:00
04/10/2012
Time


E50
Sway acceleration (g)
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1
0
-1
-2
12:00
01/10/2012
00:00
02/10/2012
12:00
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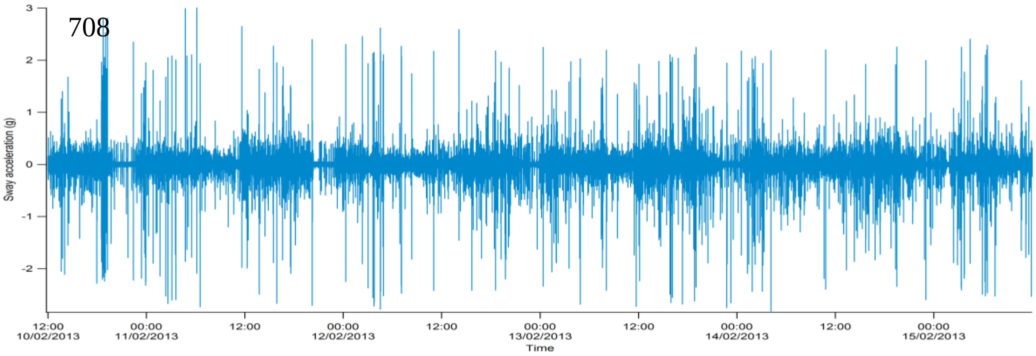
04/10/2012
12:00
Time


708
Sway acceleration (g)
3
2
1
0
-1
-2
12:00
10/02/2013
00:00
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12:00
00:00
12/02/2013
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00:00
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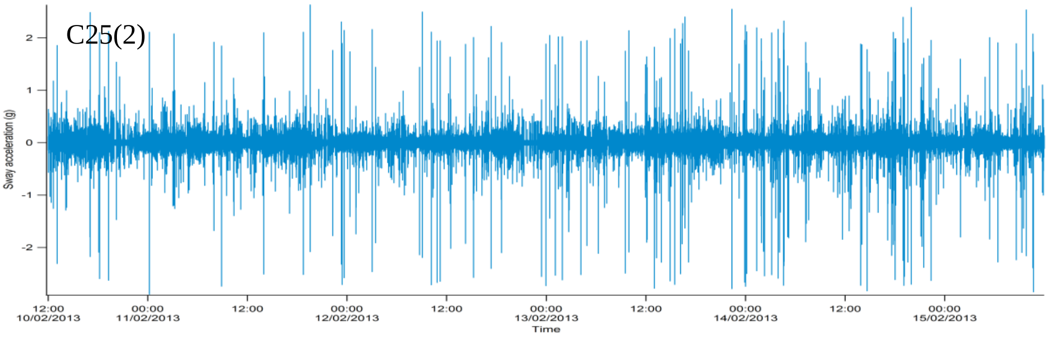
14/02/2013
12:00
00:00
15/02/2013
Time


C25(2)
Sway acceleration (g)
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0
-1
-2
12:00
10/02/2013
00:00
11/02/2013
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00:00
12/02/2013
12:00
00:00
13/02/2013
12:00
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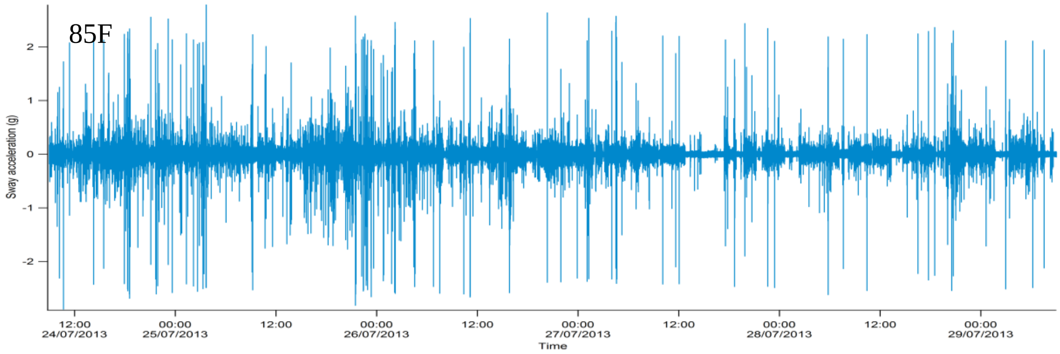
14/02/2013
12:00
00:00
15/02/2013
Time


