Feeding ecology, residency patterns and migration dynamics of bull sharks (*Carcharhinus leucas*) in the southwest Indian Ocean.

Thesis submitted in fulfilment of the requirements for the degree of

DOCTOR OF PHILOSOPHY of RHODES UNIVERSITY

by

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November 2014

Abstract

Bull sharks (*Carcharhinus leucas*) are globally distributed top predators that play an important ecological role within coastal marine communities. However, little is known about how the spatial and temporal scales of their habitat use are associated with their ecological role. In this study, a population of sub-adult and adult bull sharks were investigated within a remote subtropical marine community in the southwest Indian Ocean off the coast of southern Mozambique. The main objectives of the study were to test a minimally invasive remote biopsy sampling method; to investigate the feeding ecology of bull sharks; and to investigate the temporal and spatial scales of bull shark residency patterns and migration dynamics. Biopsy tests on free-swimming bull sharks showed that the devised sampling technique provided a minimally invasive and consistent method (biopsy retention rate = 87%) to obtain muscle tissue samples sufficiently large enough $(310\pm78mg, mean \pm SD)$ for stable isotope analysis. Results from the stable isotope analysis showed that adult bull sharks appeared to exhibit a shift towards consistently higher trophic level prey from an expanded foraging range compared to sub-adults, possibly due to increased mobility linked with size. Additionally, bull sharks had significantly broader niche widths compared to top predatory teleost assemblages with a wide and enriched range of δ^{13} C values relative to the local marine community, suggesting that they forage over broad spatial scales along the east coast of southern Africa. Results from the passive acoustic telemetry investigation, conducted over a period between 10 and 22 months, supported these findings showing that the majority of tagged adult sharks

exhibited temporally and spatially variable residency patterns interspersed with migration events. Ten individuals undertook coastal migrations that ranged between 433 and 709km (mean = 533km) with eight of these sharks returning to the study site. During migration, individuals exhibited rates of movement between 2 and 59km.d⁻¹ (mean = 17.58km.d⁻¹) and were recorded travelling annual distances of between 450 and 3760km (mean = 1163km). These findings suggest that adult bull sharks are not the sedentary species once thought to be but rather, consistently move over broad spatial scales on the east coast of southern Africa and play an important predatory role shaping and linking ecological processes within the southwest Indian Ocean.

Acknowledgements

Many aspects of this investigation first started with the statement "I have never done this before, what do you think?" and I am grateful that these words did not fall on deaf ears. The insight and advice provided in the field by Barry Skinstad, Justin Blake and Clare Keating Daly was inimitable. Even when the boat started to sink, cool heads prevailed and a motivation to get the job done in some exceptionally difficult circumstances was the difference between failure and success. There were also many others at Ponta do Ouro who provided a helping hand and support over the years including Oceans Research, Chris Vaughan-Jones, Stuart Laing, Roger Horrocks, Colin and Sam Bull, Travis and Judy Holtzhausen, Daryl Kemp, Sandra Probert, Noleen Skinstad, Laurens and Mel Koen, Simon "Mashamplane", Mathias and many others. This study would also not have been possible without the support from the Ponta do Ouro Partial Marine reserve managed by Miguel Gonçalves, as well as the Mozambican Directorate of National Conservation Areas.

Financially, this study was supported by a grant provided by the Professional Development Program of the South African National Research Foundation, administered through Bayworld Centre for Research and Education and the Port Elizabeth Museum. I am also grateful to 3 Fathoms Research, SAIAB and Rhodes University for providing invaluable financial support. Furthermore, I would also like to express my appreciation to my academic supervisors, Malcolm Smale, William Froneman and Paul Cowley (informal supervisor) who believed in me and provided me with equipment, advice and support. Without their encouragement this study would not have been possible.

I would never have had the opportunity to pursue my dreams without the support of my parents, Sean and Jean Daly. I am grateful for the sacrifices made over many years to ensure that I was able to reach this point and would never have accomplished this without their understanding, patience and motivation.

Finally, I would like to thank my wife Clare Keating Daly who first came to Mozambique to live in a tent with me whilst this study was just starting. From monkey bites to shark bites and putting up with me, there is no doubt that this study was only possible with her support and that of her family: Michael, Sally, Eliza, Eric and Madeline. Clare's support, understanding and encouragement over the years was the foundation of this study and it has been an incredible adventure. Thank you.

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Chapter 1: General introduction

1.1. Species overview

Sharks, skates, rays and chimeras make up the Class Chondrichthyes (cartilaginous fishes) and include some of the longest surviving extant species in history (Grogan et al. 2012). Of the two subclasses that make up the Chondrichthyes (Elasmobranchii and Holocephali), the elasmobranchs (sharks, rays and skates) are the most specious with 8 orders of sharks including more than 500 species (Ebert et al. 2013). Carcharhinus leucas is one of 52 shark species that belong to the family Carcharhinidae that make up one of eight families within the order Carcharhiniformes. Carcharhinidae are one of the largest and widest ranging families of sharks and can generally be distinguished by the presence of pre caudal pits, the lack of spiracles, the first dorsal fin origin above the pectoral fin and the second dorsal fin smaller than the first (Garrick 1982, Compagno 1984). Carcharhinids are often the most abundant shark family in terms of diversity and biomass in many tropical neritic and epipelagic environments (Ebert et al. 2013, Stevens et al. 2010) and as medium to large sharks (1-3m) (Ebert et al. 2013) that may feed indiscriminately on a wide variety of prey, they play an important role shaping their respective marine communities through predation and associated risk effects (Heithaus et al. 2008). Carcharhinus leucas is no exception and as a large Carcharhinid shark with a global distribution in tropical and temperate nearshore waters, it plays a key role as an apex predator in all of the world's major oceans.

Carcharhinus leucas was first described by Valenciennes (in Müller and Henle 1841) and from 1852 to 1962 at least 12 synonymies have been published in accounts ranging from the Atlantic, Indian and Pacific Ocean (Garrick 1982). These multiple synonymies are most likely due to the global distribution of the bull shark, its occurrence in freshwater, as well as the presence of a sibling species, *Carcharhinus amboinensis* (Bass et al. 1973, Garrick 1982). Investigators of early accounts from freshwater localities such as the Zambezi River (Peters 1852, in Garrick 1982), Lake Nicaragua (Gill and Bransford 1877) and the Brisbane River (Ogilby 1910) generally presumed the shark to live permanently in these freshwater systems and, therefore, described the new species in part due to its presence within these geographically isolated areas. Some of the confusion based on specimens found in freshwater was only clarified as late as 1971 when, discovering that the Lake Nicaragua sharks were also found in the Atlantic Ocean, Thorson (1971) verified that population to be *Carcharhinus leucas*. In many areas *Carcharhinus leucas* is still commonly referred to by the name originally used to describe its presence in freshwater systems. These include the Ganges River shark, Swan River shark, the Lake Nicaragua shark and as it is known locally in southern Africa, the Zambezi shark (Compagno 1984). In many Anglophone countries today, *Carcharhinus leucas* is now commonly referred to as the bull shark, in reference to its stocky appearance and potentially bellicose behaviour.

1.1.1. Reproductive biology

All Chondrichthyan reproduction employs internal fertilization resulting in small litter sizes with relatively large individual offspring (Compagno 2005). The modes of reproduction amongst elasmobranch families vary with respect to the type of foetal nutrition classified as either lecithotrophic or matrotrophic (Conrath and Musick 2012). Carcharhinids (with the exception of *Galeocerdo cuvier*) exhibit matrorophic foetal nutrition via placental viviparity whereby the yolk sac attaches to the wall of the uterus (Musick and Ellis 2005). Typically, species exhibiting this reproductive mode have gestation times of approximately one year and have litter sizes of fewer than 20 offspring (Musick and Ellis 2005). Accordingly, in the case of the bull shark, the average gestation period is between 10 and 11 months (Clarke and von Schmidt 1965), generally resulting in litter sizes of between 1 and 13 (Cliff and Dudley 1991) with offspring ranging between 56cm and 81cm at birth (Clarke and von Schmidt 1965, Bass et al. 1973, Cliff and Dudley 1991, Ebert et al. 2013).

Elasmobranchs exhibit three main reproductive cycles consisting of continuous reproduction, a partially defined annual cycle, or a well-defined annual or biennial cycle (Conrath and Musick 2012). Species-specific reproductive cycles are usually related to habitat and the employed mode of reproduction. As with most species from the genus *Carcharhinus* that occur within coastal subtropical habitats where seasonal cycles are well defined, the bull shark exhibits a defined annual or biennial cycle (Compagno 1984).

Mating typically takes place during summer months (Clarke and von Schmidt 1965, Cliff and Dudley 1991), however, there is potential for mating year round (Compagno 1984) where seasonal cycles are less defined. The seasonal reproductive cycle exhibited by the bull shark may result in seasonal migrations between areas used for mating, pupping or feeding, although little is known about locations used for mating. As with other Carcharhinids, mating behaviour of the bull shark most likely includes multiple paternity as a way to increase genetic diversity (Pratt and Carrier 2001, Conrath and Musick 2012), however, further studies are needed to confirm this.

Studies in the western Atlantic (Karl et al. 2011) and on the Australian coast (Tillet et al. 2012) suggest that female bull sharks may exhibit reproductive phylopatry with individual sharks frequenting the same estuary systems for pupping over multiple years. Although maternal gene flow may be limited amongst population subdivisions, the global population genetic structure appears to consist of three genetically distinct populations in the western north Atlantic, Indo-Australia and Fiji (Testerman et al. 2012) (Fig 1.1). This suggests that gene flow amongst populations happens over relatively large scales within ocean basins, but the relatively low fixation index values between populations suggests that there is also a low level of gene transfer over a global scale.



Figure 1.1. Three genetically distinct populations of bull sharks based on results from 470 samples using 12 bi-parentally inherited nuclear microsatellite loci. Fst = fixation index p = 0.0000 (Figure from Testerman et al. 2012).

1.1.2. Age and growth

The bull shark is a relatively large, slow growing and late maturing shark species (Compagno 1984). Typically females grow larger than their male counterparts reaching a maximum recorded length of between 257cm and 314cm TL (Clark and von Schmidt 1965, Sadowsky 1967, Bass et al. 1973, Bransttetter and Stiles 1987, Cliff and Dudley 1991, Wintner et al. 2002, Cruz-Martinez et al. 2005, Neer et al. 2005). While other reports generally suggest that larger specimens have been recorded of between 320cm TL (Garrick 1982) and 340cm TL (Compagno 1984, Heemstra and Heemstra 2005). Some of the largest recorded specimens have come from South African waters (Bass et al. 1973), and appropriately, in 2009, McCord and Lamberth (2009) described an individual female specimen of 400cm TL caught in the Breede River (Western Cape province), South Africa. The corresponding age at maximum length for the bull shark has been calculated using vertebral growth ring counts, and ranges between 21 and 29 years for males and between 24 and 32 years for females (Branstetter and Stiles 1987, Wintner et al. 2005, Cruz-Martinez et al. 2005, Neer et al. 2005). However, vertebral growth ring counts to age sharks may be susceptible to errors that lead to underestimating the age of large or long-lived sharks (Passerotti 2014). Thus, future work using alternative methods such as bomb radiocarbon dating may provide a more accurate estimate of age (Passerotti 2014).

Females grow more slowly (Cruz-Martinez et al. 2005) and reach sexual maturity slightly later than males. The reported age at sexual maturity for male and female sharks range widely, between 9 and 20 years and 10 and 21 years, respectively (Branstetter and Stiles 1987, Wintner et al. 2005, Cruz-Martinez et al. 2005). The corresponding length at sexual maturity for male and female sharks, range between 200cm to 247cm TL and 204 to 250cm TL, respectively (Bass et al. 1973, Branstetter and Stiles 1987, Cliff and Dudley 1991, Wintner et al. 2002, Cruz-Martinez et al. 2005). The variance in length and age at sexual maturity between populations suggests that some populations (such as those in the southern Gulf of Mexico) may have substantially faster growth rates as sub-adults in order to reach sexual maturity earlier. Growth rates may be influenced by various factors, most notably the effect of seasonality and temperature that changes from the equator to the poles (White and Sommerville 2010). Such a latitudinal effect has been recorded for other

elasmobranchs whose populations at lower latitudes mature earlier and grow faster (Driggers et al. 2004, Neer and Thompson 2005). Thus, this effect may account for bull shark populations that occur at lower latitudes having longer and more consistent growth periods compared to those at higher latitudes.

1.1.3. Diet

Habitat type, prey abundance, geographical location and water depth are important factors that may determine the diet of elasmobranch species (Bethea et al. 2004, Braccini et al. 2005, McElroy et al. 2006, Heithaus and Vaudo 2012). As bull sharks mostly inhabit temperate to tropical regions and have a strong affinity to coastal areas, their diet is diverse, but typically confined to prey items that occur in shallow habitats on the continental shelf. Bull sharks are capable of consuming a wide variety of prey items including teleost fish, invertebrates, elasmobranchs, turtles, dolphins and birds (Bass et al. 1973, Compagno 1984, Snelson et al. 1984, Cliff and Dudley 1991), but smaller individuals (less than 1.4m PCL) will primarily prey on teleost fish before exhibiting an ontogenetic shift towards a preference for larger prey items such as elasmobranch and mammalian prey (Cliff and Dudley 1991, Cortez 1999). The observed ontogenetic dietary shift primarily reflects the change from juvenile estuarine habitats to the coastal habitats of adults as well as the ability of adults to consume larger prey items (Snelson et al. 1984, Werry et al. 2011). On the east coast of South Africa, Cliff and Dudley (1991) showed that the predominant dietary items of bull shark populations varied according to geographical location and suggested that benthic prey are the most important prey items. Scavenging may also play a large role in the feeding strategy of bull sharks (Cliff and Dudley 1991) and reflect how individuals may take advantage of opportunistic feeding events or the most abundant prey type.

The diet of individual bull sharks may also be influenced by seasonal migrations, and accurate dietary descriptions of a species may be complicated by spatial and temporal habitat shifts (Wetherbee et al. 2012). Seasonal dietary variation has been recorded for various species of sharks (Heithaus and Vaudo 2012) where primary dietary items are likely influenced by the most abundant prey, or in some cases, by specific selection for

more energy-rich prey (Lowe et al. 1996, Estrada et al. 2006). There have been no records of specific selection for more energy-rich prey items by bull sharks, but it is likely that individuals will frequent areas with the greatest prey availability.

1.1.4. Distribution and habitat

Bull sharks typically inhabit shallow (<150m) coastal waters throughout the globe, in tropical and warm temperate oceans, including remote islands and archipelagos such as Tonga, Fiji, Azores and the Seychelles (Gadig et al. 2006, Brunnschweiler and Compagno 2008, Brunnschweiler et al. 2010) (Fig 1.2). Although bull sharks are capable of reaching depths of 200m or more (Brunnschweiler et al. 2010), they typically remain within the upper 30m of the water column (Ebert et al. 2013) and have an affinity for coastal environments with bathymetry and temperature influencing the depth at which individuals spend the majority of their time. Brunnschweiler et al. (2010) showed that adult sharks in a region with deep coastal areas spent more of their time below 20m than those that occupied shallow coastal habitats, although in both cases the temperature at which the sharks spent the majority of their time was above 25°C. Behaviour may also be a driving factor, determining the depth at which individuals occur, as they may change habitat depth to exploit a resource or undertake migrations (Carlson et al. 2010). The small eye diameter of bull sharks is strongly correlated with its shallow depth range and may reflect the adaptation to the photic environment (Kajiura et al. 2010). Bull sharks also have relatively more electro-sensory pores than other typically visual predatory sharks (e.g. white sharks) suggesting that electroreception is the primary sense employed when foraging for prey (Kajiura et al. 2010).



Figure 1.2. Bull shark global distribution with a colour scale indicating relative probability of occurrence (Figure from Aquamaps.org).

