NELSON MANDELA
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Department of Zoology

The distribution of South African loggerhead (*Caretta caretta)* sea turtles as indicated by epibionts and stable isotopes

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Plagiarism Declaration

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Declaration:

In accordance with Rule G 5.6.3, I hereby declare that this dissertation is my own work and that it has not previously been submitted for assessment to another University or for another qualification.

Signature: 

Date: 15-12-2018

Ethics Statement

All turtles handled were done so with the utmost care as to not cause unnecessary stress to the individual. Turtles were examined and worked on only once they began laying eggs as to avoid disturbing the nesting process.

This study was carried out under a research agreement entitled "Sea turtles as indicators of ocean health: evaluating nesting loggerheads and leatherbacks from South Africa as indicator species." registered with the iSimangaliso Wetland Park Authority. All sampling activities were carried out under research permits issued by the South African Department of Environmental Affairs (DEA RES2016/67, RES2017/73 & RES2018/68) and Threatened and Protected Species Permit and Institutional Standing Permit (TOPS Permit no 29516 & Standing Permit no 03315). Ethical clearance was obtained for this project from the Nelson Mandela University (Ref no. A13-SCI-ZOO-011 and A13-SCI-ZOO-012).

Summary

Many marine species undertake long-distance migrations as part of their life history strategies, and so form an important part of marine ecosystems performing a range of functions, across many habitats. However, these migratory species, including sea turtles, face multiple threats and anthropogenic impacts across their ranges and knowing their movement and distribution patterns enables more effective and appropriate conservation strategies to be devised. Satellite telemetry has provided invaluable information on spatial distribution of marine migrants, but applying this approach to a large proportion of a population is often unfeasible and costly. This study aimed to identify alternative, more cost effective methods that could assist with tracking animal movements across a larger proportion of a population of marine focal species, such as sea turtles. This study used nesting loggerhead sea turtles (*Caretta caretta*) from the iSimangaliso Wetland Park, South Africa as a model species to test these alternative methods and subsequently combine body condition, habitat use, and distribution range in the South West Indian Ocean.

First, epibiont community assemblages were investigated as a proxy to determine sea turtle body condition. A body condition index was created using plastron shape, injuries and skin deformities. Sixty turtles were classified into four body condition categories ranging from poor to very good and this was reflected in their epibiont communities as both species abundance and richness increased with a decline in body condition. A total of twenty-eight epibiont taxa were identified from a range of systematic groups including, but not limited to, Amphipoda, Cirripedia, Brachyura and Polychaeta. The barnacle *Chelonibia testudinaria* showed the greatest variation among different body conditions with an increase in abundance as turtle body condition deteriorated. These results suggest that epibiont load can be used as an indicator of body condition that is easy to implement in the field.

Second, a combination of organic $\delta^{13}C$ and $\delta^{15}N$ isotopic signatures of turtle epidermis and epibiont communities was used to infer foraging habitat. One hundred and seventy turtles were sampled for stable isotope analysis. These turtles were clustered into two groups based on $\delta^{13}C$ at -13.61 ‰ with relative depletion or enrichment indicating foraging in oceanic or neritic environments, respectively. The epibiont communities of

80 turtles closely followed this cluster grouping; turtles with depleted $\delta^{13}C$ had a higher abundance and frequency of oceanic epibiont species, such *Lepas* spp. Similarly, three neritic epibionts (*Hyale grandicornis, Hyachelia tortugae* and *Podocerus africanus*) were the other habitat-specific species driving community assemblages, with higher occurrence and abundance on turtles in the enriched $\delta^{13}C$ cluster. Additionally, the size of the dietary niche was determined by a Bayesian analysis of $\delta^{13}C$ and $\delta^{15}N$ for 46 turtles in different body condition categories. Although there was overlap among categories, individuals in very good body condition had the smallest dietary niche. These results show the complementarity of using epibionts and stable isotope analysis in determining foraging area.

Third, *Chelonibia testudinaria* barnacles on sea turtles were analysed for $\delta^{18}O$ and inorganic $\delta^{13}C$. The $\delta^{18}O$ of expected calcite fractionation was mapped for the known migration routes of eight turtles in the South West Indian Ocean. The inorganic carbon values were not very informative on movement, however, the $\delta^{18}O$ analysis of the barnacle showed the range of the turtle host moving through the isoscape. Most of the turtles migrated from the north in the Mozambique Channel, to the southern rookery in South Africa, which is in accordance with reports from tag recoveries and satellite telemetry studies. Using this approach to track migratory species that have epibiotic barnacles can provide complimentary approach to satellite tracking that can be used on more individuals within a population.

This study aids in providing alternative methods to study body condition, habitat use and regional movement of loggerhead sea turtles. These approaches can be applied to other sea turtle species and migratory marine fauna to help better understand their movement patterns thereby promoting more effective conservation strategies. Future work should consider incorporating different cohorts, examining other epibionts such as meiofauna and diatoms, including additional isotope and trace elements for analysis on habitat and improving the resolution of the isoscape data for $\delta^{18}O$ of seawater in the SWIO.

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Chapter 1

Animal migrations and tracking animal distributions

1.1 Importance and vulnerability of migrating species

Migration is a key feature in the life histories of many marine species, although this behaviour occurs at the individual level it has implications for populations (Dingle and Drake 2007). By migrating, individuals are able to exploit a greater variety of resources which would not be readily available if they were to remain in a fixed location (Dingle 2014). These movements are therefore primarily driven by ecological and biogeographic factors to access distinct resources such as food, nesting habitat or mates that support population growth through reproduction (Dingle and Drake 2007, Dingle 2009). These movements can occur over various temporal and spatial scales ranging from diel movement of zooplankton (Stich and Lampert 1981, Wall-Palmer et al. 2018) to the long-distance migrations of the arctic tern (*Sterna paradisaea*) (Egevang et al. 2010), and leatherback sea turtle (*Dermochelys coriacea*) (Shillinger et al. 2008) of over 10 000 kilometres. Although drivers of migration may vary, this phenomenon has evolved independently in many diverse taxa as a consequence of the ecological benefits/advantages obtained by undertaking these movements (Alerstam et al. 2003, Fryxell and Holt 2013).

The evolution of physiological adaptations for the endurance to withstand long-distance migrations are related to energy storage and utilization (Weber 2009). The physiological costs of movement differ between taxa that fly, swim or walk and therefore the occurrence and distance of migration are different between these various modes of transport (Tucker 1973). Swimming has been considered to be the most physiologically efficient method of migrating (Schmidt-Nielsen 1972), and biomechanical studies report that aquatic animals have high propulsion efficiencies (Taylor et al. 2003) and low fluid drag (Floryan et al. 2018). Given the spatial complexity of the marine environment and the relative ease of locomotion, many marine species undertake long-distance migrations (Robinson et al. 2009, Dingle 2014).

Migratory animals form an important part of functioning ecosystems, they do this by facilitating the transport of nutrients and propagules between distant areas while also serving as dispersal agents for epibiotic organisms (Bauer and Hoye 2014). Additionally, migratory animals can have ecological effects on resident communities through foraging on available resources, providing a prey resource to resident predators, or introducing pathogens (Zacheis et al. 2001, Altizer et al. 2011, Giroux et al. 2012). Because of the seasonality of migratory events, like turtle nesting or salmon breeding, migratory species have large, pulsed impacts on the environments they use during their journeys (Holtgrieve and Schindler 2011), and have been documented to influence energy flow and food-web dynamics, including trophic cascades (Sánchez-Zapata et al. 2007). Migratory animals influence community dynamics and through their movement connect ecological networks around the globe (Bauer and Hoye 2014).

The routes and destinations of long-distance marine migratory species often extend through the national borders of multiple countries, and as an effect of their extensive distributions these species face many anthropogenic impacts across their range (Martin et al. 2007). Socio-political boundaries can induce habitat fragmentation when national regulations and legislation are variable between countries (Dallimer and Strange 2015). Migratory species often do not receive the same level of protection across their entire distribution range (Runge et al. 2014). Furthermore, migrating populations can become vulnerable if areas that are critical for completion of their life cycle are severely impacted or disturbed by anthropogenic activities (Jensen et al. 2010, Shuter et al. 2011). In the marine environment, international legislation extends to the economic exclusion zone of 200 nautical miles offshore, but many threatened species migrate beyond this boundary which puts them at further risk (Robinson et al. 2009, Lascelles et al. 2014). Determining the movement patterns of exploited species, particularly those that migrate great distances, has been an important focus in ecological research. Similarly, understanding the migratory patterns of threatened species enables better conservation because efforts can be focused on key areas, such as at breeding, feeding and stopover sites, as well as migration corridors (Martin et al. 2007). However, tracking migratory species can be expensive and difficult and requires careful consideration to meet conservation objectives (Runge et al. 2014).

1.2 Approaches to tracking migratory species

Tracking the movement of migratory species can be done through direct monitoring of individuals using extrinsic markers, or indirectly through intrinsic markers (Rubenstein and Hobson 2004). Extrinsic markers are those attached to the animal for later retrieval and assist in a mark-recapture approach to study animal movement. Some of these markers include satellite devices, radio transmitters or simple numbered (flipper or subdermal) tags. Intrinsic markers are those derived from the animal itself such as biological or biochemical markers (Hobson 2008, Hobson and Wassenaar 2008). These markers involve the analysis of tissue that can be related to a geographical area and include genetics and stable isotopes techniques (Hobson 1999).

Extrinsic markers range from the most basic identifier tag, like a flipper or implanted tag, to a satellite device that can provide specific information of the global position (GPS) of an animal every minute, its environment (e.g. temperature) and information on behaviour (e.g. dive depth recorders). Although extrinsic methods provide invaluable information about migratory movement (Bridge et al. 2011, Hays et al. 2014), they do have limitations. Mark-recapture studies can have a low return rate of tagged individuals (Oosthuizen et al. 2010), especially for species with long migrations. Radio telemetry is useful in tracking large species or animals with short dispersal distances, but limited in tracking movement of small, cryptic animals or those with long migrations (Rubenstein and Hobson 2004). Satellite tracking is the most appropriate method for the latter. However, the feasibility of these tags is limited by the animal's body size and the expense of the equipment. There is a trade-off between size, battery power (longevity) and cost that needs to be considered when applying a satellite approach to track migratory species. Satellite tags are thus limited to tracking a small number of individuals in a population. Satellite devices may also alter the behaviour of the host and cause drag requiring additional energetic cost to movement (Thomson and Heithaus 2014, Robinson et al. 2016), or increase mortality depending on the attachment method. Satellite tags are therefore limited to tracking a small proportion of individuals in a population.

Intrinsic techniques to track migratory animals have thus become increasingly popular (Hobson and Wassenaar 2008, Robinson et al. 2010). The advantage of using intrinsic markers is that the animal does not need to be marked initially or recaptured and that

there is no bias as a result of changing behaviour due to stress involved with the tagging procedure or the possible effect of the tag on animal movement (Rubenstein and Hobson 2004). Additionally, these markers can provide both spatial and temporal information of an individual at a fraction of the cost of satellite data. Intrinsic markers do have disadvantages, which are mostly related to providing information at a much lower spatio-temporal resolution in comparison to extrinsic markers. It is therefore recommended that these techniques are used to supply complementary information for a specific target population (Vander Zanden et al. 2015, Townsend et al. 2018). The most commonly used intrinsic markers for migratory species are collected from genetics, stable isotope ratios, and epibiotic communities. An important logistical advantage of using intrinsic markers is that every capture is a recapture as all the information can be collected from the individual from the first sampling occasion (Hobson 2008).

Genetic markers have a variety of applications but are also commonly used to identify distinct population units in smaller (threatened) populations providing that there is geographic structure/isolation (Webster et al. 2002). This structure relates evolutionary history with contemporary gene flows that reproductively isolated populations have unique features. The breeding origins of individuals can be identified even when sampled on migration routes or on mixed foraging areas (Clegg et al. 2003), provided that the breeding population has been typed. So if individuals from different populations are sampled at the same foraging grounds each individual could be typed to a breeding population because they possess distinct genetic signatures in mitochondrial DNA (mtDNA), which is passed from females to their offspring (Bowen and Karl 2007). Shorebirds are one such migrant which have been successfully tracked using genetic markers (Haig et al. 1997, Wennerberg 2001). Similarly, distinct populations of sea turtles that share foraging grounds have been identified through mtDNA (Wallace et al. 2010, 2011). Although genetic markers can be effective at delineating natal origin, it cannot inform the movement of individuals during their migration (Bowen and Karl 2007). Genetic variation was therefore not used as an intrinsic marker for this study as all individuals were sampled from the same breeding population. Ideally, intrinsic markers used for tracking movement patterns should be indicative of feeding and/or nesting grounds. This requires the markers to have a distinct geographic signature and a predictable turnover time to identify distribution

pathways. Stable isotopes are potentially more informative than genetic markers in this regard.

1.2.1 Stable isotopes as intrinsic markers

The common trope for many stable isotope studies is "*you are what you* eat" (DeNiro and Epstein 1978). This is because the isotopic ratio of a consumer reliably reflects the prey it consumes (DeNiro and Epstein 1978, Post 2002, Graham et al. 2010). Isotopes are variants of the same element which differ in the number of neutrons and therefore have different atomic masses. For example, the stable isotopes of carbon are ^{12}C and ^{13}C have atomic masses of 12 and 13 Da, respecitively, and naturally occur at a ratio of circa 99 to 1 (Hobson 1999). These differences in atomic mass of the stable isotopes influence the way isotope ratios change in the environment, body tissues and metabolic pathways of an animal (Peterson and Fry 1987). The ratio of stable isotopes ^{13}C to ^{12}C ($\delta^{13}C$) and ^{15}N to ^{14}N ($\delta^{15}N$) in the tissue of the animal can therefore be used to indirectly infer the environment based on the relative enrichment (i.e. greater ratio of the heavy isotopes) or depletion (i.e. greater ratio of the lighter isotope) (Hobson 1999, Hobson and Wassenaar 2008, MacKenzie et al. 2011). Nitrogen isotope ratios are used to trace protein pathways and this enables a reconstruction of diet and trophic status (Hobson 2008). A consumer's $\delta^{15}N$ signature is typically enriched by 3-4 ‰ relative to their food sources due to metabolic activity in their bodily tissues (Peterson and Fry 1987, Post 2002). Therefore, knowledge of the prey species or baseline $\delta^{15}N$ values allows for determination of the trophic status of the animal in question. In contrast, the $\delta^{13}C$ is enriched by only 0-1 ‰ from prey to predator, and instead reflects the origin of carbon that is incorporated by primary producers (Peterson and Fry 1987, Vander Zanden and Rasmussen 2001, Post 2002).

In the marine environment, these isotope signatures can be used to identify distribution as there are distinct geographic variations in isotope signatures; conspecific individuals that feed at the same trophic level will have relatively depleted $\delta^{13}C$ signatures if they occupy habitats at higher latitudes (Hobson 1999; Takai et al. 2000). This is because the organic $\delta^{13}C$ of primary producers is strongly related to dissolved inorganic $\delta^{13}C$ which is influenced by both biological and physical processes across latitudinal gradients (McMahon et al. 2013). Similarly, $\delta^{13}C$ signatures of conspecifics that forage in offshore (pelagic) regions are also depleted in comparison to their

coastal (neritic) counterparts (Burton and Koch 1999). This is related to land-derived sources of $\delta^{13}C$ being more enriched. Geographical variability in the $\delta^{15}N$ signatures of consumers is more complex because primary producers at the base of the food web (e.g. seagrass, phytoplankton, algae) are affected by the two main sources of nitrogen: nitrogen fixation; and dentrification (Capone and Montoya 2001). Nitrogen fixation and dentrification result in relative depletion and enrichment of primary producers, respectively (Montoya 2008). Individuals foraging in inshore areas also generally have more enriched signatures, as primary producers utilize land-derived nutrients, while oceanic primary producers are reliant on nitrogen-fixing (Landrum et al. 2011). Coastal upwelling also drives enrichment of $\delta^{15}N$ and in some cases, however, this nitrogen-rich material can be transported to offshore regions (Walker and McCarthy 2012) which can potentially complicate $\delta^{15}N$ geographical patterns. Despite this, animals that forage in areas where the source of nitrogen for primary producers varies have distinctive isotopic ratios that can be used to infer movement (Hobson and Schell 1998, Seminoff et al. 2012).

Information on migration patterns and population structure can be studied through isotopic analysis of an animal's tissue (Hobson 1999, Rubenstein and Hobson 2004, Hobson 2008, Hobson and Wassenaar 2008). The development of isotope studies from tracers of origin to migratory and dispersal purposes relies on three isotopic principles (Hobson 2008):

(1) The consumer's isotopic signature reflects the food web it is associated with. If a migratory organism travels to another food web, which differs in its isotopic signature, then the tissue of the consumer can represent previous locations of foraging (Hobson 2008, Ceriani et al. 2014).

(2) The range of time that spatial information is retained for, is determined by the tissue that is chosen for comparison because the isotopic ratio is fixed at the time of synthesis. Active tissue that is replaced quickly represents ratios from recent synthesis and short time periods of information whereas, inactive tissue reflects ratios from older synthesis and longer time periods (Schell et al. 1989, Hobson 1999).

(3) Biases of isotopic signatures that is related to dietary transfers from a food source to a consumer, the energy expenditure and/or metabolic activity need to be known and their effects accounted for (Jones and Seminoff 2013).

Knowing these limitations and how to apply them is a critical step in the process of utilizing isotopes to track an animal's movement patterns.

Another application of stable isotopes in movement ecology involves constructing isotopic maps or isoscapes of spatial or temporal variability in environmental stable isotopes (West et al. 2010, McMahon et al. 2013). This is based on differences in geochemical process that occur across a land or seascape which creates a gradient for various stable isotopes. For example, atmospheric and oceanic regions are characterized by specific ratios of oxygen isotopes ^{18}O to ^{16}O ($\delta^{18}O$) due to water temperature changes as well as regional differences in evaporation and/or biogeochemical processes (Schaffner and Swart 1991, Koch 1998). The $\delta^{18}O$ ratios of seawater are closely linked with fractionation processes in the hydrological cycle as a result of various physical processes that occur across the ocean (Craig and Gordon 1965). Seawater $\delta^{18}O$ ratios are generally positively related with salinity because of the combination of evaporation and freshwater input (Epstein and Mayeda 1953). Lighter ^{18}O evaporates more readily compared to heavier ^{16}O, and in doing so increases surface water salinity and the $\delta^{18}O$ of the water (Craig and Gordon 1965). This results in a general positive latitudinal trend in $\delta^{18}O$ of seawater (LeGrande and Schmidt 2006). Greater $\delta^{18}O$ ratios of seawater are typically found in highly evaporative environments with minimal freshwater input such subtropical gyres and semi-enclosed marginal basins (Rohling and Rijk 1999, McMahon et al. 2013). The lowest seawater $\delta^{18}O$ values are found at higher latitudes and areas with high freshwater input such as large rivers (Gat 1996). Animals that migrate between different regions incorporate oxygen from the surrounding environment and preserve the isotopic signature in their tissues (Detjen et al. 2015).

The use of mapping regional differences in isotope patterns or isoscapes is particularly useful to apply to organisms such as epibiont barnacles that deposit $CaCO_3$ sequentially in their exoskeletons over time as they grow. These barnacles are common on large migratory marine vertebrates (Darwin 1854, Frick et al. 2009). Once attached to their host these barnacles grow from the inwards out, and therefore a chronology can be created from the layered $CaCO_3$ deposits. It is therefore possible to capture a record of $\delta^{18}O$ signatures of the past, corresponding to the different oceanic regions their host has travelled in during the lifespan of the barnacle (Killingley 1980, Killingley and Lutcavage 1983, Detjen et al. 2015). These isotopic ratios in the

calcite of the epibiotic barnacle can assist in tracking habitat use and regional movement.

Although stable isotope analysis (SIA) cannot provide as fine-scale information on an animal's location as satellite tags do, it has distinct benefits; carbon and nitrogen SIA is more economical at ± US$10 per sample as opposed to a satellite transmitter for ± US$3000 per animal plus additional expenses of satellite time that increase costs even further. Therefore, for studies requiring large sample sizes at a lower resolution are thus more suitable to SIA. This method informs on the type of location the animal traversed, and knowing the turnover rate of the tissue, can inform on habitat occupied previously to migration. Finally, SIA can provide information about the diet of the animal sampled (Post 2002), as well as the niche width of communities (Jackson et al. 2011).

1.2.2 Epibionts as intrinsic markers

Another potential intrinsic marker inferring the large-scale movements of migrants involves examination of their epibiotic community composition. Epibionts colonize a host when there is spatial overlap between the range of the host and the epibiont (Harder 2009). Epibionts on marine hosts are largely opportunistic, and generally recruit as pelagic larvae onto suitable substrates (Frick and Pfaller 2013) representing the most common form of a symbiotic relationship in the marine environment (Leung and Poulin 2008). Identifying the epibionts found on a marine animal and determining the distribution ranges of these epibionts can provide a map of potential occurrence for the host.

Different factors drive variation in epibiont diversity and community assemblages among individuals (Frick and Pfaller 2013). The geographic and ecological overlap between the host and their potential epibionts define the subset of community assemblages that can colonize an individual (Figure 1.1 A). Geographic overlap between the host and its epibiont is the primary requirement for epibiosis; if a host does not travel to an area where an epibiont occurs there can be no epibiosis. Conversely, range-restricted epibionts reveal more information about a host's range than cosmopolitan epibionts (Frick and Pfaller 2013). For example, differences in the epibiont assemblages have been used to identify different loggerhead populations from the Mediterranean Sea and north-western Atlantic Ocean (Domènech et al.

2015). Geographic overlap, however, does not simply imply that all potential epibionts in the region are able to colonise the host. Because of the heterogeneity of the marine environment, marine animals may utilise different habitats spatially and temporally. Therefore, successful epibiosis also requires some ecological overlap between the host and epibiont (Figure 1.1 B). The use of epibionts to examine habitat use by the host has been applied successfully to sea turtles. For example, the presence of the exclusively oceanic (pelagic) flotsam crab, *Planes spp.,* on turtles that occupy coastal (neritic) areas indicates that the host had recently arrived from oceanic environments (Limpus and Limpus 2003, Pfaller et al. 2014). Likewise the size distribution of epibiotic barnacles (*Conchoderma virgatum*) on leatherback sea turtles (*Dermochelys coriacea*) have been used to infer the time of arrival to their prerequisite tropical nesting beaches from temperate latitudes (Eckert and Eckert 1988).

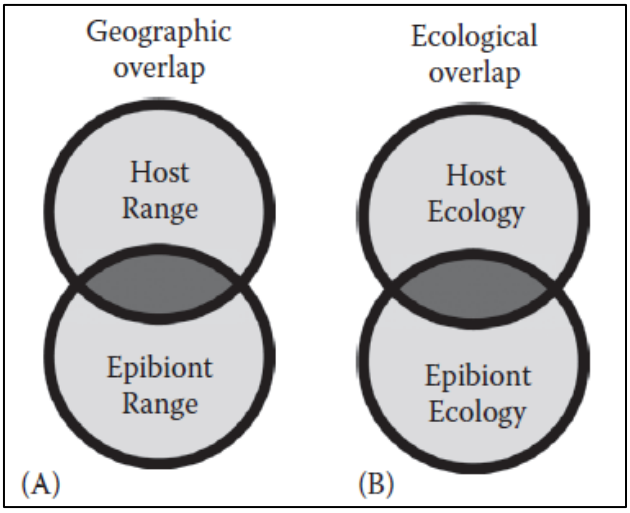

Figure 1.1: Model to conceptualise the geographical **(A)** and ecological **(B)** overlap between a host and its epibiont. From Frick and Pfaller (2013).

Besides their use to track the geographic range of hosts, epibionts have been considered a marker for assessing the general health of the host (Stamper et al. 2005). Epibiont loads vary among conspecifics and higher abundances of epibionts has been used as an indicator of poorer health. The use of epibionts to examine the health of the host has been applied to sea turtles (Deem et al. 2009, Flint et al. 2012), and whales (Apprill et al. 2014). The main benefit of using epibionts as indicators for host health is that they can be relatively easily sampled at low cost, which is particularly

useful in regions where it is not logistically or financially plausible to carry out extensive health assessments. Further, the combination of stable isotopes and epibionts can provide information on good foraging habitat for sea turtles vs those that are less productive, leaving animals in a poorer body condition and greater epibiont loads.

1.3 Sea turtles as model migratory species

Sea turtles can be used as model species to develop a methodology reliant on a suite of intrinsic markers to identify distributions that can then be applied to other migratory marine species. There are three reasons in promoting sea turtles as a model species with which to test and apply various tracking techniques: (1) Among the marine migratory species, sea turtles travel some of the greatest distances between nesting and feeding grounds (Saba 2013), and have been used to as model species to test tracking systems (Luschi et al. 2006). (2) The various intrinsic markers mentioned above can be easily collected from females once they emerge on their nesting beaches. Even if an individual does not have any epibionts SIA of their tissue can still be used to investigate habitat use. (3) Sea turtles are capital breeders and acquire their energy for reproduction in the foraging grounds months before migration to the breeding grounds where they use that store reserve (Plot et al. 2013). In reptiles the time it takes for reflection of the isotopic signature of food sources to the tissue of analysis (blood or epidermis) is in the region of months to years (Seminoff et al. 2007). All these factors mean that SIA of nesting sea turtle tissue should reflect their foraging habitats and not the nesting location, which, can be a great distance from their nesting beach (Foley et al. 2013, Schofield et al. 2013). Finally, these models can be verified using extrinsic markers, like tag returns and/or satellite tracks, on a subset of individuals.

1.4 Ecological importance of sea turtles and sea turtle health

Sea turtles have been proposed as "*sentinels of ecosystem health"* (Aguirre and Lutz 2004), because of their ability to reflect anthropogenic perturbations as they are hardy and can withstand a lot of physical damage, however, they are vulnerable to biological and chemical attack (Lutcavage et al. 1995, 1997). These anthropogenic inputs can change physiology, weaken the immune system, increase susceptibility to diseases and ultimately cause death (Aguirre and Lutz 2004). Because of the diverse range of habitats that sea turtles occupy they can be used as indicators of marine ecosystem

health across large spatial scales. Furthermore, healthy sea turtles are expected to provide a greater variety of ecological services because they can migrate longer distances, and therefore utilize a greater variety of habitats

Due to the large distributions and variety of habitats utilized, sea turtles fulfil a range of ecological roles in marine and coastal environments, acting as transporters of nutrients, predators, provision of prey, substrate for the colonization, and dispersion of epibionts, and ecosystem engineers (Bjorndal and Jackson 2002, Heithaus 2013). They are “mobile links” (Lundberg and Moberg 2003) between the marine environment and generally oligotrophic sandy beaches by transporting marine-derived nutrients from their eggs, which support predator populations and dune vegetation (Bouchard and Bjorndal 2000, Le Gouvello et al. 2017). Sea turtles have also been noted to facilitate fish foraging. Hawksbill turtles (*Eretmochelys imbricata*), help angelfish forage by biting through sponges, allowing these fish access to the inside (Blumenthal et al. 2009). Similarly, hawksbills maintain healthy coral reefs by feeding on the sponges and cnidarians, which compete for space with corals (León and Bjorndal 2002). Green sea turtles (*Chelonia mydas*) promote healthy seagrass beds through grazing by reducing decomposition time of seagrass leaves that are consumed by detritivores and thereby increases nutrient cycling (Thayer et al. 1982, Bjorndal 1997, Moran and Bjorndal 2005). Sea turtles also provide a hard substrate that epibionts can colonize (Frick and Pfaller 2013). The epibiont communities that are found on sea turtles are often diverse and contain representatives of many invertebrate taxa and algae (Pfaller et al. 2008, Fuller et al. 2010, Corrêa et al. 2014). Epibionts benefit from a reduction in competition and predation and can increase feeding, especially for filter-feeding barnacles (Frick and Pfaller 2013). Moreover, epibionts can further their range expansion and increase genetic mixing through sea turtles acting as vectors of long-distance dispersers (Harding et al. 2011).