85F
Sway acceleration (g)
2
1
0
-1
-2
12:00 24/07/2013
00:00 25/07/2013
12:00
00:00 26/07/2013
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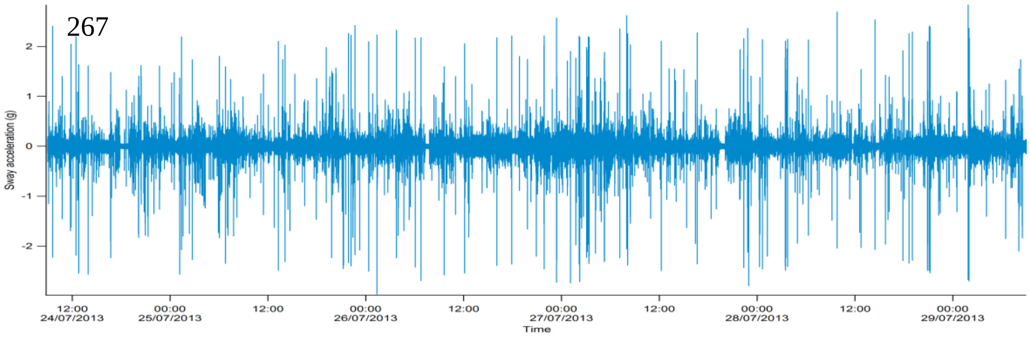
12:00
00:00 29/07/2013
Time


267
Sway acceleration (g)
2
1
0
-1
-2
12:00 24/07/2013
00:00 25/07/2013
12:00
00:00 26/07/2013
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12:00
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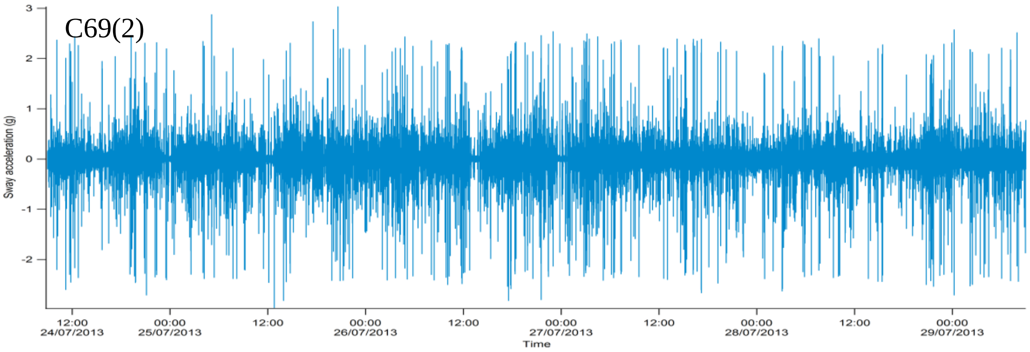
Time


C69(2)
Sway acceleration (g)
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0
-1
-2
12:00 24/07/2013
00:00 25/07/2013
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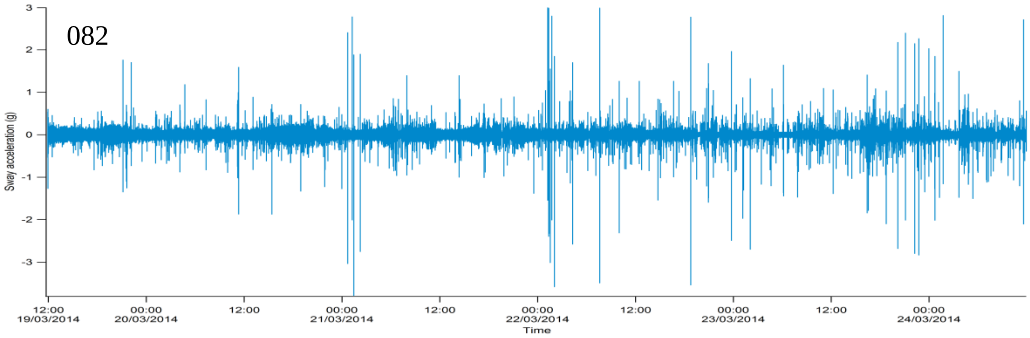
00:00 29/07/2013
Time