Although typically a tropical species, populations of bull sharks do exist either seasonally or permanently in more temperate regions and may undertake coastal migrations linked with seasonal changes in environmental variables, reproductive behaviour or ontogenetic habitat changes (Compagno 1984, White and Sommerville 2010). Reported seasonal migrations of adult sharks are typically associated with a change in sea temperature, whereby individuals move to higher latitudes during summer months, following warmer water, before retreating to lower latitudes in summer (Compagno 1984). The habitat transition of juveniles from estuarine nursery areas to coastal environments as adults is also well documented (Heupel et al. 2010, Werry et al. 2011). Similar to other Carcharhinid species, it is likely that an ontogenetic shift in movement patterns take place, with larger individuals moving greater distances and occupying a larger habitat area (Yeiser et al. 2008, Carlson et al. 2010, Werry et al. 2011). Adults are capable of moving substantial distances, with the longest recorded migration reported by Carlson et al. (2010) of 1505km within 85 days. Although in contrast to other large shark species such as the tiger shark (Galeocerdo cuvier) (Heithaus et al. 2007) and the white shark (Carcharodon carcharhias) (Bonfil et al. 2005), bull sharks have not been recorded undertaking transoceanic

migrations. However, Brunnschweiler et al. (2010) did report a bull shark moving across an open ocean channel from the Bahamas to the coast of Florida, U.S.A.

While there is evidence to suggest that bull sharks undertake seasonal migrations along the coast of southern Africa (Compagno and Smale 1986), local studies suggest that bull sharks are largely sedentary exhibiting only limited movements (Holden 1977, Cliff and Dudley 1991, Bullen and Mann 2000, Mann 2000). Historical shark net catch data from South Africa suggested that reductions in the population size of bull sharks was due to the depletion of localized or territorial populations (Holden 1977). Furthermore, Cliff and Dudley (1991) and Bullen and Mann (2000) showed that the mean distance travelled from tagged and recaptured bull sharks on the east coast of South Africa, at liberty from between 11 and 558 days, was only 28km and 71km, respectively. Additionally, catch statistics from bather protection gill nets in the same area, provided evidence to suggest that bull shark populations exhibited spatially limited habitat use (Cliff and Dudley 1991). Such evidence of persistently small-scale movement patterns is in contrast to studies in the Pacific and Atlantic Oceans (Brunnschweiler et al. 2010, Carlson et al. 2010) and there is a need to clarify the movement patterns of bull sharks within the Indian Ocean.

The unique ability to tolerate water with highly variable salinity levels allows bull sharks to penetrate freshwater systems (Pillans et al. 2008) and is an important aspect part of the species life history. Through a combination of branchial impermeability, renal conservation and hepatic urea production, bull sharks are able to effectively osmoregulate in freshwater and hypersaline conditions (Anderson et al. 2005), however, sharks appear to avoid salinity above 50.00 (Bass et al. 1973). Records exist of bull sharks as far as 3700km up the Amazon River in Iquitos in Peru (Thorson 1972) and hundreds of kilometres up other large river systems such as the Mississippi (Thomerson et al. 1977), San Juan (Thorson and Thomas 1971) and Zambezi Rivers (Bass et al. 1973). Although bull sharks have been observed surviving for extended periods in freshwater lakes, there have been no records of reproduction or population growth within these systems (Montoya and Thorson 1982, Compagno 1984). In southern Africa, bull sharks have been recorded in numerous estuarine systems from the temperate southeast coast of South Africa to the

sub-tropical east coast of South Africa and Mozambique. In the temperate regions of South Africa, adult bull sharks may penetrate the lower reaches of many larger river systems during summer such as the Breede River (McCord 2009), Fish River (Compagno and Smale 1986), Mbashe River (Plumstead 1990) and the Mzimvubu River (P. Cowley pers. com). In the sub-tropical regions of South Africa and Mozambique, adult and juvenile bull sharks may penetrate further up rivers such as the Tugela, Umfolozi, Limpopo and Zambezi (Bass et al. 1973) and have been recorded in many other smaller estuarine systems along the east coast (Whitfield 1998, Olbers 2012).

The use of estuaries by juvenile bull sharks is now well documented (Simpendorfer et al. 2005, Yieser et al. 2008, Ortega et al. 2009, Froeschke et al. 2010, Heupal and Simpfendorfer 2011, Werry et al. 2011, Matich and Heithaus 2012), as these systems constitute critical nursery areas for the species. Bull shark pups are usually born near or within estuary systems (Werry et al. 2011) where they may remain for periods of between 18 months (Heupal and Simpfendorfer 2011) and five years (Matich and Heithaus 2012). The juvenile habitat preference of elasmobranchs is typically a function of a variety of biotic and abiotic factors that are important for bioenergetics, habitat suitability, prey availability and predation risk (Grubbs 2010). Ontogenetic shifts in diet and habitat play an important role to decrease competition amongst juveniles and adults and help to limit predation from larger conspecifics (Grubbs 2010, Heupal and Simpfendorfer 2011). Dietary specialization (Matich et al. 2011) and resource partitioning between bull sharks and other predatory species that share the same nursery habitats may also be key mechanisms that decrease competition for resources (Kinney et al. 2011). The youngest individuals also have the lowest tolerance to habitat related physico-chemical variability (Werry et al. 2011) with temperature and salinity being the main factors affecting their distribution (Simpendorfer et al. 2005, Froeschke et al. 2010, Matich and Heithaus 2012). The upper limits of consistent salinity tolerable by juvenile bull sharks is approximately 50.00 (Bass et al. 1973), with the lowest consistent temperature tolerable approximately 14 °C (Matich and Heithaus 2012). However, latitudinal variability between habitats in which juveniles occur, may make a substantial difference to the limits of physical tolerance. Varying rates of spatial and temporal productivity and physico-chemical factors will exert a greater influence on sharks inhabiting estuaries at higher latitudes. Therefore, sharks in these environments may have larger activity spaces and may only be able to use these systems during summer months when temperatures are favourable (Grubbs 2010). The influence of seasonal variability on habitat availability is a consideration for populations along the southern African coast, where juveniles primarily make use of sub-tropical and temperate estuaries (Bass et al. 1973).

1.2. The ecological role and conservation of sharks

1.2.1. Ecology

Chondricthyan fish species have played a fundamental predatory role within marine communities for at least 350 million years, and large shark species have been the most persistent top marine predators throughout evolutionary time (Sepkowski 2002). Large shark species are still one of the most ubiquitous top marine predators, but currently face an increased risk of extinction due largely to escalating fishing pressure (Dulvy et al. 2014). Considering the evolutionary history and current decline in the numbers of top predatory shark species (Dulvy et al. 2014), there is an urgent need to understand the potential ecological consequences of their loss from marine ecosystems.

The ecological role of an organism can be defined simply as "what it eats and what eats it" (Heithaus et al. 2012b). However, the ecological influence of a species may extend beyond its position in the food chain to encompass more complex, interspecific interactions (Ripple and Beschta 2007). Predation has been shown to shape community structure within terrestrial ecosystems (Schmitz et al. 2004, Knight et al. 2005, Ripple and Beschta 2007), but the influence of large marine predators on their respective marine communities has been more challenging to study due to logistical constraints within the marine environment (Ferretti et al. 2010). As large shark species are often apex predators within marine communities (Cortes 1999), understanding the mechanisms and interspecific interactions of their ecological role is crucial for understanding how the structure and function of marine communities may be influenced by declining shark populations (Myers 2007).

Predators can influence their communities through direct predation or through indirect risk effects (Ripple and Beschta 2007, Heithaus et al. 2012b). Direct predation can alter the size or density of prey through mortality or consumption (Heithaus et al. 2012b) while risk effects influence community structure through anti-predator behaviour exhibited by the prey (Heithaus et al. 2009). Such anti-predator behaviour ultimately results in reduced access to resources and, therefore, a reduction of the carrying capacity of a prey population within the habitat occupied by predators (Lima and Dill 1990). Both direct predation and associated risk effects are fundamental components of the ecological influence of predators which can cause trophic cascades whereby a top predator may have several knock-on effects at various trophic levels within their respective communities. Such trophic cascades are well documented within marine communities (Frank et al. 2005, Estes et al. 1998) and sharks are often key mediators of these cascades (Myers et al. 2007, Ruppert et al. 2013).

In the context of marine communities, trophic cascades are vital mechanisms to understand. Trophic cascades are indirect interactions between predators and prey that explain how a relatively small change in the size or density of top predator populations may have farreaching consequences for the entire community or ecosystem (Baum and Worm 2009, Steneck 2012, Silliman and Angelini 2012). One of the central components of trophic cascades is the predatory release hypothesis, which predicts that the removal of top predators may allow mesopredator populations to increase (Ferretti et al. 2010). Evidence supporting this hypothesis include studies that show that the decline in catch rates of large top predatory sharks in the coastal and open ocean environments coincided with an increase in the catch rates of mesopredatory elasmobranchs (van der Elst 1979, Myers et al. 2007, Ward and Myers 2005, Shepherd and Myers 2005). In some cases, the proliferation of mesopredatory populations had knock-on effects for mesopredatory prey species and resulted in the severe decline or crash of these prey populations (Myers et al. 2007). However, the direct relationships between various species within a community may sometimes be complex and difficult to quantify and some caution needs to be taken when interpreting the conclusions of these studies (Stevens et al. 2000). Despite this, there is mounting evidence to highlight the critical role that sharks play in shaping and structuring marine communities through direct predation and associated risk effects (Lucas and Stobo

2000, Frid et al. 2007, Heithaus et al. 2008, Ferretti et al. 2010, Ward-Paige et al. 2010, Ruppert et al. 2013, Heithaus et al. 2014). As there is increasing pressure on marine ecosystems from a range of anthropogenic related disturbances such as overexploitation (Ward and Myers 2005, Jackson 2008, Worm et al. 2009), habitat loss (Dulvy et al. 2003) and climate change (Portner and Peck 2010, Lauria et al. 2012), there is an increasing need to understand the ecological role of sharks within these ecosystems (Ruppert et al. 2013).

1.2.2. Conservation status and management

The latest evaluation of the global conservation status of Chondrichthyan fish species estimate that one quarter of these species are currently threatened according to the International Union for Conservation of Nature (IUCN) Red List criteria (IUCN 2014). Furthermore, Dulvy et al. (2014) predict that the number of Chondrichthyan species listed as near threatened or threatened by the IUCN, will increase to more than half of all species (Fig 1.3) (Bradley and Gaines 2014). Relative to other vertebrate groups, the overall extinction risk for Chondrichthyan species is substantially higher with only 37.4% of all species considered to have little risk of extinction (Dulvy et al. 2014). Furthermore, there are limited data to assess the conservation status of a large proportion (46%) of Chondrichthyan species, with the largest knowledge gaps in tropical coastal seas such as the southwest Indian Ocean (Costello et al. 2012, Dulvy et al. 2014, IUCN 2014).



Figure 1.3. Chondrichthyan fishes that include sharks, rays, skates and chimaeras are predicted to be at more risk than any other vertebrate group with forecasts suggesting that more than half of the species will be listed as threatened or near threatened by the IUCN (Figure from Bradley and Gaines 2014).

The poor conservation status of Chondrichthyan species can be principally attributed to fishing pressure that has increased disproportionately to effective management policies since the start of industrial scale fishing pressure in the 1950's (Stevens et al. 2000, Mullon et al. 2005, Clarke et al. 2006, Coll et al. 2008, Dulvy et al. 2008, Garcia et al. 2008, Worm et al. 2009, Dulvy and Forrest 2010). It was only in 1994 that the parties to the Convention on International Trade in Endangered Species (CITES) mandated a review of the status and trade in sharks which up until that point were not listed on the CITES appendices and, therefore, were subject to little international concern (Stevens et al. 2000). This was mainly due to the fact that sharks were seen as less economically important compared to teleost fisheries, and there was no perceived need to manage the populations of sharks and rays that were primarily caught as by-catch. However, an increasing demand for shark fin from

a growing economy in Asia has driven a targeted shark fishing industry that is worth between 400 and 550 million USD annually (Clarke 2006a, Clarke et al. 2006b, Clarke et al. 2007). Clarke et al. (2006a) reported that an average of 38 million sharks are killed every year while Worm et al. (2013) reported that approximately 100 million and 97 million sharks were killed in 2000 and 2010, respectively. Although, varying fisheries catch data and models used to calculate these estimates of shark mortality may be subject to some error, there is general agreement that this volume of shark mortality amounts to unsustainable exploitation (Clarke et al. 2006a, Worm et al. 2013).

Chondrichthyan species are fundamentally susceptible to fishing pressure due to their conservative life history characteristics such as late maturity, low fecundity and relatively slow growth rates (Barker and Schluessel 2005, Dulvy and Forrest 2012). Bull sharks are no exception and consequently, they are listed as near threatened throughout their range by the IUCN (Simpfendorfer and Burgess 2009). Using the intrinsic vulnerability to fishing model proposed by Chueng et al. (2005) based on life history parameters and ecological characteristics, bull sharks are categorized as highly vulnerable (Chueng et al. 2005). This vulnerability is largely a consequence of the slow growth and relatively late age at which the species attains sexual maturity (Smith et al. 1998).

Globally, bull sharks are fished both recreationally and commercially throughout most of their range and targeted for their meat, liver or fins (Simpfendorfer and Burgess 2009). Although there is no evidence that bull sharks are specifically targeted in southern Africa, fisheries do exist that catch bull sharks as by-catch or as part of shark fishing operations targeting commercially valuable shark fin (Marshal and Barnett 1997, Clarke et al. 2006, McVean et al. 2006, Pierce et al. 2008). International fishing catch records show that bull sharks ranked as the seventh most frequently traded shark species in the global Hong Kong shark fin trade (Shivji 2012), suggesting that international fishing pressure on the species is relatively high.

The vulnerability of bull sharks to fishing pressure has been well demonstrated off the South African east coast where the localized fishing impact from bather protection gill nets

has caused significant population decline. Bull shark catches in bather protection gill nets declined markedly from 24.65 sharks per kilometre of net per year in 1966 to 0.87 sharks per kilometre of net per year in 1977 from where the catch remained consistently low (0.95 sharks per kilometre of net per year) up to 1990 (Cliff and Dudley 1991). Since 1990, catch rates have exhibited continued significant decline (Dudley and Simpfendorfer 2006). When compared to other shark species in the same area for which the catch rates have remained consistent (Dudley and Simpfendorfer 2006), these declines highlight the susceptibility of bull sharks to sustained fishing pressure. The recreational fishing sector in South Africa also accounts for extensive fishing pressure along the coastline, although bull sharks typically do not make up a large component of the fishery (Mann 2000, Pradervand 2007, Dicken et al. 2012). In Mozambique, the greatest fishing pressure is from subsistence fishermen, however, there is also a notable industrial and mixed artisanal and industrial sector (Afonso 2006). These fisheries are largely unregulated and reports suggest that sharks are being targeted in some areas as their valuable fins are sold directly to Chinese buyers within Mozambique (Pierce et al. 2008). The mixed subsistence and commercial shark fishery in the south of Mozambique also contributes to substantial fishing pressure on shark species, including bull sharks, and it appears that this fishery is growing and becoming more sophisticated.

Due to the general lack of data on shark populations (Dulvy et al. 2014), increased fishing pressure (Pierce et al. 2008, Worm 2009), habitat loss and alteration (Cyrus et al. 2011) and the threat of climate change (Dulvy et al. 2014), there is an urgent need to improve the management for vulnerable shark species in the southwest Indian Ocean. Based on the typically conservative life history characteristics of shark species, effective management strategies for these species should focus on maintaining reserves of reproducing adults and the protection of juvenile nursery habitats (Kinney and Simpfendorfer 2009, Dulvy and Forrest 2012). However, although high survival rates of mature adults and juveniles may mitigate population decline, the slow growth rates and long generation times of some shark species may still make them vulnerable to fishing pressure (Dulvy and Forrest 2012). Even if fished populations have existing management frameworks and regulations, it is often difficult to effectively manage populations due to species misidentification, poor catch

reports, and by-catch from non-target fisheries (Barker and Schluessel 2005). These problems are especially challenging in developing countries such as those in southern Africa, where various socio-economic factors may also influence fisheries management policies and enforcement (Barker and Schluessel 2005, Worm et al. 2013).

Increased threats to shark populations from fishing pressure also requires consideration alongside threats to marine communities such as habitat loss and climate change (Dulvy et al. 2012). The degradation of productive marine environments due to anthropogenic activities may lower the resilience of marine communities to cope with multiple stressors and the conservation management of shark populations needs to be considered in this context (Wilson et al. 2008, Ruppert et al. 2013). Effective conservation management plans should therefore consider the interplay between these threats to shark populations and their respective marine communities. For example, if forecast climate change shifts the temperature range of optimal species habitat, it needs to be taken into account when planning conservation areas (Bradshaw et al. 2008).