Despite the importance of sea turtles, their marine ecosystems are under threat from anthropogenic impacts such as development, anthropogenic induced climate change, habitat modification and pollution (Crain et al 2008, Halpern et al. 2008). Consequently, the decline of sea turtle populations globally has led to a loss of the ecological roles that they provide and has resulted in a reduction in the function of ecosystems through food web shifts and trophic cascades (Bjorndal and Jackson

2003, Bolten 2003). It is therefore important to be able to monitor these threats so that appropriate mitigation measures can be put into place.

1.5 South African sea turtles

South Africa, at the southern tip of the African continent, has incredible marine biodiversity with two ocean currents creating many unique habitats and rich biogeographic provinces (Sink et al. 2012, Branch et al. 2017, Branch and Branch 2018). The sea turtles that use the waters off Southern Africa occur at some of their most southern range limits globally, which, provides the opportunity to study the potential of range expansion for these species. Of the seven species of sea turtles globally, five occur in South African waters, namely the leatherback, loggerhead (*Caretta caretta*); hawksbill, green and olive ridley (*Lepidochelys olivacea*) (Branch et al. 2017). Of these, only two species, leatherbacks and loggerheads, nest on South African beaches. Their nesting sites are formally protected within the iSimangaliso Wetland Park World Heritage Site, and the Maputaland Marine Protected Area (Figure 1.2). South Africa maintains one of the oldest monitoring programmes for sea turtles in the world (Hughes 2010), which has aided in the recovery of the populations, especially loggerheads (Nel et al. 2013a).

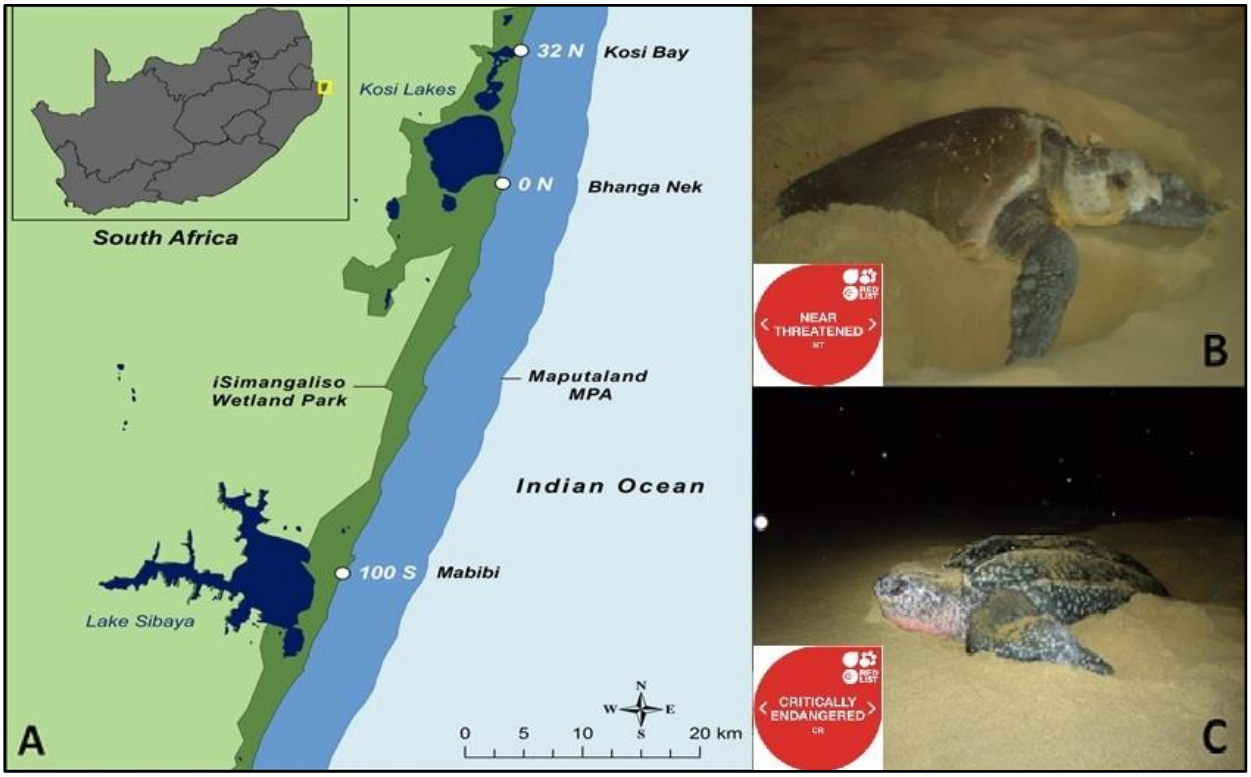

Figure 1.2: The nesting grounds of the two sea turtle species in South Africa. **(A)** The iSimangaliso Wetland Park, a UNESCO World Heritage Site. **(B)** Loggerhead sea

turtle (*Caretta caretta*) population in the Southwest Indian Ocean has been classified as near threatened by the IUCN (Nel and Casale 2015). **(C)** Leatherback sea turtle (*Dermochelys coriacea*) population in the Southwest Indian Ocean has been classified as critically endangered by the IUCN (Wallace et al. 2013).

1.5.1 Main threats and Conservation status of SWIO sea turtles

Globally all sea turtle species face threats of different types and intensities, and are all listed on the International Union for the Conservation of Nature (IUCN) Redlist for endangered species. The magnitude of anthropogenic impacts on sea turtles becomes evident when assessing stock status, with many populations being endangered or critically endangered (Mazaris et al. 2017). These globally distributed species are divided into distinct populations (Figure 1.3) which are defined both geographically and genetically (Wallace et al. 2010). This is important because even though the species might not be under threat, a population could be threatened, and this genetic distinct sub population is irreplaceable. This genetic diversity is also prevalent in the populations that nest on the South African and Mozambique coastlines (Nel 2010).

The threats facing loggerhead and leatherback sea turtles in the South West Indian Ocean (SWIO) vary at the different life stages of the species (de Wet 2012), because age classes are often spatially separated in the marine environment (Bolten 2003, Mansfield and Putman 2013, Saba 2013). On nesting beaches, the main threats are to the eggs and hatchlings though nest erosion and predation (Hughes 1974, de Wet 2012). Overall these predation rates are relatively low, owing to few natural predators and successful coastal conservation (Nel et al. 2013a). The biggest threat to leatherback sea turtles in this population is longline pelagic fisheries, which has a 41 % overlap with leatherback home ranges in the South African economic exclusion zone (de Wet 2012, Harris et al. 2018). Loggerheads, which utilize more coastal environments, are more vulnerable to threats from inshore activities such as artisanal fisheries (> 1000 mortalities per year), inshore trawling (40 mortalities a year) and shark nets (22 mortalities per year), and to a lesser extent, from pelagic longline fisheries (5 mortalities per year; de Wet 2012). Therefore, determining the areas of occurrence for the majority of both species can limit the human-turtle interaction thereby helping conserve these species.

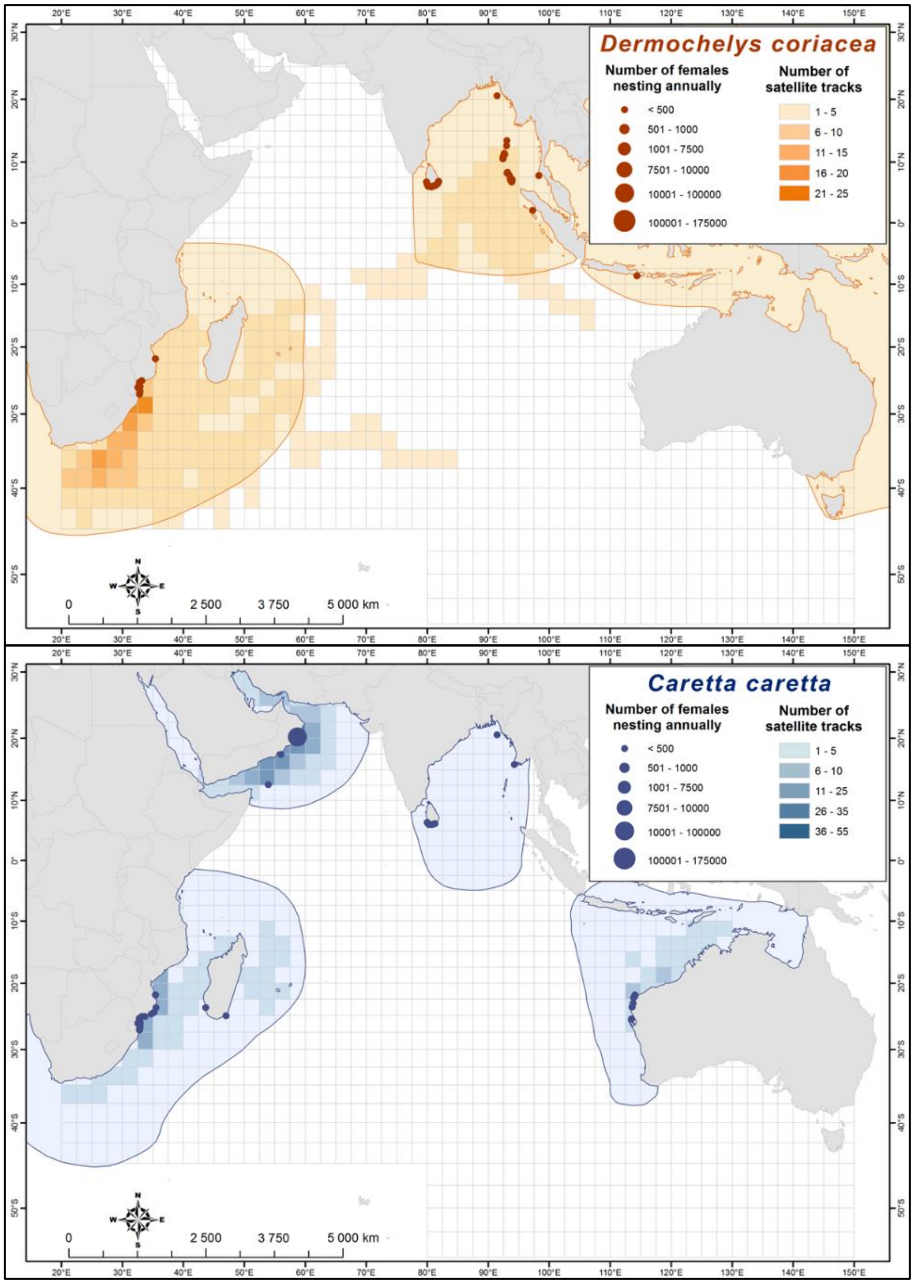

Figure 1.3: The rookeries (dots), satellite tracked distribution (shaded blocks) and boundaries (lines) of the South Western Indian Ocean Regional Management Unit (i.e. genetic populations) for leatherback (top) and loggerhead turtles (bottom) around South Africa along with other RMUs in the Indian Ocean. From Nel et al (2013b).

In South Africa, a 52-year monitoring programme has shown mixed successes in sustaining populations of locally nesting loggerhead and leatherback turtles through coastal marine protected areas (Nel et al. 2013a). The SWIO loggerhead population is listed as near threatened whereas the leatherback population is listed as critically endangered under the International Union for Conservation of Nature (Wallace et al.

2013, Nel and Casale 2015). These nesting populations have seen varying success in the recovery of their population size since their nesting habitats have received formal protection. Since the introduction of the monitoring programme along in the then Greater St Lucia Wetland Park (now iSimangaliso Wetland Park), the number of nesting loggerhead females increased at an almost exponential rate, while nesting leatherback females remained stable (Nel et al. 2013a). Sea turtles receive the same protection throughout their range in South Africa through various law and regulations (e.g. Marine Living Resources Act 1998, National Environmental Management Act 1998, and Biodiversity Act 2004). However, the regulation and enforcement of these laws often lacking in areas far from the turtle's nesting beaches. The high-use areas far offshore and away from the coast has likely led to the slow recovery of the SWIO leatherback population (de Wet 2012, Nel et al. 2013a). Conservation efforts are hindered by a lack of spatio-temporal knowledge of migration patterns and fine-scale understanding of habitat use (Wallace et al. 2011).

1.5.2 Movement in the SWIO

Tracking the movement of sea turtles in the South West Indian Ocean has mainly been through the use of satellite tag and mark-recapture studies (Hughes 1974, Luschi et al. 2003, 2006, Harris et al. 2018). The two nesting populations have a wide distribution, occurring from the Seychelles to the west coast of South Africa (Figure 1.4). Because these sea turtle populations are dependent on conservation efforts, this provides a unique opportunity to conduct scientific studies that contribute towards their conservation.

Both loggerhead and leatherback turtles in the SWIO have a degree of overlap in their distributions. However, loggerheads are more coastal than leatherbacks both in their inter-nesting movement patterns (Harris et al. 2015) and their migration routes (Luschi et al. 2006, de Wet 2012, Harris et al. 2018). Three migratory corridors have been proposed by Harris et al. (2018) for both loggerheads and leatherbacks: loggerheads use either the coast of southern Mozambique (Mozambique Channel), across the Mozambique Channel to Northern Madagascar (Malagasy Corridor), or along the South African coast to the Agulhas Banks (Agulhas Corridor), while leatherbacks utilize the extended Mozambique Corridor, Agulhas-Retroreflection Corridor, or Agulhas-Benguela Corridor."

The movement patterns of these nesting turtles in the SWIO could be assessed through the epibiont communities, and the stable isotope signatures of the turtle's epidermis tissue ($\delta^{13}C$ and $\delta^{15}N$), and epibiotic barnacles ($\delta^{18}O$ and $\delta^{13}C$). Based on the conceptual model of epibiosis (Frick and Pfaller 2013, Figure 1.1), epibiont communities should be representative of the regions in which the host turtles occupy as part of their migration to foraging grounds. Furthermore, turtles foraging in different migratory corridors will incorporate distinct isotopic signatures of these regions in their diet (Hobson 1999, 2008). For leatherback sea turtles in this region isotopic analysis of turtle epidermis tissue ($\delta^{13}C$ and $\delta^{15}N$) has been successfully used to delineate between coastal and oceanic forging (Robinson et al. 2016). Finally, isotopic analysis of the epibiotic barnacles on turtles should show movement of the host to different geographically distinct seawater isoscapes (Detjen et al. 2015). Using these complementary intrinsic methods, I aim to describe distribution nesting loggerhead sea turtles in the SWIO.

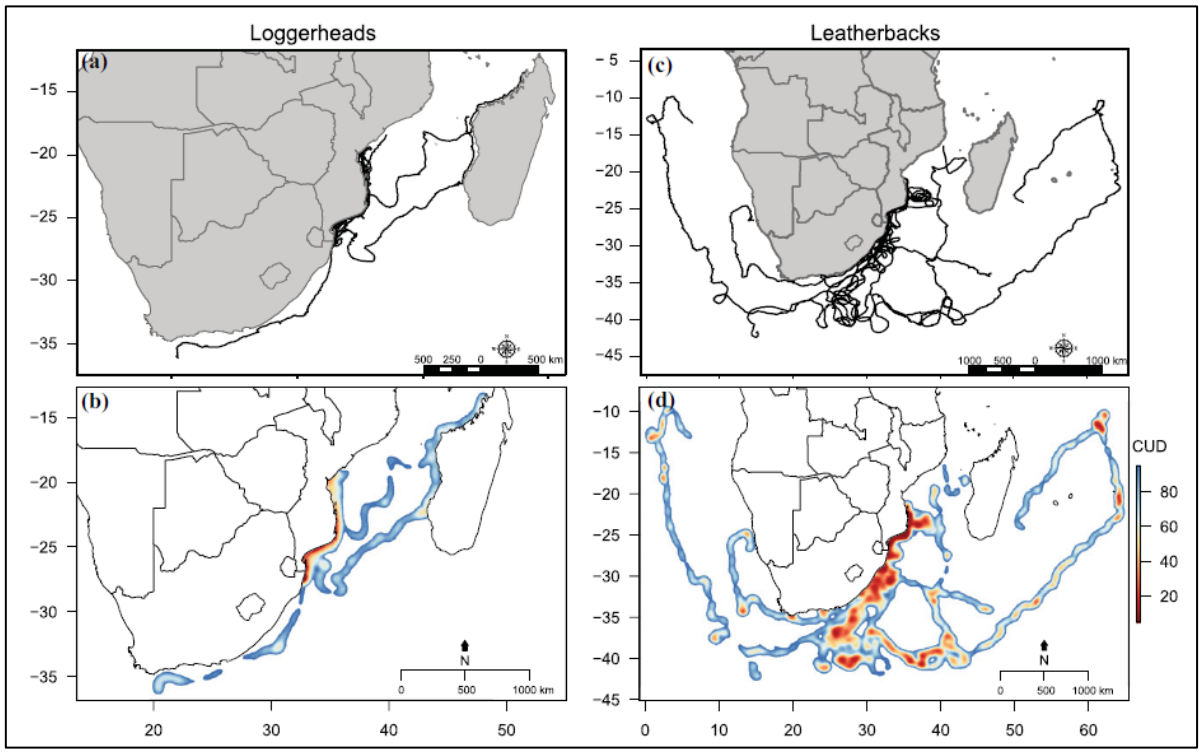

Figure 1.4: Migration routes of nesting **(A** and **B)** loggerhead (*Caretta caretta)* and **(C** and **D)** leatherback (*Dermochelys coriacea*) in the southwestern Indian Ocean represented by satellite tracks **(A** and **C)** and migratory corridors **(B** and **D).** From Harris et al. (2018).

1.6 Rationale and thesis outline

A range of methods have been applied to investigate sea turtle movement in the SWIO loggerhead sea population including mark-recapture techniques (Hughes 1974, Thorson et al. 2012), genetic methods (Shamblin et al. 2014), and satellite telemetry (Harris et al. 2015). They have led to important insights in turtle movement ecology; however, all these methods also have shortcomings. Mark-recapture studies tend to have low return rates, which decreases their accuracy for population-level estimates (Oosthuizen et al. 2010). Genetic methods are effective in resolving population structure, but do not inform about contemporary movement patterns (Bowen and Karl 2007). Satellite telemetry is very informative for tracking movement, but it is very expensive and not feasible for a large number of individuals, and losses or malfunction of loggers are common problems (Hays et al. 2007, Hebblewhite and Haydon 2010).

The limitations of tracking the movement of sea turtles both globally and in the SWIO necessitates additional studies to ascertain population-level distribution. This broader scale information should assist with the design of effective management strategies based on a larger sample size and could be expanded to represent different size class distributions. It is even more powerful when combined with information on the condition of the turtles. The results are not limited to nesting females, but any stranding or in-water study could apply the same low-cost methods. This study aims to develop a cost-effective methodology to determine turtle distribution and habitat use patterns for an enhanced fraction of nesting loggerhead and leatherback turtles nesting in South Africa. It will draw on our existing information on satellite tracked females, as well as regional maps of habitat distribution and isotherms and oxygen isoscapes. The methods will bring together different tools to fills current gaps in knowledge, driven by under-sampling due to research cost. The results of this study will be translated into GIS layers to identify proportions of the adult female population occupying different parts of the SWIO, which could assist in national and regional marine spatial planning initiatives.

This study focuses on nesting female loggerhead turtles, which are logistically more practical to sample. The health of females directly affects the fitness of the offspring (Perrault et al. 2012), which makes this cohort disproportionally important for the population. This project has three main aims: (1) To assess turtle health through body

condition and epibiont assemblages; (2) To determine habitat use through complementary assessments of epibiont communities, carbon and nitrogen isotope ratios of turtle epidermis tissue; and (3) Examine patterns using carbon and oxygen isotope ratios of epibiotic barnacle shells (Figure 1.5). The thesis structure is outlined below.

Chapter 1 provides a brief literature review outlining the current trends in tracking migratory marine fauna, specifically sea turtles and the roles they play in the ecosystem**.** The remaining thesis chapters are written as stand-alone research articles, because of this *there is some overlap with the contents of the introduction and references in each subsequent chapter*.

Chapter 2 assesses the relationship between epibiont communities of nesting loggerhead sea turtles in relation to their body condition. It is hypothesised that if physical attributes, like swimming speed or ability to self-clean, are drivers of epibiont load, that even nesting turtles in relatively poorer body condition will have a higher abundance of epibionts than those in good condition.

Chapter 3 aims to use $\delta^{13}C$ and $\delta^{15}N$ and complementary epibiont data to determine habitat patterns of loggerhead sea turtles. It is hypothesized that geographical and ecological overlap between the host and its epibionts, explains the differences in epibiont assemblages on turtles that forage in oceanic and neritic habitats. Along with this, the niche widths determined from the isotope signatures are compared among loggerheads with different body conditions. It is hypothesised that dietary differences, as indicated by the isotopic niche, drives turtle body condition where individuals foraging in distinct habitats have different body conditions.

Chapter 4 uses $\delta^{18}O$ and $\delta^{13}C$ from epibiotic barnacles to examine the movement patterns of loggerhead sea turtles. Specifically $\delta^{18}O$ will be isotopically mapped to areas of known turtle distribution. As the sea turtle migrates from feeding to nesting grounds the barnacles grow and fractionate oxygen from the surrounding seawater in their $CaCO_3$ shell. Using this method, the movement of the host can be tracked through oceanic isoscapes both spatially and temporally.

Chapter 5 concludes all the findings together in a synthesis of the above chapters. The major findings are briefly discussed in context with each other. This chapter also

looks at potential for these methods to be used on other sea turtle species and marine fauna.

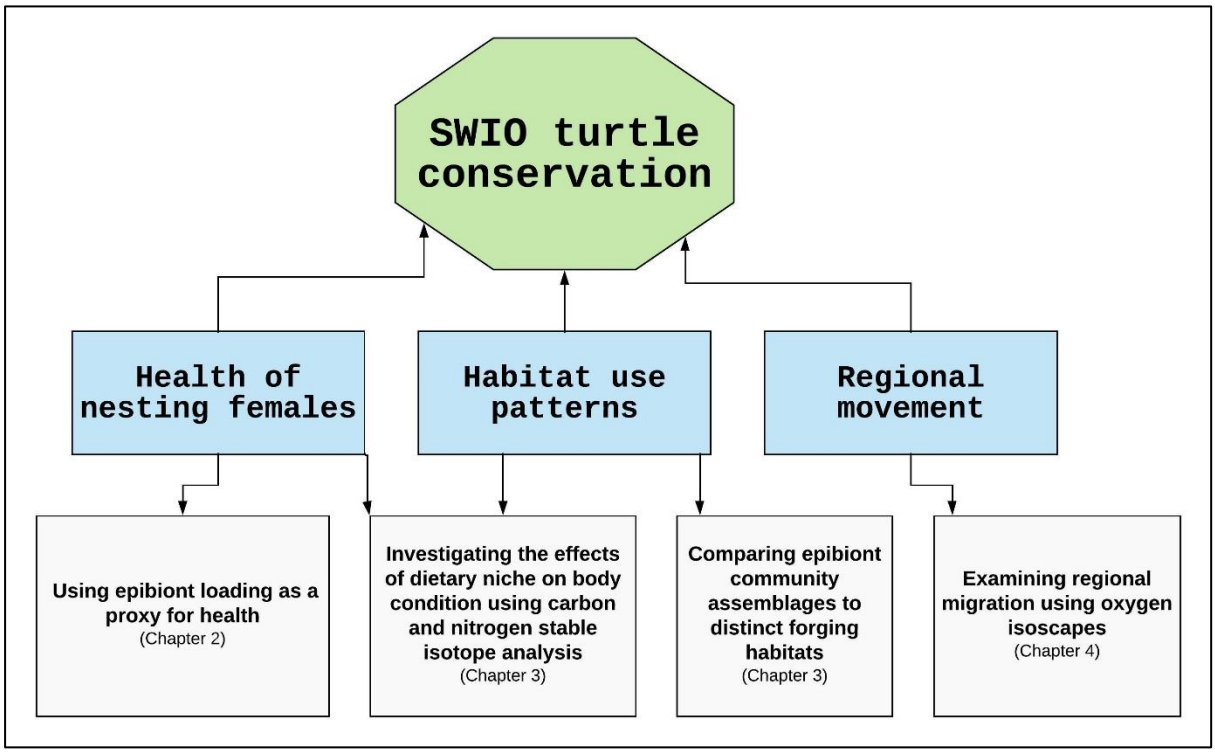

Figure 1.5: Schematic diagram outlining the main aspects of this thesis and how these will be addressed.

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Chapter 2

A cost-effective method of monitoring nesting loggerhead sea turtle (*Caretta caretta*) body condition based on their epibiont community

2.1 Abstract

Sea turtles are long-lived migratory species that are sensitive to anthropogenic impacts. Their condition reflects that of marine ecosystems and can serve as an indicator for environmental monitoring. This study examined the potential of using different characteristics of turtle epibiont communities to infer a turtle's body condition. To achieve this, 60 nesting loggerhead sea turtles (*Carretta caretta*) were sampled during the 2015/16 and 2016/17 nesting seasons and categorised into five body conditions ranging from very poor to very good. Injuries, skin deformities and plastron shape were used for this classification. Epibionts were collected from the same turtles and identified. Epibiont species richness, diversity, community structure and barnacle loading were compared among body conditions to assess their potential use as indicators of turtle body condition. Epibiont communities were influenced by body condition as both species richness and abundance increased with a decline in turtle body condition. Barnacle epibionts showed great variation among different body conditions, having significantly higher numbers on turtles in poor condition. All parameters in this study showed similar distinctive relationships with body condition of loggerhead turtles from poor to very good body condition. This emphasises the value of epibionts as effective and reliable indicator of individual health/condition. These results suggest that various characteristics of turtle epibiont communities can be used as an indicators of turtle body condition for nesting loggerhead turtles, providing a cost-effective tool that can be applied in to sea turtle monitoring programmes.

Note: The basic body condition, barnacle load and epibiont functional groups were analysed as part of my BSc Honours project. However, additional data were added and completely reanalysed for this chapter and the epibiont species were described to lowest taxonomic level.

2.2 Introduction

Sea turtles form an integral part of coastal and marine environments as they perform a variety of ecological services, including being nutrient transporters and providing habitat for epibionts (Heithaus 2013, Le Gouvello et al. 2017). However, globally sea turtle species have been severely impacted by human activities and consequently many sea turtle populations are in decline (Gibbon et al. 2000, Witherington et al., 2009). Most sea turtle monitoring programmes focus on assessing population trends (Nel et al. 2013) or causes of mortality (Seminoff et al. 2003). Assessments of the health of individual turtles are rare, despite their importance for improving conservation efforts. The health of nesting females is particularly important as this can affect reproductive output and hatchling survival (Perrault et al. 2012).