082
Sway acceleration (g)
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2
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0
-1
-2
-3
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00:00 20/03/2014
12:00
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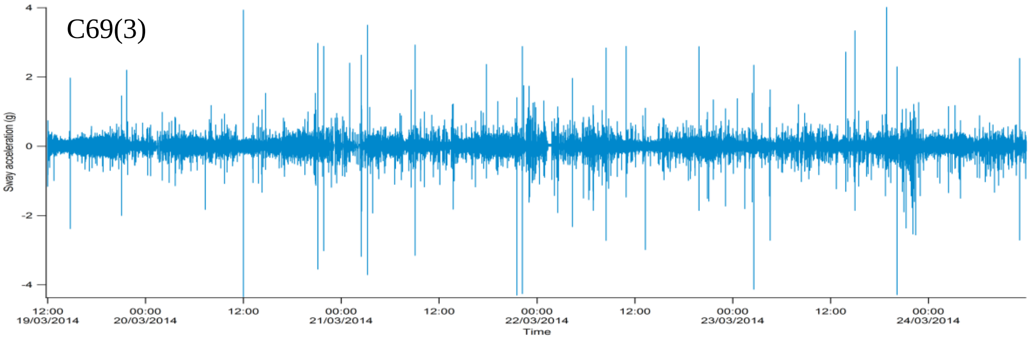
00:00 24/03/2014
Time


C69(3)
Sway acceleration (g)
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0
-2
-4
12:00 19/03/2014
00:00 20/03/2014
12:00
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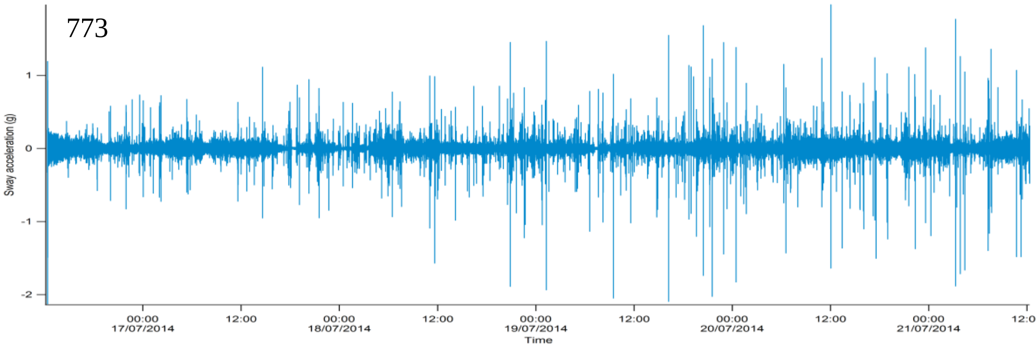
12:00
00:00 24/03/2014
Time