Ultimately, the most effective conservation management strategies for sharks should incorporate a thorough understanding of the ecology and population biology of the relevant species (Barker and Schluesell 2005). Currently, one of the major challenges for effective bull shark conservation management in the southwest Indian Ocean is the lack of data about the species ecological role, habitat use and nursery areas. While previous studies suggest that bull sharks play an important predatory role (Cliff and Dudley 1991), little is known about the comparative predatory role of this species. It has been suggested that, due to the compensatory response of teleost fish predators, the loss of shark species within a community will not impact the community as a whole (Kitchell et al. 2002). Thus, the importance of apex predatory sharks within their respective marine communities needs to be established in order to prioritize conservation measures for these groups accordingly.

It is also important to clarify the geographical distribution of bull shark populations within the southwest Indian Ocean in order to determine both the spatial extent of their ecological role and the connectivity between populations in the region. Although global studies have indicated that bull sharks can undertake substantial geographical movements (Carlson et al. 2010, Brunnschweiler 2010), it is unclear if this is the case on the east coast of southern Africa as previous research has suggested that bull shark populations are largely sedentary with limited home ranges (Cliff and Dudley 1991, Bullen and Mann 2000). Determining whether or not large-scale movements exhibited by bull sharks are indeed migrations or if their limited movements represent long-term residency is crucial when considering the design of an effective conservation management strategy for the species (Dingle and Drake 2007). It is also currently unclear where critical nursery areas for bull sharks are located due to the degradation and modification of many major southern African estuary systems (Whitfield 1998, Cyrus et al. 2011). As bull sharks exhibit reproductive phylopatry (Karl et al. 2011, Tillet et al. 2012) the localized loss of nursery habitats may deplete population level genetic diversity and, therefore, nursery habitats need to be identified and conserved. Hence, there is still a need for further understanding of bull shark populations within the southwest Indian Ocean in order to design effective conservation management measures for this vulnerable and ecologically important species.

1.3. Study site

1.3.1. Southern Mozambique

This study took place in southern Mozambique within the Ponta do Ouro Partial Marine Reserve (PPMR) (Fig 1.4). The PPMR was proclaimed on the 14th of July 2009 and encompasses 98.5km of coastline extending from the South African border in the south to the Catembe River within Maputo Bay in the north (DNAC 2011).



Figure 1.4. The study site is located in southern Mozambique within the Ponta do Ouro Partial Marine Reserve (PPMR) situated between the South African border near Ponta do Ouro in the south and Maputo Bay in the north.

This region of coast is located within a biogeographical transition area between the subtropical east coast of South Africa and the tropical coast of central Mozambique (Turpie et al. 2000) referred to as the Delagoa bioregion (Sink et al. 2004). The Agulhas Current is the dominant oceanographic feature along this coastline due to the relatively narrow continental shelf persistent along this region of coast (Schumann 1988, Ramsay 1994, Lutjeharms 2006). There is, however, a rapid widening of the shallow continental shelf approximately 200km south of Mozambique, pushing the main stream of the Agulhas Current further offshore to a distance of between 30 and 50 NM offshore of Ponta do Ouro

and Maputo (Lutjeharms et al. 2010). The region of a wider shallow shelf extends northwards until approximately Inhambane and varying current flow regimes exist in the nearshore environment similar to that of the Natal Bight (Lutjeharms et al. 2000). Investigating current flow patterns shows that the inshore shelf area of the PPMR may be highly variable and separate from the main southward flowing stream of the Agulhas Current (Lutjeharms et al. 2012). Currents and circulation patterns in this region may be largely wind driven (Saetre and Silva 1979) and highly variable both spatially and temporally (Fig 1.5).



Figure 1.5. Hybrid Coordinate Ocean Model satellite data showing the surface current speed and direction off the coast of Ponta do Ouro on the 17th of July 2012. Colours represent the speed in knots and the arrows indicate current direction. The current circulation within the nearshore shelf area off Ponta do Ouro is separate from the main stream of the Agulhas Current and is highly variable (Figure from Buoyweather.com).

Water temperatures in the area fluctuate between 22°C in winter and 29°C in summer with a semidiurnal tidal cycle with a range of between 2 and 2.4m (Kalk 1995). Water

temperature may also be influenced by upwelling events consistent with oceanographic processes such as Ekman veering (Schumann 1986) and bottom temperatures at 30m of 19°C during mid-summer have been recorded (pers. obs, unpublished data). In general, this area of the coast is subject to large south and south-westerly swells generated in the Southern Ocean during winter months (Schumann and Orren 1980) and large north and north-easterly storm swells generated in the Mozambican channel by tropical storms from February to May (Ramsay 1994, Riegl 2001). Coastal waters are generally nutrient deplete and there are limited terrestrial sources of freshwater input adjacent to the study site.

Coral reef formations within the Delagoa bioregion occur at relatively high latitude and those within the PPMR and the adjacent iSimangaliso Wetland Park in South Africa, are some of the highest latitude hard coral reefs in the world (Riegl et al. 1995). These hard coral communities are typically dominated by the genera's, *Acropora* and *Montipora*, that form a veneer on top of existing substrate (Ramsay 1994, Ramsay 1996). Reef growth primarily takes place on fossilized coastal dunes running parallel to the coast consisting of Late Pleistocene beach rock (Ramsay 1994, Ramsay 1996). Generally, hard coral coverage and colony size increases until 25m of depth and thereafter, due to light limitation, is replaced by non-photosynthetic organisms such as sponges and ascidians (Riegl et al. 1995). The general sub-tidal reef habitat is diverse with a mix of sand dominated substrate, low profile sparse reef, alcyonarian coral dominated reef or scleractinian coral dominated reef (Ramsey 1994, Pereira 2003).

The diverse habitat type of the PPMR and neighbouring iSimangaliso Wetland Park is favoured by an array of at least 354 fish species belonging to 73 families (Chater et al. 1993, Pereira 2003, Floros et al. 2012). Most fish species are representative of the subtropical and tropical Indo-Pacific region and the diversity is similar or higher than other areas within the western Indian Ocean (Floros et al. 2012). Within the southern extent of the PPMR, the most abundant fish families on shallow coral reef communities include Acanthuridae, Balistidae, Chaetodontidae, Labridae, Pempheridae and Pomacentridae (Floros et al. 2012). Deeper reefs usually have a more diverse array of pelagic and reef associated predatory fish, including the families Carangidae, Coryphaenidae

Epinephelinae, Lutjanidae, Sphyraenidae and Scombridae. The elasmobranch diversity within the PPMR and, along the coastline in general, is relatively high (Guisande et al. 2013) and includes species from the families Dasyatidae, Ginglymostomatidae, Mobulidae, Myliobatidae, Odontaspididae, Rhincodontidae, Rhinobatidae, Sphyrinidae, Stegostomatidae and Torpedinidae. Carcharhinids represent the most abundant family within the region, with at least seven commonly seen species.

1.3.2. The Pinnacle reef

The focal area for sample collection was a reef complex called the Pinnacle located approximately 3.7km offshore within the PPMR (S26° 44.934' E32° 56.083'). The Pinnacle reef is a typical fossilized coastal dune reef (Ramsey 1994) running parallel to the coast and consists of a ridge approximately 1.2km long with a series of shallower pinnacles along its spine. The reef sandstone substrate has scattered alcyonarian coral growth with limited scleractinian coral growth and is interspersed with sand patches. The southernmost extent of the reef, consisting of a shallow mound (<35m) adjacent to a deeper offshore area (>40m), was where the majority of shark observations took place. Strong currents (>1 knot) persisted within the area of the Pinnacle reef, flowing predominately in a northerly or southerly direction.

To understand the extent and structure of the Pinnacle reef, a bathymetric survey was conducted. Firstly, a grid course over the known area of the reef was plotted using a navigational chart plotter (Garmin Homeport Version 2.2.1). A boat equipped with a GPS / Sonar combination device (Garmin GPSMAP 441) was then used to navigate the plotted course. The GPS antennae was positioned directly above the sonar transducer, and the GPS unit was set to log depth and position every meter and record this data onto a removal memory device. This resulted in 3175 data points that were converted from Garmin GPX format into CSV format. This data was then imported into the visualisation software Surfer (Version 10, Golden Software) and plotted as a grid map with graphic overlay to produce Figure 1.6. This bathymetric map allowed us to effectively design and implement specific



aspects of this study (see Chapter 4), as well as provide a detailed visual representation of the study site (Fig 1.6).

Figure 1.6. The bathymetry of the Pinnacle reef with a colour key representing depth and grid numbers representing latitude (x-axis) and longitude (y-axis).

At the onset of summer (November), large numbers of Carangid species start to congregate at the Pinnacle reef, including shoals of giant trevally (*Caranx ignoblis*) and yellow spotted kingfish (*Carangoides fulvoguttatus*) (Fig 1.7). The arrival of these fish usually coincides with the first appearance of multiple bull sharks at the site (pers. obs.). By the end of December the large aggregations of giant trevally have usually dispersed, but multiple bull sharks remain at the site throughout summer. As with many other productive reefs in the area, other pelagic fish and shark species are commonly seen, but the Pinnacle is the only reef in the area where schools of giant trevally's and bull sharks have been observed together. Other shark species commonly encountered at the Pinnacle reef include the tiger shark (*Galeocerdo cuvier*), the blacktip shark (*Carcharhinus limbatus*), the grey reef shark (*Carcharhinus amblyrhyncos*), the silvertip shark (*Carcharhinus albimarginatus*) and the scalloped hammerhead shark (*Sphyrna lewini*).



Figure 1.7. A bull shark associated with an aggregation of giant trevally (*Caranx ignobilis*) at the study site on the Pinnacle reef (Photograph by Ryan Daly).

The Pinnacle reef is situated within the PPMR boundaries and as such is subject to the reserve fishing regulations that state only up to 10 pelagic fish species per person per day may be caught (DNAC 2011). There are no recreational regulations pertaining to the capture of sharks (DNAC 2011), but local tourism operators generally do not support the capture or killing of sharks. Fishing pressure at the Pinnacle reef is mainly from recreational tourists that primarily fish during holiday periods in South Africa and Mozambique (pers. obs, unpublished data). The PPMR management has begun to collect fisheries catch data from the recreational sector, but the amount and accuracy of this information is still limited. In the south of the PPMR artisanal fishing by locals is limited to shore based effort that targets only teleost fish. In the north, however, there are small
fishing vessels at Machangulo that target the calmer waters on the eastern side of Maputo bay catching mainly *Sillaginidae*, *Mullidae* and some *Sparidea* species (de Boer 2000). There are also small commercial fishing vessels from Maputo that fish in the area occasionally and there have been sightings and reports of larger long line fishing vessels that do pass through the area (Pierce et al. 2008), although these vessels do not seem to exert any sustained fishing pressure. However, due to the remoteness of the area there may be a substantial amount of illegal commercial fishing that remains unreported.

1.4. Thesis overview

Chapter one provides a general introduction to bull sharks and includes a summary of the current literature on bull sharks covering reproductive biology, age and growth, diet, distribution and habitat. The literature review is followed by a discussion on the ecological role of predatory shark species in the marine environment, the significance of the decline in predatory shark numbers within marine communities and the current conservation status of Chondrichthyans in general, with specific focus on the bull shark. The chapter ends with a description of the study site, including the geographical location and a bathymetric map of the Pinnacle reef where all fieldwork for this study was conducted.

Chapter two provides a description of the remote biopsy probe developed for collecting muscle tissue from free-swimming bull sharks for stable isotope analysis. The chapter describes the design of the biopsy probe and presents results of the field experiments conducted to obtain samples from bull sharks for this study. The usefulness of the biopsy probe, together with laser photogrammetry and underwater video as a non-lethal and minimally invasive alternative sampling method is discussed, and the potential limitations and applications of these methods are summarized.

Chapter three examines the comparative feeding ecology of sub-adult and adult bull sharks as well as top predatory teleost fish. The chapter presents the results of the stable carbon and nitrogen isotope analysis, which showed that sub-adult and adult bull sharks had a significantly larger niche width than co-occurring top predatory teleost assemblages and, therefore, played an important predatory role within their respective marine communities. Furthermore, these results showed that adult bull sharks in particular had a wide range of enriched δ^{13} C values relative to the local marine community, suggesting that they forage over a broad spatial scale on the east coast of southern Africa. This is discussed in the context of what is known about the foraging range of bull sharks in southern Africa and it is proposed that bull sharks play an important predatory role over a substantial geographical range in this region. Lastly, the influential predatory role of bull sharks is discussed in the context of the future need to improve the conservation management plans for the species.

Chapter four presents the findings of a passive acoustic telemetry study on the movement patterns and migration dynamics of bull sharks within the study region. Results from this aspect of the study supported evidence from Chapter three which suggested that bull sharks forage over broad spatial scales on the east coast of southern Africa. The majority of tagged bull sharks were found to undertake frequent and spatially significant geographical migrations along the coast of Mozambique. These results are the first to show that bull sharks undertake return migrations and suggest that bull sharks in southern Africa are not as sedentary as once thought. The need for a further understanding of bull shark migration dynamics are discussed and it is proposed that future conservation strategies need to consider the broad spatial scales of bull shark habitat use.

Finally, Chapter five provides a synthesis of the study and highlights future research and conservation priorities.

1.5. Ethics Statement

All research in this investigation was conducted under the permit number 0002/2010 issued by The Mozambican Directorate of National Conservation Areas. The Animal Ethics Committee of the Department of Zoology and Entomology at Rhodes University approved the research protocol used in this study (ethical clearance number ZOOL-14-2012).

Chapter 2: Evaluation of an underwater biopsy probe for collecting muscle tissue samples from free-swimming bull sharks.

*This chapter is a modified version of the published manuscript: Daly R and Smale MJ (2013) Evaluation of an underwater biopsy probe for collecting tissue samples from bull sharks, *Carcharhinus leucas*. African Journal of Marine Science 35: 129-132.

2.1. Introduction

Recent literature suggests the need for non-lethal and fishery-independent sampling strategies for large apex predatory shark species due to population declines and conservation measures (Hammerschlag and Sulikowski 2011). Although there are cases where certain data may only be obtained using lethal sampling (Heupel and Simpfendorfer 2010), with the growing number of large shark species at risk (Myers et al. 2007, Ferretti et al. 2008, Dulvy et al. 2008), there is a need to investigate alternative sampling methods.

With the successful employment of methods used to investigate free-swimming elasmobranchs (Deakos 2010, Rohner et al. 2011, Marshall and Pierce 2012), there is a need for complementary *in situ* sampling methods. Important information on the biology, ecology, habitat use and conservation status of sharks may be obtained using muscle tissue for stable isotope investigations (Hussey et al. 2011, 2012, Carlisle et al. 2012, Shiffman et al. 2012, Trueman et al. 2012) and genetic analysis (Sandoval-Castillo et al. 2004, Schmidt et al. 2009, Clua et al. 2010, Karl et al. 2011, Tillet et al. 2012). In some circumstances, these would best be collected with minimal stress and impact on the species under study.

To date, the development and application of least-invasive methodologies to obtain tissue samples from free-swimming marine species has largely focused on cetaceans. Winn et al. (1973) provided the first published description of the use of a biopsy system for large cetaceans and subsequently several other studies have improved and applied the biopsy

design for a range of cetacean species (Jahoda et al. 1996, Weller et al. 1997, Reeb and Best 2006). These studies describe how biopsy sampling can be used to effectively obtain tissue samples from free-swimming marine mammals while causing minimal disturbance.

Applying a remote biopsy probe to sample shark species has, thus far, been limited to whale sharks (*Rhinocodon typus*) (Castro et al. 2007, Ramirez-Macias et al. 2007, Schmidt et al. 2009), white sharks (*Carchardon carcharias*) (Gubili et al. 2009, Carlisle et al. 2012) and relatively small carcharhinid shark species (Robbins 2006, Clua et al. 2010). Most of these biopsy probes have relied on modified cetacean biopsy designs, which primarily employ a barbed mechanism designed for relatively soft dermal tissue. This study evaluates a biopsy probe designed for penetrating the tough dermal denticle layer of the bull shark that, instead, relies on a suction mechanism to retain tissue samples.