Ecologists have commonly resorted to using body condition indices as an estimation of an animal's nutritional state and a proxy for its health (Ullman-Culleré and Foltz 1999) or reproductive status (Guinet et al. 1998). Health is a clinical examination involving laboratory tests as oppose to body condition which is a general examination that can be readily determined. Monitoring sea turtle health using physiological or metabolic approaches can be logistically challenging in remote areas and prohibitively expensive for developing countries. It is therefore useful to develop low-cost methods to monitor sea turtle body condition as a proxy of their health, which are logistically feasible yet reliable and accurate. A number of methods have been implemented to determine the body condition of sea turtles. These include mass-length relationships (Bjorndal et al. 2000; Jessop et al. 2004), blood sample analysis (Stamper et al. 2005) and epibiont load (Nájera-Hillman et al. 2012; Deem et al. 2009). Weighing adult hard-shelled turtles in the field is not easy, and for leatherbacks near-impossible, whereas blood sample analysis is expensive. Sampling of epibionts is thus considered logistically most feasible and cost effective.

The iSimangaliso Wetland Park, a UNESCO World Heritage Site, is situated on the east coast of South Africa and protects the nesting beaches for leatherback (*Dermochelys coriacea*) and loggerhead (*Caretta caretta*) sea turtles. Little is known about the body condition of nesting females, despite carapace length and width being routinely monitored, and to date no assessment of health or body condition has been made. Furthermore, sea turtle epibionts from this region are poorly studied with only

one report by Hughes (1974), which provides a brief description of six epibiont species. A comprehensive assessment of epibiont communities for nesting sea turtles in this region therefore provides a valuable biodiversity baseline in its own right and strengthens insights into the ecological roles of sea turtles as distributing agents and substrates for other species.

The aim of this study was to assess the relationship between body conditions and macrofaunal epibiont communities of nesting loggerhead sea turtles and develop recommendations for a reliable sampling protocol to monitor turtle body condition as a proxy of turtle health. It is hypothesised that if physical attributes, like swimming speed or ability to self-clean, are drivers of epibiont load, that even nesting turtles in relatively poorer body condition will have a higher abundance of epibionts than those in good condition. This was tested through three objectives: (1) To quantify turtle body condition through the development of a body condition index; (2) To assess the relationship between turtle body condition and epibiont community assemblages based on species richness, diversity, and abundance; and (3) To assess the relationship between body condition and epibiotic barnacle loading. This is the first study to report on these trends for loggerhead turtles in the South West Indian Ocean region.

2.3 Methods and materials

2.3.1 Sampling area and seasons

The northern beaches of the iSimangaliso Wetland Park, South Africa (Figure 1.2), are frequented by loggerhead sea turtles during their nesting periods in the austral summer (November – February). This study focused on the area with highest density of nesting loggerhead turtles, north of the Bhanga Nek field station (Nel et al. 2013). Nesting loggerhead turtles encountered during nightly monitoring patrols along a 6-km stretch of beach were sampled for this study. These patrols took place over two nesting seasons from 1st of December 2015 to 26th of January 2016, and again from 28th of November 2016 to 10th of February 2017.

2.3.2 Calculation of a turtle body condition index

For each female loggerhead turtle encountered, location and basic morphometrics including straight and curved carapace length and width were recorded following a

standardized protocol used in the South African turtle monitoring programme since 1973 (Nel et al. 2013). Where present, flipper tags and internal passive integrated transponders were used to identify individuals so as to avoid re-sampling.

The body condition of the nesting loggerheads was quantified using a composite index, incorporating information on: (1) the shape of the plastron; (2) injuries; and (3) visible skin conditions. For each turtle, these parameters were scored on a scale from 1 to 5 using specific criteria (Table 2.1) with 1 being an individual in suboptimal body condition and 5 reflecting optimal body condition. The composite body condition index was calculated by adding weighted scores for plastron shape, injuries and skin abnormalities to contribute 65, 25 and 10% respectively. The parameters were weighted based on the perceived effect on body condition, as plastron shape is the is the best predictor (Thomson et al. 2012) it contributed the most to determining body condition. The injuries and skin abnormalities have been used elsewhere to classify healthy and unhealthy individuals (Deem et al. 2009, Flint et al. 2010). Each sea turtle was thereby assigned to a body condition class as either very poor, poor, average, good or very good (Table 2.1).

Plastron shape was used because Thomson et al. (2009) demonstrated that this is a good proxy for turtle body condition, and a suitable alternative to conventional mass/length ratios, Plastron shape was measured, since weighing turtles was not logistically feasible during foot patrols. Turtles with concave (hollow) plastrons have a lower mass to length ratio than those individuals with convex plastrons. The extent of limb amputations and/or any other apparent wounds, or injuries were also identified and recorded. Injuries that were recent such as small bleeding wounds and superficial scratches were not incorporated in this study as these occurred recently and would not result in a sudden change in body condition. Where possible, the cause of injuries was identified as either natural - shark attacks - or anthropogenic - boat strikes - (serial cuts or cracked carapace) or interactions with fisheries (foul hooks, line or net chafing). Skin abnormalities were categorised on severity based on the size and number of growths as well as rashes.

Table 2.1: The composite body condition index calculated as weighted sum after classifying each loggerhead sea turtle (*Caretta caretta*) according to plastron shape, injuries and skin condition, each scored as five categories (1 to 5) according to set criteria.

	Criteria			Overall condition based on composite scores
Body Condition (Weighting)	A: Plastron Shape (65%)	B: Injuries (25%)	C: Skin Condition (10%)	
1: Very Poor	Very concave	A whole flipper, or more missing	Multiple large lumps or cancers (fibropapillomatosis)	1 – 1.5
2: Poor	Concave	50% to 75% of flipper missing	A medium to large lump or any signs of fibropapillomatosis	1.5 – 2.5
3: Average	Straight	50% to 25% of flipper missing	Small lump(s) or rashes	2.5 – 3.5
4: Good	Convex	25% of flipper missing	Minor rashes or skin conditions	3.5 – 4.5
5: Very good	Bulging, convex	No injuries present	No lumps or skin conditions	4.5 – 5
Index calculation	Score for A* 0.65 + Score for B * 0.25 + Score for C * 0.10			

2.3.3 Epibiont community sampling and processing

Epibionts were carefully scraped from the right side of each turtle's carapace using a plastic paint scraper and stored in vials of 70% ethanol for later identification. Where necessary, sand was washed off the carapace before collection using sea water. After collection, the epibionts were counted and identified to the lowest possible taxonomic level using suitable literature (Day 1967, Griffiths 1976, Monroe 1979, Barnard and Karaman 1991, Epibiont Research Cooperative 2007, Branch et al. 2017).

To examine the relationship between barnacle load and body condition, each turtle was systematically photographed (whole carapace, front left/right, back left/right), and the percentage cover of barnacles on the carapace was estimated using ImageJ software (Schneider et al. 2012).

2.3.4 Statistical analyses

To investigate the relationship between epibiont community assemblages and body condition, a number of univariate and multivariate analyses were used. Only epibiotic macrofauna was used in these analyses because of the ease to quantify abundance (number of individuals per taxa). Species richness was determined for each turtle from the number of taxa per individual. To test for differences in species richness among the different body condition classes, a nonparametric Kruskal-Wallis test was performed since the data failed the assumption of normality (Shapiro-Wilk test; $p < 0.05$). Following a significant test result, the Nemenyi-test with chi-squared approximation for independent samples was performed as a post-hoc test to elucidate the significant differences in species richness between each of the body conditions. The Shannon-Wiener Index was used to assess epibiont community diversity among different body condition groups. This diversity index is a quantitative measure reflecting the species richness and relative abundance of each taxa. Shannon-Weiner Index was calculated per body condition category using untransformed abundance data and number of taxa following the methods of Robinson et al. (2017). Epibiont community data (species-abundance) were transformed using the square root function to weight the contributions of common and rare taxa (Clarke and Warrick 2001). These data were used to create a resemblance matrix based on Bray-Curtis similarities. A non-metric Multi-Dimensional Scaling (MDS) plot was used to visualise similarities among epibiont communities and their relationship with to turtle body condition categories. Differences in community structure between body condition categories were compared using a one-way permutational multivariate analysis of variance (PERMANOVA). The PERMANOVA design employed a Bray-Curtis index of dissimilarity and used 999 permutations of the residuals, with body condition as a fixed factor. This test was chosen because of its robustness to differences in multivariate dispersion (Anderson and Walsh 2013). A SIMPER analysis was used to examine the contribution of individual epibiont taxa to dissimilarities between significant clusters identified between body condition categories (Clarke and Gorley 2006).

To determine if there were differences in barnacle loading (carapace % cover) among the different body condition categories, a nonparametric Kruskal-Wallis test was performed because the data failed the assumption of normality (Shapiro-Wilk test; $p < 0.05$). Following a significant test result, the Nemenyi-test with chi-squared

approximation for independent samples was performed as a post-hoc test to elucidate the significant differences in barnacle loading between each of the body conditions.

All results with $p < 0.05$ were considered statistically significant. The PERMANOVA+ add-on (Anderson et al. 2008) to PRIMER v6 software (Clarke & Gorley 2006) was used to carry out multivariate analyses. Univariate statistical tests were performed in R version 3.5.1 (R Core Team 2018).

2.4 Results

2.4.1 Body condition

A total of 60 nesting loggerhead sea turtles were assessed for their body condition (and epibiont assemblages) in this study, 48 and 12 from the 2015/16 and 2016/17 nesting seasons, respectively. None of them had a very poor body condition, 11 turtles were categorized as poor, 21 as average, 18 as good and 10 as very good condition. Eight individuals (13 %) had up to 25 % of a flipper missing. Of the injuries that were identified, two were attributed to boat strikes and three to shark attacks. None of the turtles showed external symptoms of fibropapillomatosis, however, 8 of the individuals (13 %) had minor skin conditions that ranged from red patchy skin to small growths.

2.4.2 Epibiont community assemblages

From the 60 nesting loggerhead sea turtles sampled, we collected a total of 28 distinguishable epibiont species from nine systematic groups (Table 2.2). Epibiont species richness varied among turtle body conditions (Kruskal-Wallis $X^2 = 13.154$, $df = 3$, $p < 0.005$; Figure 2.1), showing an increase of species richness with deterioration in body condition. The post-hoc Nemenyi-test showed significant differences between poor and very good ($p < 0.005$), and average and very good ($p < 0.05$) body conditions. The Shannon-Wiener Index indicated an increase in diversity of epibiont communities with an increase in body condition (Table 2.3). The high Shannon–Wiener Index (2.38) of epibiont communities on very good body condition turtles suggests that the epibionts on the carapace had higher evenness than other groups. There was an increase in the abundance of epibionts per host with a decrease in body condition (Table 2.3).

Table 2.2: Epibiont identification and abundance (mean ± standard deviation) per host condition category (poor, average, good, very good) of loggerhead turtles (*Caretta caretta*) nesting in the iSimangaliso Wetland Park, South Africa.

Systematic Group and epibiont taxa		**Mean ± Standard Deviation of Epibionts**			
		Poor *n* = 11	**Average *n* = 21**	**Good *n* = 18**	**Very Good *n* = 10**
Annelida					
Polychaeta	Nereidae	0.18 ± 0.40	0.05 ± 0.22	0.18 ± 0.33	0.20 ± 0.63
	Phyllodocidae	0	0.10 ± 0.44	0	0
	Syllidae	0	0.05 ± 0.22	0.06 ± 0.24	0.1 ± 0.32
Crustacea					
Amphipoda	*Ampithoe falsa*	0.45 ± 1.04	0.05 ± 0.22	0.24 ± 0.97	0.2 ± 0.63
	Caprella equilibra	0	0.38 ± 1.02	0	0
	Ericthonius ledoyeri	0.91 ± 2.21	0	0	0
	Hyachelia tortugae	2.09 ± 4.50	1.00 ± 3.77	2.18 ± 4.41	1.00 ± 2.16
	Hyale grandicornis	4.82 ± 12.77	5.43 ± 11.27	6.76 ± 10.26	0.50 ± 1.58
	Hyale maroubrae	1.18 ± 2.27	0	0.12 ± 0.49	0.30 ± 0.95
	Podocerus africanus	18.27 ± 49.66	5.47 ± 14.27	0.82 ± 3.15	0.40 ± 0.97
	Podocerus pyurae	0.36 ± 1.21	0.14 ± 0.65	0.76 ± 1.86	0.40 ± 1.26
	Stenothoe adhaerens	0	0	0.35 ± 0.86	0
Brachyura	*Planes major*	0.27 ± 0.65	0	0	0
Cirripedia	*Balanus amphrite*	4.00 ± 5.03	1.14 ± 1.46	0.59 ± 0.94	0.80 ± 1.93
	Chelolepas cheloniae	1.09 ± 1.58	0.14 ± 0.65	0	0
	Chelonibia caretta	1.00 ± 1.41	0.71 ± 1.06	0.29 ± 0.69	0.20 ± 0.63
	Chelonibia testudinaria	36.00 ± 28.67	12.48 ± 10.41	2.94 ± 2.33	1.70 ± 1.15
	Conchoderma virgatum.	0.45 ± 1.50	0.10 ± 0.43	0.29 ± 0.69	0
	Lepas spp.	0.73 ± 1.27	0.90 ± 1.34	1.00 ± 1.77	0.40 ± 0.84

	Platylepas hexastylos	0	0.24 ± 0.7	0.35 ± 1.06	0.40 ± 1.26
Copepoda	*Balaenophilus manatorum*	2.36 ± 3.23	0.62 ± 0.92	1.29 ± 1.99	0.8 ± 1.93
Isopoda	*Exosphaeroma* sp.	0.45 ± 0.69	0.05 ± 0.22	0	0.20 ± 0.63
Tanaidea	*Hexapleomera robusta*	2.09 ± 4.08	0.81 ± 1.21	0.41 ± 0.94	0
Mollusca					
Gastropoda	Unidentified gastropod	0.18 ± 0.60	0.95 ± 0.30	0	0
Chlorophyta	*Ulva* sp. [#]	4	7	7	2
	Unidentified green algae [#]	2	1	0	2
Phaeophyceae	*Ectocarpus* spp. [#]	10	16	15	9
Rhodophyta	Corallinales [#]	10	9	4	3

[#] - indicates presence only. Individual counts were not undertaken

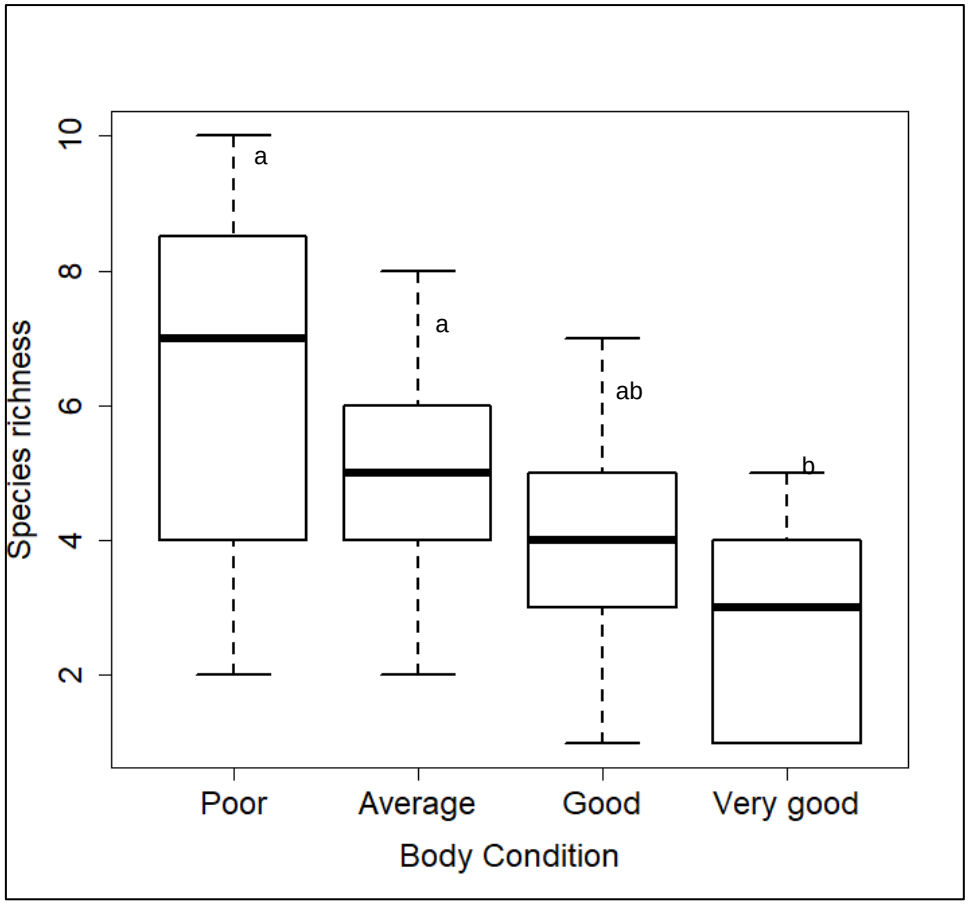

Figure 2.1: Box plot of epibiont species richness on the carapace of nesting loggerhead sea turtles (*Caretta caretta*) categorized by body condition. Bold lines indicate medians, boxes represent 25th and 75th percentiles, respectively and the upper and lower whiskers extend to data points that are 1.5 times the interquartile range from the box. Lowercase letters indicate post hoc groupings.

Table 2.3: Total number of taxa, mean abundance of epibionts and diversity index of epibiont communities of loggerhead sea turtles (*Caretta caretta*) with different body conditions nesting in the iSimangaliso Wetland Park, South Africa.

Body condition	Total number of taxa	Mean number of epibionts per host	Shannon-Weiner Index
Poor	17	76.27	1.73
Average	18	29.81	1.81
Good	17	18.59	2.14
Very Good	14	7.4	2.38

Epibiont community structure gradually changed with the deterioration of host turtle body condition (MDS; Figure 2.2). PERMANOVA identified statistically different epibiont communities among the four turtle body condition groupings (df = 3, 56, F= 2.8172, $p < 0.005$). Additionally, post-hoc pairwise comparisons of the PERMANOVA test revealed that epibiont communities of poor condition turtles were statistically different from good ($p < 0.005$) and very good ($p < 0.005$) body condition turtles. While, turtles with average body conditions were statistically different from good ($p < 0.05$) and very good ($p < 0.005$). However, there were no significant differences between poor and average, and good and very good body conditions (Table 2.4), suggesting a gradual change of community structure with the deterioration of turtle condition (Figure 2.2). SIMPER analysis showed that the dissimilarity between turtle body condition groups was mainly due to five epibiont taxa: *Chelonibia testudinaria*; *Podocerus africanus*; *Hyale grandicornis; Balanus amphrite*; and *Balaenophilus manatorum* (Table 2.5). These species were thus driving the changes in epibiont community structure among body conditions.

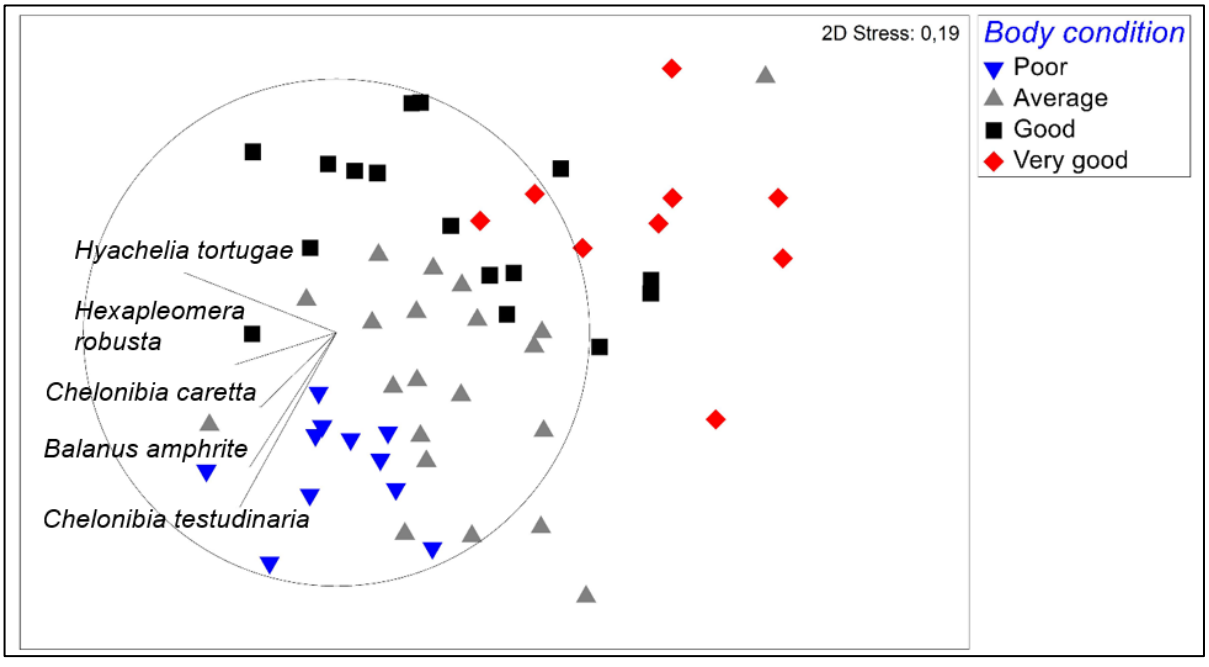

Figure 2.2: Multidimensional scaling (MDS) of epibiont community abundance data (based on Bray-Curtis similarities) collected from the carapaces of nesting loggerhead sea turtles (*Caretta caretta*) grouped according to different body condition categories (poor, average, good, very good). The lines indicate which epibiont taxa most influenced the grouping based off Spearman Rank correlations. Longer lines correspond to higher R^2 values and indicate which species had the greatest influence on driving dissimilarities among communities.

Table 2.4: *P* values for the PERMANOVA pairwise comparisons of epibiont communities of nesting loggerhead sea turtles (*Caretta caretta*) with different body conditions (poor, average, good, very good).

Body condition	Average	Good	Very Good
Poor	0.1339	0.001	0.001
Average		0.016	0.001
Good			0.2498

Table 2.5: Results of the SIMPER used to identify the relative contribution of the top three epibiont species causing dissimilarity between nesting loggerhead sea turtle (*Caretta caretta*) epibiont communities with different body condition categories (poor; average; good; very good).

Body condition comparison	Epibiont taxa	Average dissimilarity	Contribution % to similarity
Poor vs average	*Chelonibia testudinaria*	10.49	17.37
	Podocerus africanus	9.38	15.54
	Hyale grandicornis	6.44	10.66
Poor vs good #	*Chelonibia testudinaria*	17.38	23.65
	Podocerus africanus	8.35	11.36
	Hyale grandicornis	8.04	10.93
Poor vs very good #	*Chelonibia testudinaria*	20.45	26.63
	Podocerus africanus	9.42	12.26
	Balanus amphrite	6.46	8.41
Average vs good #	*Chelonibia testudinaria*	12.01	17.67
	Hyale grandicornis	11.02	16.22
	Podocerus africanus	6.69	9.84
Average vs very good #	*Chelonibia testudinaria*	14.92	20.37
	Hyale grandicornis	8.75	11.94
	Podocerus africanus	7.73	10.54
Good vs very good	*Hyale grandicornis*	12.30	16.87
	Chelonibia testudinaria	7.66	10.52
	Balaenophilus manatorum	7.39	10.14

#- These communities were significantly different from each other as indicated by the PERMANOVA pair-wise comparisons.

2.4.4 Epibiotic barnacle load

The percentage cover of barnacles also differed significantly among turtle body condition categories (Kruskal-Wallis $\chi^2 = 23.798$, $df = 3$, $p < 0.001$; Figure 2.3), with significant differences between poor and good, and poor and very good conditions (Nemenyi-test, $p < 0.05$). These results reflect a gradual increase in barnacle loading with a subsequent deterioration in turtle body condition.

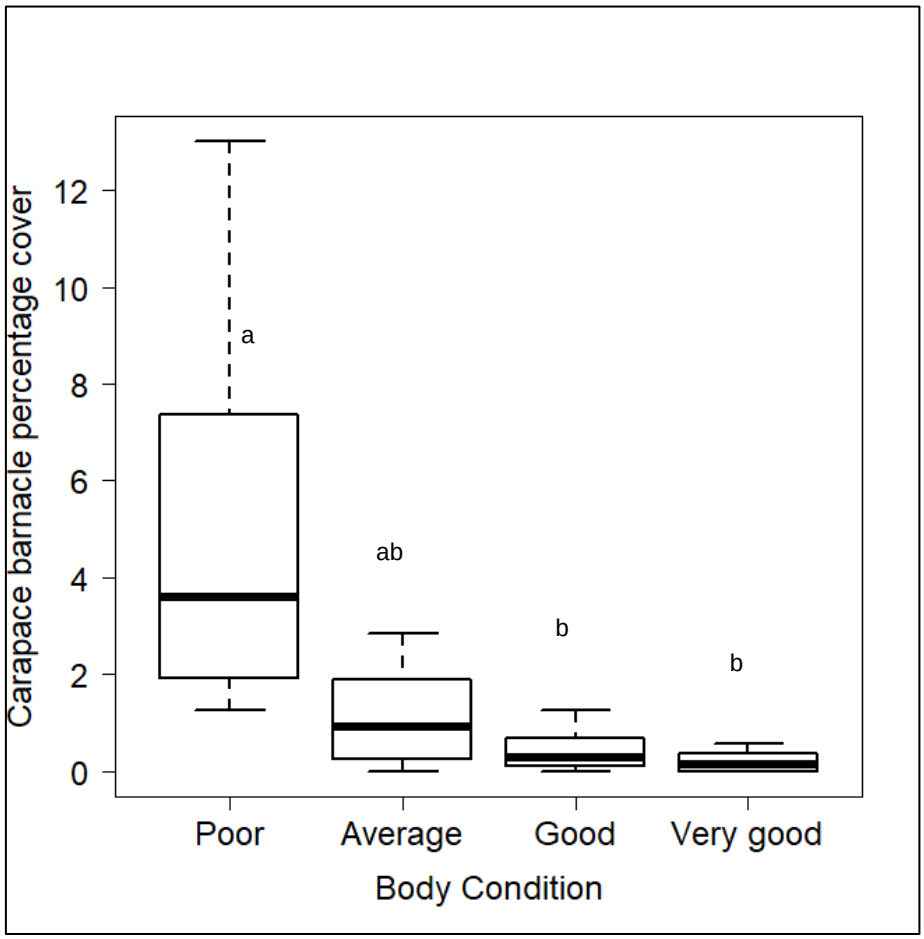

Figure 2.3: Box plot of percentage coverage of the turtle barnacle *Chelonibia testudinaria* on the carapace of nesting loggerhead sea turtles (*Caretta caretta*) categorized by turtle body condition. Bold lines indicate medians, boxes represent 25th and 75th percentiles, respectively and the upper and lower whiskers extend to data points that are 1.5 times the interquartile range from the box. Lowercase letters indicate post hoc groupings.

2.5 Discussion

This study is the first in the South-Western Indian Ocean (SWIO) region to estimate body condition of nesting loggerhead sea turtles using external characteristics. It was found that epibiotic species richness, diversity, community structure and barnacle cover gradually change in a consistent manner with deteriorating turtle body condition. The hypothesis that turtles in compromised body conditions have higher epibiont loading was shown to hold true even for nesting females, which are in generally good health, since the total abundance of epibionts as well as barnacle loading gradually increased as body condition deteriorated. The probable mechanism of these patterns is that a turtle of poor health swims slower, which facilitates the settlement of free-swimming larvae on its surface, thereby increasing epibiont loading. Turtles in poor condition also have a reduced ability to self-groom (Deem et al. 2009) by actively rubbing their carapace against hard structures, such as reefs or rocks, to remove epibionts (Heithaus et al. 2002). This study therefore provides supportive evidence for the use of turtle epibionts as cost-efficient and reliable indicators of sea turtle condition and health.