773
Sway acceleration (g)
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-1
-2
00:00
17/07/2014
12:00
00:00
18/07/2014
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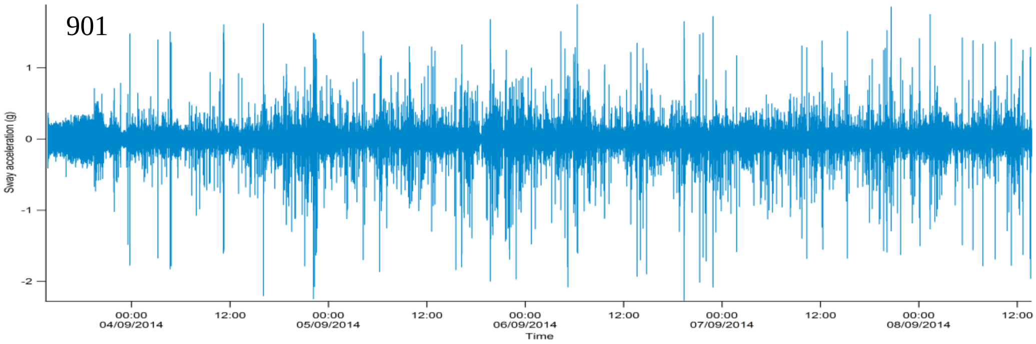
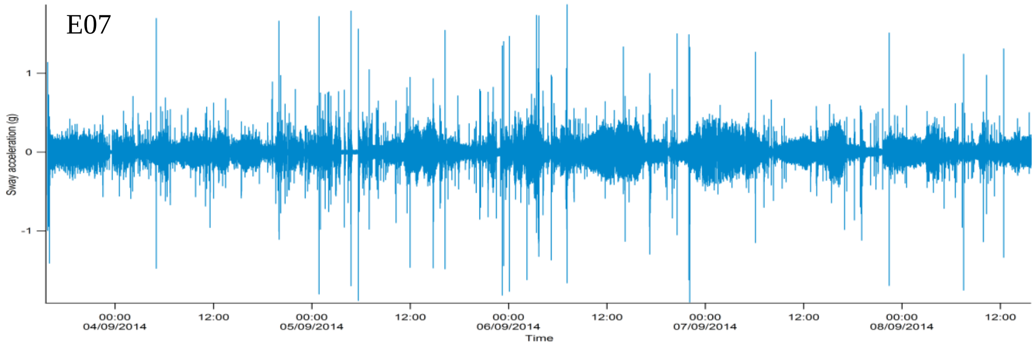
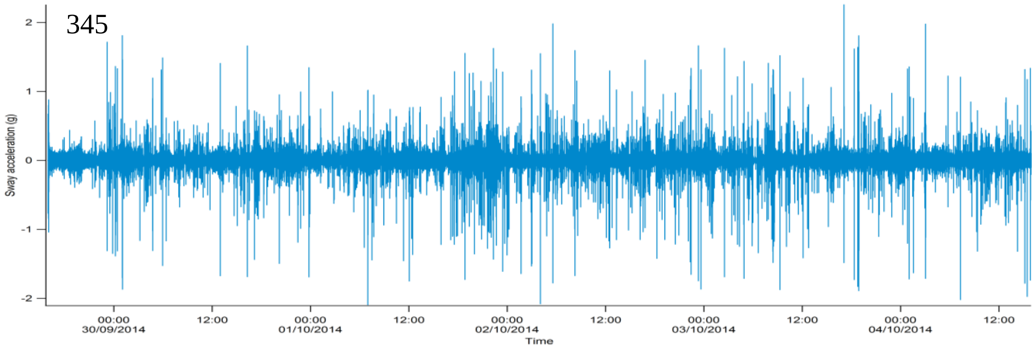
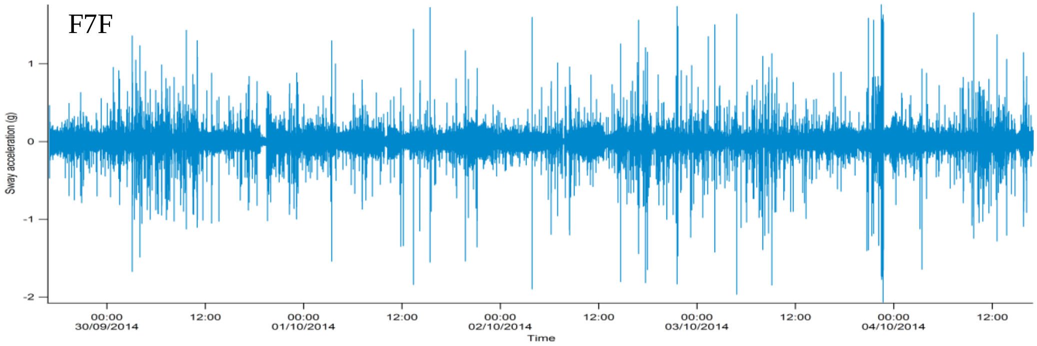
19/07/2014
12:00
00:00
20/07/2014
12:00
00:00
21/07/2014
12:00
Time


901
Sway acceleration (g)
1
0
-1
-2
00:00
04/09/2014
12:00
00:00
05/09/2014
12:00
00:00
06/09/2014
12:00
00:00
07/09/2014
12:00
00:00
08/09/2014
12:00
Time


E07
Sway acceleration (g)
1
0
-1
00:00
04/09/2014
12:00
00:00
05/09/2014
12:00
00:00
06/09/2014
12:00
00:00
07/09/2014
12:00
00:00
08/09/2014
12:00
Time


345
Sway acceleration (g)
2
1
0
-1
-2
00:00
30/09/2014
12:00
00:00
01/10/2014
12:00
00:00
02/10/2014
12:00
00:00
03/10/2014
12:00
00:00
04/10/2014
12:00
Time


F7F
Sway acceleration (g)
1
0
-1
-2
00:00
30/09/2014
12:00
00:00
01/10/2014
12:00
00:00
02/10/2014
12:00
00:00
03/10/2014
12:00
00:00
04/10/2014
12:00
Time