2.2. Material and Methods

2.2.1. Manufacturing and design

Biopsy probes were manufactured by Rob Allen Dive Factory (www.roballen.co.za) in Durban, South Africa, and can be ordered directly from the factory (divefac@iafrica. co.za). The biopsy tip is made from high-grade stainless steel and fitted to a 1.3 m long spring steel spear, which was shot from a 1.1 m long Rob Allen speargun powered by a 20 mm diameter elastic rubber, set to 49 kg of force when loaded. The biopsy probe design relies on a sharpened front edge to penetrate the skin. A series of four ventilation holes with a diameter of 3 mm are drilled at the base of the probe and covered with a 6 mm elastic rubber band. This allows the probe to penetrate the skin, forcing the water above the skin out of the ventilation holes and thereby creating a suction effect when the probe exits and the elastic band seals the ventilation the holes. The probe has a diameter of 6 mm and extends 26 mm from the outer front edge to the outer base of the stopper ring (Fig 2.1).



Figure 2.1. Lateral view of the biopsy probe to scale showing the penetrating barrel (A), the stopper ring (B), the elastic band around the ventilation holes (C), and the ventilation hole position (D).

2.2.2. Controlled biopsy tests

Before conducting *in situ* tests, the efficacy of the new biopsy design was compared with a biopsy probe of similar proportions relying on three rearward facing barbs. This test took place in a confined body of water using the carcass of a blacktip shark (*Carcharhinus limbatus*) measuring 1.6 m total length (TL). Direct comparison of each biopsy design took place over 40 trials from a distance of 1 and 2m, targeting the dorsal musculature of the

shark between the first and second dorsal fins.

2.2.3. In situ biopsy tests and sampling

In situ sampling, using the new biopsy design, targeted large (1.7–2.2 m TL) bull sharks in the coastal waters of southern Mozambique between January 2010 and January 2011. The dorsal musculature of the shark between the origin of the first dorsal and the origin of the second dorsal fin was targeted and the probe was fired from a perpendicular angle to the shark at an approximate range of between 1 m and 2 m. When sampling successive sharks, the time between sampling events was recorded. When possible, biopsied sharks were photographed, sexed and accurately measured using paired-laser photogrammetry (Deakos 2010, Rohner et al. 2011) (Fig 2.2). Parallel lasers were fixed on an aluminium frame so that the beams were 300 mm apart and calibrated *in situ*. The lasers were then attached to an underwater camera with a 35 mm lens. Photographs were edited in Adobe Lightroom 3 and the PixelStick software package was used to measure the distance between on screen pixels. The precaudal length (PCL, mm) of the shark was calculated using the following equation:

$$PCL = 300 \left(\frac{Y}{X}\right)$$

where Y is number of pixels between the tip of the snout and the precaudal notch, X is the number of pixels between the laser points and 300 is the distance between the laser points in millimetres (Fig 2.2). The data were converted to cm and TL was calculated using the equation in Wintner et al. (2002) for males and females respectively.

Tissue samples were removed from the front end of the biopsy tip using a pair of sharp nose forceps and placed in an Eppendorf test tube. Biopsy probes were cleaned and visually inspected between sampling events to prevent cross contamination. Samples were stored and frozen at $-20 \square C$ for subsequent analysis in the laboratory at Rhodes University, South Africa. In the laboratory, the tissue samples were weighed (wet weight in grammes) using a Mettler Toledo PB3002 electronic balance.



Figure 2.2. A free-swimming bull shark (*Carcharhinus leucas*) being photographed using laser photogrammetry after being biopsied. X represents the distance between laser points, Y shows the precaudal length of the shark being measured and Z shows the biopsy scar (Photograph by Ryan Daly).

2.3. Results

2.3.1. Controlled biopsy tests

The preliminary control test of the barbed biopsy probe design on the blacktip shark carcass had a 0% tissue retention success rate after 20 attempts from distances of 1 m and 2 m. In contrast, the new biopsy design had a 100% tissue retention rate after 20 attempts from both 1 m and 2 m distance with a mean tissue sample weight from the combined attempts of 211 mg (SD = 118).

2.3.2. In situ biopsy tests and sampling

The *in situ* tests using the biopsy probe design presented in this study retained tissue samples from bull sharks 87% of the time. Biopsied sharks exhibited a short aversion response directly after the biopsy event, but in most cases shortly resumed normal behaviour and remained within the area. Measured bull sharks had a mean length of 190 cm TL with a range between 170 cm and 220 cm TL. *In situ* observations identified the sex of 63% of biopsied sharks and there were no differences in the tissue retention rates or mean sample sizes between male ($315 \text{ mg } \pm 79$, mean \pm SD) and female ($313 \text{ mg } \pm 102$, mean \pm SD) sharks. When sampling successive specimens, the mean turnaround time between a sampling event and the next opportunity for sampling was four minutes. The mean wet weight of the biopsy tissue samples from bull sharks was 310 mg (SD = 78) and ranged between 200 mg and 430 mg. All samples contained dermal denticles (~5%), dermal tissue (~40%) and muscle tissue (~55%).

The biopsy scar on the shark was easily visible for at least two weeks and helped to identify biopsied sharks and prevent double sampling within this period. Five known individual bull sharks were monitored over six months and, in all cases, the biopsy wound healed completely within six weeks with no visible persistent scar.

2.4. Discussion and conclusion

2.4.1. Biopsy efficacy

The biopsy probe tested during this investigation effectively collected dermal and muscle tissue from large free-swimming bull sharks. The high retention rate (87%) of the biopsy probe along with a short time interval (less than five minutes) between sampling events allowed for efficient fieldwork. The mean size of the tissue samples obtained (310 mg) was substantially more than that of Robbins (2006) (~55 mg) and the tissue sample size range (200–430 mg) was similar to studies using tissue samples from captured sharks for stable isotope (Abrantes and Barnett 2011, Drymon et al. 2012, Speed et al. 2012) and genetic

analysis (Sandoval-Castillo et al. 2004, Clua et al. 2010, Tillet et al. 2012).

Although no differences in tissue retention rates or mean sample sizes between individuals of different sex or size classes were recorded in this study, further investigation is needed to determine if the thicker dermal layer of larger sharks may affect tissue retention. Large (>250 cm TL) sexually mature female sharks may have an especially thick dermal layer, associated with mating (Pratt 1979), which may reduce the retention rate of the biopsy probe. The most important variable determining the tissue retention rate in this study was most likely variability associated with the open ocean and study subject. *In situ* observations suggest that the 13% tissue retention failure was due to the poor aim of the biopsy probe, resulting from the unexpected swimming escape movement of the shark.

2.4.2. Biopsy applications and limitations

The application of this biopsy probe may be of interest when considering sampling sharks whose population is at risk or when resources or regulations do not permit capture. Other sampling methods such as photo ID (Marshall and Pierce 2012) and laser photogrammetry (Deakos 2010) that do not involve the capture or constraint of sharks can be used in combination with biopsy sampling in order to provide researchers with alternatives to invasive sampling methods. Such multifaceted and minimally invasive sampling approaches may become increasingly important when sampling large and rare sharks in order to mitigate the risk of killing sharks from capture-related stress (Morgan and Burgess 2007, Skomal 2007, Brooks et al. 2012).

Obtaining biopsy samples from sharks *in situ*, however, does have limitations. Research may be confined to shallow water (<40 m) where the water is clear and the sharks are approachable. There may also be circumstances where the shark may present a threat to the investigator and, in these cases, knowledge and prior experience diving with the species in the specific environment may mitigate these risks.

In summary, the biopsy probe tested here provides researchers with a novel method for obtaining dermal and muscle tissue samples from a large carcharhinid shark species under suitable marine conditions of clear, shallow waters. If the environmental variables permit, the use of this biopsy probe may be a selective and efficient way to obtain tissue samples with minimal disturbance to the subject under investigation. This may allow researchers to conduct stable isotope or genetic analysis on large sharks that cannot be captured due to logistical or conservation constraints.

Chapter 3: Comparative feeding ecology of bull sharks inferred from stable isotope analysis.

*This chapter is a modified version of the published manuscript: Daly R, Froneman PW and Smale MJ (2013) Comparative feeding ecology of bull sharks (*Carcharhinus leucas*) in the coastal waters of the southwest Indian Ocean inferred from stable isotope analysis. PLoS ONE 8: e78229.

3.1. Introduction

Large shark species are often top predators within their respective marine ecosystems and consequently, play an important role shaping community dynamics (Chapter 1). Yet, habitat loss and over-fishing increasingly threaten shark populations (Dulvy et al. 2008, Worm et al. 2013), and the ecological consequences of their removal from marine communities have been substantial (Myers et al. 2007, Ferretti et al. 2010). Sharks may shape their communities through direct predation or associated risk effects (Heithaus et al. 2009), but the understanding behind broad spatial and temporal scales over which these processes operate is poor. Often it is difficult to conduct long-term studies on large sharks throughout their range because they are rare, difficult to observe, highly mobile and often widespread. The application of stable isotope analysis (SIA) applied to investigate the trophic ecology of large shark species has, however, provided new insights into the processes through which they shape their communities (Hussey et al. 2012).

Traditionally, investigating the trophic ecology of large sharks has relied on stomach content analysis from captured dead sharks (Hyslop 1980, Cortes 1999). The limitations of this method arise from the snapshot nature of recently consumed dietary items, the logistical difficulties associated with sampling, and conservation related concerns of sampling threatened populations (Hammerschlag and Sulikowski 2011). Although SIA may not provide the taxonomic resolution of stomach content analyses, it does provide information on the assimilated diet of the consumer over time (Estrada et al. 2003). Refined by recent progress in the application of stable carbon and nitrogen isotope analysis to study elasmobranchs (Hussey et al. 2010, Logan and Lutcavage 2010, Kim and Koch 2012, Kim

et al. 2012), the associated methodology is a robust and complementary tool for investigating the trophic ecology of elasmobranchs. The application of SIA provides insight into various population, species and individual level processes, including the trophic position (Estrada et al. 2003, Hussey et al. 2011), niche width and overlap (Vaudo and Heithaus 2011), ontogenetic dietary shifts (Estrada et al. 2006, Kim et al. 2012), species foraging strategies (MacNeil et al. 2005) individual foraging strategies (Kim et al. 2012, Matich et al. 2011), and habitat use (Abrantes and Barnett 2011, Carlisle et al. 2012, Speed et al. 2012) of large sharks.

Juvenile bull sharks occur in estuarine nursery areas that are typically accessed more easily than the coastal marine environment and, as such, have been the focus of many previous studies (Bass et al. 1973, Simpfendorfer et al. 2005, Ortega et al. 2009, Curtis et al. 2011, Heupel and Simpfendorfer 2011). However, less is known about the habitat use and trophic ecology of sub-adult and adult populations in the southwest Indian Ocean, specifically in Mozambique, where studying these populations remains logistically challenging. The diet of bull sharks typically includes a wide variety of prey items (Cliff and Dudley 1991), but the spatial and temporal scales over which individuals forage is unclear. Bull sharks are known to exhibit periods of extended site fidelity (Cliff and Dudley 1991, Brunnschweiler and Barnett 2013) that may be linked to resource availability, however, individual home ranges may vary over broad spatial and temporal scales (Brunnschweiler et al. 2010, Carlson et al. 2010). Bull sharks are capable of ranging over wide geographical areas (Brunnschweiler et al. 2010, Carlson et al. 2010) and may undertake seasonal migrations (Compagno 1984, Bass et al. 1973, Hammerschlag et al. 2012), yet little is known about how bull shark foraging behaviour links with these processes. There is also uncertainty related to how foraging behaviour may vary within a population or between individuals. Recent studies suggest that juvenile bull sharks exhibit some level of individual dietary specialization (Matich et al. 2011), however, there is little knowledge regarding individual dietary specialization of larger sharks and how factors such as ontogeny, gender or habitat use affect the trophic dynamics of individuals or populations.

This study employed SIA to investigate the trophic ecology of bull sharks within a coastal marine community. The aims of this investigation were (1) to investigate the isotopic composition of the sampled marine community at the study site, (2) to investigate potential bull shark dietary sources at the study site, (3) to determine and compare the niche width of sub-adult and adult bull sharks and co-occurring predatory teleost fish assemblages, and (4) to calculate the δ^{15} N based trophic position of the sampled bull shark population. As bull sharks are especially vulnerable to increasing pressure from overfishing and habitat loss globally (Dulvy et al. 2008) due to their affinity to coastal habitats and low intrinsic rebound potential (Smith et al. 1998), information on their predatory role within the sampled marine community may help to establish a more effective conservation strategy for the species.

3.2. Materials and methods

3.2.1. Sampling

Visual assessments of the fish community took place over the course of 111 dives at the study site. Based on these observations, samples of predatory fish species were obtained by selectively spear fishing the most numerically abundant species. Other fish species sampled were primarily acquired from artisanal fishermen at the study site and opportunistic biopsy samples were collected from blacktip sharks (*Carcharhinus limbatus*) at the study site (Table 3.1). Sampling effort was focused on austral summer months (November to April) between January 2009 and March 2012. A total of 59 samples from 16 teleost fish species and two small shark species were collected and white muscle tissue was used for stable isotope analysis. Fish species were grouped into categories based on primary habitat type, trophic position and primary prey items known from the literature (Table 3.1) (Randall and Bishop 1967, Hobson 1974, Harmelin-Vivien 1981, Sudekum et al. 1991, Bachok et al. 2004, Heemstra and Heemstra 2004, Froese and Pauly 2009).

Table 3.1. Sampled teleost fish and shark species assigned to four groups based on their primary habitat, primary dietary items and trophic position (TP) based on stomach content established from www.fishbase.org.

Species	Common name (n)	TP Primary		Primary	Assemblage
		(SE)	Habitat	Diet	
Group 1					
Acanthocybium solandri	Wahoo (8)	4.4 (0.8)	Offshore /	Fish /	Pelagic Top
Coryphaena hippurus	Dorado (4)	4.4 (0.8) Pelagic		Invertebrates	Predator
Istiophorus platypterus	Sailfish (1)	4.5 (0.8)			
Euthynnus affinis	Kawakawa (1)	4.5 (0.8)			
Group 2					
Scomberomorus commerson	King mackerel (10)	4.5 (0.8)	Coastal /	Fish /	Coastal
Carangoides fulvoguttatus	Yellowspotted trevally (6)	4.4 (0.8)	Pelagic	Invertebrates	Top Predator
Seriola dumerili	Greater amberjack (3)	4.5 (0.8)			
Caranx sexfasciatus	Bigeye trevally (4)	4.5 (0.8)			
Sphyraena jello	Pickhandle barracuda (4)	4.5 (0.8)			
Group 3					
Lutjanus gibbus	Humpback snapper (3)	3.6 (0.6)	Coastal /	Invertebrates	Coastal
Plectorhinchus playfairi	Rubber lips (4)	3.3 (0.52)	Benthic		Consumer
Umbrina robinsoni	Slender baardman (1)	3.4 (0.42)			
Balistapus undulates	Orange-striped trigger fish (1)	3.4 (0.42)			
Rhabdosargus sarba	Natal stumpnose (1)	3.4 (0.6)			
Chrysoblephus Puniceus	Slinger (1)	3.5 (0.46)			
Oplegnathus robinsoni	Natal knifejaw (1)	3.2 (0.42)			
Group 4					
Carcharhinus limbatus	Blacktip shark (4)	4.2 (0.7)	Coastal /	Fish /	Elasmobranch
Rhizoprionodon acutus	Milk shark (2)	4.3 (0.8)	Pelagic	Generalist	

Shark muscle tissue samples for stable isotope analysis were obtained from free-swimming sharks using a biopsy probe attached to the tip of an underwater spear gun (Chapter 2). Samples from 18 bull sharks were obtained and the respective gender was identified *in situ* when possible and confirmed from video footage. The presence of claspers confirmed male sharks, but in some cases, the gender of sub-adult sharks was not clear due to the relatively small size of the claspers and, in total, only 11 individuals were positively sexed. All sampled sharks were measured *in situ* using laser photogrammetry (Chapter 2) and ranged in size from 1.6 - 2.5m TL ($2m \pm 0.3$, mean \pm SD). Bull sharks were then grouped into sub-adults (1.6m-2.2m) and adults (2.2m-2.5 TL) based on the approximate length at sexual maturity (c. 2.2m TL) according to the literature (Bass et al. 1973, Branstetter and Stiles 1987, Wintner et al. 2002, Cruz-Martinez et al. 2005).

