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RESIDENCY AND TROPHIC ECOLOGY OF JUVENILE WHALE SHARKS (*Rhincodon typus*) IN THE WESTERN INDIAN OCEAN

by

Clare E. M. Prebble

A thesis submitted to the University of Southampton in accordance with the
requirements for the degree of Doctor of Philosophy

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ABSTRACT

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The whale shark (*Rhincodon typus*) is the largest fish in the world, and while whale sharks are popular, charismatic megafauna supporting a major tourist industry, the global whale shark population has decreased by >50% in the past decade. Whale sharks are now listed as Endangered on the IUCN Red List of Threatened Species. Effective conservation of whale sharks depends on accurate and reliable information describing their trophic and spatial ecology.

Whale sharks are found in predictable aggregations in certain coastal and island areas in the tropics and subtropics, presumably for feeding. ‘Whale shark season’ typically lasts for a short period at each site, and is often associated with an ephemeral food source, with individual sharks present for varying lengths of time. Conservation assessment of whale sharks through their entire range is difficult due to limited knowledge of their behaviour following dispersal from these coastal aggregation sites, as well as the poor understanding of their movements and geographical connectivity on a timeframe that is relevant to management.

Whale sharks are relatively rare, oceanic, pelagic animals. The difficulty of observing the behaviour of mobile animals at sea, particularly in the open ocean, has encouraged the use of indirect biochemical methods to infer aspects of trophic and spatial ecology. Stable isotope analysis of consumer tissues provides a useful tool to investigate the retrospective movement and trophic ecology of mobile animals, and fatty acid analysis provides further information on diet.

In this thesis I use stable isotope and fatty acid markers coupled with direct data on residency and movement of known individual whale sharks, derived through photo-identification to examine the trophic and spatial ecology of three male-dominated whale shark aggregations in the Western

Indian Ocean and Arabian Gulf. I have assembled samples from 186 individuals, representing an estimated 7 to 55% of all individuals in their respective aggregations.

Comparing the isotopic composition of whale shark tissues with known isotopic latitudinal gradients in diet, paired with long-term photo-identification data, I demonstrate limited latitudinal movement of individual sharks between important whale shark feeding aggregations in Mozambique, Tanzania, and Qatar. The relatively large isotopic dataset allows the first realistic assessment of isotopic niche breadth in whale shark feeding aggregations. Based on these results, I argue that eastern African and Arabian whale sharks should be treated as separate management units until their functional connectivity is shown to be significant.

For the Tanzanian sharks, I have applied both stable isotope analysis and fatty acid analysis to this comparatively resident aggregation over a multi-year timescale. Stable isotope analysis indicates that the sharks forage primarily within the local food web. Fatty acid results show epipelagic feeding, and population-level responses to seasonal environmental changes. However, highly distinctive lipid class compositions within the local food web also suggest preferential routing of lipids in whale shark tissues. Preferential routing of essential fatty acids may be an unrecognised source of variance in elasmobranch dietary ecology, particularly filter feeding elasmobranchs, which urgently requires further study.

Lastly I have used stable isotope analysis and dietary mixing models to investigate the coastal and offshore habitat use of an unseasonal whale shark aggregation in Mozambique. Here, isotope results suggest that whale sharks are feeding primarily on epipelagic zooplankton, and the sharks' presence is tied to ocean-scale dynamics, with possible dietary contributions from epipelagic and deep-water sources. Mixing models imply the existence of an un-sampled dietary item, potentially from offshore, oligotrophic waters and / or dietary routing in whale sharks.

Obtaining information on mobile marine animals is challenging. I also discuss the uses and limitations of stable isotope analysis techniques applied to whale sharks over varying spatial and temporal scales. I have shown that stable isotope analysis when used in conjunction with other methods can prove a valuable tool to shed light on their trophic and spatial ecology, but that interpretation of biochemical data is challenging particularly in the absence of experimental studies validating physiological and biochemical assumptions.

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Academic Thesis: Declaration Of Authorship

I, Clare Prebble declare that this thesis and the work presented in it are my own and has been generated by me as the result of my own original research Residency and trophic ecology of juvenile whale sharks (*Rhincodon typus*) in the Western Indian Ocean.

I confirm that:

1. This work was done wholly or mainly while in candidature for a research degree at this University,
2. Where any part of this thesis has previously been submitted for a degree or any other qualification at this University or any other institution, this has been clearly stated,
3. Where I have consulted the published work of others, this is always clearly attributed,
4. Where I have quoted from the work of others, the source is always given. With the exception of such quotations, this thesis is entirely my own work,
5. I have acknowledged all main sources of help,
6. Where the thesis is based on work done by myself jointly with others, I have made clear exactly what was done by others and what I have contributed myself,
7. None of this work has been published before submission, Chapter 2 has been submitted after revision.

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Chapter 1 Introduction

1.1 Project Overview

In this thesis I describe investigations into the trophic and spatial ecology (trophic geographgy) of whale sharks (*Rhincodon typus*) at their major feeding areas in the Western Indian Ocean. In this introduction I will summarise basic knowledge on the ecology of whale sharks, indicating methodological complexities associated with gathering information on this enigmatic species. I then outline the principle tools used in this thesis: stable isotope and fatty acid biomarker analysis before describing the geographic context of my study sites.