2.5.1 Turtle body condition

The body condition of an animal is a reference to its energetic state and performance, since an individual in good condition is assumed to have higher energy reserves than one in poor condition (Labrada-Martagon et al. 2010). Body condition is thus a proxy for an animal's health condition and can be quantified by assessing external parameters of an animal (Bjorndal et al. 2000, Labrada-Martagon et al. 2010). By contrast, quantifying the health state of an individual refers to its physiological state and would require a much more intensive veterinary assessment, including blood testing (for haematological levels, plasma biochemistry, pathogen sampling etc.) (Li et al. 2015). It is therefore often not logistically feasible to address the health of wild animals directly, but instead use indicators and proxies, such as body condition to assess if a population thrives or diminishes based on the health of its individuals (Stevenson and Woods 2006). In this context, it is key to create suitable and unambiguous criteria to determine the body condition of wild animals.

In this study, turtle body condition was determined using plastron shape, injuries and skin condition. This method is based on observations that turtles with a concave

plastron generally have a lower than average mass-length ratio, while those with a bulging, convex plastron generally have a higher than average mass-length ratio (Thomson et al. 2009). It has also been suggested that the tissue bulk around the neck and shoulders can be used to rapidly assess the body condition of turtles by measuring neck circumference and skin thickness (Whiting et al. 2007, Flint et al. 2010). This method was originally also applied in this study, but results are not presented here, since both of these measurements were highly biased by the skill of the researcher and the behaviour and position of the turtle. The overall body condition of nesting turtles was rated as “good” based on physical examinations of the nesting females and that none of the females sampled had “very poor” body condition. This was not surprising as nesting females represent a ‘healthy’ cohort of the population, since they have to build up energy reserves to undergo migration from feeding to nesting grounds and develop eggs (Rivalan et al. 2005). Although this study only examined nesting individuals, notable differences in body condition were still observed.

Anthropogenic threats to sea turtles like boat strikes, fisheries interactions and pollution are a cause for concern and can lead to a decline in population numbers (Bolten et al. 2011). However, the results from this study showed that nesting turtles from South Africa did not show excessive signs of these impacts. Anthropogenically-induced injuries from boat strikes or fisheries were low at ~2.5 %, which is similar to records from other regions. Seminoff et al. (2003) and Denkinger et al. (2013) reported anthropogenic injuries to green turtles (*Chelonia mydas*) in Mexico and the Galapagos at a rate 4 % and 3.7 %, respectively. The rate of detection of severe injuries like boat strikes is often lower in nesting turtles than that of stranded individuals (Denkinger et al. 2013), as strandings include more species and a larger proportion of the population than the relatively healthy sub-sample of nesting females. Our results thus do not reflect the full extent of anthropogenic impacts, but provide a benchmark for future monitoring.

None of the turtles sampled in this study showed external symptoms of fibropapillomatosis and to date only one confirmed case of fibropapillomatosis has been reported for a South African green turtle (Nel, unpublished data). Fibropapillomatosis occurs more frequently in green turtles although it has been observed in the other chelonid species, particularly those that frequent polluted areas or of poor water quality (Herbst and Jacobson 2002, Greenblatt et al. 2004). In Moreton

Bay (Eastern Australia), where levels of terrestrial runoff and other pollutants are high (Aguirre et al. 1999), 40-70% of green and loggerhead individuals are affected by fibropapillomatosis. The absence of this disease here is thus suggestive of a reasonably healthy ocean environment.

2.5.2 Epibionts assemblages and body condition

In a global context, the epibiont species richness for the South African loggerhead population was found to be low compared to other regions. In this study, 28 epibiont species were recorded, while 86 have been recorded from Georgia, USA (Frick et al. 1998), and 40 from the Mediterranean Sea (Domenech et al. 2015). These differences are likely due to abiotic and biotic factors. Rough sea conditions might decrease the ability of attachment of epibionts, thereby only allowing the hardiest epibionts to occur in dynamic regions, such as the There was a vast increase in the epibionts found from this region when comparing this study (28 species) to the historical report by Hughes (1974) who reported 6 species occurring on nesting females. However, this could be due to sampling effort as this study actively searched for epibionts and used a microscope to identify species.

The diversity of epibiont communities among the groups was different, with the turtles in very good body condition having the highest Shannon-Weiner Index. However, the index is based on the number of taxa and their relative abundances. Ecological theory describes the intermediate disturbance hypothesis as a mechanism that promotes diversity (Connell 1978). This hypothesis states that in a community, diversity is maximised when there is intermediate frequency and intensity of disturbances. Sea turtles in very good body condition could expose their epibiont communities to these types of disturbances through self-grooming. This mechanism prevents species becoming dominant, thereby promoting evenness. The abundance of all epibiotic taxa on turtles in very good body condition was comparatively low which can result in a high Shannon-Weiner Diversity number (Tuomisto 2012). Overall the diversity index in this study was relatively high when comparing among other Cheloniidae from the Eastern Tropical Pacific. Green and olive ridley (*Lepidochelys olivacea*) turtles in this region had Shannon-Wiener Diversity indices of 0.93 and 1.37, respectively (Robinson et al. 2017).

We found notable differences in the epibiotic community structure that correspond with differences in the body condition of their sea turtle hosts. The SIMPER analysis identified *Balanus amphrite, Chelonibia testudinaria, Hyale grandicornis,* and *Podocerus africanus* as the main species driving the community assemblages between significantly different body condition categories. These species occur in relatively higher abundances and frequencies on all body condition categories. Of all the epibiont groups examined, barnacles contributed most to the dissimilarity between the body conditions. Higher loads of *Chelonibia testudinaria*, both in terms of abundance and carapace percentage cover, were associated with a poorer turtle body condition, which has also been noted in other studies. Green (1998) observed emaciated, near-dead and dead green turtles "smothered" in barnacles. While, Flint et al. (2010) found that immature green sea turtles with more than 20 barnacles on the plastron were more likely to be unhealthy than those individuals absent of barnacles. The barnacle epibiont loading on the sea turtles sampled in this study was however considerably low, ranging from 0 to 93 individuals per turtle. In comparison, migratory and resident loggerheads in North Carolina, USA, were found to have accumulations of *Chelonibia testudinaria* on their carapace ranging from 6 to 386 and 97 to 3667 in abundance, respectively (Stamper et al. 2005). There was an increase in the frequency of occurrence of *Chelonibia testudinaria* from this study with that of Hughes (1974). Hughes (1974) found a 47 % occurrence while this study found an 91 % occurrence. By contrast, *Chelonibia testudinaria* was found to be omnipresent on nesting loggerheads in Georgia, USA (Frick et al. 1998).

The validity of the relationship between health of a turtle and its epibiont/barnacle loading has been debated in previous assessments for other sea turtles (Stamper et al. 2005, Deem et al. 2009, Flint et al 2010, Nájera-Hillman et al. 2012). Determining the factors that affect epibiont frequency and occurrence on sea turtles is challenging because turtle behaviour, which is influenced by predation, physical stress and competition, can also influence epibiont assemblages (Frick et al. 2000, Nájera-Hillman et al. 2012). Understanding the cause and effect relationship between a high epibiont load and body condition is thus difficult to determine *in situ*, and the data from this study are interpreted in a correlative manner and do not intend to draw conclusions on causality.

Epibiont community succession on the turtle's carapace can explain why turtles with a poor body condition have a greater number of epibionts and higher species richness. Using *in situ* experiments, Frick et al. (2002) examined how epibiont communities change temporally over a nesting season. The epibiont community is generally initiated when barnacles colonize a turtle, they do this by identifying suitable habitat through chemical receptors of the carapace (Zardus et al. 2008). These are "pioneer" species that facilitate the colonization of other epibionts through the creation of microhabitats by increasing surface area and altering water flow patterns on the carapace (Pfaller et al. 2006), secondary taxa such as algae and hydrozoans can then colonize. Additional taxa such as amphipods and tanaids can colonize the carapace as they have a strong association with algal mats (personal observation). This community succession can be identified in the MDS plot that shows gradual change in epibiont assemblages over body condition categories. One of the main drivers of this change was *Chelonibia testudinaria*, which was also identified as causing the most dissimilarity in body condition.

It has been suggested that heavy infestations by epibionts may be detrimental to turtle health (Greenblatt et al. 2004) as epibionts increase the energetic cost of the host to swim and forage (George 1997). Ultimately, turtle health can be compromised by increased weight and drag, which in some cases is excessively large. For example, green turtles in Uruguay were found to have bio-fouling from an invasive gastropod (*Rapana venosa*) that accounted for up to 20 % of the turtle's mass (Lezama et al. 2013). For reproductive females, the associated cumulative energetic cost is particularly compromising during long migrations of thousands of kilometres between feeding and nesting habitats. Furthermore, burrowing barnacles embedded in host turtles can cause deep-tissue wounds that cause erosion/necrosis of the underlying bone (Frick and Zardus 2010). When barnacles occur on the beak, this can cause deformations that compromise foraging ability and in severe cases even cause death (Green 1998). The potential effects of epibionts to sea turtle health noted in this study were: (1) the accumulation of epibionts that could increase drag; (2) burrowing barnacles that were deeply buried in the carapace; and (3) barnacles on the beak causing difficulty in foraging.

2.5.3 Future research and applications

Application of epibiont sea turtle research has a wider scope than just assessing the health status of the host (Frick and Pfaller 2013). For example, epibionts can be applied to study the migratory routes or habitat use of their host (Pfaller et al. 2014, Domenech et al. 2015), since epibiont communities reflect the biogeographic regions the turtle has been to (see Chapters 3). Furthermore, certain epibionts, such as barnacles, contain traceable isotopic signatures in their shells that can be matched with specific ocean regions, which allows to trace a turtle's migratory history (Detjen et al 2015, Chapter 4). Sea turtles can and should be studied as potential vectors of invasive species (Harding et al. 2011), especially those populations that have long migratory routes between nesting and foraging grounds.

2.5.4 Conclusion

This study has shown a consistent relationships between epibiont species richness, epibiont community structure and barnacle loading and turtle body condition. These findings thus suggest that the sampling of epibionts is a non-invasive, logistically easy and cost-effective method to determining the body condition of nesting loggerhead turtles in the South West Indian Ocean. Because of the difficulties in counting and identifying different macrofauna, barnacles can be used as a simple indicator of a sea turtle's body condition. Data collected in this study thereby provide a baseline for future monitoring of turtle and ecosystem health. In a changing ocean environment, it is important to establish baseline observations on ecosystem and organism health in order to detect the impacts of environmental change. As sea turtles are "sentinels of ecosystem health" (Aguirre and Lutz 2004), the early detection of increased injuries or outbreaks of diseases would be indicative of a deterioration in ocean health, especially in regions where water quality is not routinely monitored, and this can facilitate the early mitigation of further impacts.

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Chapter 3

Neritic and oceanic foraging habitats for nesting loggerhead (*Caretta caretta*) sea turtles: Evidence from epibiont community composition and stable isotope analysis

3.1 Abstract

Loggerhead sea turtles (*Caretta caretta*) occupy a wide array of marine habitats throughout their life. Understanding their habitat use provides important information to design effective conservation strategies for this threatened species. This study used a combination of isotopic analysis of turtle epidermis tissue and epibiotic community structure to examine habitat use patterns of females that nest in the iSimangaliso Wetland Park, South Africa. Two foraging groups were identified, based on relative enrichment/depletion of $\delta^{13}C$. Epibiont community structure was significantly different between these two groups, with the open-ocean pelagic species *Lepas* spp. occurring in higher abundances on individuals with depleted $\delta^{13}C$. Three neritic epibionts (*Hyale grandicornis, Hyachelia tortugae* and *Podocerus africanus*) were the other habitat-specific species driving community assemblages, with higher occurrence and abundance on turtles with enriched $\delta^{13}C$. Both isotope and epibiont data thus suggest that nesting loggerheads of the South West Indian Ocean population exhibit a bimodal foraging strategy. The isotopic niche width of nesting females was large as determined by the high variability in $\delta^{13}C$ and $\delta^{15}N$ signatures of the turtles' tissue. While isotopic niches overlapped among turtles with different body conditions, turtles in very good condition had a significantly narrower isotopic niche width than those in poorer condition. This could indicate that very healthy turtles are food specialists and more compromised turtles utilise a wider range of food items. These results show the complementarity of using stable isotopes and epibiont communities, which can be applied effectively to a large number of individuals within a population and, in combination, becomes a useful tool for conservation planning of a variety of migratory species.

3.2 Introduction

For sea turtles, like many other marine vertebrates, long-distance migration is a main feature of their life cycle. This generates important research, conservation and management challenges (Godley et al. 2008). Sea turtles have a complex life history and occupy a multitude of marine environments. Loggerhead turtles (*Caretta caretta*) change their diet and feeding area from open-ocean to coastal regions at the ontogenetic shift from juveniles to adults around seven to 12 years of age (Bjorndal et al. 2000, Bjorndal et al. 2003, Bolten 2003). It was originally hypothesised that this ontogenetic shift from an oceanic to neritic habitat was unidirectional (Carr 1986), however, reports have indicated that some individuals can either undergo a second shift back to oceanic habitats, or never leave these environments, except to reproduce (Hatase et al. 2002, Reich et al. 2010). As adults their foraging grounds can occur thousands of kilometres from their nesting grounds (Mansfield and Putman 2013, Saba 2013). Furthermore, individuals within the same population can occupy different foraging habitats during their adult life (Hatase et al. 2002, Reich et al. 2010, Seminoff et al. 2012).

The variety of habitats used by loggerheads is an important consideration in conservation planning. Four marine habitats have been identified for loggerheads throughout their life cycle: (1) neritic, those areas close to the coast with water depths that are less than 200 m; (2) benthic, the bottom of the ocean floor; (3) oceanic, waters where depths exceed 200 m; and (4) pelagic, the upper most layer of the ocean, closest to the surface. Because loggerhead turtles spend a majority of their time in the uppermost 200 m of the water column (Polovina et al. 2003, Freitas et al. 2018), these marine habitats are often complementary (neritic/benthic and oceanic/pelagic). As a result of complex habitat usage, loggerhead turtles are generalised in their feeding, consuming a variety of organisms from seagrass to fish (for further details see review by Jones and Seminoff 2013).

Studying the movement patterns of migratory marine species is often logistically, and financially, constrained. However, unlike other marine species, sea turtles are easily accessible while at their nesting beaches. This, coupled with the fact that they can occupy distinct ocean habitats, makes them ideal species to gain insight into various tracking techniques, which can then be applied to other marine fauna. Stable isotope

analyses are increasingly being used to gain insights into the ecology and movement patterns of mobile and resident sea turtles (Ceriani et al. 2014, Vander Zanden et al. 2015). Most isotope analysis studies in ecology have focused on stable carbon ($^{13}C/^{12}C = \delta^{13}C$) and nitrogen ($^{15}N/^{14}N = \delta^{15}N$) ratios, which are the most informative elements for trophodynamic studies, and can be applied to characterise habitat use patterns (Peterson and Fry 1987, Hobson 2008). These biogeochemical markers are metabolically integrated into an organism through its food source and therefore reflect trophic patterns (DeNiro and Epstein 1978, 1981, Post 2002). This is based on the fact that isotopic signatures of consumers do not reflect the exact isotopic values from the food available to them, but instead there is a predictable enrichment due to metabolic fractionation at every step of the food chain. Furthermore, individuals that have broad diets will have large variability in their isotopic niche (Bolnick et al. 2002, Bearhop et al. 2004). Because the enrichment of carbon through the food web is minimal, this element is not necessarily a good indicator of the trophic level of an organism, but is more useful to describe different carbon sources and flow pathways (DeNiro and Epstein 1978, Peterson and Fry 1987). Comparably, nitrogen has a larger enrichment factor and is thus more useful to identify the trophic level of an organism (DeNiro and Epstein 1981). In the marine environment, primary producers have easily distinguishable δ15N signatures depending on different biological processes and the source of the nitrogen utilized such as through N_2 fixation, denitrification and nitrification (Montoya 2007).

When examining the isotopic signatures of many individuals within a given population or community, these values provide information on the trophic variability, ecological niche width, foraging area (Araújo et al. 2007, Jackson et al. 2011), and nutrient transport within a given ecosystem (Vander Zanden et al. 2012). A recommended approach to evaluate trophic dynamics is through a quantitative measure of the isotopic niche space (Layman et al. 2007, Jackson et al. 2011). Variability of $\delta^{13}C$ and $\delta^{15}N$ signatures for the consumer in question allows for the conceptualization of its ecological niche (Layman et al. 2007, Jackson et al. 2011). Both the size and overlap of isotopic niches for different groupings within a community can be statistically compared, therefore allowing interpretations relating to dietary overlap and resource partitioning (Jackson et al. 2011).

The epibiont composition of a turtle reflects the environment an individual has been moving through and can be used as complementary evidence to stable isotope analysis in determining a turtle's habitat use and movement patterns (Casale et al. 2004, Hobson 2008, Reich et al. 2010, Frick and Pfaller 2013, Pfaller et al. 2014). Epibionts that originate from areas outside the sampled range show previous occupation by the host. During movement into oceanic environments, sea turtles may be colonised by communities of pelagic organisms typically associated with drifting flotsam and jetsam (Frick and Pfaller 2013). The majority of these pelagic organisms found on sea turtles include pedunculate barnacles (*Lepas* spp. and *Conchoderma* spp.) and grapsid crabs (*Planes* spp.). The presence of such epibiont species on nesting sea turtles provides evidence for the recent occupation of oceanic habitats by turtle hosts prior to their migration to coastal nesting grounds (Reich et al. 2010, Frick and Pfaller 2013).

South Western Indian Ocean (SWIO) loggerhead turtles nest primarily in South Africa and Mozambique (Nel and Casale 2015). Tag recoveries revealed that post-nesting females tend to migrate long distances from the breeding to feeding grounds, travelling as far north as the Seychelles and as far south as the Agulhas Bank at the southwestern tip of South Africa (Luschi et al. 2003, 2006). These dispersed foraging areas can cause differences in morphology and physiology of loggerhead turtles, since oceanic environments are generally oligotrophic (Lugendo et al. 2006) in comparison to neritic environments. Quality and quantity in the diet of turtles that occupy different marine environments can also contribute to differences in their growth rate (Bjorndal 1985, Kubis et al. 2009) and potentially body condition. In different regions, adult loggerheads from the same population that forage in pelagic environments were found to be significantly smaller compared to individuals foraging in the neritic environment, as determined by stable isotope analysis (Hatase et al. 2002, Reich et al. 2010). The proportion of nesting loggerhead turtles in the SWIO that forage in neritic or oceanic environments is currently not known. The complementary use of stable isotope signatures and epibiont composition is a potential approach to investigate this research gap, and was therefore chosen for this study.

The aim of this study is to combine various lines of evidence to determine the location of foraging habitats of loggerhead sea turtles nesting on the beaches of the iSimangaliso Wetland Park, South Africa. It is hypothesised that differences in the

foraging habitats of turtles, such as between oceanic and neritic foraging areas, determine patterns in their associated epibiont assemblages. To test this notion, I identified patterns in stable isotope signatures ($\delta^{13}C$ and $\delta^{15}N$) of turtle epidermis tissue and related these to patterns in epibiont community structure. It is further hypothesised that dietary differences, as indicated by the isotopic niche, drive turtle body condition where individuals foraging in distinct habitats have different body conditions. This study therefore has three objectives: (1) examine the stable isotope signatures of turtle epidermis and epibiont community structure, and identify feeding habitats; (2) compare the isotope signatures and epibiont community structure for complementarity; and (3) determine if body condition is a function of foraging habitat based on stable isotope signatures comparing Bayesian metrics.

3.3 Methods and Materials

3.3.1 Sample collection

This study was conducted in iSimangaliso Wetland Park on the northeast coast of South Africa (Figure 1.2). Samples were collected during two nesting seasons (2016/17 and 2017/18) in the austral summer, between November and February of each year. Breeding for both nesting species takes place along the 230 km coastline of the Park, however, the highest densities occur in the northernmost section along a 56 km stretch of beach (Nel et al. 2013). Approximately 1000 loggerhead (*Caretta caretta*) females nest each summer in the Park (Nel 2010). Nightly foot patrols were undertaken along the beach and each turtle encountered was opportunistically sampled once egg-laying commenced. Each female was identified from a titanium flipper tag (or tagged if she was untagged) and sampled only once per season to avoid re-sampling.

3.3.2 Tissue sampling and preparation for stable isotope analysis

Skin samples for stable isotope analysis were collected from 170 nesting loggerhead turtles, from 51 and 119 individuals in the 2015/16 and 2016/17 nesting seasons, respectively. These skin samples were collected from the front flipper using a sterile 6 mm biopsy punch (Thomson et al. 2012) and stored in a cryovial with 95% ethanol at room temperature. Ethanol was used for sample preservation because it has been shown to not significantly alter the isotopic signatures of sea turtle epidermis (Barrow

et al. 2008). After the skin sample was taken, the biopsy wound was sterilized with an antiseptic spray to avoid infection. In the laboratory, the upper layer of the skin (stratum corneum) was removed from the dermis using a scalpel blade. The remaining tissue was rinsed in distilled water and dried at 60°C for at least 48 hours. The dried samples were ground into a homogeneous powder using a mortar and pestle, and 0.45-0.55 mg was weighed into tin capsules.

Samples were analysed at the Stable Isotope Analysis Laboratory of the Mammal Research Institute (Pretoria, South Africa). The stable isotope ratios of ^{13}C to ^{12}C and ^{15}N to ^{14}N were determined using a mass spectrometer Flash EA 1112 Series elemental analyser coupled through an interface (Conflo III) to a Thermo Fisher Scientific Delta V Plus (all equipment supplied by ThermoFischer, Bremen, Germany) (Le Gouvello et al. 2017). Results are expressed in delta notation using a per mill scale using the standard equation (Coplen 2011):

$$\delta x = ([\frac{Rsample}{Rstandard}] -1) * 1000)$$

Where X is ^{13}C or ^{15}N and R_{sample} and $R_{standard}$ is the ratio of heavy to light isotope of the relative element in the sample and standard, respectively. A laboratory running standard (Merck Gel: $\delta^{13}C = -20.57‰$, $\delta^{15}N = 6.8‰$, $C\% = 43.83$, $N\% = 14.64$) and blank sample were run after every 12 samples. All results are referenced to Vienna Pee-Dee Belemnite for carbon isotope values, and to air for nitrogen isotope values. The standard used was Pee Dee Belemnite (PDB) limestone for $\delta^{13}C$ and atmospheric nitrogen for $\delta^{15}N$. The precision of the analyses was 0.13 ‰ for $\delta^{13}C$ and 0.14 ‰ for $\delta^{15}N$. To observe isotopic variation between the samples each epidermis sample was run in duplicate.

3.3.3 Epibiont collection

Epibiont sampling and processing is fully described in Chapter 2. In short, sand was washed off the carapace of the egg-laying female and epibionts collected off the right side of the carapace using a plastic paint scraper and placed in 70% ethanol. Macrofaunal invertebrate species were identified under a microscope to the lowest possible taxonomic level and counted. Epibionts were collected for 80 turtles with complementary stable isotope samples.

3.3.4 Body condition classification

The method to sample and define body condition for each nesting loggerhead female was described fully in Chapter 2. In short, three criteria (plastron shape, injuries and skin condition) were used to classify each female into poor, average, good and very good body conditions. Forty-six turtles, with stable isotope skin samples, were categorised into body condition: poor; average; good; and very good.

3.3.5 Statistical analyses

To evaluate if there were any differences between the nesting seasons (2015-16 and 2016-17) a one-way permutational Multivariate Analysis of Variance (PERMANOVA, Anderson 2001) was used for both stable isotope values ($\delta^{13}C$ and $\delta^{15}N$). To identify distinct turtle foraging grounds, the number of clusters that best represented the distribution of the isotope signatures was determined through a k-means clustering analysis (Hartigan and Wong 1979). Euclidean distance was used to calculate dissimilarity and mean silhouette widths to determine the optimal fit for the number of clusters. The silhouette width indicates the strength of a cluster, with a higher value being a stronger representation of the data. The k-means cluster analysis and silhouette widths were calculated first using the data for each isotope signature separately ($\delta^{13}C$ and $\delta^{15}N$), and then again with the entire dataset.

The presence of macrofaunal epibionts on the carapace of turtles was used to further examine if habitat specific epibionts were matched with the clustering of loggerheads based on stable isotope analysis of the turtle. Individual epibionts were assigned a category as being a habitat specialist or generalists (following the classification by Reich et al. 2010); specialist occupy either (1) oceanic/pelagic, or (2) neritic/benthic habitats, whereas the generalists (3) occur in both (1) and (2), using appropriate literature (Day 1967, 1969, Griffiths 1976, Barnard & Karaman 1991, Frick et al. 2003, 2004, 2006, Foster et al. 2004, Frick and Pfaller 2013, Pfaller et al. 2014 and Branch et al. 2017). Chi-square tests were used to assess if the occurrence of either oceanic/pelagic or neritic/benthic epibionts on loggerhead sea turtles were different between the isotopic clusters. Furthermore, a two-sample t-test (or non-parametric variety) was used to compare $\delta^{13}C$ signatures between turtles with oceanic/pelagic and neritic/benthic epibionts, respectively. Only $\delta^{13}C$ was used here as it is better at

delineating oceanic versus neritic environments. Generalist epibionts in category 3 were not useful for habitat identification and thus disregarded in this analysis.

To visualise the relationship between isotopic signatures of the turtles and their epibiotic communities a Non-metric Multi-Dimensional Scaling (MDS) was used. All the epibiont species-abundance data were transformed using the square root function and a resemblance matrix based on Bray-Curtis similarities was created. To test for significant differences in epibiont community structure between turtle foraging habitats (as reflected in isotope clusters), a one-way permutational multivariate analysis of variance (PERMANOVA). The PERMANOVA design employed a Bray-Curtis index of dissimilarity which was assessed using 999 permutations of the residuals with isotope cluster as a fixed factor (fixed factor, two levels: enriched; depleted clusters). This test was chosen because of its robustness to differences in multivariate dispersion (Anderson and Walsh 2013). A SIMPER analysis was then used to examine the contribution of individual epibiont taxa to any separation between isotopic clusters (Clarke and Gorley 2006). All macrofaunal epibiont taxa identified from the sea turtles were used in these multivariate analyses.

To address the hypothesis that turtles foraging in distinct habitats exhibit differences in body condition, $\delta^{13}C$ and $\delta^{15}N$ isotopic signatures were compared among a subset of nesting loggerhead turtles with different body conditions (poor, average, good and very good) using a one-way PERMANOVA. The $\delta^{13}C$ and $\delta^{15}N$ data were first normalized and subsequently combined into a resemblance matrix (Euclidean distance). Differences between body conditions (random factor, four levels: poor, average, good and very good) were tested using 999 permutations of the residuals. Differences in the feeding niche among different body condition categories of nesting loggerhead turtles were tested using Stable Isotope Bayesian Ellipses in R (SIBER, Jackson et al. 2011). The R package “mvnormtest” (Jarek 2015) was used to test the SIBER assumption of a multivariate normal distribution for each group. SIBER creates isotopic niches around specified groups or communities and these niches can be examined visually and tested for area and overlap. The Bayesian inference method allows error associated when fitting ellipses to groups to be calculated using the number of samples as well as the distribution of data. The standard ellipse area (SEA) for a set of bivariate data were calculated using a Markov Chain Monte Carlo (MCMC) algorithm for sampling from a probability distribution to generate a distribution of

covariance matrices that designate data in terms of likelihood. The standard ellipse area for small sample sizes (SEA_C), which contains 40 % of the data regardless of sample size, was used to compare niche widths between groups. A Bayesian estimate of the standard ellipse area (SEA_B) was used to test the probability a group ellipse is smaller or larger than the others. Ellipse areas were subsequently compared using pair-wise tests to calculate probability that the posterior distribution of one group is larger, or smaller than another. This allows significant differences in niche size among the body conditions to be inferred. Additionally, convex hull area (TA) – although more sensitive to sample size – was also used to compare among groups and their overlap (Layman et al. 2007).