3.2.2. Sample preparation

All tissue samples used for stable isotope analysis were frozen at -20°C before transport to Rhodes University, South Africa. In the laboratory samples were oven dried at 60°C for 48 hours or until constant weight was reached and were then homogenized into a fine powder using a Crescent Wig-L-Bug. Samples were then weighed to approximately 1mg using a Sartorius micro-balance with a precision of 0.01mg and placed in 6x4mm tin capsules before being sent to Iso-Environmental at Rhodes University in South Africa. Samples were analysed using a continuous flow Europa Scientific 20-20 IRMS linked to an ANCA SL Prep Unit. Each batch of 96 combustions contained 29 internal standards (beet sugar and ammonium sulphate) and 5 certified protein standards (Casein calibrated against IAEA-CH-6 and IAEA-N-1). Stable isotope ratios were expressed in the delta notation where δ^{13} C or δ^{15} N = [(R_{sample}/R_{standard})-1] × 1000 and R is 13 C/ 12 C or 15 N/ 14 N and nitrogen, δ^{15} N.

As the presence of lipids in the muscle tissue samples may lead to depleted δ^{13} C values (Hussey et al. 2012, Post et al, 2007), samples were checked for skewed C:N ratios. All samples had low C:N ratios (3.28 ± 0.31 , mean \pm SD) which were not correlated with δ^{13} C (R²=0.26) suggesting that lipid content in the samples was negligible (Post et al. 2007). Bull shark samples specifically had a low C:N ratio (3.1 ± 0.18 , mean \pm SD) confirming that lipid content was low. The presence of urea in elasmobranch tissue may also lead to depleted δ^{15} N values (Hussey et al. 2012, Kim and Koch 2011), but for the purposes of this investigation it was not accounted for due to the variable and possibly negligible effects on elasmobranch muscle tissues (Logan and Lutcavage 2010).

3.2.3. Data analysis

Differences in mean and variance of the carbon and nitrogen isotope values of bull sharks between males and females and adults and sub-adults were investigated. The mean difference and variance in carbon and nitrogen isotope values between bull sharks and fish groups 1-3 were also calculated. In all cases the data were tested for normality using a Shapiro-Wilk test and square root transformed where applicable. To test for differences between groups a t-test for independent samples (for normally distributed data) or a Wilcoxon Signed Rank test (for non-normally distributed data) was used. To investigate variance between groups a Bartletts test for homogeneity of variances was performed in the statistical package R (CRAN 2009). A linear regression analysis was also conducted to investigate the relationship between shark size (TL) and δ^{13} C and δ^{15} N values.

3.2.4. Dietary analysis

The relative contribution of different fish groups to the diet of bull sharks was estimated using the isotope mixing model SIAR (stable isotope analysis in R) (Parnell et al. 2010) in the R statistics platform (CRAN 2009). The model was run using sub-adult and adult bull sharks as the consumers and teleost fish groups 1-3 and shark group 4 were used as sources. Although fish groups 1 and 2 were grouped separately *a priori* due to known differences between habitat preference and primary prey items, they exhibited no significant differences between δ^{13} C and δ^{15} N values. In order not to confound the mixing model, groups 1 and 2 were combined for this analysis. Trophic fractionation values for bull sharks used to run the model were 2.29‰, ± 0.22 (mean, ± SD) for Δ 15N and 0.90‰, ± 0.33 (mean, ± SD) for Δ 13C (Hussey et al. 2010). Concentration factors were not incorporated into the model because the variation between the source values of carbon and nitrogen isotopes was negligible (Phillips and Koch 2002, Caut et al. 2009). The results of the mixing model showing the calculated bull shark dietary proportions were represented as box plots indicating the 25, 75 and 95% confidence intervals.

3.2.5. Niche width analysis

Niche widths and overlap for bull sharks and fish groups 1 and 2 were calculated using SIBER (Stable Isotope Bayesian Ellipses in R) metrics (Jackson et al. 2011) in the R statistics platform (CRAN 2009). Small sample size corrected Bayesian ellipses were employed to account for potential bias between sample sizes when performing comparative analysis between groups. The area of the small sample size corrected ellipses was used to

represent niche width. To compare differences in niche width between groups, the proportion of Bayesian ellipses (initially calculated using the model in the SIBER package) that were larger or smaller relative to the compared group were calculated and represented as a probability value between 1 and 100.

3.2.6. Trophic position

To determine the trophic position of bull sharks the following equation was used: $TP = \lambda + (\delta^{15}N_{consumer} - \delta^{15}N_{base}) / \Delta_n$, where λ is the trophic position of the consumer used as a baseline, $\delta^{15}N_{base}$ is the mean $\delta^{15}N$ of this baseline, $\delta^{15}N_{consumer}$ is the $\delta^{15}N$ value of bull sharks and Δ_n was the fractionation value of the consumer (Post 2002). The value chosen for the base consumer was the mean nitrogen isotope value of fishes from group 3 (11.01‰) as this group accounted for a high proportion of potential dietary items (as calculated by the mixing model) with a mean trophic position of 3.4 (Table 3.1). Trophic positions for each fish species were obtained from www.fishbase.org (Froese and Pauly 2009) and were based on analyses performed on stomach contents of the respective fishes. The fractionation value used for muscle tissue of $\delta^{15}N$ for bull sharks was 2.29 (Hussey et al. 2010).

3.3. Results

3.3.1. Community isotopic composition

Figure 3.1 shows the results from the stable isotope analysis plotted in isotopic niche space representing the mean (±SD) δ^{13} C and δ^{15} N values of groups 1-4 and individual bull shark samples representing sub-adult and adult sharks. Fish groups 1 and 2 exhibited similar mean δ^{15} N values and group 2 had slightly more enriched mean δ^{13} C values with no significant (p>0.05) difference in variance between the δ^{15} N and δ^{13} C values of these groups (Table 3.2). Fish group 3 had relatively depleted δ^{15} N values, but a significantly greater variance (p<0.05) of more enriched δ^{13} C values relative to groups 1 and 2 (Table 3.2). Elasmobranch group 4 exhibited relatively enriched δ^{13} C and δ^{15} N values relative to groups 1-3 with δ^{13} C values within the same range as those exhibited by bull sharks with a more enriched δ^{15} N mean value (Table 3.2).



Figure 3.1. A dual isotope plot representing the sampled fish community and individual bull sharks. Fish groups represented by the mean δ^{13} C and δ^{15} N values (±SD). Adult individual bull sharks are represented by squares and sub-adults are represented by triangles.

3.3.2. Bull shark stable isotope analysis

No significant differences in δ^{13} C and δ^{15} N between male and female bull sharks were apparent (p > 0.05). Additionally, there was no significant relationship between δ^{13} C (R² = 0.2, p>0.05) and δ^{15} N values (R² = 0.01, p>0.05) and shark size. However, adult sharks did exhibit significantly greater variance (p<0.05) of more enriched δ^{13} C values (range = 4.8‰) compared with sub-adult values (range = 2.6‰). By contrast, the δ^{15} N values did not exhibit significant variance (p>0.05), but were narrower and more enriched in adult (range = 1.3‰) compared to sub-adult sharks (range = 2.5‰) (Table 3.2, Fig 3.2).

Table 3.2. A summary of the mean δ^{15} N and δ^{13} C values for all groups and calculated niche widths of adult and sub-adult bull sharks and fish groups 1 and 2.

Group	Mean δ¹⁵N (‰)	Mean δ ¹³ C (‰)	Niche
	(Range)	(Range)	Width
All bull sharks	13.5 (2.9)	-14.6 (5.11)	3.1
Adult bull sharks	13.8 (1.3)	-13.9 (4.8)	3.1
Sub-adult bull sharks	13.3 (2.5)	-15.0 (2.6)	2.1
Group 1	12.5 (1.1)	-17.2 (2.2)	1.1
Group 2	12.6 (4.3)	-17.0 (2.1)	0.9
Group 3	11.0 (2.1)	-16.1 (4.4)	-
Group 4	14.65 (0.8)	-14.81 (1.0)	-

3.3.3. Mixing model

The mixing model suggested that dietary items from group 3 make up the largest proportion of both sub-adult (84.2%) and adult (73.8%) bull shark diet. Sub-adult bull sharks had smaller contributions from combined groups 1 and 2 (10.1%) and group 4 (0.05%) relative to adult bull sharks that had greater contributions from combined groups 1 and 2 (16.0%) and group 4 (10.1%). Figure 3.2 indicates the credibility intervals at 25, 75 and 95% associated with these mean values.



Figure 3.2. Boxplots from the SIAR mixing model showing the relative dietary contributions of potential prey sources (Groups 1-4) to the diet of sub-adult and adult bull sharks. Groups 1 and 2 were combined for the analysis due to similar stable isotopic signatures between these groups. The dietary proportions indicate the credibility intervals at 25, 75 and 95%.

3.3.4. Niche width

Bayesian ellipse areas represent the niche widths of bull sharks and fish groups 1 and 2 in isotopic niche space (Fig 3.3). The niche width of bull sharks was significantly larger than

both fish in group 1 (0.98 probability) and group 2 (0.99 probability). The variance of δ^{13} C values exhibited by bull sharks was, in both cases, significantly greater than groups 1 (p<0.05) and 2 (p<0.05), accounting for the majority of the difference between niche widths. In contrast, the variance of δ^{15} N values exhibited by bull sharks and fish groups 1 (p>0.05) and 2 (p>0.05) were not significantly different. There were no significant differences in niche width between group 1 and 2 (0.67 probability) and group 1 exhibited a 61% overlap with group 2 in niche space (Fig 3.3).



Figure 3.3. A dual isotope plot representing the niche widths of bull sharks (black line), fish group 1 (red line) and fish group 2 (green line). Calculated niche width is represented by the small sample size corrected ellipses (solid lines) and displayed in a δ^{13} C- δ^{15} N niche space.

The niche width of adult bull sharks (3.1) was greater than sub-adults (2.1) (0.85 probability) and exhibited a degree of niche overlap (adult niche width overlapped sub-adult niche width by 60%) (Fig 3.4). Neither the δ^{13} C nor the δ^{15} N values were significantly different (p>0.05 in both cases) between sub-adult and adult sharks. The variance of δ^{13} C

values was significantly different (p<0.05), but the variance of $\delta^{15}N$ values was not significantly different (p>0.05) between sub-adult and adult sharks.



Figure 3.4. A dual isotope plot representing the niche widths of adult (black line) and subadult (red line) bull sharks. Calculated niche width is represented by the small sample size corrected ellipses (solid lines) and displayed in a δ^{13} C- δ^{15} N niche space.

3.3.5. Trophic position

The mean calculated TP_{SIA} of bull sharks was 4.5 (± 0.3). Adult sharks (4.6 ± 0.2 , mean \pm SD) exhibiting a higher TP_{SIA} compared with sub-adult bull sharks (4.4 ± 0.3 , mean \pm SD).

3.4. Discussion and conclusion

3.4.1. Community isotopic niche space

The range of δ^{13} C values exhibited by all fish groups in the sampled community were within the range of baseline samples obtained from the same area by a previous investigation (Hill et al. 2006). The broad range of δ^{13} C values exhibited by group 3 suggests that the various species within the group had a relatively varied diet as suggested by the literature (Randall and Bishop 1967, Hobson 1974, Harmelin-Vivien 1981, Sudekum et al. 1991, Bachok et al. 2004, Heemstra and Heemstra 2004, Froese and Pauly 2009). In contrast, fish groups 1 and 2 had a more specialized diet with a similarly depleted and relatively narrow range of δ^{13} C values, suggesting that species from groups 1 and 2 are foraging primarily on a δ^{13} C depleted prey source, most likely consisting of small planktivorous fish species that are typically δ^{13} C depleted relative to inshore sources (Hussey et al. 2011, Hill et al. 2006). In situ observations supported this theory and suggested that there was a common association between fish species from group 1 and 2 and an abundance of small planktivorous fish species mainly from the family Caesionidae. The δ^{13} C and δ^{15} N values exhibited by elasmobranch group 4 were more enriched than fish groups 1 and 2 with a range of δ^{13} C values more similar to those of the sampled bull shark population. Group 4, however, did have a relatively more enriched $\delta^{15}N$ value range compared to bull sharks and this suggested a dietary contribution from prey with enriched δ^{15} N signatures. Although bull sharks and species from group 4 may have had overlapping diets to some extent, it is likely that group 4 had a relatively higher contribution of a more enriched pelagic dietary source. This is supported by the literature (Bass et al. 1973) and in situ observations that suggest that to some extent the blacktip shark (Carcharhinus *limbatus*) in group 4 may prey on similar dietary items as bull sharks, but they are typically more pelagic than bull sharks (Bass et al. 1973).

3.4.2. Bull shark niche space

Bull sharks with isotopic values that diverged most from the values exhibited by the fish community at the study site appeared to have more enriched δ^{13} C values implying that their prev originates from a more enriched δ^{13} C community. Considering the biogeographic gradients along the southern African east coast suggested by Hill et al. (2006), these sharks most likely forage further south of the study site and / or primarily inshore. The isotopic values of some sharks were, in fact, similar to the coastal base source near Durban, South Africa (Hussey et al. 2011), although it would be difficult to account for as other factors such as estuarine habitat use may have contributed to a change in isotopic ratios (Werry et al. 2011, Vorwerk and Froneman 2009). The relatively slow isotopic turnover rate of elasmobranch muscle tissue (Logan and Lutcavage 2010) may also mean that sharks foraging between different habitats will not reach isotopic equilibrium with one habitat which makes the interpretation of these data difficult. This may mean that the isotopic signatures reflected by these muscle tissues are, in fact, a mix of the dietary contributions from all frequented habitats (Abrantes and Barnett 2011) and may not reflect specific dietary contributions from the study site. However, these signatures may still provide a relative measure between individual bull sharks that reflect the differences between foraging ranges. Considering that bull sharks are capable of substantial movements (Brunnschweiler et al. 2010 and Carlson et al. 2010) it is likely that a proportion of the population forages over a wide geographical range on the east coast of southern Africa. This is supported by records of bull sharks from the Eastern Cape coast (Compagno and Smale 1986) and the migration of a male adult bull shark from the Western Cape coast to the Mozambican coast over a distance of approximately 2000km (M. McCord pers. com).

3.4.3. Bull shark dietary contributions

The mixing model suggested that fish species from group 3 made up the largest proportion of potential bull shark diet within the sampled community. The relatively high contribution of similar mesopredatory fish species to the diet of large bull sharks is consistent with previous studies (Cliff and Dudley 1991, Werry et al. 2011), but the relatively small

contribution of elasmobranchs in the diet of adult sharks contrasts those sampled on the east coast of South Africa (Cliff and Dudley 1991). However, differences were expected in the dominant dietary items between the study site and the east coast of South Africa, as there is a steep biogeographical gradient along this coast resulting in substantial habitat differences between the study site and the lower east coast of South Africa. Although stable isotope analysis may not provide the taxonomic resolution of stomach content analysis, it does suggest that mesopredatory fish species do constitute a consistently important portion of bull sharks diet over time. Dietary contributions from top predatory fish species and shark species from groups 1, 2 and 4 were not as important as group 3, however, the mixing model suggested previtems from group 1, 2 and 4 made up a higher proportion of adult bull sharks diet. This is consistent with the increased ability of larger sharks to consume larger prey items from higher trophic levels, including other elasmobranch species (Cliff and Dudley 1991, Lowe et al. 1996). Considering the diverse number of prev items that bull sharks are known to consume (Compagno 1984, Cliff and Dudley 1991), it is possible that their prey spectrum at the study site may have been under represented. These may include other species of elasmobranchs that are known to constitute an important part of the prey of adult bull sharks caught in bather protection nets on the east coast of South Africa (Cliff and Dudley 1991). Though, the snapshot nature of stomach content analysis probably reflected the recent foraging behaviour of those sharks and may have been biased towards prey from nearshore habitats where the bather protection nets are situated (Cliff and Dudley 1991).