1.2 Whale Sharks

1.2.1 Introduction

Whale sharks are the world's largest extant fish (Chen et al. 1997) and have a circumtropical distribution (Rowat & Brooks. 2012), extending seasonally into temperate regions (Duffy. 2002, Turnbull & Randell. 2006). The majority of whale sharks are thought to be found in the Indo-Pacific (Pierce & Norman, 2016). Since the first description of the species by Smith (1828), little was known about whale shark biology and ecology aside from stranding locations and fisheries catches until the 1990s (Rowat & Brooks. 2012). *Rhincodon typus* belongs to the monotypic family Rhicodontidae within the Order Orectolobiformes, which has 42 species including Stegostomidae (leopard sharks), Ginglymostomatidae (nurse sharks), and Orectolobidae (wobbegongs). These groups share several morphological and anatomical similarities including skeletal anatomy, tooth and dermal denticle morphology, fin placement and barbel morphology (Compagno 1973). Whale sharks are one of only three filter feeding shark species, along with the more temperate basking shark (*Cetorhinus maximus*), and deep-water megamouth shark (*Megachasma pelagios*). All of whom have independently evolved this feeding strategy (Sanderson & Wasserug 1993). Planktivorous elasmobranchs comprise of only 14 species of mainly large-bodied sharks and rays from 5 genera including other warm water planktivores such as mobula rays.

Despite displaying little evidence of sociality, whale sharks can be found in relatively high densities at some sites. Areas where several hundred individuals have been documented include, but are not restricted to, the Arabian Gulf and the Gulf of Oman (Robinson et al. 2016), Ningaloo Reef in Western Australia (Wilson et al. 2006, Norman, Holmberg, Arzoumanian, Reynolds, Wilson, Rob, Pierce, Gleiss, de la Parra, et al. 2017), Darwin Island in the Galapagos (Acuña-Marrero et al. 2014), the Yucatan Peninsula in Mexico (de la Parra Venegas et al. 2011), southern Mozambique (Norman, Holmberg, Arzoumanian, Reynolds, Wilson, Rob, Pierce, Gleiss, de la

Parra, et al. 2017), various sites in the Philippines (Schleimer et al. 2015), and Mahe in the Seychelles (Brooks et al. 2010, Rowat & Brooks. 2012).

Although whale sharks are now a comparatively well-studied elasmobranch (Stevens, 1986, Colman. 1997, Martin. 2007, Rowat & Brooks. 2012), details of their basic ecology remain elusive, such as breeding behaviour.

1.2.2 Ontogenetic Habitat Use

Reliable coastal feeding aggregations have provided researchers with an opportunity to predictably locate the species, which was formerly viewed as sparse, oceanic and difficult to study. Whale sharks can commonly be found at a small number of these coastal, seasonal aggregation sites where they exploit ephemeral bursts in productivity and food availability, such as fish spawning events and zooplankton blooms (Heyman et al. 2001, Meekan et al. 2006, 2009, de la Parra Venegas et al. 2011, Rowat et al. 2011, Rohner 2012, Robinson et al. 2013, 2016, Rohner, Armstrong, et al. 2015a), in an otherwise oligotrophic ocean environment. Aggregation sites appear to be characterised by shallow, relatively warm waters close to steep topographic features (Copping et al. 2018). The topographic contrasts may encourage upwelling-related productivity in shallow waters, or provide thermoregulatory compensation in warm waters allowing deep diving into cold mesopelagic layers (Brunnschweiler et al. 2009). The overwhelming majority of coastal aggregation sites are characterised by populations of whale sharks showing pronounced sex- and size-based segregation, dominated by juvenile males, with an average total length (TL) of all sharks of 4-8 m (Rohner, Richardson, et al. 2015, Norman, Holmberg, Arzoumanian, Reynolds, Wilson, Rob, Pierce, Gleiss, de la Parra, et al. 2017). This suggests that there are strong ontogenetic and sex-specific habitat or dietary shifts in place for the species. Based on clasper morphology, male length at maturity is between 7-9 m (Rohner et al. 2011, Rowat et al. 2011, Rowat & Brooks 2012, Rohner, Armstrong, et al. 2015a, Robinson et al. 2016), with some variation between geographic regions (Pierce and Norman 2016). Size at maturity of female sharks is approximately 9 m TL based on visual and laser photogrammetric measures and assumed pregnancy status based on visual outside examination of their bellies (Ramírez-Macías et al. 2012, Acuña-Marrero et al. 2014).

Until recently, large whale sharks have been particularly elusive as they are generally absent from the coastal aggregation sites. Now, larger sharks are have been found in oceanic waters (Ketchum et al. 2012) with some evidence they are targeting different prey, or foraging in different habitats (Borrell et al. 2011). A feeding area at Al Shaheen in Qatar is dominated by mature male sharks, with a small number of adult females seen (Robinson et al. 2016). Darwin Arch in the Galapagos Islands boasts seasonal sightings of pregnant female sharks (Acuña-Marrero et al. 2014), and there are seasonal sightings of pregnant females in the Gulf of California (Ramírez-macías et al. 2012). Both mature male and pregnant female sharks have been observed at St Helena Island in the Atlantic (Clingham, Brown, et al. 2016).