All results with $p < 0.05$ were considered statistically significant. The PERMANOVA+ add-on (Anderson et al. 2008) to PRIMER v6 software (Clarke & Gorley 2006) was used to carry out multivariate analyses. Univariate statistical tests were performed in R version 3.5.1 (R Core Team 2018).

3.4 Results

The isotopic signatures did not differ between sampling seasons (PERMANOVA; Pillai's trace = 0.014, $F_{1, 2} = 0.013$, $p = 0.10$), hence the samples were pooled for subsequent analyses. The average $\delta^{13}C$ was -14.20 ± 1.2 ‰ (range -16.18 to -10.11), and $\delta^{15}N$ value was 11.21 ± 1.38 ‰ (range 7.96 to 14.72; Figure 3.1).

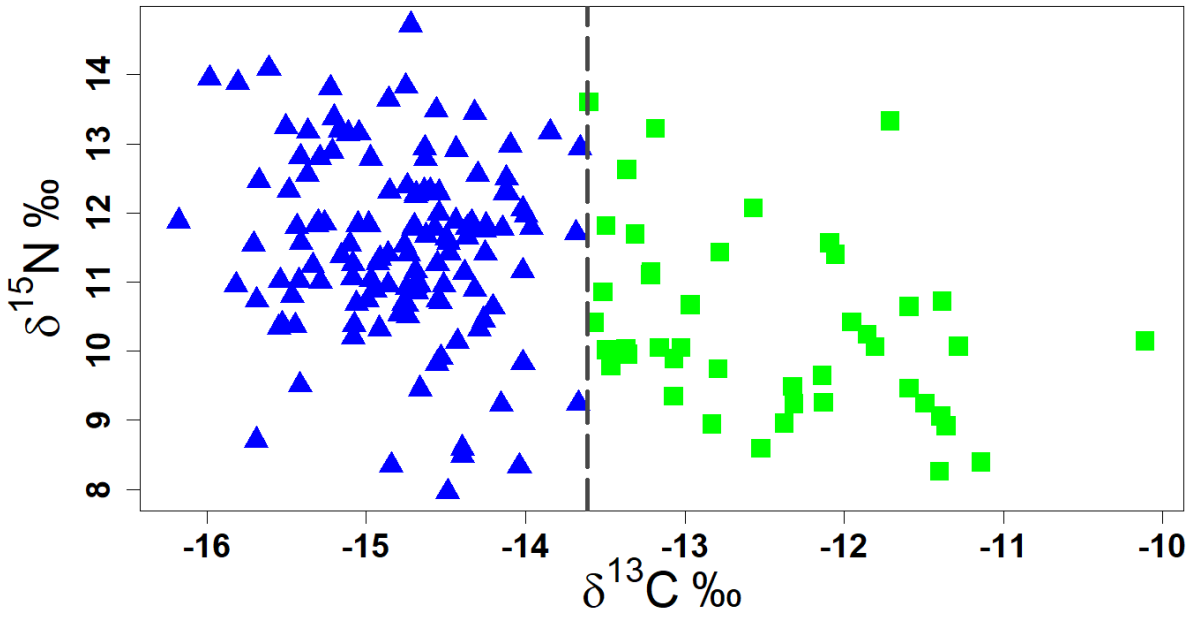

Figure 3.1: Distribution of $\delta^{13}C$ and $\delta^{15}N$ stable isotope signatures from nesting loggerhead sea turtles (*Caretta caretta*). Clusters for $\delta^{13}C$ were determined using k-means cluster analysis of silhouette width. The enriched cluster (green squares, *n* = 43) is separated at $\delta^{13}C$ = -13.61 ‰ (dashed grey line) from the depleted $\delta^{13}C$ cluster (blue triangles, *n* = 127).

3.4.1 Foraging regimes based on isotopes

Two $\delta^{13}C$ clusters, based on silhouette width with k-means clustering were identified for the 170 loggerhead isotope samples (Table 3.1). The silhouette width of 0.684 for two clusters based on $\delta^{13}C$ indicates the best fit clustering structure. The relatively depleted $\delta^{13}C$ cluster (n = 127; $\delta^{13}C$ mean = -14.80 ‰, SD = 0.52; $\delta^{15}N$ mean = 11.53 ‰, SD = 1.30) is separated at $\delta^{13}C$ = -13.61 ‰, from the relatively enriched cluster (n = 43; $\delta^{13}C$ mean = -12.44 ‰, SD = 0.85; $\delta^{15}N$ mean = 10.28 ‰, SD = 1.21; Figure 3.1).

Table 3.1: Determining the number of clusters based on average silhouette widths. The clusters were calculated for $\delta^{13}C$, $\delta^{15}N$, and both isotope signatures combined. The bold value ($\delta^{13}C$, two clusters) indicates the best fit.

Number of clusters	$\delta^{13}C$ and $\delta^{15}N$	$\delta^{13}C$ only	$\delta^{15}N$ only
2	0.489	**0.684**	0.551
3	0.405	0.555	0.554
4	0.346	0.600	0.565
5	0.340	0.585	0.547
6	0.363	0.552	0.558

3.4.2 Epibiont community structure associated with foraging habitat

Epibionts were collected and identified from 45 and 35 sea turtles in the depleted and enriched $\delta^{13}C$ clusters, respectively. Twenty-four macrofaunal epibiont taxa were identified from 80 loggerhead females. Of the identified epibiont species, 16 were used for the assignment of habitat types (Table 3.2). Three of the species were classified as oceanic/pelagic and 13 as neritic/benthic based on literature describing their distributions and habitat. Neritic epibionts occurred more frequently on nesting females in the enriched $\delta^{13}C$ cluster compared to those in the depleted $\delta^{13}C$ (Table 3.2, Figure 3.2). Of the 80 loggerhead sea turtles sampled, 46 had only neritic epibionts and 30 had oceanic and neritic epibionts present on the carapace. Four sea turtles were found with no epibionts present on the carapace and therefore, were excluded from the epibiont community analysis. The occurrence of oceanic/pelagic epibionts was significantly higher (chi-square, $df = 1$, $\chi^2 = 13.075$, $p < 0.001$) on nesting females in the depleted $\delta^{13}C$ cluster, whereas, the occurrence of neritic/benthic epibionts was significantly higher (chi-square, $df = 1$, $\chi^2 = 14.922$, $p < 0.001$) on nesting females in the enriched $\delta^{13}C$ cluster. There was also a significant difference (Mann-Whitney $U = 293$, $p < 0.05$) between the $\delta^{13}C$ of sea turtles which had neritic/benthic only epibionts ($\delta^{13}C$ mean = -13.20 ± 1.51 ‰) compared to those with oceanic/pelagic epibionts ($\delta^{13}C$ mean = -14.83 ± 0.79 ‰) present (Figure 3.2).

Table 3.2: Species occurrence of epibionts found on the carapace of nesting loggerhead sea turtles (*Caretta caretta*). The epibionts were classified into two distinct habitats: oceanic/pelagic (highlighted in grey) and neritic/benthic, the occurrence of these are presented relative to the isotopic cluster of the sea turtle.

		Occurrence of epibiont	
Taxon	**Species**	Depleted cluster (*n*=46)	Enriched cluster (*n*=30)
Cirripedia	*Conchoderma virgatum*	8	1
	Lepas spp.	24	2
Brachyura	*Planes major*	2	0
Amphipoda	*Ampithoe falsa*	2	3
	Caprella equilibra	1	4
	Ericthonius ledoyeri	1	3
	Stenothoe adhaerens	0	2
	Hyachelia tortugae	6	18
	Hyale grandicornis	12	21
	Hyale maroubrae	1	7
	Podocerus africanus	9	20
	Podocerus pyurae	2	5
Isopoda	*Exosphaeroma* sp.	0	4
Polychaeta	Nereidae	1	4
	Phyllodocidae	0	2
	Syllidae	1	0

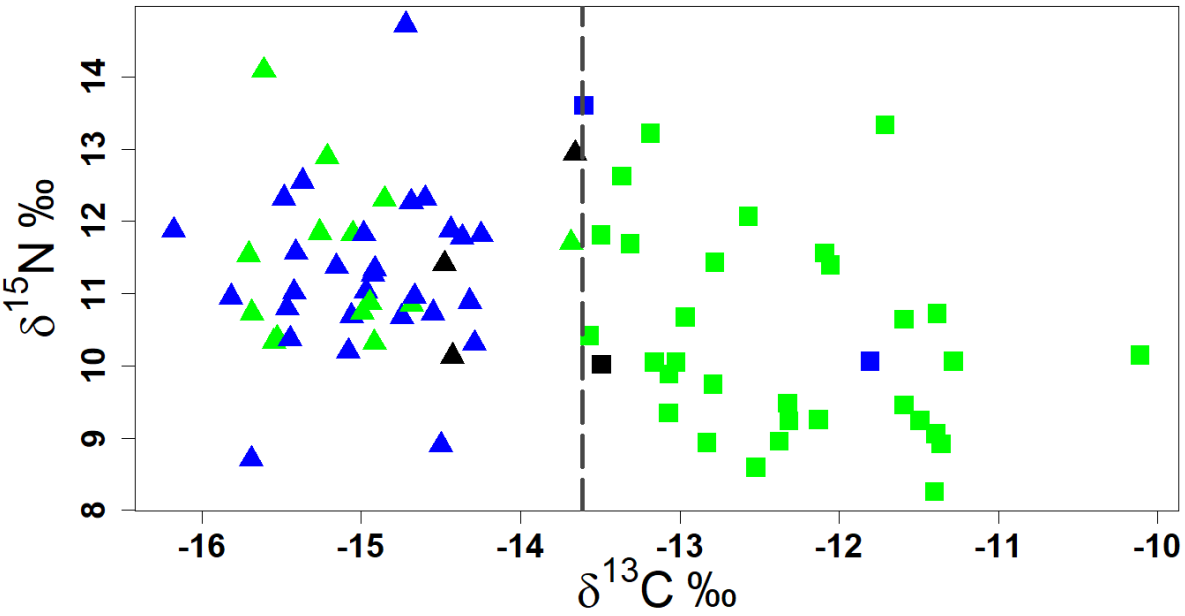

Figure 3.2: Distribution of $\delta^{13}C$ and $\delta^{15}N$ stable isotope signatures from nesting loggerhead sea turtles (*Caretta caretta*) with groupings of their epibionts. Blue points indicate occurrence of oceanic/pelagic epibionts and green points represent the occurrence of individuals with only neritic/benthic epibionts. Squares are sea turtles in the enriched $\delta^{13}C$ cluster and triangles are those in the $\delta^{13}C$ depleted cluster. Black points are individuals with no epibionts present on the carapace. The dashed grey line represents the sectioning of the $\delta^{13}C$ isotope clusters at $\delta^{13}C$ = -13.61 ‰.

There were significant differences in epibiont community structure between the two isotope clusters (Figure 3.3; PERMANOVA: *df* = 1, 75, *F* = 6.9083, *p* < 0.001). The SIMPER analysis indicated that five taxa (*Chelonibia testudinaria, Podocerus africanus, Hyale grandicornis, Hyachelia tortugae* and *Lepas spp.)* contributed to 75 % dissimilarity between the isotopic clusters (Table 3.3). *Chelonibia testudinaria* is the only non-habitat-specific epibiont among them, which was identified from the SIMPER, whereas *Lepas* spp. is a pelagic specialist, and the other three are found in the neritic environment.

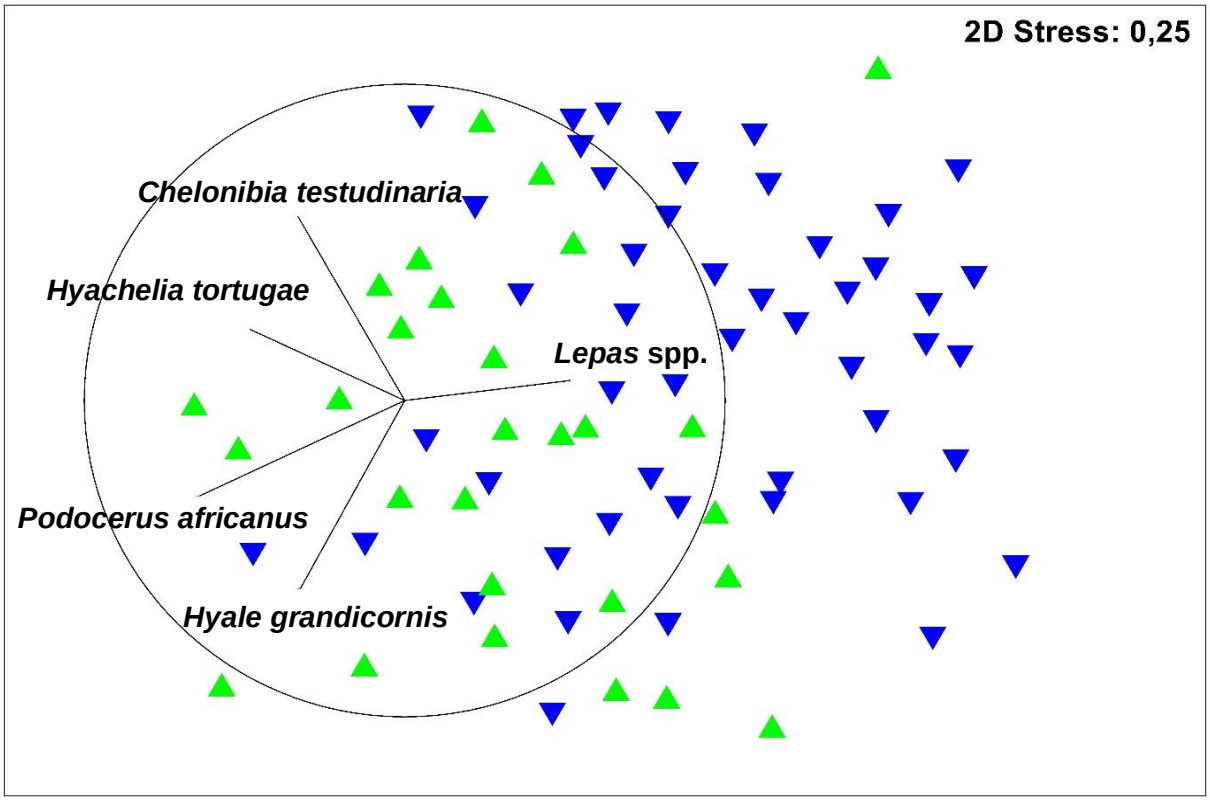

Figure 3.3: Multidimensional scaling (MDS) using epibiont species-abundance data of nesting loggerhead sea turtles (*Caretta caretta*). Sea turtles were grouped by feeding habitat as determined by stable isotope signatures: green triangles represent the $\delta^{13}C$-enriched cluster and blue triangles the $\delta^{13}C$-depleted cluster. The lines indicate those epibiont taxa that most influenced the grouping based off Spearman Rank correlation, longer lines are those with higher R^2.

Table 3.3: Results of the SIMPER analysis used to identify the relative contribution of the top five epibiont species driving dissimilarities between epibiont communities between different feeding habitats based on relative enriched and depleted $\delta^{13}C$ clusters.

Taxa	**Average abundance**		**Dissimilarity**	
	Depleted	**Enriched**	**Average**	**Contribution**
Chelonibia testudinaria	15.03	12.94	19.73	25.22
Podocerus africanus	13.97	7.78	15.61	19.96
Hyale grandicornis	13.07	7.06	14.56	18.62
Hyachelia tortugae	4.03	0.98	5.59	7.14
Lepas spp.	0.17	2.53	5.13	6.56

3.4.3 Body condition

The $\delta^{13}C$ and $\delta^{15}N$ isotope composition of loggerheads did not vary significantly with body condition (Pillai's trace = 0.121, $F_{3, 6}$ = 1.856, p = 0.09, Table 3.4). The SIBER analyses indicated that there was significant overlap among all the sea turtles with different body conditions (Figure 3.4). Therefore sea turtles with different body conditions do not occupy different isotopic niches. However, there were significant differences in the size of the isotopic niche of sea turtles with different body conditions (Figure 3.4, Table 3.5 and Table 3.6). The area metrics reveal that TA, SEA and SEA_C of sea turtles with very good body conditions were significantly smaller than those of the other body condition categories (e.g. SEA_C of the very good body condition category was 2.99, 3.42, 3.42 times smaller than the poor, average and good categories; Table 3.6).

Table 3.4: Mean (±SD) $\delta^{13}C$ and $\delta^{15}N$ isotope ratios of nesting loggerhead sea turtles (*Caretta carettta*) per body condition category.

Body Condition	**$\delta^{13}C$ (‰)**	**$\delta^{15}N$ (‰)**
Poor (n= 11)	-13.96 ± 1.29	11.24 ± 1.03
Average (n= 16)	-14.39 ± 1.35	11.27 ± 1.80
Good (n= 13)	-14.32 ± 1.42	11.05 ± 1.43
Very Good (n= 6)	-14.70 ± 0.46	10.59 ± 0.66

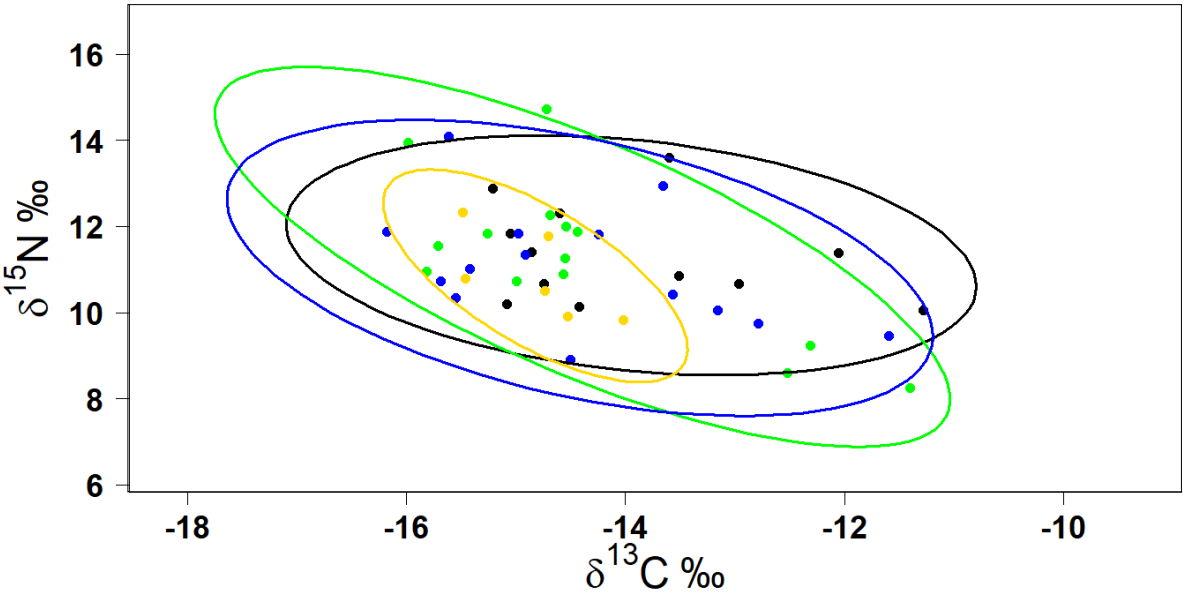

Figure 3.4: Variation in $\delta^{13}C$ and $\delta^{15}N$ of nesting loggerhead sea turtles (*Caretta caretta*) for different body condition categories (poor = black, average = green, good = blue and very good = gold). Standard ellipses were based on maximum likelihood estimates and were corrected for small sample sizes.

Table 3.5: Pairwise comparisons of the Bayesian estimate of the standard ellipse area (SEA_B) for isotopic composition ($\delta^{13}C$ and $\delta^{15}N$) of nesting loggerhead sea turtles (*Caretta carettta*) in different body condition categories. Values represent the probability that the SEA_B for one body condition group is smaller than another.

Pair-wise comparison	Poor	Average	Good	Very Good
Poor <	0	0.914	0.797	0.043
Average <	0.086	0	0.274	0.004
Good <	0.203	0.726	0	0.14
Very good <	0.957	0.996	0.760	0

Table 3.6: Convex hull area (TA), standard ellipse area (SEA) and standard ellipse area for small sample size (SEA_C, 40% credible interval) of nesting loggerhead sea turtles (*Caretta caretta*) in different body condition categories.

	TA	SEA	SEA_C
Poor (n= 11)	9.04	4.44	4.88
Average (n= 16)	12.51	5.14	5.57
Good (n= 13)	13.12	5.15	5.58
Very Good (n= 6)	1.85	1.30	1.63

3.5 Discussion

The primary aim of this study was to identify broad marine foraging habitats of loggerhead sea turtles nesting in the iSimangaliso Wetland Park, South Africa, and relate them to distinct habitats in the SWIO. To do this, complementary analyses of $\delta^{13}C$ and $\delta^{15}N$ ratios from the turtle's tissue and epibiont communities were used. Stable isotope analysis revealed two foraging groups for nesting loggerheads, as reflected in $\delta^{13}C$ enriched and $\delta^{13}C$ depleted clusters. These clusters were associated with distinct epibiont assemblages that represent neritic and oceanic foraging habitats, as indicated by epibiont species and a known depletion of $\delta^{13}C$ from neritic to oceanic habitats (Cherel and Hobson 2007, Newsome et al. 2007). The results from this study confirm that nesting loggerhead sea turtles exhibit a bimodal foraging strategy by foraging either in oceanic/pelagic or neritic/benthic habitats, as shown in similar studies in Cape Verde (Hawkes et al. 2006), Florida, USA (Reich et al. 2010), and Japan (Hatase et al. 2002). Lastly, the isotopic niche of individuals with different body conditions were compared. There was significant overlap between the isotopic niches among the body condition categories. However, turtles that were in very good condition had significantly smaller niches than the rest of the categories (poor, average and good). This did not support the hypothesis that sea turtles foraging in different habitats will have different body condition categories, but that increased food specialisation is associated with very good body condition. The results from this study supports the hypothesis that sea turtles foraging in different habitats have stable isotope signatures and epibiont assemblages resembling these environments.

Corroborative evidence from isotope analysis and epibiont community assemblages suggests that these differences are a result of loggerhead sea turtle movement patterns and habitat use at these distinct neritic and oceanic foraging grounds.

3.5.1 Habitat use inferred from isotopes and epibionts

Epidermal isotopic composition of the sea turtles sampled in this study reflect signatures from foraging areas visited in the past months, which are distinct from those at the recently visited nesting grounds (Vander Zanden et al. 2014). Metabolic processes record and integrate the isotopic signature of nutrients consumed into different tissues (Remien 2015). The isotopic values of the tissue can take a period of time (ranging from a few days to a year) to reach equilibrium with that of the diet (Martínez del Rio et al. 2009, Wolf et al. 2009). This delay in isotopic signature response is due to tissue turnover rate. The turn-over rate is the period of time that the animal tissue takes to assimilate the isotopic signature of the food item the animal is feeding on (Fry & Arnold 1982, Hesslein et al. 1993, Vanderklift & Ponsard 2003). Recent diet can be inferred using tissues with a high metabolic activity and quick turnover rates like liver or whole blood (a week), and tissue with slower metabolic activity like bone or scutes, can represent the diet over the life of the consumer (Tieszen et al. 1983, Reich et al. 2007). Since sea turtles are capital breeders, they obtain all energy for migration and reproduction (and hence $\delta^{13}C$ and $\delta^{15}N$ signatures) in distant foraging areas, which occur months before coming to their nesting beaches (Miller 1997, Plot et al. 2013). Opportunistic feeding during the reproductive season has been reported for loggerheads (Schofield et al. 2006), but the isotopic signatures will not be reflected in the tissue due to the assimilation time required. For reptiles, including sea turtles, the assimilation of isotopic signatures from habitat and resource use is generally only reflected in the epidermis months to years later as a result of the slow metabolic processes (Seminoff et al. 2007, Reich et al. 2008).

The SWIO loggerhead population exhibited distinct foraging habitats according to $\delta^{13}C$ values. While there were two $\delta^{13}C$ clusters, similar to the findings by Reich et al. (2010), this does not indicate that there are only two foraging areas. These authors suggested that the complex nature of the $\delta^{13}C$ gradients in the marine environment make it difficult to elucidate polymodal foraging areas. To validate this, the known feeding grounds and migration corridors of loggerheads in the SWIO region should be

considered when interpreting the results of the current study (e.g. Luschi et al. 2003, Harris et al. 2018). More generally, the marine environment has four identified $\delta^{13}C$ gradients ranging from enriched to depleted (reviewed in Reich et al. 2010) and interactions between these can confound the isotope results, making determining exact foraging areas difficult. These four gradients are (1) a depletion of $\delta^{13}C$ from neritic to oceanic environments; (2) a depletion of $\delta^{13}C$ from benthic to pelagic marine zones; (3) a depletion of $\delta^{13}C$ from low latitudes to high latitudes; or (4) an enrichment of $\delta^{13}C$ from low trophic position organisms to higher ones. This study only identified two isotopic ($\delta^{13}C$) clusters, which correspond to a distinction of marine gradients (1) and (2).

As sea turtles move offshore, the prevalence of oceanic/pelagic epibionts increases and thus makes them more susceptible to colonization from these organisms (Frick and Pfaller 2013). The isotopic clustering and epibiont assemblages from this study validated this relationship. Sea turtles foraging in oceanic/pelagic habitats (as identified by their stable isotope signatures) had a higher abundance of those oceanic epibionts. This was complemented by the MDS and SIMPER analyses that identified *Lepas* spp. as one of the main species driving the differences between the epibiont communities of turtles feeding in oceanic and neritic habitats. *Lepas* spp. was the only oceanic epibiont driving the difference in the community structure between sea turtles foraging in neritic and oceanic habitats. The other oceanic epibionts (*Planes major* and *Conchoderma virgatum*) were rare and therefore did not contribute to the dissimilarity. The neritic epibionts that were driving differences in community assemblages (*Podocerus africanus, Hyale grandicornis, Hyachelia tortugae*) were more abundant in sea turtles with enriched $\delta^{13}C$, and therefore foraging in neritic habitats.