3.4.4. Predatory niche width

Although top predatory fish assemblages (group 1 and 2) and bull sharks both had similarly high trophic positions, the significantly smaller niche widths of predatory fish assemblages, represented by groups 1 and 2 relative to the sampled bull shark population, suggest that bull sharks have a greater influence on their respective marine communities. Bull sharks exhibited a significantly larger range of δ^{15} N values and δ^{13} C values compared to the predatory fish assemblages suggesting that as a population they forage on a wider diversity of prey from various trophic levels. While predatory teleosts are also capable of substantial migrations (Mann 2000), bull sharks are more likely to forage amongst more diverse habitats, such as estuaries, and may exhibit individual foraging strategies (Matich et al. 2011). Bull sharks are thought to be generalist predators (Cliff and Dudley 1991, Werry et al. 2011) that consume a variety of prey items, but foraging behaviour of individuals may be more complex. Matich et al. (2011) suggested that juvenile bull sharks exhibit a degree of dietary specialization individually, but as a population, they consume a variety of prev items more typical of a generalist species. Foraging variability may also be influenced by the seasonal availability of resources and individuals or populations may adopt different foraging strategies according to changing environmental variables (Matich and Heithaus 2013). Even though data from this study were not sufficient to investigate individual specialization or diet variability, these studies (Matich et al. 2011, Matich and Heithaus 2013) investigated juvenile sharks within the confines of an estuary and, therefore, it is likely that the foraging strategies employed by adults would be different as they are more mobile, encounter a more diverse range of habitats and are not as constrained by predator risk effects (Heithaus et al. 2009). In an environment where different food webs have a high degree of geographical overlap, predators may be more likely to utilize the most abundant food source regardless of the food web in which the resource occurs (Quevedo et al. 2009). At the study site in this investigation, the pelagic and coastal food webs were not spatially distinct and bull sharks were easily able to move between them (pers obs.). Therefore, it is unlikely that individuals would exhibit foraging strategies limited to only one food web or resource pool. This would account for the broad range of δ^{13} C values exhibited by bull sharks in this study and supports the argument that adult bull sharks are not dietary specialists albeit that foraging strategies of individuals may be complex (Bolnick et al. 2011) and deserve further investigation.

3.4.5. Adult vs sub-adult bull shark niche space

Due to challenges associated with sampling at the remote study site, it was only possible to obtain a small sample size (n=9) from adult bull sharks. In order to account for this, the small sample size corrected Bayesian ellipse analysis was employed (Jackson et al. 2010) which dealt well with the limited sample sizes. However, it is also acknowledged that

typically sample sizes smaller than 10 can lead to increased variance in the Bayesian model output and may lead to an enlarged ellipse area estimate. Taking this into account, it was still apparent that adult sharks exhibited a shift in niche space towards a more enriched δ^{15} N diet with a wider range of δ^{13} C values. Evidence from the mixing model supports this hypothesis, suggesting that larger predatory fish species made a large contribution to the diet of adult sharks. The higher calculated trophic position of adult sharks also reflected this. Additionally, supporting evidence from the literature suggests that adult sharks consume a greater proportion of larger prey from higher trophic levels (Cliff and Dudley 1991, Werry et al. 2011). Adult sharks also exhibited a significantly wider range of δ^{13} C values implying that they source their dietary items from a more diverse habitat range than sub-adults. These results were also consistent with the published literature that suggests larger sharks typically range over broader geographical areas (Curtis et al. 2011, Werry et al. 2011, Werry et al. 2012). This suggests that bull sharks not only undergo an expanded dietary range related to prey size (Cliff and Dudley 1991), but may also exhibit a niche shift consistent with an expanded foraging area (Hammerschlag-Peyer et al. 2011). In many marine communities, the increased mobility of adults is an important mechanism through which spatially separated communities are connected, and ensures energy transfer that link ecological processes which may maintain the functionality of these systems (Gaines et al. 2007). Factors such as gender and individual foraging behaviour may, however, require additional investigation in order to further elucidate the ecological role of bull shark populations.

3.4.6. Bull shark trophic position

The range of δ^{15} N values used to calculate the trophic position (TP_{SIA}) for bull sharks was similar to other studies that investigated large shark species (Estrada et al. 2003, Hussey et al. 2011, Carlisle et al. 2012, Werry et al. 2011). The lack of an ontogenetic relationship between the size of bull sharks and δ^{15} N was, however, in contrast to some of these studies (Hussey et al. 2011, Abrantes and Barnett 2011, Borrell et al. 2011). This was most likely due to the limited size range of individuals sampled in this study and the absence of juveniles from the study site. The narrow range of δ^{15} N values exhibited by adult bull sharks also suggests that these sharks are close to a δ^{15} N plateau and that they all feed at a consistently high trophic level (Kim et al. 2012), consequently decreasing the size based variation in δ^{15} N values.

The mean TP_{SIA} for bull sharks (4.5) based on stomach content analysis alone (TP_{SCA}) was slightly higher than the value of 4.3 reported by Cortes (1999). Although previous studies (Hussey et al. 2011, Boyle et al. 2012, Drymon et al. 2011) reported a similar range of difference between TP_{SIA} and TP_{SCA} when calculating TP_{SIA}, Hussey et al. (2012) found that TP_{SIA} values typically are higher than TP_{SCA} values. Calculated TP_{SIA}, however, can vary considerably depending on the assigned TP of the base consumer, the chosen fractionation rate and the type of tissue sampled (Hussey et al. 2012, MacNeil et al. 2006). Therefore, while the absolute value of calculated TP_{SIA} should be used with caution, the relative value calculated in this study provided a meaningful comparison between bull sharks and fish assemblages within the sampled community. As expected, bull sharks occupied a high trophic position within the sampled community consistent with the trophic positions of other large shark species calculated from stable isotope analysis (Estrada et al. 2003, Hussey et al. 2011).

3.4.7. Conclusions

This study suggests that although top predatory teleost fish and large shark species may have similarly high trophic positions, their predatory role within their respective marine communities may be functionally different. A single large shark species such as the bull shark may exert a disproportionately large predatory influence within and amongst various marine communities due to their ability to prey on a broad diversity of prey items over a wide geographical range. The predatory influence of adult sharks may be particularly important as they increasingly consume prey from higher trophic levels and from a greater foraging range. Unlike predatory teleost fish assemblages, large shark species may be able to link important ecological processes within and between a diverse range of marine habitats. However, these processes may be complex and a further understanding of interpopulation and inter-individual variability will elucidate the roles of such large shark species further. As bull sharks are slow growing and late maturing, they have an intrinsic vulnerability to population decline (Smith et al. 1998, Myers et al. 2007) typically driven by fishing pressure (Dulvy et al. 2008, Worm et al. 2013, Ferretti et al. 2010) and habitat degradation (Werry et al. 2012, Lee et al. 2006). Therefore, understanding how the spatial and temporal scales of these threats are linked to the variable feeding ecology of shark populations are important conservation management considerations (Chapter 5).

Chapter 4: Bull shark residency patterns and migration dynamics.

*This chapter is a modified version of the published manuscript: Daly R, Smale MJ, Cowley PD and Froneman PW (2014) Residency patterns and migration dynamics of bull sharks (*Carcharhinus leucas*) on the east coast of southern Africa. PLoS ONE 9: e109357.

4.1. Introduction

The spatial ecology of apex predatory shark species is important to understand when considering their role in structuring marine communities and proposing effective conservation strategies (Speed et al. 2010, Abrantes and Barnett 2011, Simpfendorfer et al. 2011, Speed et al. 2012). Adult bull sharks are known to consume a variety of prey from multiple trophic levels (Cliff and Dudley 1991, Chapter 3) and may influence marine communities through predation over a broad geographical range (Chapter 3). However, little is known about the long-term (>1yr) temporal and spatial scales over which adult bull sharks typically move. Previous studies suggest that adult bull sharks exhibit high levels of residency interspersed with some degree of short to medium scale geographical migrations linked with seasonal change (Compagno 1984, Brunnschweiler et al. 2010, Carlson et al. 2010). On the east coast of South Africa, bull sharks are known to undertake seasonal migrations into more temperate latitudes during summer (Compagno 1984, Compagno and Smale 1986), but have been thought to be largely resident with limited home ranges (Cliff and Dudley 1991, Mann 2000).

For a slow growing and late maturing species such as the bull shark (Winter et al. 2002), exhibiting periods of residency and undertaking large-scale migrations are important life history characteristics that may make the species especially vulnerable to both fishing pressure and habitat loss (Smith et al. 1998, Barnett et al. 2011). Evidence suggests that bather protection nets have already depleted bull shark populations on the east coast of South Africa (Cliff and Dudley 1991, Dudley and Simpfendorfer 2006) and understanding the residency patterns and migration dynamics of this species will help to improve

conservation strategies (Warkentin and Hernández, Shuter et al. 2011). In this study, passive acoustic telemetry was employed to investigate the habitat use of adult bull sharks at a remote natural aggregation site in southern Mozambique that is minimally influenced by human activity. The aim was to investigate the temporal and spatial dynamics of adult bull shark residency patterns at the study site and quantify the timing, frequency and distance of migration events.

4.3. Methods

4.3.1. Shark capture and tagging

Between January 2012 and February 2013, 18 bull sharks were captured and tagged at the Pinnacle reef. Sharks were captured from a 6m vessel using baited Mustad 18/o circle hooks connected to 450 pound carbon coated steel trace attached to 20m of nylon rope and two 15-liter surface buoys. Once a shark was hooked, it was left to tire for 20-30 minutes before being brought alongside the vessel where it was inverted to induce a state of tonic immobility and then secured alongside the vessel while remaining partially submerged. Captured sharks were first measured (TL) and then surgically equipped with a VEMCO V16 coded acoustic transmitter (VEMCO Ltd., Halifax, Canada). The tags were implanted into the peritoneal cavity via a 2-3cm incision in the abdominal wall that was subsequently sutured closed using a surgical needle and thread (Stevens 1999). Tags were deployed in four main batches with a battery life span of between 1625 and 3650 days. All tags transmitted at 69 kHz at low power with a nominal delay of 120 seconds (range: 80-160 seconds). Before release, a uniquely coded plastic dart tag (ORI, South Africa) was externally attached to the base of the first dorsal fin to identify internally tagged individuals. To release the shark, the hook was removed from the mouth before turning the shark into an upright position and releasing it. The time from securing the shark alongside the vessel to its release ranged between 10 to 15 minutes. Tagged sharks ranged between 195 and 283cm TL (mean TL = 254.4cm) with a M:F ratio of 11:7 (Table 4.1). Based on the measured length, all sharks were considered sexually mature adults (Cliff and Dudley, Wintner et al. 2002) and all males (with the exception of ID # 13 which may not have been sexually mature) had calcified claspers.

Table 4.1. Summary of all bull sharks tagged with acoustic transmitters at the Pinnacle reef. TL represents total length. Total detections refer to all detections on all receivers at the study site over the length of the study.

Shark	Date	TL		Total	Total number	Residency	
ID	tagged	(cm)	Sex	detections	of days detected	Index (RI)	
1	21.01.2012	250	М	4135	172	0.26	
2	23.01.2012	220	F	4649	86	0.13	
3	30.01.2012	260	М	19	5	0.01	
4	02.02.2012	280	F	117	15	0.02	
5	23.11.2012	282	М	884	76	0.21	
6	24.11.2012	270	М	4	1	< 0.01	
7	07.12.2012	253	F	24	7	< 0.01	
8	09.12.2012	244	М	1142	36	0.10	
9	18.12.2012	263	М	1134	96	0.28	
10	20.12.2012	264	М	1667	45	0.13	
11	24.01.2013	250	F	1790	40	0.12	
12	26.01.2013	262	М	128	8	0.03	
13	29.01.2013	195	М	126	12	0.04	
14	30.01.2013	251	F	145	21	0.04	
15	31.01.2013	283	М	1677	76	0.26	
16	03.02.2013	261	F	1546	44	0.15	
17	18.02.2013	241	F	996	23	0.08	
18	19.02.2013	251	М	10960	169	0.61	

4.3.2. Receiver deployment

An array of three VEMCO VR2 acoustic receivers was deployed at the study site (Fig 4.1) between November 2011 and January 2012. All receivers were attached directly to the reef

substrate using a 4mm stainless steel anchor chain connected to a 3m long 14mm nylon rope suspended by a 200mm trawl float with the receiver attached vertically to the rope 2.5m from the substrate. The deployment of three receivers on the Pinnacle reef occurred in locations where reef profile was low and within safe diving limits. These three receivers collected data continuously from November 2011 to November 2013. Two additional receivers were deployed inshore of the Pinnacle reef approximately 700m from the shore and spaced 2.4km apart. The inshore receivers were maintained from January 2012 to November 2013 although data were retrieved from only one inshore receiver due to equipment failure.



Figure 4.1. The Pinnacle reef complex with the colour scale indicating the corresponding depth of the reef and numbered flags represent the approximate location of three acoustic receivers on the reef.

In addition to the local receiver array, data were also obtained from VEMCO VR2W acoustic receiver arrays along the coast and within estuaries in South Africa maintained by the Acoustic Tracking Array Platform (Hout Bay, False Bay, Gansbaai, Mossel Bay, Algoa Bay, Port Alfred, Dwesa MPA, Port St Johns, South Africa / Mozambique border) and the Marine Megafauna Foundation (Tofinho, Pomene and Bazaruto) as well as an array at Aliwal Shoal maintained by the Department of Environmental Affairs: Oceans and Coasts (Fig 4.2). All receivers at the above mentioned locations were operational over the same period as data were collected for this study.



Figure 4.2. Map of southern Africa with the location of the study site at Ponta do Ouro in southern Mozambique indicated in red. All other listed locations represent acoustic receiver sites on the coast (represented by a circle) or within an estuary (represented by an x) from which additional bull shark detection data were obtained.

Two temperature loggers (Hobo Water Temp Pro v2), set to record temperature every hour, were deployed at the Pinnacle reef from November 2011 until November 2013 (on-going). One logger was attached to receiver number 1 (Fig 4.1) approximately 2m from the bottom.

The second logger was located 3km inshore of the Pinnacle reef at a depth of 16m, approximately 2m from the bottom.

4.3.3. Range testing

Range tests were conducted by positioning an acoustic transmitter (VEMCO V16-6L) at various distances from a receiver (5m, 100m, 200m, 300m, 400m) and depths (bottom = 25m deep, surface =10m deep) for a period of four days per interval and depth. When possible, environmental conditions (wind direction and strength, swell height, sea surface temperature, current direction and strength) at the study site were recorded during the range test. Detections recorded by each receiver were grouped into bins representing "day" and "night" hours. Individual detection probabilities were then calculated for each period (day and night) at each position (surface and bottom) from each distance (5m, 100m, 200m, 300m, 400m) based on the total number of recorded detections expressed as a percentage (0-100%) of the known number of transmissions from the tag. The mean detection rates at each time of day, depth and distance were compared using a one-way ANOVA.

4.3.4. Data analysis

4.3.4.1. Presence / absence data analysis

Detection data from the Pinnacle reef study site was downloaded and initially analysed using the software package VUE version 2.0.6 (VEMCO 2013). Data from all receivers were combined to investigate overall presence and absence at the study site. Daily detections were plotted on a time line. A shark was considered present on a certain day when at least one detection was recorded within 24 hours. Potentially false detection data were examined by considering realistic detection time frames and potential tag collisions and any erroneous data were removed. Combined daily detection data from all Pinnacle receivers for males and females were analysed for differences using a Students t-test.

4.3.4.2. Temporal residency patterns

Data from all three receivers at the Pinnacle reef were combined to investigate the temporal use of the offshore reef habitat. Detection data from individual sharks were binned into hourly periods and represented in rose diagrams using the statistical software package Oriana (version 4, Kovach Computing Services). Rao's Spacing Test (Batschelet 1981) was used to test for uniformity in the temporal detection data by investigating the null hypothesis that the recorded temporal detection data are evenly distributed throughout each hour of a 24-hour period. The level of statistical significance is determined from a table of simulated critical points (Russell and Levitin 1995) with the statistical significance set to p<0.05. The time of sunrise ranged between 04:51 h and 06:39 h and the time of sunset ranged between 17:07 h and 18:44 h during the length of this investigation.

4.3.4.3. Residency

Overall residency indices (RI) at the Pinnacle reef were calculated for each shark by dividing the number of days each shark was detected by the total number of days each shark was monitored (Bond et al. 2012, Welsh and Bellwood 2012). This measure standardizes the detection data for each shark regardless of the monitoring period by providing a figure of between 0 (shark detected on zero days that it was monitored) and 1 (shark detected on every day that it was monitored) that allows us to compare residency indices between sharks that were monitored over different time periods. An overall residency index was calculated for the entire study period, as well as the mean monthly indices. To investigate any potential bias between the monitoring periods of individual sharks and the calculated residency indices of males and females were compared using a Student's t-test. A single linkage hierarchical cluster analysis was also employed to investigate groupings within the sample population based on overall residency indices. Finally, a regression analysis and one-way ANOVA were employed to investigate the relationship between monthly residency indices and mean monthly sea temperatures.
4.3.4.4. Migration

Migration events were defined as directed persistent one-way movements between two receivers over large spatial scales (Dingle and Drake 2007). The overall migration distances for sharks were measured using Garmin Homeport (version 2.2.1) and calculated as the minimum distance along the coast between receiver locations (Bansemmer and Bennett 2011). The rate of travel (km.day⁻¹ and km.h⁻¹) by a shark was calculated from the minimum distance moved between two receivers (km) divided by the time at liberty (days) during a migration event. Regression analyses with a one-way ANOVA were employed to investigate any relationships between shark length and migration distance and speed.