1.2.3 Breeding and Reproduction

Little is known about whale shark breeding and reproductive life history (Rowat & Brooks 2012). Large pregnant females are rarely sighted in most areas (Eckert & Stewart 2001, Ramírez-macías et al. 2012, Acuña-Marrero et al. 2014, Clingham, Brown, et al. 2016) and the only pregnant whale shark that has been physically examined was a single female caught off Taiwan (Joung et al. 1996). This female had 304 pups in her uterus in various stages of development ranging from 42-64 cm in length. The larger pups were free of their egg cases and appeared fully developed. This remains the largest litter size reported for any shark species (Joung et al. 1996), and indicated that whale sharks are aplacental viviparous. Genetic analysis of the pups showed that there was likely to have been a single father (Schmidt et al. 2010), suggesting that females may be able to store sperm. Whale shark pups are likely to vary considerably in size at birth, and can be viable anywhere from 46 cm (Aca & Schmidt 2011). Only a handful of whale sharks <1.5m have been reported from the wild (Rowat & Brooks 2012) so it remains unclear whether whale sharks have specific pupping areas. Reproductive periodicity is also unknown, as re-sightings of pregnant females are rare even in areas where they are observed (Norman, Holmberg, Arzoumanian, Reynolds, Wilson, Rob, Pierce, Gleiss, de la Parra, et al. 2017)

1.2.4 Feeding Ecology

The whale shark is one of only three filter-feeding shark species, all of which are among the largest extant marine vertebrates. Whale sharks are the largest (Che-tsung et al. 1997), followed by the more temperate basking shark (*Cetorhinus maximus*), and the deeper-water megamouth shark (*Megachasma pelagios*). Whale sharks have a pad-like filtering apparatus that differs from the basking and megamouth sharks, which have a more bristle-like gill rakers (Martin & Naylor 1997, Sims 2008). Whale sharks are able to passively ram feed or actively feed by suction depending on circumstance (Motta et al. 2010). This enables them to target more mobile prey while remaining well-suited to exploit dense prey aggregations (Rohner, Armstrong, et al. 2015a). Whale sharks target a wide variety of prey items including sergestid shrimp (Rohner, Armstrong, et al. 2015a), tuna spawn (Robinson et al. 2016), copepods, chaetognaths, euphausiids, fish larvae (Motta et al. 2010) and various other surface zooplankton species (Rowat & Brooks 2012), as well as small schooling fishes (Rowat & Brooks 2012). The whale sharks' feeding system allows a lot of flexibility in prey choice, and they can 'catch' anything from < 1.2 mm fish eggs up to > 100 mm long baitfish (Heyman et al. 2001, Motta et al. 2010). However, prey biomass rather than prey size is likely to be the main driver behind feeding behaviour (Heyman et al. 2001, Rohner, Armstrong, et al. 2015a). Feeding, especially active feeding, is energetically costly, and a threshold prey biomass may need to be reached to trigger feeding to ensure a net energy gain (Rohner, Armstrong, et al. 2015a, Armstrong et al. 2016). Whale sharks have also demonstrated learned feeding behaviours in response to anthropogenic activities. Whale sharks in Oslob, Philippines have learned to be hand fed 'uyap', a mixture of fish and shrimp, as a tourist attraction (Thomson et al. 2017), and the sharks will target lift-nets of fishermen in Cenderawasi Bay, Indonesia where they 'suck' the small baitfish out of the holes in the nets (Himawan et al. 2015).

Previous biochemical and stomach content analysis on whale sharks has also inferred that a component of whale shark diet may also derive from deep-water sources and emergent zooplankton (Rohner, Couturier, et al. 2013). Stomach contents of four stranded sharks from Mozambique and South Africa were dominated by mysids and sergestids. The dominant mysids were demersal zooplankton that migrate into the water column at night, suggesting night time feeding. Fatty acid analysis of whale shark dermal connective tissue had high levels of bacterial fatty acids, inferring a detrital link potentially via dermersal zooplankton. Fatty acid profiles also showed high levels of oleic acid in whale sharks similar to zooplankton and myctophid fishes from the meso- and bathypelagic zone, suggesting foraging in deep-water (Rohner, Couturier, et al. 2013). Deep-water resources could be an important resource for whale sharks during longer open-ocean movements where their food is patchily distributed.

Whale sharks are opportunistic feeders and show a wide range of individual variability in feeding preferences within seasons and across time. Fatty acid analysis of whale sharks in Western Australia showed a considerable amount of intraspecific variability in whale shark fatty acid profiles within aggregations and between years of study (Marcus et al. 2016) suggesting that they were feeding over a wide range of habitats.

Isotope analysis of whale shark tissues indicates some ontogenetic and sexual differences in feeding behaviour (Borrell et al. 2011, Marcus 2017). Whale sharks from Gurjurat, India showed similar overall isotope signatures to local planktivorous fishes suggesting they were feeding on similar prey. However there was a positive relationship between total length and both δ^{15}N and δ^{13}C, indicating that either the larger sharks might be feeding at a higher tropic level overall, and / or in different locations. Additionally there were small differences between the isotopic ratios of male and female whale sharks. While the sex difference was not significant in Gurjurat sharks, a similar trend was reported in whale sharks from Western Australia (Marcus 2017) supporting the idea of sex-related feeding preferences. Though the size range was smaller for the whale sharks in Western Australia, Marcus (2017) also reported similar size-related feeding preferences, especially in females.

1.2.5 Tourism

The docile nature, charismatic status and predictable occurrence of whale sharks has spurred on the development of popular and lucrative ecotourism industries in several countries (Rowat et al. 2006, Pethybridge et al. 2007, Cagua et al. 2014, Haskell et al. 2014, Pierce & Norman

2016). Whale shark tourism can benefit developing countries, and the resulting high economic value has also encouraged varying levels of protection (Cagua et al. 2014, Graham 2003). However, if left unregulated, disturbance of the sharks by snorkelers and boats can lead to short-term stress, as evidenced by the sharks exhibiting avoidance behaviours (Pierce et al. 2010b, Haskell et al. 2014).