Latitudinal foraging, or the third $\delta^{13}C$ gradient, could not be detected by either the isotope or epibiont data collected in this study. Even with the analysis of all epibionts only a gradual change in epibiont community was observed on the MDS plot. Therefore, geographic origin of specific epibionts could not be distinguished based on these data. Results by Robinson et al. (2016b) for the same loggerhead population reported $\delta^{13}C$ values that were both more depleted and enriched (range -19.0 to -9.4 ‰) than the values obtained in the current study (range -16.18 to -10.11 to ‰). This depletion in $\delta^{13}C$ for loggerhead sea turtles was attributed to a southerly migration

towards the Agulhas Bank region, as the signatures were similar to those of open ocean leatherback sea turtles (*Dermochelys coriacea*) that had been satellite tagged occupying this region. This discrepancy in the $\delta^{13}C$ range could, therefore, be related to the location at which the turtles migrated to. Robinson et al. (2016b) collected stable isotope samples from nesting loggerhead sea turtles across their entire range within the iSimangaliso Wetland Park, while the current study only focussed on those in the northern-most section. These results suggest that loggerhead sea turtles nesting on beaches in the southern region of iSimangaliso may be more likely to migrate offshore and further south. Other studies have also found that $\delta^{13}C$ signatures vary with nesting beach location; higher latitudes are correlated with more depleted signatures (Reich et al. 2010, Ceriani et al. 2014, Vander Zanden et al. 2015). Epibionts could be used to elucidate this gradient. Southern Africa has six coastal biogeographic provinces (Lombard 2004, Sink et al. 2012), of which four occur in known sea turtle foraging areas (Agulhas, Natal, Delagoa, and Indo-West Pacific). These biogeographic regions are well defined by fauna and flora and occur along a latitudinal gradient representing distinct sea surface temperature (Griffiths et al. 2010). Although not described in the current study, future research can validate this gradient through examination of region-specific epibionts. In addition to macro-epibionts, diatom communities, which are present on all sea turtles (Robinson et al. 2016a, Majewska et al. 2017), and meiofauna communities (Corrêa et al. 2014) could be investigated to supplement current analyses.

The fourth $\delta^{13}C$ gradient is determined by the base source of $\delta^{13}C$ in the food web, which could potentially explain why there are individuals in the depleted cluster without oceanic/pelagic epibionts. Inshore seagrass beds, for example, have enriched $\delta^{13}C$ signals compared with more depleted phytoplankton-based food webs in the pelagic zone (Peterson and Fry 1987). Satellite studies show loggerheads that nest on South African beaches frequent areas off southern Mozambique (de Wet 2012, Harris et al. 2018). These feeding grounds overlap with coastal seagrass habitats at Inhaca Island, Bazaruto and Maputo Bay (Gullström et al. 2002, Scheren et al. 2016). However, foraging grounds also occur along the wave-dominated eastern coastline of South Africa (de Wet 2012, Harris et al. 2018). These areas do not support vast seagrass beds (Green et al. 2003), and the source of $\delta^{13}C$ at the base of the food web is

relatively depleted. Therefore, sea turtles foraging in these neritic environments can have relatively depleted $\delta^{13}C$ and no apparent oceanic epibionts.

Although $\delta^{13}C$ increases with an increase in trophic position, it does so to a lesser degree than $\delta^{15}N$ (Post 2002, Bearhop et al. 2004a, Michener and Kaufman 2007). Both $\delta^{13}C$ and $\delta^{15}N$ are primarily determined by the dietary sources, but metabolic processes lead to a discrimination between isotopic ratios of a consumer and its diet (Vanderklift and Ponsard 2003). These discrimination values are typically 3-4 ‰ for $\delta^{15}N$ and can be used to determine trophic position on the food web and 1 ‰ for $\delta^{13}C$ (Post 2002). The $\delta^{15}N$ was not utilized for distinguishing foraging area because it did not cluster as well as $\delta^{13}C$ and there was no correlation with epibiont community. However, $\delta^{15}N$ can still be informative on other aspects of sea turtle ecology. Loggerhead sea turtles have a broad diet, consuming a wide variety of prey including jellyfish, cephalopods, and fish (Bjorndal 1997). The wide range in $\delta^{15}N$ values from this study supports this, indicating this population forages across a range of trophic levels and on a variety of prey items. Nevertheless, $\delta^{15}N$ has been used to examine habitat use by sea turtles, Seminoff et al. (2012) reported a foraging dichotomy for Indonesian leatherback sea turtles, with individuals that occupied eastern and western Pacific foraging areas having a relatively enriched and depleted $\delta^{15}N$ values respectively. A similar trend was found for nesting leatherbacks from the eastern Pacific in Costa Rica in comparison to those from the western Atlantic at St Croix (Wallace et al. 2006) and for loggerheads off southern Peru (Pajuelo et al. 2010). Nitrogen isotopes were useful in these systems because of the different sources of nitrogen across an entire ocean basin or between ocean basins. The different patterns are likely due to denitrification resulting in enriched $\delta^{15}N$ values, compared to N_2 fixation which causes depleted $\delta^{15}N$ values (Deutsch et al. 2007).

3.5.2 Turtle body condition

Loggerhead sea turtles with different body conditions were not foraging at distinct locations, since there was significant overlap between their isotopic niches. However, turtles in a very good body condition had a significantly reduced isotopic niche, with a narrower range for both $\delta^{13}C$ and $\delta^{15}N$ as indicated by the convex hulls (TA) and standard ellipse area (SEA) (Layman et al. 2007, Jackson et al. 2011). It has been suggested that groups within a population that forage over a larger geographic scale

exhibit greater variation in isotope ratios than groups with a limited distribution (Bearhop et al. 2004a, Ceia et al. 2014). Results from this study suggests that individuals in better body condition have a small feeding range and animals in sub-optimal body condition exploit a greater range of prey species or foraging areas. The epibionts of turtles in very good body condition also showed low epibiont species richness and almost no oceanic/pelagic epibionts (Chapter 2).

Wider isotopic ranges for turtles suggests greater plasticity of their foraging and trophic niches, as demonstrated for juvenile and adult hawksbill sea turtles (*Eretmochelys imbricata*; Ferreira et al. 2018). In this study different niche widths were obtained for nesting females that represent the same age class of the population in different body condition categories. However, the variability in $\delta^{15}N$ among body conditions indicated that these groups were still feeding at the same trophic level, since tissue enrichment between different trophic positions in $\delta^{15}N$ would be 3-4 ‰ (Post 2002). Although, nesting loggerheads with a very good body condition had the lowest $\delta^{15}N$ of all groups, the next closest group was only 0.6 ‰ enriched in comparison.

Foraging area, prey items and thus the niche of individual turtles, can affect their body size. For some populations of adult loggerhead sea turtles, foraging dichotomy is linked to body size with smaller females occupying predominantly oceanic habitats and large individuals utilizing mainly neritic habitats (Hatase et al. 2002, Hatase et al. 2010). A study done in parallel to this (Le Gouvello unpublished data) showed that different size groupings of nesting loggerheads from the same population had a strong isotopic niche overlap. It did, however, detect a decrease in the isotopic niche width with an increase in the size of individuals.

3.5.3 Future research

This study only investigated nesting females of the SWIO loggerhead population and thus the generality of these results across the population is uncertain. Future investigations on habitat use should also include adult males and juveniles. Southern Africa is also home to three other sea turtle species that do not nest in the region: hawksbills, greens and olive ridleys (*Lepidochelys olivacea*) with limited ecological or population information on these species. Research to date has focussed on the nesting species in the region (loggerheads and leatherbacks). Different sea turtle species use different marine environments (Luschi et al. 2006, Godley et al. 2008)

which can be assessed through stable isotope (Robinson et al. 2016b) and epibiont community assemblage analyses (Robinson et al. 2017). By understanding habitat use of different species one can infer ecological benefits on regional and global scales.

There are many other stable isotopes used in movement ecology that were not incorporated in this study (Hobson 1999). The most common isotopes used for sea turtle research is $\delta^{13}C$ and $\delta^{15}N$ (Pearson et al. 2017), however, incorporation of other isotopes could prove invaluable in delineating habitat use further. Sulphur and lead can differentiate anthropogenic inputs into the environment (Krouse and Grinenko 1991, Komárek et al. 2008), which could be useful for managing endangered marine species, like sea turtles, with a strong spatial overlap with humans.

3.6 Conclusion

This study revealed two foraging habitats for loggerhead sea turtles nesting in the iSimangaliso Wetland Park, South Africa, on the East African seaboard. This bimodal foraging strategy of using either oceanic or neritic environments is similar to reports from other regions. However, the complex nature of $\delta^{13}C$ gradients and their interactions confounded the resolution of this study, and it is likely that there are more clearly defined foraging areas, as evident from tag-recovery studies and migration corridors (Hughes 1974, Luschi et al. 2006, Harris et al. 2018). Because of the large proportion of nesting loggerhead sea turtles in the $\delta^{13}C$ depleted cluster (oceanic foragers) this provides further support against the generalization that adult sea turtles almost exclusively occupy neritic environments (Carr 1986).

The results here show that epibiont community patterns corroborate clusters based on stable isotope analyses. Furthermore, the isotopic niche of nesting loggerheads in the SWIO is broad as indicated by the variability of $\delta^{13}C$ and $\delta^{15}N$. However, there were notable differences in the niche widths of body condition groups in this population, with those individuals in very good body condition having a smaller isotopic niche and compared to other groupings.

The use of a combination of stable isotope analyses and epibiont community composition to conduct research on sea turtle habitat utilization has been shown to be beneficial in this study as well as others (Reich et al. 2010). Additional community analysis of cryptic epibionts such as meiofauna and diatoms may reveal regional use not currently elucidated in this study. The use of these techniques is non-invasive and

cost-effective and can therefore be employed on a large number of individuals within a population. This stable isotope and epibiont approach is therefore ideal for conservation programmes trying to understand the habitat use of large-scale, migratory marine species

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Chapter 4

Stable isotope analysis of epibiotic barnacles to track movement of loggerhead sea turtles (*Caretta caretta*)

4.1 Abstract

Sea turtles provide a substrate to a diverse range of epibionts that can be used to study a host's ecology. Turtle barnacles are particularly useful, because they can live for up to three years on a host and store chemical signatures of the environment in which they grew. These chronologies are stored in different growth layers of their calcareous shell and reflect migratory routes of the host turtle. Known isotopic gradients of $\delta^{18}O$ occur in the ocean, which can be used for tracking species that migrate between distinct isotopic areas. This study analysed stable isotopes of $\delta^{18}O$ and inorganic $\delta^{13}C$ from the shells of *Chelonibia testudinaria* barnacles sampled from eight nesting loggerhead sea turtles (*Caretta caretta*) in the iSimangaliso Wetland Park, South Africa. Isoscapes of $\delta^{18}O$ corresponding to barnacle calcite were mapped for the known distribution range of South African loggerheads, showing a strong positive gradient in $\delta^{18}O$ of barnacle calcite with an increase in latitude. Isotopic signatures from layered barnacle calcite deposits were used to construct isotopic profiles that reflected environmental variability during the lifespan of the barnacle. Some turtles' re-migratory movements between nesting seasons were detected from the barnacle's isotopic profiles. The range of $\delta^{18}O$ measured from the barnacles was then used to map the movement of the turtle host's ranges, which indicated most of them migrated north from the nesting beaches. Although the spatial resolution is relatively low, these maps show movement in areas that are consistent with distribution ranges identified by satellite telemetry studies. The isoscape approach shown in this study can be applied to track movement patterns of a wide range of marine migratory species. It is a complementary method to satellite tracking of individuals and can be run at a fraction of the cost on a larger proportion of the population. Understanding migratory routes of endangered species is key in developing more effective conservation strategies.

4.2 Introduction

Sea turtles provide a specific, mobile, ecological substrate for a diverse range of invertebrate epibionts, including barnacles, amphipods, isopods, cnidarians and polychaetes (Pfaller et al. 2006, Fuller et al. 2010, Chapter 2). Epibionts have been used as a proxy to study host ecology, evolution and movement (Nieberding and Olivieri 2007, Pfaller et al. 2014) and can provide alternative cost-effective and less invasive approaches for research on the hosts themselves. The presence (or absence) of particular epibionts is a reflection of host migration routes since species assemblages differ between biogeographical regions (Caine 1986, Casale et al. 2004, Domenech et al. 2015). Barnacles are particularly useful for tracking movement patterns, because when growing they incorporate information about local geochemical environments in their $CaCO_3$ shell in distinct growth layers (Killingley 1980, Killingley and Lutcavage 1983, Detjen et al. 2015). Barnacles are found commonly on the carapace and skin of sea turtles, where they attach as larvae and can live for up to three years (Hayashi and Tsuji 2008, Ewers-Saucedo et al. 2015, Doell et al. 2017,). This timeframe is useful to delineate migration routes between nesting and foraging grounds of the hosts, which for sea turtles is generally 2-4 years (Lohmann et al. 1999).

Stable isotope analysis has become a popular tool to infer an animal's movement in the environment from the isotopic composition of their tissue (Hobson 1999, Hobson 2008a, 2008b). The large-scale geographic variation in biogeochemical isotopic processes results in spatial patterns of stable isotope ratios known as isoscapes (Graham et al. 2010, West et al. 2010, McMahon et al. 2013). The latitudinal gradients of stable oxygen isotope ratios ($\delta^{18}O$) in the marine environment has been used as a spatial tool for inferring the movement of migratory marine species from calcite-shelled organisms (Killingley 1980, Killingley and Lutcavage 1983, Detjen et al. 2015). The isotopic composition of carbonate shells during deposition is a function of the ambient temperature and isotopic composition of the seawater, which it precipitates (Epstein et al. 1953). Seawater $\delta^{18}O$ values are correlated with salinity because of the combined effects of evaporation and freshwater input either from precipitation or rivers (Epstein and Mayeda 1953). Enriched $\delta^{18}O$ isotopic signatures in seawater are usually observed in highly evaporative environments and semi-enclosed basins, such as the Mediterranean Sea ($\delta^{18}O$ = 1.7 ‰, Rohling and Rijk 1999). On the other hand depleted $\delta^{18}O$ values are found at high latitudes and areas with extensive freshwater input, such

as the Arctic Ocean ($\delta^{18}O$ = -20 ‰, McMahon et al. 2013). Sea surface temperature also has a well-established, large-scale latitudinal gradient. Estimates of the spatial range in which the isotopic composition of a carbonate shell was formed can therefore be determined once corrections for biological processes have been applied (Killingley and Newman 1982, Detjen et al. 2015).

Biomineralization, the process of generating minerals to harden existing tissue in carbonate shells, has primarily been investigated for paleontological studies on past climatic environments by assessing temperature-associated variations in the process (Urey 1947, Epstein et al. 1953). However, isotopic fractionation through biological processes can result in enrichment or depletion of the ratio from the ambient environment (Crippa 2015). Originally, the equations to estimate paleotemperature using this approach were based on calcite organisms which fractionate oxygen isotopes in equilibrium with the environment (Epstein et al. 1953, Crippa 2015). For barnacles this is however not the case, and during biomineralization an enrichment of about 1.3 ‰ for $\delta^{18}O$ has been reported, which could be attributed to the metabolic processes required to produce a complex shell (Killingley and Newman 1982). Because of fractionation of $\delta^{18}O$ from sea water to barnacle calcite, there is an inverse in the ratios, such that barnacles growing in enriched $\delta^{18}O$ waters will have a depleted $\delta^{18}O$ signature in their calcite, and the opposite occurs for barnacles growing in depleted $\delta^{18}O$ waters (Killingley and Newman 1982).

Similarly, variability in the inorganic carbon signature ($\delta^{13}C$) can also be incorporated into estimates of spatial origin as dissolved inorganic carbon in surface ocean water is influenced by physical and biological processes that create latitudinal gradients (McMahon et al. 2013). There is a general latitudinal trend in dissolved inorganic $\delta^{13}C$ with more enriched isotopic signatures around the equator and depleted values towards higher latitudes (McMahon et al. 2013). This pattern is related to areas of outgassing, such as equatorial upwelling regions, where the surface waters become enriched in $\delta^{13}C$ (Lynch-Stieglitz 1995). However, as the air to sea gas exchange fractionation of CO_2 is driven by temperature, this confounds the latitudinal trend to moderately small geographic ranges (Gruber et al. 1995, Lynch-Stieglitz 1995). This restricts the usefulness of inorganic $\delta^{13}C$ variability as an independent indicator of large-scale spatial origin in oceanic basins. In contrast, strong inorganic $\delta^{13}C$ gradients are observed between freshwater and marine environments (Gruber et al. 1995). This

has been used to identify spatial utilization of estuaries, lagoons, and coastal embayments at the regional scale by sea turtle hosts with epibiotic barnacles. This is possible as barnacle growth in a bay or estuarine environment is associated with a depleted $\delta^{13}C$ ratio of the calcite. Comparatively, barnacle growth in marine environments coincides with enriched $\delta^{13}C$ levels in the dissolved inorganic carbon component (Killingley and Lutcavage 1983).

This study uses the isotopic composition ($\delta^{18}O$ and $\delta^{13}C$) of barnacle shells to examine the movement patterns of loggerhead sea turtles (*Caretta caretta*) that nest on the beaches of the iSimangaliso Wetland Park, South Africa (Figure 1.2). Previous tracking techniques to determine the movement of loggerhead sea turtles in the South West Indian Ocean (SWIO) has mainly used of satellite tags and mark-recapture. These studies have shown that this population has a wide distribution, occurring from the Seychelles to the west coast of South Africa (Hughes et al. 1974, Luschi et al. 2003, Harris et al. 2018). However, the majority of turtles appear to migrate north from the nesting beaches along the coast (Harris et al. 2018). The $\delta^{18}O$ signatures in barnacle shells will likely reflect two main migratory routes, of those individuals that travel northwards from the nesting grounds and those that travel southwards. Additionally, barnacle calcite is inert and does not change over time. Therefore each new layer added represents the environment that a barnacle was growing in, this can be used to possibly examine temporal movement between nesting and feeding grounds.

The overall aim of this study was to apply the isoscape mapping method to provide a cost-effective assessment of turtle movement in the SWIO. Published information of regionally specific ocean oxygen isotope ratios and temperature of sea water will be used to map isotopically distinct geographical areas. Using the known $\delta^{18}O$ fractionation values for barnacles (Killingley and Newman 1982), an isoscape of expected $\delta^{18}O$ of barnacle calcite can then be generated (Detjen et al. 2015). The isoscape will then be used to track nesting loggerhead sea turtles both spatially and temporally through the life of their epibiotic barnacles, *Chelonibia testudinaria*. The specific objectives are to: (1) create an isoscape of $\delta^{18}O$ barnacle calcite in the SWIO region using available data on $\delta^{18}O$ of seawater and sea surface temperature; (2) link $\delta^{18}O$ isotope signatures measured from loggerhead's epibiotic barnacle shell with respective ocean isoscapes; and (3) assess whether the chronology of barnacle shells

can be used to track the migratory history of turtles. The results from this study can be used to provide complementary information on loggerhead movement in the SWIO. This study represents the first application of these techniques to study sea turtle movement in the SWIO region and therefore forms an important baseline for any future research.

4.3 Methods

Chelonibia testudinaria barnacles were collected from nesting loggerhead sea turtles (*Caretta caretta*) in the iSimangaliso Wetland Park, South Africa (Figure 1.2) during the 2015-16 and 2016-17 nesting seasons, respectively. To avoid disturbing a turtle during nesting, barnacles were taken only once egg-laying was near completion. The two largest barnacles were collected from the carapace of each individual sea turtle and frozen. A total of 16 barnacles were analysed from eight turtles.

4.3.1 Barnacle sampling and isotope analysis

In the laboratory, sand, algae, dwarf male barnacles and other epifauna were removed from the calcareous shell which was then rinsed in distilled water. Height and rostro-carinal length were measured to determine the age of each barnacle (Doell et al. 2017) and the 4th, 5th and 6th wall plates were removed and used for analysis (Figure 4.1 A). The shell was sliced along the growth axis (Killingley and Lutcavage 1983, Biasatti 2004, Detjen et al. 2015) of the wall plates (Figure 4.1 B) at the Centre for High Resolution Transmission Electron Microscopy, Nelson Mandela University, South Africa. A 0.4 mm diamond blade saw was used to cut the barnacle into different layers 0.5 mm thick (Figure 4.1 C). The calcite deposit around the edge of each layer was removed using a Dremel® micro-drill (Figure 4.1 D), because this thickened layer is exposed to physical damage and in many cases had been colonized by encrusting algae. Each layer represents a distinct time of deposition giving a chronology from the oldest layer on top to the youngest layer on the bottom (Bourget and Crisp 1975, Crisp and Bourget 1985).

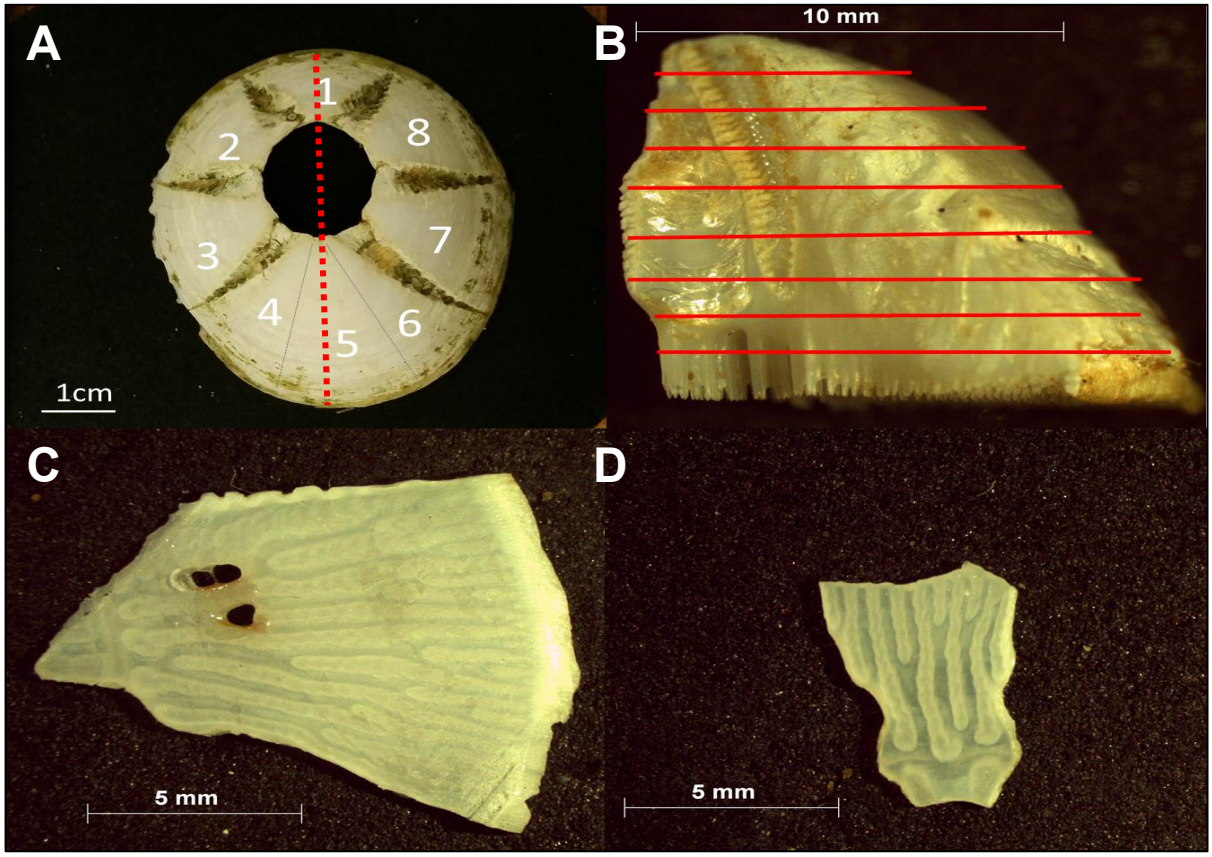


Figure 4.1: *Chelonibia testudinaria*: **(A)** with its eight wall plates (adapted from Blick et al. 2010), the red dotted line indicates the rostro-carinal length used to determine the age of the barnacle (Doell et al. 2017); **(B)** the cross section and layering of the barnacle for isotopic analysis with the oldest layer on top and the most recent layer at the bottom; **(C)** a distinct growth layer before; and **(D)** after the outer edges' thick calcite deposit was removed using a Dremel micro-drill.

Each layer was crushed into a fine powder using a mortar and pestle, put into tin boats and placed under vacuum at 200°C to remove any volatiles and/or organic material (Detjen et al. 2015). The samples were then packed into Eppendorf tubes and sent for analysis at the Stable Isotope Analysis Laboratory of the Mammal Research Institute (Pretoria, South Africa). Aliquots of approximately 0.025 to 0.030 mg of the pre-treated barnacle samples were weighed into borosilicate tubes with a septa lid. These were then flushed with helium, prior the injection of a standardized amount of ortho-phosphoric acid via an acid pump (Fluid Metering Inc, supplied by Thermo).

The stable isotope ratios of ^{13}C to ^{12}C and ^{18}O to ^{16}O were determined using a gas bench (Finnigan GBII) with a GC PAL autosampler, coupled to a Delta V Plus stable

light isotope ratio mass spectrometer via a ConFlo III system (all equipment supplied by Thermo Fischer, Bremen, Germany). All results are referenced to Vienna Pee-Dee Belemnite for carbon isotope values and VSMOW for oxygen isotope values. The stable isotope signature is expressed in delta (δ) in parts per thousand (‰) using the standard equation (Coplen 2011):

$$\delta x = ([\frac{Rsample}{Rstandard}] -1) * 1000)$$

Where X is ^{13}C or ^{18}O and R_{sample} and $R_{standard}$ is the ratio of heavy to light isotope of the relative element in the sample and standard, respectively. The precision for carbon and oxygen isotopes is derived from the standard deviations obtained for USGS35. The precision for Carbon was < 0.04 ‰ and for Oxygen < 0.05 ‰.

4.3.2 Creating isoscape maps

ArcGIS (V 10.6) was used to create an isoscape map showing the barnacle calcite $\delta^{18}O$ signatures that are expected for the SWIO to provide geographical context for results of $\delta^{18}O$ analysis of the turtle's barnacle. To match the isotopic signatures of barnacle shells with those of sea water, the values of the ocean isoscape map were converted to $\delta^{18}O$ signatures of calcite according to a formula designed by Epstein et al. (1953) and modified by Killingley and Newman (1982) with a correction of +1.3 ‰ for barnacle calcite (for further details see Detjen et al 2015). This formula requires three variables: sea surface temperature (T); $\delta^{18}O$ signatures of the water (W); and the $\delta^{18}O$ signature of the calcite (C), and was originally intended to convert water and barnacle calcite $\delta^{18}O$ signatures into expected sea surface temperatures. Rearranged, it can serve to predict C from T and W, using the following equation (Detjen et al. 2015):

$$C = \frac{-[-0.28\mathrm{W} - 4.3] - \sqrt{[-0.28\mathrm{W} - 4.3]\,2 - 4\,\mathrm{x}\,[0.14]\,\mathrm{x}\,[0.14\mathrm{W}2 + 4.3\mathrm{W} - \mathrm{T} + 16.5]}}{0.28}$$

The map used geospatial data of temperature (˚C) and the $\delta^{18}O$ of seawater. Average annual sea surface temperature data (T) were obtained from NOAA archives (NOAA 2005), and $\delta^{18}O$ signatures of the water (W) from measurements in 2006 (LeGrande and Schmidt 2006, Figure 4.2). Using ArcGIS, separate layers of mean sea surface temperature (Figure 4.3 A) and $\delta^{18}O$ signature of the sea surface water (Figure 4.3 B)

were created. These were converted to raster data and the above equation applied using the Raster calculator tool to convert values to equivalent barnacle calcite signatures of $\delta^{18}O$ expected for the waters around South Africa and Mozambique, where the loggerheads have previously been recorded (Luschi et al. 2003, 2006, Harris et al. 2018). Based on the range of $\delta^{18}O$ isotopic signatures measured for 16 barnacles, maps were generated reflecting the movement of the turtles.