Unless otherwise stated, all data analyses were conducted using the statistical package R (CRAN 2009)

4.4. Results

4.4.1. Range testing

Summary results from the range test experiment are presented in Table 4.2 and Figure 4.3. Combined mean detection rates at 5m were 79.5% (SD = 14.0), at 100m were 15.4% (SD = 8.7) and at 200m no detections were recorded. Detection rates were significantly higher (p<0.05) during the day than at night when the tag transmitted from the bottom position (25m deep) at 5m and 100m distance from the receivers. The detection rate was also significantly higher (p<0.05) during the day compared to night when the tag transmitted from the surface (10m deep) at 100m from the receiver, but there was no significant difference (p<0.05) between day and night when the tag transmitted from a distance of 5m. Bottom (25m deep) tag detection rates were significantly higher (p<0.05) than surface detection rates (10m deep) during the day and night when the tag transmitted from a distance of 5m, but there was no significant difference (p<0.05) in detection rates when the tag transmitted from a distance from a distance of 5m, but there was no significant difference (p<0.05) in detection rates when the tag transmitted from a distance from a distance of 5m, but there was no significant difference (p<0.05) in detection rates when the tag transmitted from a distance of 5m, but there was no significant difference (p<0.05) in detection rates when the tag transmitted from a distance of 5m, but there was no significant difference (p<0.05) in detection rates when the tag transmitted from 100m distance during day or night. In all cases there was a significantly higher (p<0.05) detection rate for all tags transmitting from 5m compared to

100m.

Table 4.2. Range test results represented as mean detection rates expressed as percentages. Distance corresponds to the distance of the receiver from the transmitter (5m, 100m, 200m, 300m, 400m), Day or Night corresponds to the time of day detections were recorded and Surface (10m) or Bottom (25m) corresponds with the depth at which the transmitter was positioned in the water column. Standard deviations are presented in parenthesis.

Distance	Day	surface	Day	bottom	Night	surface	Night	bottom
(m)	(%)		(%)		(%)		(%)	
5m	64.1 (5.6)		95.5 (2.2)		72.2 (10.1)		85.9 (2.3)	
100	25.6 (8.1)		17.6 (10.4)		4.6 (7.8)		13.6 (10.5)	
200	0		0		0		0	
300	0		0		0		0	
400	0		0		0		0	



Figure 4.3. Range test results. Proportion of transmissions received vs the expected detections received represented as a percentage (0-100%) from tags deployed at the study site at varying distances, depths and times of day. Error bars represent standard deviation.

Environmental conditions recorded during the range test varied widely with wind direction (north, northeast, east, south and southwest), wind strength (5-25 knots), swell height (1.5-3m), current direction (north, south) and current strength (0 to >2 knots) all typical for the study site.

4.4.2. Presence / absence at the study site

Bull sharks were monitored at the study site for 664 days between January 2012 and November 2013 (Fig 4.4). All sharks were detected on the receiver array at the Pinnacle reef for at least one day after tagging. Individual tag detection totals ranged between 3 and 10958 detections (1724.1 \pm 2664.6, mean \pm SD) over a period of 1 to 172 days (50.3 \pm 52.9, mean \pm SD) (Table 4.1). Shark presence was variable with some sharks (ID # 1,2,5,8,9,15,16,18) exhibiting continuous periods of presence for over one month that ranged between 46 and 158 days (Fig 4.4). Although there was not a significant difference (t-test, DF = 6, p>0.05) in the total number of days detected between males and females, males (62.8 ± 62.0 , mean \pm SD), on average were detected for more days than females (30.6 ± 27.9 , mean \pm SD). Most sharks (72%) were detected at the inshore receiver for an average of 5.8 (SD = 9.0) days, but nearly all of these individuals (97.1%) were also detected at the Pinnacle reef on the same day.



Figure 4.4. Daily bull shark detection data from all receivers at the study site. Detection data are represented by black squares and presented on a timeline from January 2012 to December 2014 for all 18 tagged sharks. The first detection data point for an individual shark corresponds to the day the shark was tagged at the study site.

The majority (15 out of 18) of tagged sharks were also detected at the array of five receivers located at the border of South Africa and Mozambique, approximately 12km south of the Pinnacle reef. Detections at the border receiver array only occurred over periods when sharks were also detected at the Pinnacle reef without exceptions. The total number of days that sharks were detected on the border receiver array ranged between 1 and 102 days (16.8 \pm 29.3, mean \pm SD) and the proportion of days that sharks were detected at both the border receiver array over the same monitoring period ranged widely between 0.01% and 100% (49 \pm 41.6, mean \pm SD).

4.4.3. Temporal residency patterns

Figure 4.5 represents the temporal detection patterns of all sharks with sufficient data (n = 10) at the Pinnacle reef. Hourly detection frequencies for all sharks (except ID # 15 and 18) exhibited non-homogeneous distributions (Rao's spacing test, p<0.01). There was some overlap in temporal use of the Pinnacle reef between individuals (ID # 1, 2,5,8,9,16) that appeared to be present primarily from morning (~7am) until early evening (~8pm). Other individuals, however, were primarily present during the evening (ID # 10 and 11) whilst others were present throughout the day and night (ID # 15 and 18). Most sharks (except ID # 15 and 18) exhibited increased habitat use at the Pinnacle reef over a 12-hour period followed by a 12-hour period of absence.





Figure 4.5. Circular plots showing the temporal detection frequency of bull sharks at the Pinnacle reef. The temporal distribution of most sharks (except 15 and 18) was significantly non-homogenous (Rao's spacing test, p < 0.01). Individual sharks not represented had insufficient data for the analysis.

4.4.4. Residency

Bull sharks monitored at the Pinnacle reef exhibited residency indices (RI) ranging between 0.001 and 0.67 (0.14 ± 0.15 , mean \pm SD) over the duration of the study (Table

4.1). Results from a hierarchical cluster analysis (Fig 4.6) suggest that there were three main groupings of sharks based on their overall residency indices. Five of 18 tagged sharks (ID # 18,5,1,9,15) exhibited relatively high overall residency indices (RI > 0.2) with periods of prolonged (>3 consecutive months) high monthly mean residency indices (>0.3) interspersed with relatively short periods of absence (Fig 4.4) and appeared to be seasonal residents. One individual (ID # 18) appeared as an outlier within the seasonal resident group with an overall residency index (RI = 0.6) substantially higher than other individuals within the group. The second group of seven individuals (ID # 3,6,7,13,14,4,12) exhibited very low resident indices (RI<0.1) with only a few detections over the entire study period (Fig 4.4). The third group of sharks (ID # 17,16,8,11,2,10) exhibited intermediate residency indices (RI = 0.3) interspersed with long periods of absence (Fig 4.4).



Figure 4.6. Cluster analysis results. Dendrogram showing the groupings of individual sharks based on their calculated residency indices at the study site.

There were no significant relationships between the total monitoring periods of individual sharks and the total number of detections or total days detected or calculated residency indices (p>0.05). There was no significant (p>0.05) difference in the overall residency indices exhibited by female or male sharks, however, the mean male residency index (0.18 \pm 0.18, mean \pm SD) was higher than the mean female residency index (0.08 \pm 0.06, mean \pm SD) and only male sharks exhibited residency indices over 0.2 (n=5). Monthly mean residency indices were highest during summer (Dec-Feb) (mean = 0.33) and autumn (Mar-May) (mean = 0.46), compared with winter (Jun-Aug) (mean = 0.08) and spring (Sep-Nov) (mean = 0.24), and showed a positive correlation (R² = 0.84, p<0.05) with increased water temperature at the study site (Fig 4.7).



Figure 4.7. Mean monthly residency indices and mean monthly sea temperature. Bar charts represent the mean monthly residency indices from all tagged bull sharks detected at the Pinnacle reef. Error bars representing standard deviation. The dashed line represents the mean monthly sea temperatures (°C) recorded at the study site.

4.4.5. Migration

Ten out of 18 tagged sharks undertook large-scale migrations (>400km) primarily in northerly direction (Fig 4.8) with only 1 shark (ID # 7) undertaking a southward migration of 450km to Aliwal Shoal, South Africa (Fig 4.2). No other detections were recorded at any of the receivers located in South African coastal waters during the monitoring period (Fig 4.2). Eight out of ten sharks returned to the study site after migration events (ID # 2,4,7,10,11,14,17,18) and one shark undertook multiple return migrations (ID # 14). Individuals that undertook migrations ranged in size between 220 and 280cm TL (mean = 258.8cm TL) and 70% were females. The minimum distance that sharks travelled during migration events (persistent and directed one-way movements between receivers) ranged between 433km and 709km (533 \pm 122.8, mean \pm SD) with daily rates of movement ranging between 2.0 km.day⁻¹ and 59.1 km.day⁻¹ (17.6 \pm 15.5, mean \pm SD) and with corresponding speed ranging between 0.08 and 2.5 km.h⁻¹ (0.75 ± 0.65 , mean \pm SD). Total annual minimum distances travelled by sharks ranged between 450 and 3760km (mean = 1163km). There was no significant relationship (p>0.05) between total length and distance travelled or speed. Nor were there any significant relationships (p>0.05) between gender and distance travelled or speed, although females did exhibit a mean travelling distance $(991.7 \text{km} \pm 327.3 \text{km}, \text{mean} \pm \text{SD})$ greater than that of males $(770 \text{km} \pm 266.7 \text{km}, \text{mean} \pm 10.2 \text{km})$ SD). Northward migration events took place between January and September with the majority (75%) during austral autumn and winter (March-August).



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Figure 4.8. Bull shark migration events along the coast of Mozambique. The x-axis represents a time line showing months and year. The y-axis shows distance along the coast in kilometres (starting at Ponta do Ouro) corresponding with the coastal map of southern Mozambique. The solid square markings show annual bull shark detection events at the study site and other locations within Mozambique.

4.5. Discussion

Evidence provided in this study suggests that the Pinnacle reef in southern Mozambique is an important aggregation site for adult bull sharks and that they exhibit prolonged periods of residency, primarily during the austral summer months. Importantly, this study highlights the geographical scale of movement that adult bull sharks are capable of undertaking along the coast of southern Africa, and suggests that they are not as sedentary as previously thought (Holden 1977, Cliff and Dudley 1991, Mann 2000). This study also documents the first return migration events for the species along this coast and emphasizes the need for greater understanding of bull shark migration dynamics.

4.5.1. Range testing

Receiver range testing was conducted at the Pinnacle reef to help interpret the detection data at the study site (How and de Lestang 2012, Kessel et al. 2013). Results from the range test experiment suggested that the detection range of receivers in this study was relatively

poor although within the same range as previously reported (How and de Lestang 2012, Welsh et al. 2012). Previous studies (How and de Lestang 2012, Brunnschweiler and Barnett 2013) using the same tag specifications (VEMCO V16-6L) in similar depths to this study reported mean detections rates of less than 60m (Brunnschweiler and Barnett 2013) and 80% detection rates at 0m which were reduced to approximately 35% at 100m and 10% at 200m (How and de Lestang 2012). Although due to the scale of this experiment it is not possible to determine the actual detection threshold and caution needs to be taken when interpreting these results. The range test experiment did suggest that increased biological noise from the Pinnacle reef at night might have negatively impacted the detection range of transmitters, especially when the tag transmitted from a position near to the reef. This was expected as nocturnal biological reef noise has been known to lower detection rates, specifically in tropical environments due to increased acoustic interference (Welsh et al. 2012). Reef rugosity and profile at the study site may have further compounded detection interference when tags transmitted close to the reef as acoustic signals may have been reflected or refracted by the reef (Welsh et al. 2012). Surface wave and wind action may also negatively impact detection rates due to increased acoustic interference, but the range test experiment did not adequately control for the potential interference of absolute transmitter and receiver distance from surface interference.

Although the range test experiment provided evidence to suggest that the detection range at the study site was poor, the actual detection range and performance of the acoustic receiver array appeared to be adequate for the study subject. When investigating the actual detection data from tagged sharks that showed some sharks (44%) were detected by the receiver array at the Pinnacle reef for continuous periods of between 46 and 158 days. Periods of shark absence are further confirmed by detections from locations other than the study site for 10 out of 18 tagged sharks. Based on 111 observations at the study site it was evident that sharks swam slowly relatively close the reef and had a preference for shallow reef areas. This suggests that the most significant detection interference may come from reef noise and profile, but the sharks slow swimming speed and preference for shallow areas where receivers were located are important considerations that may have improved detection rates. In this study the range test experiment gave us a proxy for receiver performance, although the actual receiver performance is still largely dependent on the

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study subject and associated behaviour relative to the site-specific acoustic receiver array.

4.5.2. Presence / absence

Data from this study suggests that the Pinnacle reef is an important habitat for adult bull sharks with tagged individuals exhibiting periods of prolonged presence. Detection data from the nearby receiver array at the south African / Mozambican border also suggests that bull sharks at the study site do undertake medium-scale movements (<12km), but the frequency, direction and range of these movements may vary widely between individuals. The majority of tagged sharks (72%) also undertook inshore forays at the study site, but further investigation is required to determine the importance of inshore habitat use.

4.5.3. Temporal residency patterns

The majority of tagged sharks appeared to exhibited diel patterns in habitat use during this investigation, which is consistent with previous observations of multiple shark species across a range of habitats (Heupel et al. 2012, Barnett et al. 2012, Filmalter et al. 2013). Previous studies investigating diel patterns in bull shark habitat use found that sharks exhibited increased diurnal site fidelity followed by absence or broader nocturnal habitat (Brunnschweiler and Barnett 2013). Findings from this investigation showed that the majority of individuals appeared to demonstrate similar diel patterns, although some individuals exhibited higher nocturnal site fidelity while others exhibited equal habitat use throughout a 24-hour cycle. The behavioural drivers behind diel habitat use may vary and include foraging (Speed et al. 2011) predator avoidance (Heupel and Simpfendorfer 2005) and thermal regulation (DiGirolamo et al. 2012). In the absence predator constraints, it is likely that foraging behaviour is an important driver of temporal habitat use. The significantly biased and varied temporal habitat use patterns of sharks at the study site suggest that individuals may employ a variety of temporally segregated foraging strategies.

4.5.4. Residency

The overall range of residency indices recorded for bull sharks during this study were lower than a previous investigation in Fiji (Brunnschweiler and Barnett 2013), although in this study there was no food provisioning; the receiver array was smaller and; the study site was located in an environment with greater seasonal change. There appeared to be individual variation in the levels of residency behaviour that could be categorized into three general groupings (Fig 4.6), but overall austral summer and autumn residency indices were substantially higher for individuals and mean monthly sea temperatures appeared to be a major driver of habitat use at the study site. Bull shark seasonal habitat use is well documented and may be more pronounced in sub-tropical environments such as the study site (Cliff and Dudley 1991, Compagno and Smale 1986, Brunnschweiler and Baensch 2011).

Although the seasonal change in water temperature appeared to be an important driver of residency at the study site, the wide range of residency indices suggests other drivers, such as reproductive activity and foraging strategies, may also be important factors determining habitat use by bull sharks (Barnett et al. 2011, Weng et al. 2005, Papastamatiou et al. 2013, Werry et al. 2014). The onset of increasing levels of residency exhibited by bull sharks at the study site appeared to be associated with large aggregations of Carangid fish species (mainly *Caranx ignobilis*, but also *Carangoides fulvoguttatus*, *Carangoides gymnostethus* and *Caranx sexfasciatus*), known to be bull shark prey items (Cliff and Dudley 1991). However, it was not clear if bull sharks were responding to increased prey abundance or if both the sharks and the fish aggregations were responding to environmental cues. Prey availability is a known driver of shark habitat use (Fitzpatrick et al. 2012, Kock et al. 2013), thus it is likely that the increase in seasonal potential prey abundance at the study site influences the foraging behaviour and associated residency patterns of bull sharks at the study site.

4.5.5. Migration

Evidence from this study confirmed that bull sharks are capable of substantial migrations (Carlson et al. 2010) and provided the first description of the distance and speed bull sharks travel on return migration events. Migration distances recorded for individuals in this investigation were within range reported for bull sharks (Carlson et al. 2010), but present the longest travel distances recorded for bull sharks in southern Africa. Previous studies in South Africa using conventional tagging (Mann 2000) and catch data (Cliff and Dudley 1991) showed that bull sharks were a sedentary species occupying small home ranges. In contrast, this investigation shows that it is more likely that bull sharks along the southern African coast exhibit periods of residency interspersed with substantial geographical migration events.