1.2.6 Genetic Population Structure

Current knowledge on long term, global scale connectivity of whale shark populations is based largely on genetic work (Castro et al. 2007, Schmidt et al. 2009, Vignaud et al. 2014) which has found a lack of spatial population structure between the Indian and Pacific ocean basins, but with some separation of the Atlantic subpopulation. The last tropical connection between the Atlantic and Indo-Pacific oceans was 3.5 million years ago, before the rise of the Isthmus of Panama. Vignaud et al. (2014) proposed that connectivity and breeding between these ocean basins has since been infrequent enough to allow genetic drift (i.e. connectivity of less than a few individuals every 2-4 years). Genetic structure is not significant among aggregations within the Indo-Pacific, which led to the suggestion that an ocean basin wide conservation strategy is required (Castro et al. 2007, Schmidt et al. 2009). Although gene flow is sufficient to standardise populations, it is unlikely to be enough to re-establish populations (Schmidt et al. 2009). Therefore this timescale of connectivity may be too long to consider in isolation for management purposes of this endangered species. Monitoring regional scale movements of individual fish is therefore an important, and perhaps more relevant part of a practical conservation strategy as there is potentially some population structure yet to be identified.

1.2.7 Conservation Status

As well as ecotourism, the predictable occurrence of whale shark aggregations has led to directed fisheries (Che-tsung et al. 1997, Speed et al. 2008, Ramírez-Macías et al. 2012, Araujo et al. 2014). While most of the major commercial fisheries have now closed, chiefly due to economically unsustainable reductions in catches, fishery and trade management is still required (Pierce & Norman 2016). Their surface-feeding habit, and the proximity of most aggregations to the coast, have also left the species susceptible to human threats such as boat strikes and net entanglements (Robinson et al. 2013, Rohner, Richardson, et al. 2018).

Declines in sightings and catch per unit effort have been reported from both the Atlantic (>30%) and Indo-Pacific (>50%). These data, combined with the slow growth rates and late maturity of whale sharks have resulted in an “Endangered” listing on the IUCN Red List for the Indo-Pacific subpopulation, and globally (Pierce and Norman 2016). Their high mobility has also led to a listing on Appendix I on the Convention on Migratory Species (CMS 2017).

Although whale sharks benefit from better international protection, on paper, than most shark species, significant gaps remain in our understanding of their biology and ecology. This makes the identification and management of separate geographical stocks difficult at present, and little is known on the movement and occurrence of whale sharks while in pelagic waters (Rowat & Brooks 2012), and the threats they may face there (Pierce and Norman 2016).

1.2.8 Horizontal Movements

Whale shark movements have been tracked using a number of approaches, although as with the majority of whale shark research, attention is largely focussed on coastal juvenile aggregation sites. Short term movements, swimming patterns, and site residency have been examined using active acoustic tracking (Gunn et al. 1999), and passive acoustic arrays (Cagua et al. 2015). Satellite tagging (Berumen et al. 2014, Robinson et al. 2017, Rohner, Richardson, et al. 2018) has demonstrated significant geographical movements of whale sharks away from feeding areas and across international boundaries from national Exclusive Economic Zones (EEZ) into the high seas (Berumen et al. 2014, Tyminski et al. 2015). Satellite tagging has recorded median horizontal movement rates of 24-38 km per day (Hueter, Tyminski, & de la Parra 2013, Hearn et al. 2016). Although whale sharks are clearly capable of travelling 1000's of kilometres away from aggregation sites they show high site fidelity to many of these aggregations and are often re-sighted in subsequent years, for up to 20 years in some cases (Norman and Morgan 2016). Individuals rarely swim between neighbouring feeding hotspots (Brooks et al. 2010, McKinney et al. 2017), but rather return to the same site each time (Norman et al. 2017).

1.2.9 Diving Behaviour

Whale sharks spend most of their time in the epipelagic zone (0-200 m), and much of the daytime in the top 10 m of water (Rowat & Brooks 2012), but have also been recorded diving up to 1928 m deep (Tyminski et al. 2015). Thus, whale sharks also dive to mesopelagic (200-1000 m) and bathypelagic depths (>1000 m) tolerating temperatures down to 2.2°C (Wilson et al. 2006), though little time is spent at these depths. There is evidence of crepuscular and diel patterns in diving behaviour showing that generally greater depths are reached during the daytime as opposed to night time, and this behaviour is sometimes reversed in shallow water (Brunnschweiler & Pratt 2008, Motta et al. 2010, Tyminski et al. 2015, Ramírez-Macías et al. 2017). Diving behaviour appears to change abruptly at dawn and dusk (Wilson et al. 2006, Tyminski et al. 2015). The purpose of these deep dives made to mesopelagic and bathypelagic depths is unclear, but is suspected to be related to feeding or foraging behaviours, especially when crossing the open ocean with low productivity oligotrophic waters (Brunnschweiler & Pratt 2008, Tyminski et al. 2015). Deep diving could also relate to geomagnetic navigation (Brunnschweiler et al. 2009), and energy saving locomotion by gliding slowly down and forward with no effort followed by steep ascents (Gleiss et al.) Although electronic tagging technology is continuously improving, some challenges still remain. There are usually a small number of individuals in tagging studies, relative to population size, largely as a result of the expense of hardware and satellite time. High rates of tag loss and early detachments also remain common (Hays et al. 2007, Hammerschlag et al. 2011, Sequeira et al. 2013). Most tags only allow us to infer behaviours (Tyminski et al. 2015, Rohner, Richardson, et al. 2018), although multi-sensor tags are increasingly being used, some with integrated cameras (Gleiss et al. 2011). These limitations of telemetry studies mean that an integrated approach is needed to fully understand the feeding ecology of whale sharks in environments where they cannot be directly observed.