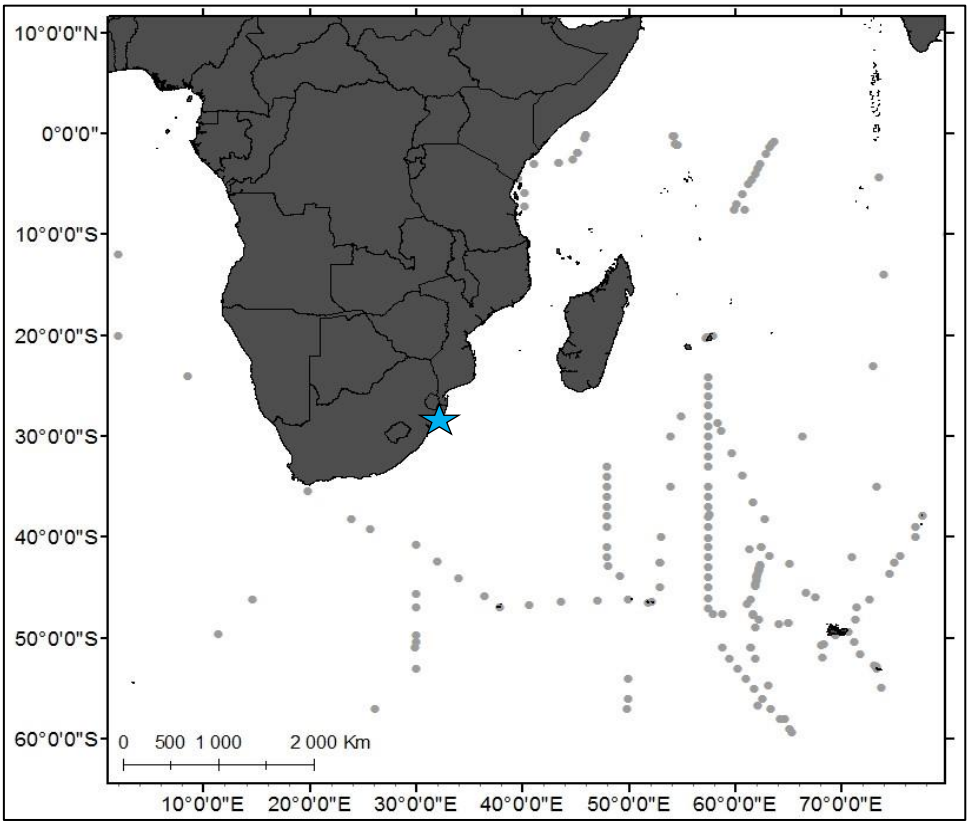


Figure 4.2: Area of known distribution of South Western Indian Ocean loggerhead turtles. Grey dots represent positions where records of $\delta^{18}O$ isotopes and sea surface temperature coincide (NOAA 2005, LeGrande and Schmidt 2006), and which were interpolated to calculate ocean isoscapes in this study. The blue star marks the study site in the iSimangaliso Wetland Park, where loggerhead sea turtles (*Caretta caretta*) were sampled during nesting.

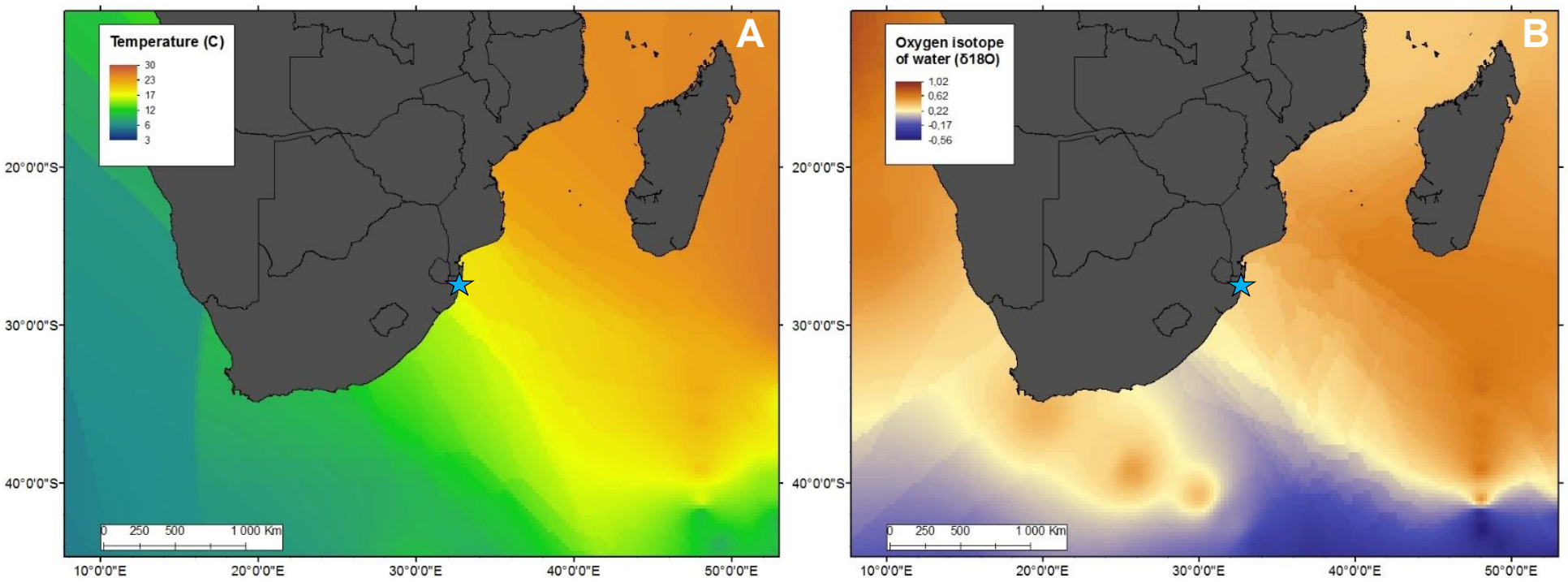


Figure 4.3. (A) Annual mean sea surface temperature of the South West Indian Ocean and South East Atlantic Ocean based on NOAA (2005) records. **(B)** Oxygen ($\delta^{18}O$) isoscape of the South West Indian Ocean and South East Atlantic Ocean (LeGrande and Schmidt 2006). The blue star marks the study site in the iSimangaliso Wetland Park, where loggerhead sea turtles (*Caretta caretta*) were sampled during nesting.

4.3.3 Data analyses

To test the validity and accuracy of using $\delta^{18}O$ from barnacles to track turtle movement, two replicate barnacle shells were analysed per turtle. Isotopic signatures for $\delta^{18}O$ and $\delta^{13}C$ were plotted in chronological order from the base of the barnacle, which represents the youngest layer, to characterize how the isotopic profiles reflect the environmental parameters experienced by each barnacle throughout its life. Additional metadata collected for each individual were examined in conjunction with the isotope signatures from the barnacles. These data include $\delta^{13}C$ signatures from the epidermis of the turtle (Chapter 3) and, where known, the frequency of inter-nesting events from an ongoing mark-recapture study (Nel, unpublished data). The $\delta^{13}C$ signatures of the epidermis from the turtle were compared to the last deposited layer from the barnacle, since both are estimated to contain signatures of the turtle foraging grounds, which are distinct from nesting grounds (Chapter 3). This comparison was done using a Spearman Rank correlation, after the data failed the assumption of normality (Shapiro-Wilk: $p < 0.05$).

4.4 Results

A total of 130 layers from 16 *Chelonibia testudinaria* barnacles were analysed for $\delta^{18}O$ and $\delta^{13}C$. These layers varied in number per barnacle, between six and 11 layers, depending on the size and height of each individual. A summary of each barnacle's length, height, and the range of $\delta^{18}O$ and $\delta^{13}C$ isotopic signatures (for both barnacles and their turtle host) are provided in Table 4.1. The data used to create the oxygen isoscape (Figure 4.3), did not have adequate records north of Madagascar, which limited the geographical extent of the isoscape in the SWIO.

Table 4.1: *Chelonibia testudinaria* collected from loggerhead sea turtles (*Caretta caretta*). The two barnacles for each turtle are reported as A and B, respectively.

Barnacle ID	Height (mm)	Length (mm)	$\delta^{18}O$ barnacle range (‰)	$\delta^{13}C$ barnacle range (‰)	$\delta^{13}C$ of turtle (‰)
1 A	12.6	51.0	-2.13 to -0.48	-0.19 to 0.89	-15.45
1 B	13.5	53.6	-1.63 to -0.89	-0.07 to 2.42	
2 A	10.1	41.2	-1.48 to -0.46	0.05 to 1.47	-11.28
2 B	12.2	46.4	-1.65 to -0.44	0.14 to 0.97	
3 A	8.0	35.0	-1.69 to -0.11	-0.06 to 0.70	-15.08
3 B	8.2	33.1	-2.23 to -0.91	-0.22 to 0.29	
4 A	9.9	38.9	-2.85 to -0.53	-0.79 to 0.90	-15.06
4 B	8.5	32.1	-1.44 to -0.34	-0.38 to 1.46	
5 A	10.4	46.1	-1.94 to -0.48	0.14 to 1.00	-14.66
5 B	10.0	42.6	-1.82 to -0.68	0.14 to 0.96	
6 A	12.2	48.5	-1.56 to -0.33	0.28 to 1.39	-14.55
6 B	12.7	47.0	-1.06 to -0.59	-0.50 to 1.32	
7 A	9.5	36.1	-0.68 to -0.11	0.34 to 2.04	-11.8
7 B	8.9	29.1	-0.43 to -0.08	0.15 to 1.78	
8 A	13.7	51.2	-0.53 to 0.28	0.56 to 1.77	-14.98
8 B	10.0	41.6	-0.83 to -0.24	0.77 to 1.47	

Chronologies of oxygen isotope signatures derived from analysis of different layers of a barnacle shells revealed that there were variabilities in the $\delta^{18}O$ and in $\delta^{13}C$ signatures, reflecting environmental change during the lifespan of the barnacle (Figure 4.4). The isotopic profiles from different barnacles collected from individual turtles were generally similar in their range. However, in some cases the profiles showed deviation in isotopic chronologies, between layers that were sampled at the same distance from

the base for each barnacle. For example, barnacles 4A and 4B from turtle 4 showed similar isotopic profiles in the youngest layers, however, there are large oscillations in 4A that are not apparent in 4B.

Oxygen ($\delta^{18}O$) isotopic signatures in barnacle shells reflect an increase with latitude (Figure 4.5). The maps for each barnacle from the same turtle varied, although significant overlap existed, suggesting that confidence in a turtle's core movement range increases with the analysis of replicate barnacle shells (Figure 4.4). The range of $\delta^{18}O$ values (Table 4.1) was used to infer the extent of the turtle's movement during the life of the barnacle. Although $\delta^{13}C$ was analysed from the barnacle shells it was not informative for habitat or area use, as the ranges of the signatures were limited. Furthermore, there were no clear relationships between inorganic $\delta^{13}C$ of the barnacle and organic $\delta^{13}C$ of the turtle epidermis (Spearman Rank correlation, R_s = 403.07, p = 0.12, R^2 = 0.17).

Turtles numbered 1, 3 and 4 were recorded to have also nested the season prior to having their barnacles collected. The $\delta^{18}O$ isotopic chronologies of the barnacles for these turtles indicate this remigration to the nesting area (Figure 4.4). The oscillation of isotopic chronologies indicates migration with depleted and enriched $\delta^{18}O$ signatures indicating foraging and nesting grounds, respectively. Turtles 7 and 8 had narrow isotopic ranges, as reflected by their isotopic profiles. These individuals also migrated the furthest south from the nesting beach (Figure 4.5).

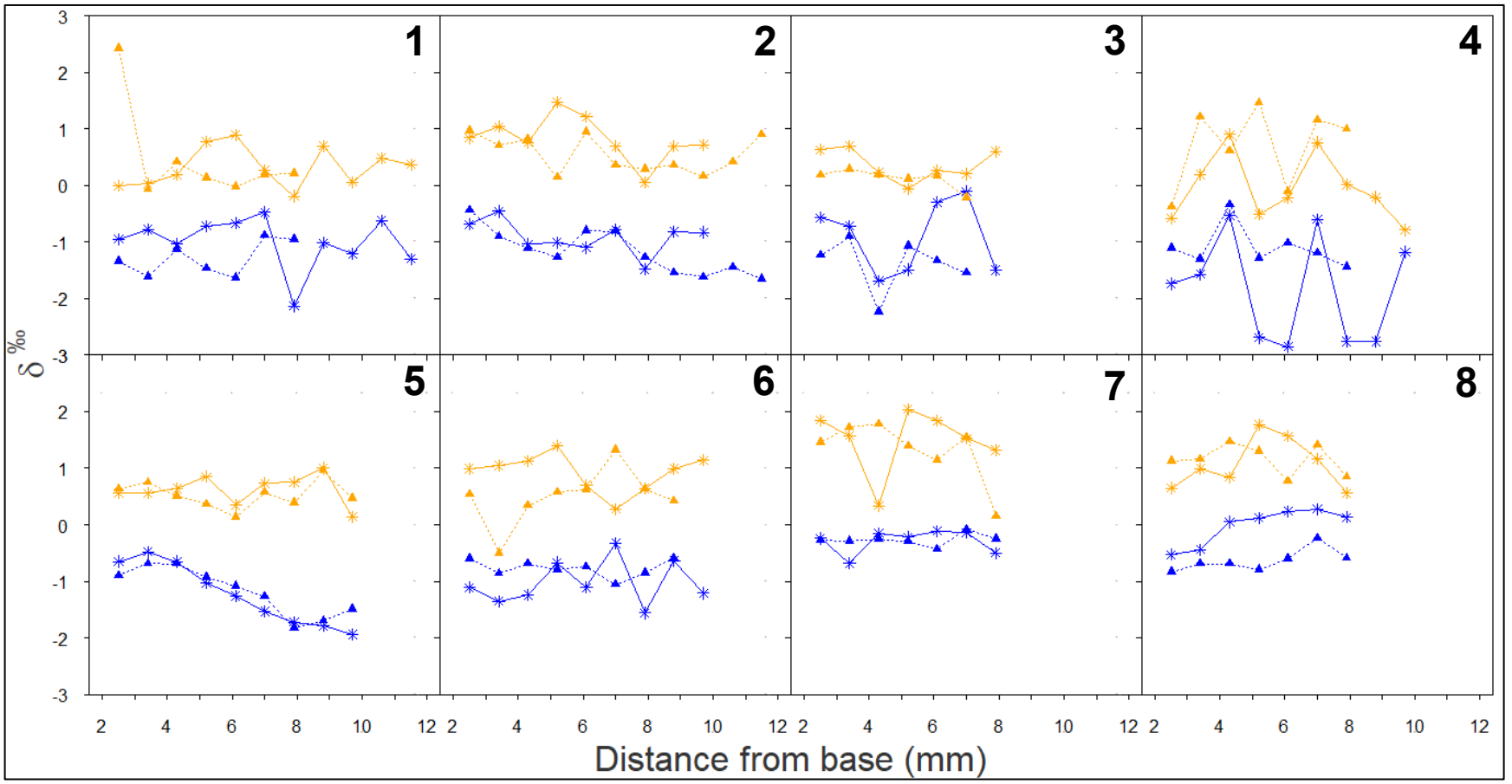
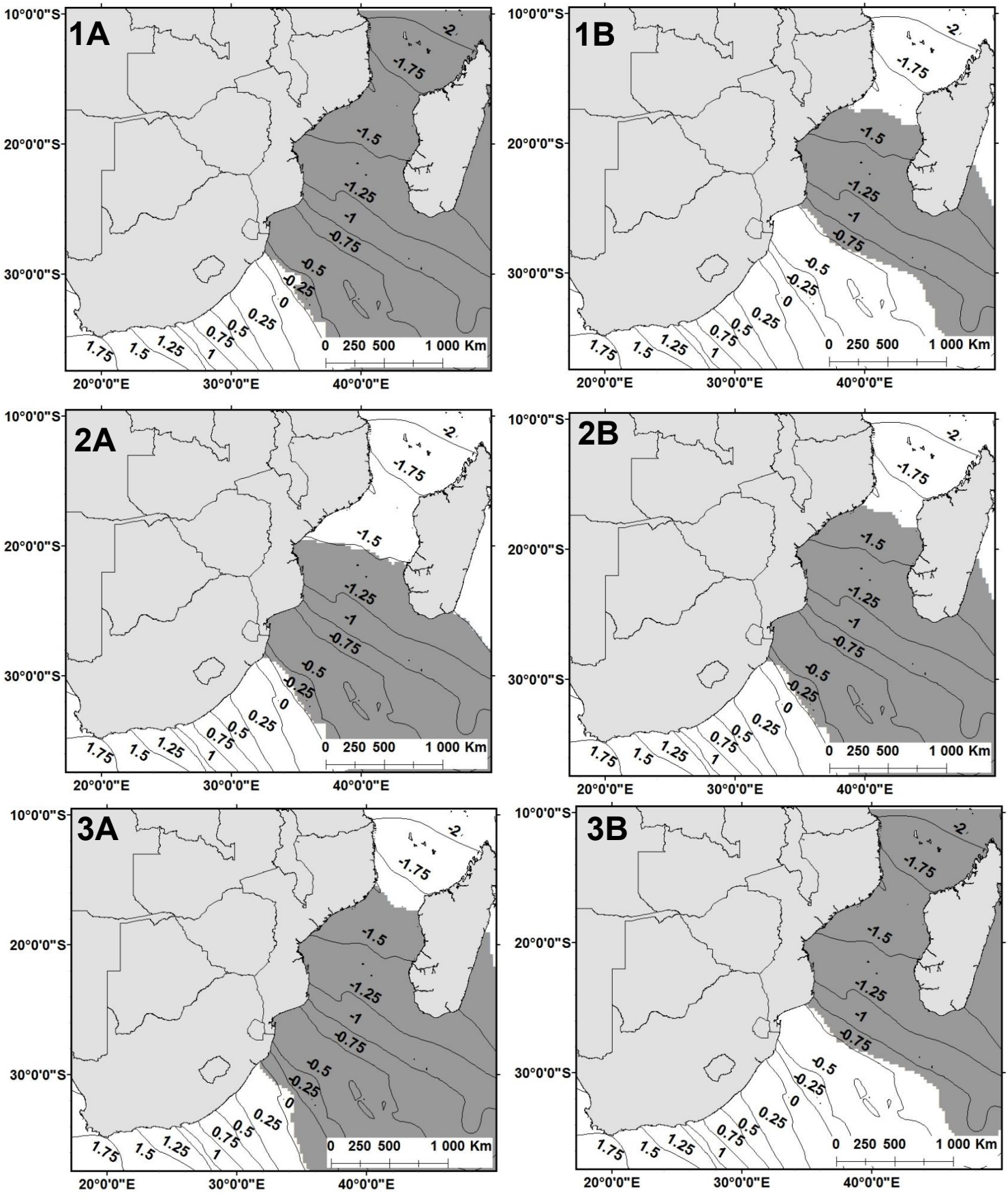


Figure 4.4. $\delta^{18}O$ (—) and $\delta^{13}C$ (—) isotopic profiles of *Chelonibia testudinaria* collected from eight nesting loggerhead sea turtles (*Caretta caretta*). The two barnacles per turtle are represented as stars (Barnacle A) and triangles (Barnacle B), respectively. The distance from the base (mm) represents the age of calcite deposit, with layers further away representing older deposits.


1A
1B
2A
2B
3A
3B
10°0'0"S
20°0'0"S
30°0'0"S
20°0'0"E
30°0'0"E
40°0'0"E
-2
-1.75
-1.5
-1.25
-1
-0.75
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1
1.25
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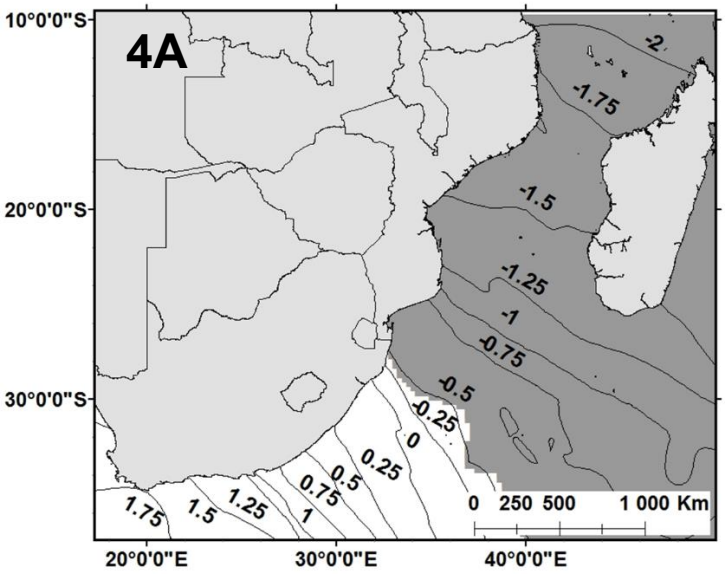
4A
10°0'0"S
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30°0'0"E
40°0'0"E
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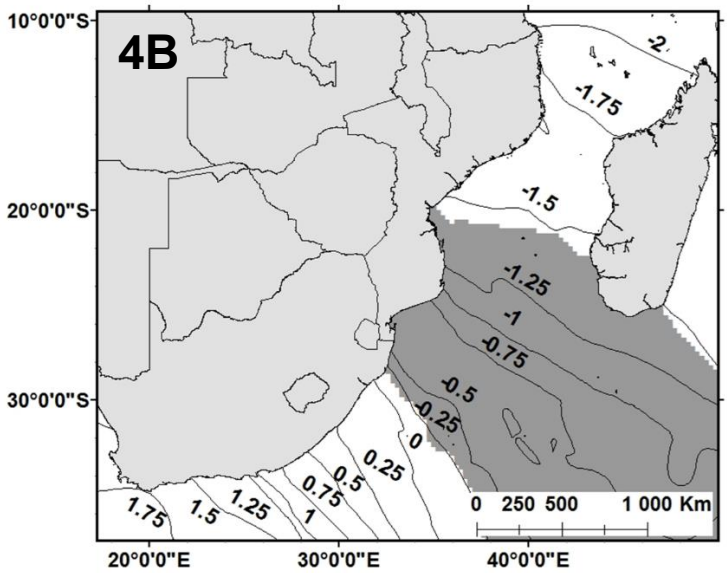
4B
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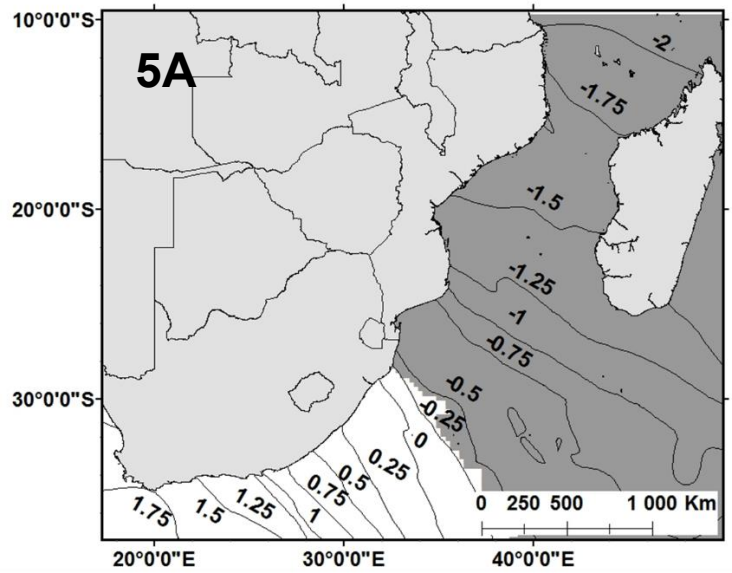
5A
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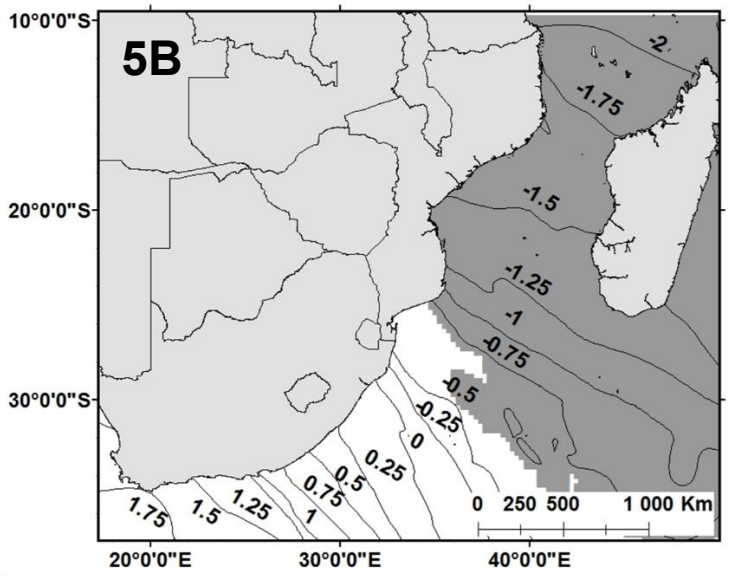
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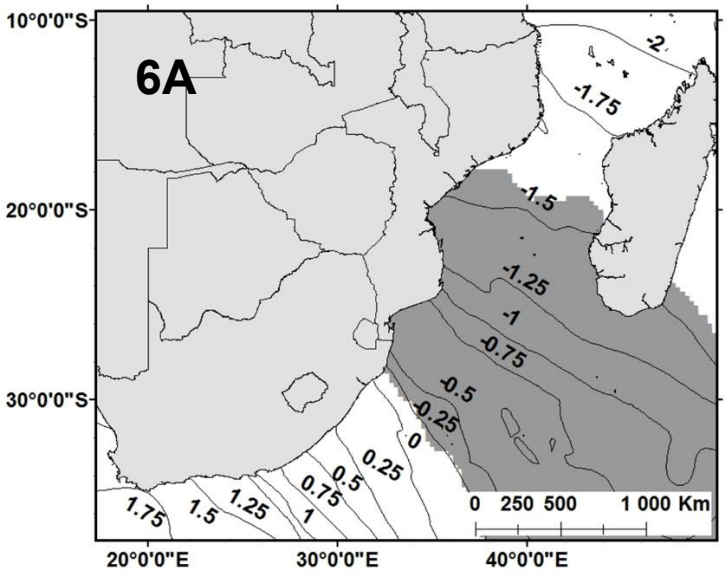
6A
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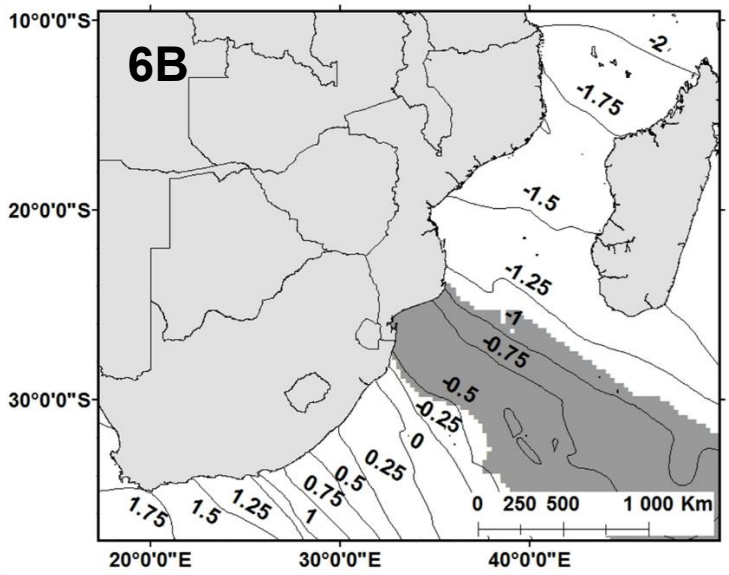
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-1
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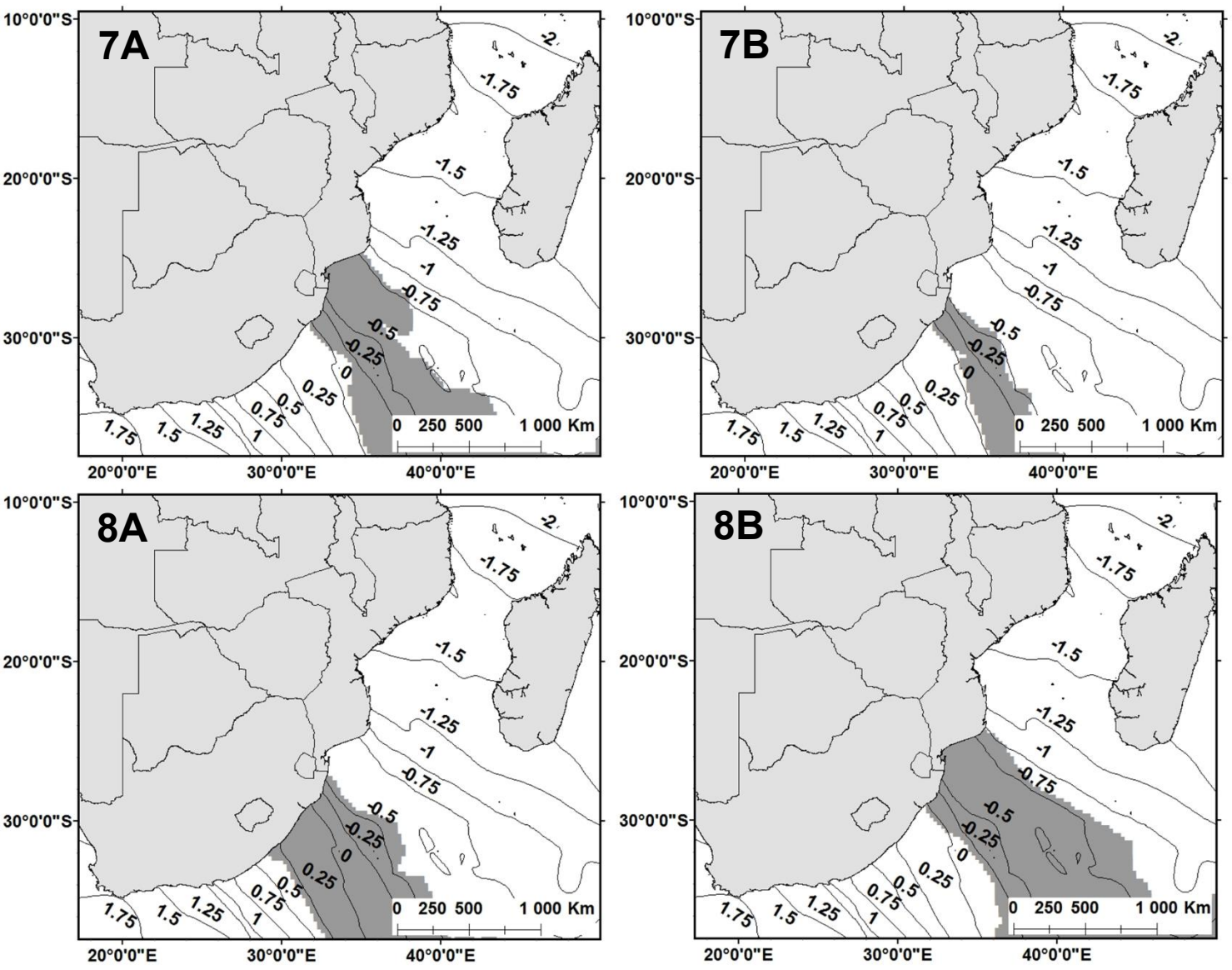