Most migration events recorded in this investigation were linked with seasonal change at the study site with a northward migration to warmer latitudes during austral winter and spring and a southward movement into more temperate latitudes during summer. This is consistent with previous literature that has shown bull sharks may be more abundant at higher latitudes along the east coast of South Africa during warmer austral summer months (Cliff and Dudley 1991). However, one shark (a mature female, ID # 14) undertook multiple return migration events over multiple seasons, suggesting that seasonal change may not be the only driver of migration events. Other large shark species may undertake migrations driven by reproductive activity (Bansemer and Bennett 2011) and prey availability (Kock et al. 2013). As female sharks in this investigation were recorded migrating further and undertaking the majority of migration events (70%), it is possible that gender specific migration linked to reproductive cues were a factor that determined the distance, direction and frequency of migration events.

Evidence from this study also indicate that bull sharks are capable of covering substantial distances over short periods of time with sustained mean (0.74km.h⁻¹) and maximum (2.46km.h⁻¹) rates of movement faster than those previously recorded for bull sharks (Carlson et al. 2010). Recorded rates of movement were still, however, substantially slower

than tiger (3 - 4km.h⁻¹) and white sharks (4.7km.h⁻¹) undertaking sustained movements (Holland et al. 1999, Bonfil et al. 2005, Heithaus et al. 2007), but showed that within a year, adult bull sharks are capable of covering a broad geographical range that may include coastal waters of multiple countries. This supports evidence from a previous investigation that showed adult bull sharks have a geographically broad foraging range along the east coast of southern Africa (Chapter 3). Although in this investigation bull sharks were only detected as far north as Bazaruto, it is possible that some sharks travel substantially further north and additional investigation is required to establish the extent of bull sharks northward range.

4.5.6. Summary

Previous investigations using telemetry to monitor adult bull shark habitat use have shown that bull sharks have an affinity for coastal environments, exhibit periods of residency and are capable of substantial geographical migrations (Brunnschweiler et al. 2010, Carlson et al. 2010, Brunnschweiler and Barnett 2013, Hammerschlag et al. 2012). Results of this study generally supported these findings and provided further understanding of the residency patterns and migration dynamics of bull sharks in southern Africa with the first description of return migration events for the species. This study also provided evidence to suggest that coastal migrations are a frequent and important component of bull sharks habitat use. Migrations appeared to be driven primarily by seasonal change, but further investigation is required to understand how spatiotemporally fluctuating resources and reproductive activity may influence bull shark residency patterns and migration dynamics (Dingle and Drake 2007). Improving our understanding of bull shark migration dynamics is important to elucidate the spatial scales of their ecological role (Holdo et al. 2011) and plan effective conservation management strategies for this species (Shuter et al. 2011). A broad range of management approaches encompassing trans-boundary cooperation may be required to effectively conserve critical bull shark habitat.

Chapter 5: Synthesis

5.1. Overview

The main aim of this study was to investigate a population of bull sharks in a remote region of southern Mozambique by employing non-lethal methods in order to improve the regional and global understanding of the ecology and movement patterns of these apex predators. Although bull sharks have been the subject of numerous studies that have investigated the reproductive biology, growth, diet and habitat use (Chapter 1), little is known about the spatial and temporal dynamics of adult bull sharks ecological role within marine ecosystems. To date, there have been no studies in Mozambique that have investigated large predatory shark species and such studies in South Africa have primarily relied on data from culling programs (Cliff and Dudley 1992). This study has used innovative methods (Chapter 2) to help fill important gaps in the regional and global knowledge about the functional ecological role that bull sharks play within their respective marine communities (Chapter 3). Additionally, this study has shown that bull sharks in southern Africa are not as sedentary as once thought but, in fact, are capable of undertaking spatially significant migrations within the southwest Indian Ocean (Chapter 4). Although, further investigation is required to determine the complete spatial and temporal scales of bull shark movements and associated ecological roles in the region, this new knowledge has important implications for the conservation management of bull sharks.

5.1.1. Non-lethal sampling

The motivation to develop a novel non-lethal sampling method to collect data for this investigation was primarily based on practical constraints and the concern for using lethal sampling methods to study a vulnerable species. Currently there is an urgent need to collect data on shark species, many of which are currently data deficient (Dulvy et al. 2014), in order to improve conservation measures. However, many shark species are also subject to

increasing threats, and alternative non-lethal sampling methods may help to mitigate additional mortality from research practices (Hammerschlag and Sulikowski 2011, Dulvy et al. 2014). Certain scientists still advocate the need to lethally sample sharks, citing that the sacrifice of some will contribute to the ultimate conservation for the whole (Heupel and Simpfendorfer 2010). However, this approach to sampling threatened shark species may soon become as redundant as research employing lethal sampling methods for threatened terrestrial apex predators. There are currently a variety of non-lethal sampling alternatives to obtain data on many important aspects of a shark species life history and biology, although there is a need for further application and innovation (Hammerschlag and Sulikowski 2011).

The development of a biopsy probe to collect muscle and skin tissue samples from freeswimming bull sharks in this study showed how a simple design made it possible to collect data, when combined with the appropriate analysis, on the species feeding ecology (Chapter 3). It is important to note, however, that the biopsy probe was used in conjunction with other non-invasive methods, including laser photogrammetry and underwater video (Chapter 2). The use of such methods are becoming increasingly common, but there is a need to improve the data collection protocol associated with these methods in order to improve the accuracy and reliability of the data (Marshall and Pierce 2012). Such methods may also have their limitations (Chapter 2), although they can be incorporated into part of a multifaceted study in order to mitigate the risks associated with more invasive sampling methods (Chapter 2). Until a few decades ago, collecting data on free-swimming shark species was a novelty (Nelson 1977), but such practice, along with non-invasive sampling methods, should now be seen as part of an increasingly innovative approach to collecting important data from sharks that will contribute to effective conservation management plans (Hammerschlag and Sulikowski 2011). Further research should focus on improving existing non-lethal sampling methods and developing new methods for studies that address important gaps in the knowledge of threatened shark species.

5.1.2. Feeding ecology

Determining the diet of top predatory sharks from stomach content data may offer some insight into the range of prey items that these sharks consume, however, one cannot determine the complete ecological role of a predator based solely on its direct predatory influence (Chapter 1). There are various aspects of a predators influence to consider when assessing their ecological role within an ecosystem (Ripple and Beschta 2007, Heithaus et al. 2012b). Yet, it is often difficult to study the complete range of predatory influences that large shark species may exert on their respective prey and communities due to their natural environment and elusive nature. Therefore, a relatively novel tool (stable isotope analysis) was employed to investigate the trophic position, niche width and comparative predatory role of bull sharks within their respective marine community. This method did not allow the characterisation of the complete predatory influence (i.e. risk effects were not investigated) that bull sharks may exhibit within their marine community, however, it did provide insight into the comparative predatory role that bull sharks play within their marine community.

This study found that both co-occurring top predatory teleost fish assemblages and bull sharks had similarly high trophic positions, suggesting that each group exerts important direct predatory influence on their marine community (Chapter 3). Bull sharks, however, appeared to have a functionally different predatory role to the top predatory teleost fish with a significantly broader niche width, suggesting that they have a greater influence on a larger proportion of their respective marine community. This highlighted the difference between the ecological roles of these top predators and provided further evidence to support the fact that large sharks play a critical role shaping and structuring marine communities (Lucas and Stobo 2000, Ward-Paige et al. 2010, Ruppert et al. 2013, Heithaus et al. 2014). Specifically, evidence from this aspect of the investigation suggested that adult sharks may play an especially important role linking ecological processes over significant geographical scales along the east coast of southern Africa.

5.1.3. Movement patterns

The movement patterns and migration dynamics of bull sharks in the southwest Indian Ocean have not been the subject of recent or thorough investigation (Holden 1977, Cliff and Dudley 1991, Bullen and Mann 2000). Therefore, there was an incentive to investigate this subject in order to characterise the residency patterns and clarify the spatial scale of bull sharks movement in this region. As data from the feeding ecology study (Chapter 3) suggested, bull sharks are capable of foraging over broad spatial scales within the region, probably on the same scale as bull sharks in the north Atlantic (Carlson et al. 2010) and Pacific (Brunnschweiler et al. 2010).

This study provided evidence to suggest that bull sharks exhibited periods of residency at the study site and the Pinnacle reef appeared to constitute critical habitat for the studied population, particularly during austral summer (Chapter 4). Although the levels of residency amongst individuals did vary, there was a significant link between residency and seasonal change mediated primarily by sea temperature (Chapter 4). These results supported previous studies that showed bull shark habitat use might be linked with seasonal change (Compagno and Smale 1986, Brunnschweiler and Baensch 2011) and that individual residency within a population may vary (Brunnschweiler and Barnett 2013). This study also indicated that migration events were a relatively frequent and consistent component of bull shark habitat use in the region (Chapter 4) supporting evidence from previous studies in the Atlantic and Pacific (Brunnschweiler et al. 2010, Carlson et al. 2010), but also provided the first evidence of return migration for the species. This supports evidence from Chapter 3, suggesting that bull sharks may play an important predatory role linking ecological processes over broad spatial scales on the east coast of southern Africa.

Characterising bull shark migration dynamics is important, as migration is an essential component of the life history and ecological niche of a species (Dingle and Drake 2007, Cresswell et al. 2011). Understanding the spatial and temporal scales of bull shark migration may also have important implications for elucidating the ecological role of these sharks within a range of marine communities in the southwest Indian Ocean. However, in

order to characterise the migration dynamics of a species or population, its mechanism and function needs to be understood (Dingle and Drake 2007). This study has provided some evidence to suggest that seasonal change, foraging and reproductive activity are most likely the drivers behind bull sharks migratory movement patterns, but further research is required. It is likely that a suite of complex drivers act together on an individual level to guide the migration dynamics of bull sharks. Thus, future research to understand these drivers should focus on foraging dynamics and reproductive activity as these are known to influence the habitat use and migration dynamics of shark species (Bonfil et al. 2005, Grubbs et al. 2005, Barnett et al. 2011, Bansemer and Bennett 2011, Kock et al. 2013).

Understanding the spatial and temporal extent to which bull sharks influence marine communities within the southwest Indian Ocean has crucial conservation implications for marine communities within this region. Migratory species may have complex and multi-faceted influence within and between multiple marine communities as they link and shape ecological processes (Gaines et al. 2007). The predatory influence that bull sharks have within one community is a vital aspect of the balance and function within that community, but the migratory dynamics of top predatory species may have even more wide-ranging consequences for multiple communities if their abundance or range becomes altered (Holdo et al. 2011). Thus, the conservation of top predatory migratory species such as the bull shark should be a priority when considering effective marine conservation management strategies for the region.

5.2. Conservation

This study fills essential gaps in the regional knowledge of bull shark movement patterns and the associated predatory role that are key for planning thorough conservation management strategies. Importantly, this study has highlighted the crucial ecological role that bull sharks play within their respective marine communities and suggests that the predatory role of bull sharks is functionally different from that of co-occurring predatory teleost fish species (Chapter 3). Thus, the decline or loss of bull sharks may substantially alter the structure or function of their respective marine communities (Stevens et al. 2000, Myers et al. 2007). Therefore, the conservation of bull sharks within the southwest Indian Ocean should be prioritised in order to preserve the integrity of coastal marine communities.

In light of increased fishing pressure for sharks within the southwest Indian Ocean (Marshall and Barnett 1997, Pierce et al. 2008), improving fisheries regulation and enforcement must be an important component of an effective conservation management strategy for the species. However, improving fishing regulations within this region will not be without its challenges as shark fishing is largely unregulated, and the majority of catches go unreported (Pierce et al. 2008). With increased market demand for shark products by a burgeoning Asian market, along with an increase in trade and cooperation between Africa and China (Clarke et al. 2007, Dell'Apa et al. 2014), it is likely that targeted fishing will become one of the largest threats to shark populations within the southwest Indian Ocean. As traditional fishing regulation practices (Barker and Schluessel 2005) may not be applicable to a large sector of the artisanal fishing community in Mozambique (Pierce et al. 2008), alternative approaches must be considered. This may include improving the participation of the artisanal fishing sector in the management of local fisheries resources through educational programs and tourism initiatives (Barker and Schluessel 2005). Considering the stunted economic development status of fishing communities in developing countries such as Mozambique (Daly 2014) the best conservation approach may be to provide these communities with economic alternatives to fishing. The sustainable economic benefit of shark related tourism has been demonstrated to encourage the participation of local communities and governments in the conservation of this resource (Anderson and Waheed 2001, Gallagher and Hammerschlag 2011) with studies suggesting that a single shark may be worth as much as 2.64 million USD in tourism revenue in its lifetime (Clua et al. 2011). Bull sharks in particular may attract high-value tourism as evident in Fiji (Brunnschweiler and Baensch 2011), and may have the potential to generate significant sustainable income whilst providing an alternative economic incentive to fishing communities.

In order to design effective conservation management strategies for bull sharks within the southwest Indian Ocean, their complex habitat use patterns (Chapter 4) need to be considered. Whilst marine protected areas may provide protection for specific areas where sharks exhibit periods of residency (Knip et al. 2012) they may be less effective during periods of migration (Werry et al. 2014). Furthermore, marine protected area management plans must consider ontogenetic changes in the habitat use patterns of sharks and may only be effective for specific stages of a shark species life (Werry et al. 2011). However, if marine protected areas provide sharks with protection for a critical habitat or life stage, they may be an effective conservation tool (Knip et al. 2012) when paired with conservation measures that incorporate shark migration (Shuter et al. 2011). Effective migratory conservation measures also have to consider trans-boundary cooperation and policy alignment within the habitat range of the targeted species or population (Werry et al. 2014). This may be accomplished through multilateral arrangements and regional fisheries management organizations and is a crucial aspect of an effective conservation management plan for bull sharks in the southwest Indian Ocean (Barker and Schluesell 2005).

Applying effective conservation measures for bull sharks in southern Mozambique should start with improving the current regulations of the Ponta do Ouro Partial Marine Reserve (PPMR). This study has provided strong evidence that the Pinnacle reef provides both a unique and critical habitat for mature adult bull sharks, specifically during austral summer (Chapter 4). However, fishing regulations outlined by the new (partially implemented) management plan (DNAC 2011) do not make specific reference to regulations for sharks even though it does recognize that shark populations in general are under threat (p.16, DNAC 2011). Addressing this disparity and dictating specific regulations that prevent the recreational fishing of bull sharks and other shark species would be appropriate as a first step towards enforcing the conservation values of the PPMR. The effective conservation of bull sharks within the PPMR will ultimately make it an important refuge area for bull shark populations in this region that currently face various localised threats including habitat loss (Cyrus et al. 2011), culling programs (Cliff and Dudley 1991) and commercial fishing pressure (Pierce et al. 2008).

5.2.1 Future research priorities

Further research important for the improvement of the conservation management of bull shark populations in the region should focus on identifying migration routes and potential nursery areas. Although this study has shown that bull sharks do undertake frequent and geographically substantial migrations, additional research is required to quantify the spatial patterns and full extent of these migrations. Understanding the spatial and temporal scales of bull sharks movement patterns is necessary for evaluating the exposure of threats that this population of bull sharks are subject to and, therefore, improve conservation management plans accordingly. Furthermore, if bull sharks are frequently travelling across political boundaries, then trans-boundary management strategies must be aligned. Thus, future research could investigate gene flow between sub-populations and fine-scale patterns in relatedness or familial structure of the sharks within the region. Finally, critical nursery areas for bull shark populations within the region need to be identified and incorporated into the regional conservation management planning for the species. Estuaries are known to play an important nursery role for bull sharks, yet many estuary systems in southern Africa have been subject to degradation and anthropogenic modification (Whitfield 1998, Cyrus et al. 2011). Therefore, there is a need to establish which estuaries in the region are currently playing a key nursery role for the species. Such research priorities may be most effectively achieved by trans-boundary collaborations that investigate large-scale regional research questions covering the entire range of the studied population of bull sharks. Such a regional West Indian Ocean approach to future research could be achieved by improved collaboration by existing research groups but would also need sufficient funding to tackle some of the proposed research that may need to take place over large spatial and temporal scales.

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