1.2.10 Ecological Role

Whale sharks inhabit a lower trophic position than most shark species (Christiansen et al. 2015) and are thus not keystone predators. Although few species-specific data on the role of whale sharks in ocean ecosystems are available, inferences can be made from ecologically comparable marine megafauna species, such as baleen whales (Estes et al. 2016). Whale sharks are likely to influence the marine system as consumers, as prey, as detritus, and through energy storage and transport (Estes et al. 2016). Whale sharks consume large quantities of biomass (Motta et al. 2010, Rohner, Armstrong et al. 2015a, Tyminski et al. 2015). The large size of whale sharks, along with their extensive vertical and horizontal movements suggest that whale sharks would be important vectors transporting energy, nutrients, and other materials through ocean ecosystems (Estes et al. 2016). In areas that are resource-limited, phytoplankton growth is encouraged, perpetuating energy and growth up the food chain (Estes et al. 2016). After death, whale shark carcasses will sink to the seafloor where they would likely provide food and habitat for deep-sea organisms (Higgs et al. 2014, Estes et al. 2016). Given the inherently short-lived nature of vent systems, whale falls, and thus whale shark falls, are likely to also be important stepping-stones in the spatial ecology of the deep sea (Estes et al. 2016).

The role of pelagic species in cycling nutrients between the surface and the mesopelagic is greater than previously thought (Roman & McCarthy, 2010, Saba & Steinberg, 2012). With the ever-increasing commercial interest in deep-sea resources there is a need for a greater understanding of how deep diving pelagic species, like whale sharks, interact with and impact the mesopelagic food web, and vice versa (Anderson et al. 2018a).

1.3 Methods

From the discussion above it is clear that gathering information on the trophic and spatial ecology of whale sharks is challenging. Away from juvenile aggregation sites whale sharks are presumably highly dispersed in the open pelagic ocean, potentially feeding at depth, and encounters are rare.

Breeding and nursery sites are unknown and the location and ecology of females and adult males is poorly understood. Life history ecology of whale sharks cannot be understood purely from direct observation, and a range of indirect methods are needed to infer aspects of movement and trophic ecology. In this section I briefly introduce three methods used in this thesis: Photo identification, stable isotope analysis and fatty acid analysis.

1.3.1 Photo-Identification

Photo-identification (photo-ID) is often used as a method for long term population monitoring of whale sharks (Graham & Roberts, 2007, Holmberg, Norman & Arzoumanian, 2009, Brooks et al. 2010, Norman et al. 2017). Whale sharks possess unique and stable skin colouration patterns on their dorsal surfaces (Arzoumanian et al. 2005, Marshall & Pierce 2012), allowing accurate identification and recognition of individuals across time and space. This method of mark-recapture has been used to assess population structure and inter-annual abundance at several feeding areas, including Mozambique and Western Australia (Brooks et al. 2010, Rohner et al. 2013, Cagua et al. 2015), and to assess the occurrence and scale of individual-level interchange among feeding sites (Brooks et al. 2010, McKinney et al. 2012, Andrzejaczek et al. 2016, Norman et al. 2017, Robinson et al. 2017). Photo-ID is an easy to execute, low cost and non-invasive technique, and facilitates the sampling of many individuals giving a more robust conclusion for population scale movements. A review of global photo-ID data revealed a high rate of fidelity of individual whale sharks to aggregation sites and very low levels of movement (Norman et al. 2017) despite the established capabilities to do so (Sequeira et al. 2013, Berumen et al. 2014, Tyminski et al. 2015). The analysis included over 30,000 whale shark encounters with reports ranging from 1992 to 2014. Data were collected from 54 countries with more than 6000 individual whale sharks identified. Examination of the global scale photo-ID data revealed high site fidelity among individuals, with limited movements of sharks between neighboring countries and no records of large ocean basin-scale migrations. Photo-ID data has also been used to generate residency models and investigate residency patterns within and between aggregation sites (Whitehead 2001). Photo identification has been used to estimate individual residency times in the Gulf of California, and the Philippines (Ramírez-macías et al. 2012, Araujo et al. 2014) and other places including Ningaloo in Western Australia, where a more than 20 year re-sighting was recorded (Norman & Morgan 2016).

A caveat of using photo-ID is spatial bias, as work is often focused at aggregation sites where whale sharks are readily seen, but spend a relatively small proportion of their time. Almost all aggregation sites are seasonal and individuals are often transient (Rowat et al. 2009, 2011, Fox et al. 2013). As such, photo-ID data should not be considered in isolation when investigating the whole geographic range of a population.

1.3.2 Stable Isotopes as Biological Tracers

Biochemical 'tags' offer a cost-effective complement to photo-ID data. Some light, structural elements such as carbon, nitrogen and sulfur have naturally occurring stable isotopes, and in general the heavier isotopes are far rarer in the biosphere than the lighter isotopes. Heavy and light isotopes of an element behave differently in biochemical reactions due to the higher activation energy associated with bond formation and bond cleavage for heavier isotopes. Consequently heavy and light isotopes react at different rates, leading to kinetic fraction in biosynthetic reactions that do not proceed to completion such as photosynthesis. In marine ecosystems this means that there is a natural variation in the ratios of heavy to light isotopes in phytoplankton across the global ocean and among different ecosystems (Hobson 1999).