Figure 4.5: Movement of eight nesting loggerhead sea turtles (*Caretta caretta*) in the South West Indian Ocean based on the $\delta^{18}O$ signatures from analysis of *Chelonibia testudinaria* shells. Two barnacles were analysed for each turtle, represented as A and B. The barnacle oxygen isoscape is based on sea surface temperatures and $\delta^{18}O$ water signatures. The limit of the turtle movement (shaded region) is based off the range of $\delta^{18}O$.

4.5 Discussion

For the use of an effective ecogeochemical approach to study animal movement, it is recommended to apply all of the following criteria (Hobson et al. 2010, McMahon et al. 2013): (1) Create a geochemical map or isoscape that characterizes distinct isotopic signatures of different areas; (2) understand the turnover rates of different tissue types that determine the period of spatial assimilation of isotopic signatures; and (3) distinguish the isotopic fractionation factors between a consumer and its diet, or between animals and the environment, that may modify the signature from the baseline isoscape. This study followed the above approaches and found that $\delta^{18}O$ analysis of barnacle calcite could be used to define the movement of the sea turtle migrations during the lifetime of the barnacle at a relatively coarse spatial scale. The isoscape maps, which reflect the range of $\delta^{18}O$ signatures of each barnacle, provide a visual representation of the spatial resolution at which this method can inform on turtle movement. The accuracy of this method can only be validated by collecting data from individuals with satellite tags. Most of the turtles investigated in this study had foraging habitats north of the nesting beaches, which was also found by satellite and tag recovery studies of this population (Luschi et al. 2003, 2006, Harris et al. 2018).

Carbon isotope ratios were not informative in delineating movement ranges of loggerhead turtles throughout the life of the barnacles, however, the narrow range in $\delta^{13}C$ values suggests that the turtles exclusively inhabit fully marine environments (Cherel and Hobson 2007). A large change in $\delta^{13}C$ would represent growth of a barnacle in environments with large freshwater input, where dissolved inorganic carbon signatures are depleted relative to ocean values (McMahon et al. 2013). Although movement into freshwater environments could not be determined, $\delta^{13}C$ values from the barnacles suggest that none of the individuals had occupied these areas. Isotopic analysis of *Chelonibia testudinaria* in Chesapeake Bay reported $\delta^{13}C$ values as depleted as -6 ‰, which was interpreted to reflect barnacle growth in brackish waters, such as estuaries with high freshwater input (Killingley and Lutcavage 1983). In the present study there were no trends between inorganic $\delta^{13}C$ of barnacles and organic $\delta^{13}C$ of turtle epidermis. However, fractionation rates differed between organic and inorganic $\delta^{13}C$ and this could

account for the lack of correlation. The lack of variability in $\delta^{13}C$ signatures of barnacle shells can be supplemented with organic $\delta^{13}C$ of turtle tissue, which better depicts habitat use as either neritic or oceanic (Reich et al. 2010, Chapter 3).

Isotopes are commonly used in studies of habitat and area use of marine vertebrates, and have primarily focused on organic $\delta^{13}C$ and $\delta^{15}N$ signatures. Although these are useful in delineating habitat use (Reich et al. 2010, Robinson 2016, MacKenzie et al. 2011), results can be complicated by trophic and/or physiological factors, as well as baseline variations that occur over a short temporal scales (Fourqurean et al. 1997, Riera et al. 1997, Lorrainet al. 2015). In contrast, oxygen isotopes have minimal temporal variation and have been used to record past climatic events over geological time periods (Schöne and Gillikin 2013, Leng and Lewis 2016). Although $\delta^{18}O$ can be affected by evaporation, rainfall and salinity (Epstein and Mayeda 1953), variability as a result of these physico-chemical factors can be accounted for at regional geographic scales. Utilizing other common isotopes along with oxygen to infer animal movement can add more information about habitat use.

In this study, replicate barnacles collected from an individual turtle were of similar size, yet there were differences between their isotopic chronologies. These differences could be explained by the age of the barnacles. *Chelonibia testudinaria* are estimated to live up to three years (Hayashi and Tsuji 2008, Ewers-Saucedo et al. 2015, Doell et al. 2017), however their growth is not uniform. *Chelonibia testudinaria* exhibit a von Bertalanffy growth curve having rapid growth the first year with a slow, but steady increase thereafter (Sloan et al. 2014, Ewers-Saucedo et al. 2015, Doell et al. 2017). Using the growth curve described in Doell et al. (2017) and the length of the barnacles from turtle 4 (Table 1) the estimated age for 4A is 222 days (95% CI 120 - 422) and for 4B is 160 days (range 95% CI 92 - 308). While these two barnacles are not much different in terms of their average estimated age based on their size, they could have grown at different rates making the age estimation less reliable. Furthermore, the ambient flow rate of water has been shown to influence rocky intertidal barnacles, where those with intermediate flow rates grew faster because of an increase in food availability (Crisp 1960, Eckamn and Duggins 1993). The carapace of turtles has different flow patterns and drag (Logan and Morreale

1994, Scharer 2001), which is known to affect settlement of epibiotic barnacles (Pfaller et al. 2006, Najera-Hillman et al. 2012). Furthermore, epibiotic growth on barnacles has been shown to negatively influence the growth of the host barnacle (Barnes 1955). It is thus likely that replicate *Chelonibia testudinaria* barnacles analysed in this study exhibited variability in growth rates, which could not feasibly be controlled for.

Although the isotope data showed movement ranges, the accuracy of the underlying isoscape could be vastly improved with higher spatial data coverage. Obtaining $\delta^{18}O$ of seawater and temperature from South Africa to Seychelles (via the Mozambique Channel), could improve isoscape resolution and accuracy and potentially allow for turtle movements from coastal to offshore environments to be delineated. Furthermore, the isoscape calculated in this study did not extend past Mozambique due to a lack of oceanic data for this region. The isoscape gradient shows a steady depletion of $\delta^{18}O$ further north. Extrapolating this and the $\delta^{18}O$ values from turtles 1, 3, 4 and 5 it is possible that these individuals migrated further north than indicated by the isoscape.

The method employed here to distinguish nesting individuals occupying different areas can also be applied to turtles at their feeding grounds to determine whether individuals that feed in the same area are from genetically distinct populations. For example, loggerhead turtles from the northwestern Atlantic and Mediterranean populations share a foraging ground in the Mediterranean but remain genetically distinct from one another (Carreras et al 2011). A genetic study on overlapping populations can be verified by stable isotope analysis of the turtles' barnacles to show individual movement to nesting grounds. Regional Management Units (RMUs) have been drawn up for all seven sea turtle species, with overlap of area for many populations (Wallace et al. 2011). Understanding the proportion of individuals that belong to specific RMUs where there is overlap is an important conservation priority (Hamann et al. 2010).

Chelonibia testudinaria occur on all hard-shelled sea turtles (Frick et al. 1998, Lazo-Wasem et al. 2011), as well as sirenians (Zardus et al. 2014), crocodilia (Cupul-Magana et al. 2011) and brachyura (Cheang et al. 2013). Similar barnacle species occur on whales (Hayashi et al. 2013) and leatherback sea turtles (*Dermochelys coriacea*) (Robinson et al 2017). The method developed in this study could be particularly useful for assessing

regional movement patterns of leatherbacks in the SWIO, as this species has a larger range and more migration corridors than loggerheads (Harris et al. 2018). This could provide additional valuable information for this population, which is classified as critically endangered (Wallace et al. 2013). With barnacles occurring on a wide array of species, the methods used here can be applied to these organisms as complementary approach to satellite tracking. There are many whale species that use the waters off southern Africa as breeding grounds and barnacles collected off these individuals could be used to map feeding habitats, typically in the Southern Ocean. This has been done for Pacific gray whales (*Eschrichtius robustus*), however, an isoscape approach was not implemented here due to the lack of appropriate geographic mapping software at the time (Killingley 1980). The isoscape created for this region could be directly applied to barnacles occurring on other species without correction, because the +1.3 ‰ correction was observed in a range of Balanomorpha (Killingley and Newman 1982).

The use of isotopic analysis of epibiotic turtle barnacles provides an alternative to satellite tracking. With a finer scale isotopic map of $\delta^{18}O$ for barnacle calcite it will be possible to delineate a turtle's movements before reaching the nesting grounds, and where possible, identify migrations between nesting events. Although the accuracy and spatial resolution of using this isotopic approach is low relative to satellite tracking, it is a far cheaper alternative. The total cost to analyse 16 barnacles from eight turtles was about $1200, compared to a satellite tag study, which would have cost of more than $10 000. This makes barnacles an excellent proxy to study spatial ecology of their host, which can be utilized on a greater proportion of the population.

4.6 Conclusion

Although the data available for creating the isoscape of the SWIO region were limited, the inferences about loggerhead turtle movement were largely consistent with field observations from satellite and tag recovery studies (Luschi et al. 2003, 2006, Harris et al. 2018). The use of $\delta^{18}O$ can provide valuable information on turtle movement ranges over the life of the barnacle, which is typically 3 years (Hayashi and Tsuji 2008, Ewers-Saucedo et al. 2015, Doell et al. 2017). This approach provides low-resolution information that is valuable for regional management of sea turtle populations at both their nesting

and foraging areas. The main advantage of using this method is the low cost compared to satellite tracking, which makes it particularly suitable for application in developing regions. With more spatial data of water $\delta^{18}O$ and temperature, a refinement of the isoscape for the region can be achieved, giving more resolution of turtle movement. The methods used here may then prove valuable to conservation efforts, not only for sea turtles but also for many other marine species.

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Chapter 5

General conclusions

Determining the movement patterns of migratory species has been an important factor to meet some of the grand challenges of organismal biology (Schwenk et al. 2009, Bowlin et al. 2010). This has been achieved to various degrees of success using intrinsic and extrinsic markers (Rubenstein and Hobson 2004). Marine turtles are appropriate candidates to test different extrinsic and intrinsic methods used to track marine migratory species' distributions. They are circumglobal in distribution, inhabit most oceans, but the different species and populations occupy a variety of ecological niches with distinct foraging and nesting grounds (Wallace et al. 2010). Sea turtles consequently fulfil a range of ecological roles in the marine environment (Heithaus 2013), despite many populations being severely depleted in numbers, with some still threatened and/or in decline (Wallace et al. 2011). In order to develop effective spatial conservation tools, their habitat use, including movement during breeding and non-breeding periods, and feeding patterns need to be understood. Furthermore, these different feeding patterns could affect the body condition of individual turtles frequenting these environments and could be used as parameters to infer health.

Proposed research priorities for sea turtles have focused on five key research themes (Hamman et al. 2010). One of these is biogeography, which can be used to inform on the other research themes, specifically population ecology, threats and conservation strategies. This research project contributes new information within this theme for the South West Indian Ocean (SWIO) loggerhead sea turtle population. Previously in the SWIO, knowledge of South African turtle migration was limited to data from a small number of satellite tracking devices and mark-recapture studies (Hughes 1974, Luschi et al. 2003, 2006, Harris et al. 2018). This study aimed to enhance the understanding of the body condition and movement of loggerhead sea turtles (*Caretta caretta*) in the SWIO through: (1) assessing the relationship between epibiont community composition and turtle body condition; (2) $\delta^{13}C$ and $\delta^{15}N$ stable isotope analysis of turtle epidermis and epibiont community structure to examine foraging area; (3) $\delta^{18}O$ and $\delta^{13}C$ stable isotope

analysis of epibiotic barnacles to show regional movement of the turtles host; and (4) examining the effect of foraging area on body condition.

5.1 Epibionts as a proxy for sea turtle health

The condition of marine species, like sea turtles and their populations, reflects the health of ecosystems, as organisms are highly dependent and therefore closely linked to their environment (Aguirre and Lutz 2004). Anthropogenic disturbance, like pollution and habitat modification (Mrosovsky et al. 2009, Witherington et al. 2009) and changing ocean conditions, partly due to climate change (Chaloupka et al. 2008, Van Houtan and Halley 2011), are important threats to turtle habitats, which affect the distribution and abundance of populations. Therefore, monitoring sea turtle condition efficiently with minimal costs was an important goal of this study. Body condition is a well-recognized parameter of sea turtle health (Bjorndal et al. 2000, Labrada-Martagon et al. 2010), and epibionts have been used as an alternative technique to classify individuals into body condition categories. **The results from this study demonstrated that epibionts can be used to elucidate body condition of nesting females, despite them being assumed to be in 'good' health.** Community structure of epibionts on the carapace showed a strong relationship with turtle body condition; individuals in a poorer condition had greater species richness and relative abundance of epibionts (Chapter 2). These results are in line with the hypothesis that if physical attributes, like swimming speed or ability to self-clean, are drivers of epibiont load, even nesting turtles in relatively poorer body condition will have a higher abundance of epibionts than those in good condition. The use of epibionts is thus a quick method to examine the (body) condition of an individual nesting turtle. This method can easily be applied and modified to different turtle populations.

Habitat quality of sea turtles feeding in different foraging areas likely affects body condition and growth rate and ultimately health (Balazs and Chaloupka 2004). The effect of feeding niche, determined by stable isotope analysis, on body condition was also investigated (Chapter 3). **It was found that there was significant niche overlap with body condition, however turtles in very good body condition had a narrower isotopic niche.** The results do not support the hypothesis that dietary differences, as indicated by the isotopic niche, drives turtle body condition where individuals foraging in

distinct habitats have different body conditions. It is plausible that those individuals in very good condition have narrow isotopic niches because they do not forage far from the nesting area and therefore do not expend excessive amounts of energy during foraging.

It should be noted that none of the SWIO loggerheads examined showed signs of fibropapillomatosis, a disease in sea turtles that is characterized by the occurrence of epithelial fibropapillomas (Herbst and Jacobson 2002, Greenblatt et al. 2004), or any similar looking type of disease. This disease is an unambiguous indicator of sea turtle health and has reached epidemic proportions in other regions of the world (Aguirre and Lutz 2004). Fibropapillomatosis commonly occurs in turtles that inhabit or frequent areas that are severely impacted by anthropogenic inputs, particularly marine pollution, like in Moreton Bay, Australia where 40-70% of green and loggerhead individuals are affected (Aguirre et al. 1999). It is therefore important to monitor nesting turtles for the occurrence of this disease. If fibropapillomatosis is discovered on a turtle one can use the epibiont community composition, and stable isotope analysis of the turtle's skin (Chapter 3) and epibiotic barnacles (Chapter 4) to show where the turtle was previously, and possibly determine the origin.

Overall, the nesting loggerheads in the South African population were relatively healthy, with no individuals being categorised as having a very poor body condition. Because this sea turtle population has seen increases in the number of nesting individuals (Nel et al. 2013), and the prevalence of anthropogenic threats was low or non-existent (i.e. boat strikes, interaction with fisheries, fibropapillomatosis) it can be assumed that this region is relatively undisturbed. Ultimately, to make assumptions about the entire populations, all different cohorts would need to be examined. The body condition index developed in this study could be used for other Cheloniidae as epibiont loading and health deterioration has been noted in green sea turtles (*Chelonia mydas*) (Flint et al. 2010, Najera-Hillman et al. 2012). Using epibionts, particularly barnacle cover, is an easy method which can be applied in the field, providing a quick assessment of a turtle's condition. The fact that all parameters investigated in this study, including epibiont assemblage structure and diversity, as well as barnacle loading, showed distinctive relationships with the body

condition of loggerhead turtles, emphasised the value of epibionts as effective indicators for population and ecosystem health.

5.2 Epibiont communities to infer turtle habitat use

Besides their use as a proxy to infer health on turtles, epibionts can be used to examine the broad distribution, and son indications of habitat utilization, by the host. Hosts that move between geographically distinct areas acquire unique epibionts from these regions (Frick and Pfaller 2013). In this study, epibionts were used to infer inshore versus offshore foraging habitats of the host. **The results showed that turtles foraging habitats reflected the epibiont community structure on the carapace. Individuals that forage inshore had a higher frequency of neritic epibionts (e.g. *Podocerus africanus, Hyale grandicornis, Hyachelia tortugae*) compared to open ocean foragers, which had a higher occurrence of oceanic and pelagic organisms (e.g. *Lepas* spp.)** (Chapter 3). An analysis of $\delta^{13}C$ of the skin corroborated this relationship, revealing turtles with isotopic signatures below -13.61 ‰ had a greater proportion of oceanic/pelagic epibionts compared to individuals with more enriched $\delta^{13}C$ values. These results are in line with the hypothesis that geographical and ecological overlap between the host and its epibionts, explains the differences in epibiont assemblages on turtles that forage in oceanic and neritic habitats.

The only other report on sea turtle epibiosis in the region found six species of epibionts on loggerhead hosts (Hughes 1974). This study found that the species richness (28 species) and occurrence of those listed species is far higher than previously reported for the region. *Chelonibia testudinaria*, for example, was only found on 47% of adult loggerheads in 1974, compared to 91% today (Chapter 2). This discrepancy could be because the number of nesting turtles has increased in this time (Nel et al. 2013) and resulted in a stronger association between the epibiont and its host. A higher population density of hosts allows for increased larval settlement of this turtle barnacle possibly due to a higher spatial overlap of available habitat for the epibiont. This relationship has been reported for a declining leatherback population in the East Pacific, where *Stomatolepus elegans* is not present even though it occurs on other populations that are stable (Robinson et al. 2017). The report by Hughes (1974) also noted a change in epibionts

with a shift in size. Sub-adult loggerhead turtles had predominantly *Lepas* spp., a pelagic barnacle, however, as the size of the turtle increased the rate of this organism on the carapace decreased. This is possibly indicative of the turtle's ontogenetic shift from juveniles to adult moving to occupy more neritic environments (Bolten 2003). However, the results from this study indicate that a proportion of the nesting population forage in oceanic environments.

The use of epibionts to study various aspects of sea turtle ecology has increased in popularity over the last decade (see review by Frick and Pfaller 2013), and is likely going to grow with new tools such as genetic analysis (Rawson et al. 2003, Pinou et al. 2013). Epibionts provide an alternative method to document body condition (Chapter 2), and determine the foraging habitat of the host (Chapter 3). The resolution of this tool can be enhanced with smaller and more diverse epibionts being investigated. Meiofauna (Corrêa et al. 2014) and diatoms (Majewska et al. 2017, Robinson et al. 2016) are organismal groups that were not incorporated in this study but have the potential to enhance the understanding of sea turtle ecology.

5.3 Stable isotope analysis to determine habitat use and regional movement of turtles

The use of stable isotope analysis to study various aspects of sea turtle ecology has increased dramatically since the pioneering paper by Killingley and Lutcavage (1983). The stable isotope ratios of $\delta^{18}O$ and inorganic $\delta^{13}C$ from loggerhead epibiotic barnacles were used by Killingley and Lutcavage (1983) to differentiate turtles using estuarine and marine environments along the east coast of the USA. The stable isotope approach used in this study allowed identification of foraging area and large-scale regional movement across the SWIO because turtles using the same area had similar isotopic values.

The time period that an isotopic signature represents is dependent on the organism's tissue and the metabolic process, resulting in different tissues representing a different time of assimilation (Tieszen et al. 1983, Hobson and Clark 1992). Some tissues are replaced quickly, such as plasma (a few weeks), while others, such as skin and muscle, have a longer turnover rate and therefore represent longer time periods (months to years). The epidermis tissue used to determine foraging habitat of the SWIO loggerheads

(Chapter 3) was expected to have a relatively slow turnover rate and indicated the last three to six months of foraging (Reich et al. 2008). The stable isotope analysis of the epidermis tissue was therefore appropriate at making assumptions of the foraging habitats of turtles sampled at their nesting grounds. **The results indicated that nesting loggerhead sea turtles in the SWIO have a bimodal foraging strategy feeding in either neritic or oceanic habitats.** Stable isotopic carbon signatures were particularly useful at delineating foraging habitat among different turtles. However, due to the complex nature of $\delta^{13}C$ ratios in the marine environment it is likely that there are more foraging areas and that this population actually exhibits a polymodal forging strategy (Reich et al. 2010).

Mapping extensive geographic patterns of isotope values in the marine environment is a novel branch of isotope ecology (Hobson et al. 2010, Graham et al. 2010, McManhon et al. 2013). With advances in software and methods in spatial-data analysis (e.g., ArcGIS, QGIS, Google Earth, and some R packages), researchers are able to apply geographic locations to stable isotope signatures and create maps of similar values (isoscapes). **The isotopic mapping of $\delta^{18}O$ in the SWIO showed a strong latitudinal gradient that was useful to track the past movements of nesting loggerheads by analysis of the shells of their epibiotic barnacles** (Chapter 4). Mapped movement ranges showed that most turtles migrated north from the nesting beaches, which is supportive of the main migration corridors for loggerheads in the region (Luschi et al. 2003, 2006, Harris et al. 2018). However, the oxygen isotope data of seawater used for this were sparse for this region and greater resolution of isoscape patterns is required to be able to delineate the extent of the turtle's range and possibly show neritic-oceanic movement.

The use of other intrinsic makers, such as trace elements, in addition to stable isotope analysis to infer movement patterns can provide more resolution of habitat use. Trace elemental analysis has been shown to be useful in other areas in linking the contribution of oceanic habitats to neritic foraging grounds over large scales (Lopez-Castro et al. 2013). The presence of individual turtles using large estuaries adjacent to coastal foraging areas in the SWIO could be delineated through trace elements. Furthermore, some tissues are inert after synthesis, such as hair, bones, and tusks, and can represent longer

time periods in animals, sometimes throughout the life of the individual (Beltran et al. 2015, Ramirez et al. 2015). Sea turtle scutes represent the long-term diet of an individual and isotope analysis of this tissue can indicate a shift in habitat use – from oceanic to neritic – as has been recorded elsewhere (Reich et al. 2007, Pajuelo et al. 2012, López-Castro et al. 2014).

5.4 Way forward and concluding remarks

Climate predictions over the next 50 years reveal that a trend of increasing temperatures is likely to continue at a more rapid rate (Pachauri et al. 2014). The combination of rising temperatures and El Nino variability causes physiological stress for species. Due to changes in climate which have already been recorded and are still predicted to increase over the next century, it is important to predict, and track, *inter alia*, species range shifts. These predictions can assist with the identification of future conservation areas (Wilson et al. 2005), and will predict species at risk. SWIO sea turtles could experience a shift in breeding and feeding habitats. It is expected that rookeries may extend or shift south, possibly leading to local extinctions at the northern range limits, but it is unclear how foraging areas would be affected. It may follow the same southward movement as is predicted for marine ecosystems (Burrows et al. 2011). However, turtles may respond quicker to climatic changes compared to habitat forming organisms that they interact with, such as coral reefs or sea grass beds (Walther et al. 2002, Parmesan 2006). As these turtles are at some of their southernmost limits, understanding how climate change will impact them can be used to understand population shifts in other regions.

In summary, this study demonstrated that loggerhead turtles in the SWIO are in relatively good health, with no nesting individuals from South Africa being in a very poor body condition, and that the turtles have a wide distribution utilising many habitats. Conservation management of migratory species requires an understanding of their regional movement and habitat use patterns, and of the connectivity between different populations. Therefore, the findings of this study have implications for the use of epibionts and stable isotopes in conservation management of various marine migratory species. Future applications of the methods described and used in this study are not limited to the region or target species. Effective protection of loggerhead sea turtles in the region can

be achieved if conservation efforts are extended further into their oceanic environments. Moreover, management needs to be applied at a regional scale, across international boundaries to all areas that the sea turtles utilize.

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