The isotopic composition of primary production at the base of the food chain environment is transferred through food webs, as individuals foraging in specific environments incorporate the temporally and spatially averaged isotopic composition of their prey. Retrospective analysis of the isotopic composition of organisms is therefore useful in trophic and movement ecology studies as it is a reflection of their food sources, giving time-integrated information on the assimilated diet (Graham et al. 2010).

The stable isotope composition of nitrogen and carbon (expressed as $\delta^{15}N$ and $\delta^{13}C$ values respectively) are predominantly used as trophic and spatial markers in the marine environment as they vary dynamically in space and time as a result of changing environmental variables (Trueman et al. 2012). Stable isotope studies can be used in multiple ways, and on different spatial scales to infer retrospective behaviour about a consumer. Within a food web, $\delta^{13}C$ values are traditionally used to provide information on location or nutrient sources, while $\delta^{15}N$ values are often used primarily to infer trophic level. However diet source, location and trophic level influence both $\delta^{13}C$ and $\delta^{15}N$ values, and the relative importance of these driving variables differs among ecosystems and consumers.

During trophic interations biosynthesis of nitrogen containing excretion products (e.g. urea, ammonia, uric acid) favours the light isotope of nitrogen (^{14}N) so that the remaining consumer body N pool is relatively enriched in the heavier isotope of nitrogen (^{15}N) compared to the assimilated food. The relationhsip between the isotopic composition of nitrongen of a consumer's tissue and its diet is termed 'trophic enrichment' and the absolute difference between the consumer and the prey is the trophic discrimination factor (TDF), expressed in per mil units ($\delta^{15}N$). TDF for N vary among consumers but compilations of experimental studies yield mean values of approximately 3.4‰ (Post 2002). It is well known that TDF's vary systematically among consumers, most likely linked to the simlarity in amino acid composition between a consumer and its prey, so that herbivores and omnivores tend to have higher TDF than carnivores and especially pisciverous fishes (Hussey et al. 2013, McMahon et al 2010). Accurate estimation of TDF requires controlled feeding studies, which are difficult to achieve in large, slow growing organisms such as whale sharks.

Within food webs, stable isotopes of carbon show little trophic enrichment with alteration of ~0-1‰ between trophic levels. Consumer $\delta^{13}C$ values are therefore commonly assumed to closely represent the source of primary productivity (Davenport & Bax 2002). Large differences in $\delta^{13}C$ values among consumers are interpreted as reflecting feeding in food webs fuelled by different primary production types, for example inshore and benthic habitats, as mangrove systems show more enriched $\delta^{13}C$ values compared to offshore pelagic habitats (Hobson et al. 1994, Hobson 1999, Tanakaa et al. 2009). Inshore habitats generally have higher nutrient content and are therefore more productive. This is usually a result of detrital carbon input from benthic algae and sea grass, and can be enhanced by upwelling and phytoplankton blooms (McMahon et al. 2013). $\delta^{15}N$ values too can vary significantly on smaller spatial scales through the addition of terrestrial anthropogenic nitrogen sources, such as fertilisers and agricultural waste. The resulting eutrophication can cause an increase in productivity, followed by denitrification and a more enriched $\delta^{15}N$ signal (McMahon et al. 2013).

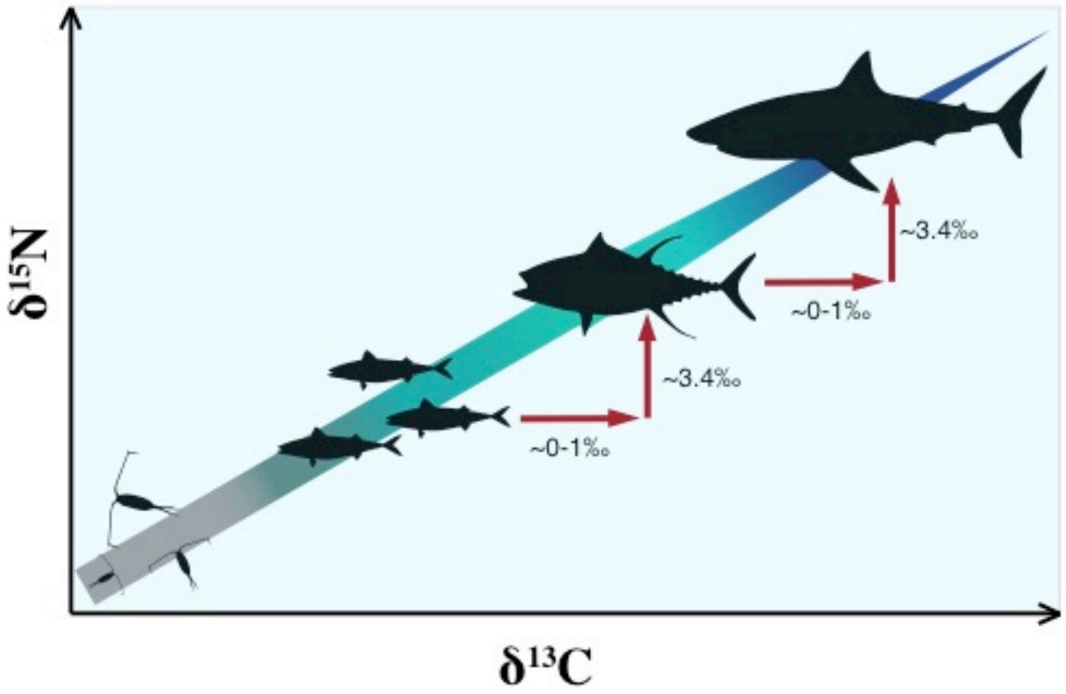

Figure 1.1 Schematic demonstrating the trophic enrichment of $\delta^{13}C$ and $\delta^{15}N$ values in a marine food-web.

$\delta^{13}C$ values in phytoplankton are predominantly controlled by the concentration and isotopic composition of dissolved CO_3 and phytoplankton growth rates, both terms highly dependent on sea surface temperature (SST). Consequently $\delta^{13}C$ values in phytoplankton broadly vary with latitude with increased spatial gradients at high latitude regions (e.g. Magozzi et al. 2017). Regions of higher productivity, associated with inshore and upwelling zones, tend to have increased $\delta^{13}C$ values. In areas where nutrients are not limiting ^{12}C is preferentially taken up during photosynthesis to maintain high growth rates (Popp et al 1998). Dissolved CO_2 is controlled by SST and water stratification. Lower water temperatures can hold more dissolved nutrients and CO_2 leading to more negative $\delta^{13}C$ values as ^{12}C is preferentially taken up. Stratification at higher SST can limit nutrient and CO_2 availability and with higher growth rates generally leads to more negative $\delta^{13}C$ values. Over ocean-basin scales this produces a latitudinal gradient. Higher latitude, pelagic ecosystems typically have much lower $\delta^{13}C$ values than inshore lower latitude ecosystems (Cherel & Hobson 2007, Graham, Koch, Newsome, Mcmahon, Aurioles, et al. 2010, Jaeger et al. 2010).

Broadly speaking, spatial patterns in phytoplankton $\delta^{15}N$ values are controlled by nitrate (NO_3^-) source size and composition, isotopic composition of the ambient water column, and the degree of NO_3^- uptake by phytoplankton community. However, nitrogen is present in the marine environment in multiple forms, and isotopic fractionation occurs when each form is converted to another. Thus the processes controlling the composition of marine nitrogen isotopes are more complex than in carbon. These processes are described in detail by Ryabenko (2013), briefly summarised here.

Nitrogen fixing bacteria (diazotrophs), such as the cyanobacterium *Trichodesmium* fix atmospheric N_2. During nitrogen fixation the lighter ^{14}N is preferentially incorporated. However nitrogen fixation has a relatively small isotopic fractionation effect (-2 to +2‰, (Ryabenko, 2013)), so newly fixed organic nitrogen has $\delta^{15}N$ values close to atmospheric N by definition 0‰). Nitrogen fixation is energetically costly, and is inhibited in areas where fixed nitrogen is high. Therefore fixation is highest in warm, well-lit ocean regions with relatively low amounts of fixed nitrogen (Montoya 2007, McMahon et al. 2013, Ryabenko 2013). Marine diazotrophs are thought to be limited by light, and potentially iron sources (Somes et al. 2010). As such nitrogen fixation is highest in warm, well-lit ocean regions rich in atmospheric iron (Somes et al. 2010). The subtropical Atlantic Ocean in particular appears to be a region of relatively intense nitrogen fixation, and therefore relatively low $\delta^{15}N$ values in POM.

Where fixation adds nitrogen to the oceanic N pool, it is balanced by the loss of nitrogen through denitrification. Denitrification largely occurs in sub-oxic conditions where microbes use nitrate (NO_3^-) instead of O_2 during respiration. Nitrate is converted to gaseous N_2O and N_2 which escape to the atmosphere (Somes et al. 2010). Denitrification strongly discriminates in favour of ^{14}N, with a fractionation factor of ~20-30‰, leading to enrichment of ^{15}N in the remaining dissolved nitrate pool. Dentrification predominantly occurs in oxygen minimum zones of the eastern tropical North Pacific, the eastern tropical South Pacific and the northern Arabian Sea.

Phytoplankton can assimilate nitrogen (either as ammonium, nitrate or nitrite) directly from the water column and preferentially incorporate ^{14}N (Graham et al. 2010). Nitrification is the oxidisation of reduced ammonia (NH_4^+) to nitrite (NO_2^-) and nitrate (NO_3^-) with varying isotopic fractionation effects. The isotopic effect associated with nitrification is greatest in ammonia-oxidising bacteria (~14.2 to 38.2‰ (Casciotti et al. 2010)), compared to nitrite-oxidising (-12.8‰). The combined kinetic isotopic effect of nitrification from ammonia to nitrate may vary depending on the external conditions and microbial community, but a global average isotope effect associated with nitrate assimilation is frequently estimated at 5‰ (Somes et al. 2010). The processes above combine to produce systematic variations in spatial distributions of $\delta^{15}N$ values, with nitrate-rich upwelling areas followed by nitrification. Spatial patterns are also determined by the ambient isotopic composition of regions before upwelling. The Atlantic waters, and particularly sub-tropical Atlantic waters are characterised by relatively low $\delta^{15}N$ values, reflecting high rates of nitrogen fixation, balanced by relatively positive $\delta^{15}N$ values in the eastern Pacific and northern Arabian Sea associated with denitrification.

Ideally, to be able to infer information about a consumer's movement or migration their isotopic composition needs to be compared to both the sampling location, and if they are not consistent, a spatially explicit reference to determine possible foraging locations. Sampling on ocean basin scales, and in the open ocean in general, is logistically difficult. The mechanisms of large-scale isotopic variation and oceanographic environmental variables models can instead be used to predict variability in space and time (Magozzi et al. 2017). These spatially explicit isotopic gradients are called 'isoscapes' (Graham, Koch, Newsome, Mcmahon, Aurioles, et al. 2010) and can be used to study animal migrations.

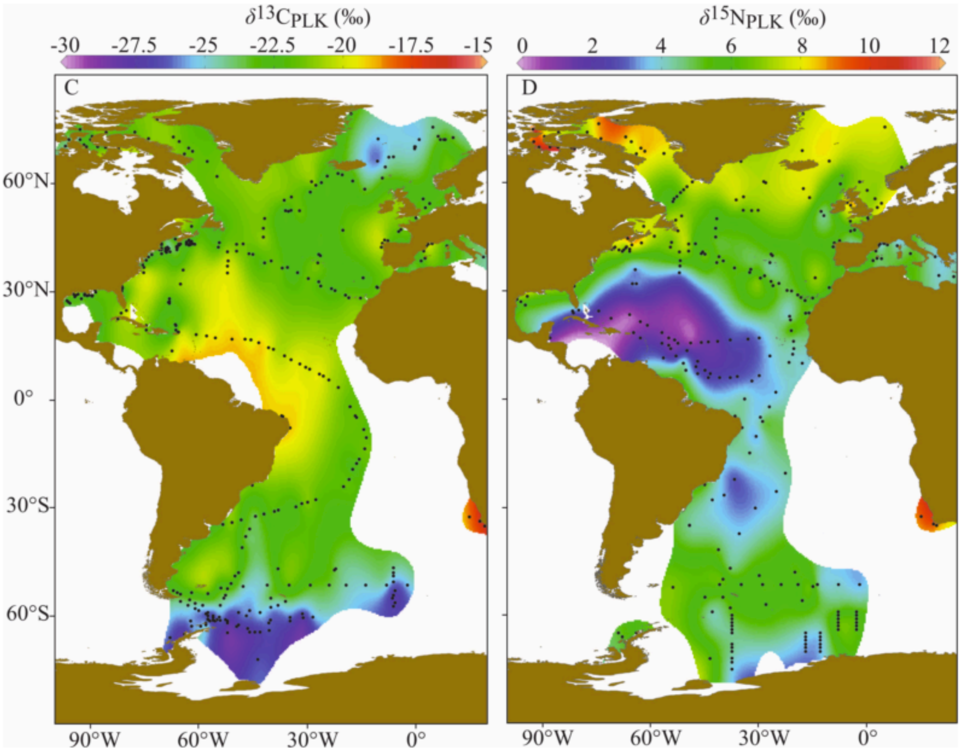

Figure 1.2 Adapted from McMahon et al 2013. Figure shows modelled $\delta^{13}C$ and $\delta^{15}N$ isoscape for the Atlantic Ocean.

Thus $\delta^{15}N$ and $\delta^{13}C$ values in consumer tissues can, depending on their application, can be used to investigate within food web trophic ecology and dietary preferences, small-scale habitat preferences and residency, and large ocean basin scale movements. However, working with isotopes in the marine environment is inherently challenging because of the many complex variables influencing the baseline isotopic signature over large geographical scales (Graham et al. 2010, Trueman et al. 2012), coupled with uncertainty introduced by biochemical fraction within food webs and potential movement of consumers and prey among isotopically distinct regions or over time. As $\delta^{15}N$ and $\delta^{13}C$ vary over such large scale and changeable ocean features there are currently very few spatio-temporal isoscape models for the world's oceans (Trueman et al. 2012, McMahon et al. 2013). Despite this limitation, broad-scale patterns in $\delta^{15}N$ and $\delta^{13}C$ isotopes can still provide a useful tool in mapping the long term movements of mobile marine species (Reich et al. 2007, Carlisle et al. 2012, Cherel & Hobson 2007, Trueman et al. 2012), and stable isotope analysis (SIA) has already been used successfully to investigate different aspects of trophic and spatial ecology for several marine species (McMahon et al. 2013) including whale sharks (Borrell et al. 2010). There are nevertheless additional considerations when interpreting data from elasmobranchs.

1.3.3 Applying stable isotope analysis to elasmobranchs

The challenges of SIA interpretation extend beyond the temporal changes in baseline SI profiles. Researchers must also consider species and individual level differences in metabolic strategy, tissue turnover rates and sample preparation (Dalsgaard, St. John, et al. 2003, Caut et al. 2009, Trueman et al. 2012, Marcus et al. 2016) when using and interpreting isotopes.

Within an organism, lipids are depleted in ^{12}C relative to proteins and carbohydrates, resulting in more negative $\delta^{13}C$ values, so variation in lipid content of sampled tissues can introduce bias and misinterpretation of results. This can be dealt with either chemically, before analysis, or mathematically afterwards (Post et al. 2007a). The benefit of mathemaical normalisation is that it reduces variables and preserves the samples for $\delta^{15}N$ analysis, which is not affected by lipid content.

Urea is an additional issue that needs consideration when dealing with elasmobranch tissue samples. Elasmobranchs have a unique physiology compared to other marine vertebrates, notably the retention of urea and trimethylamine oxide (TMAO) in their tissues for osmoregulation (Olson 1999). Urea is a waste product of metabolism and as such is expected to be enriched in ^{14}N. So high concentrations of nitrogenous waste in tissues, especially muscle tissue, can influence and artificially depress $\delta^{15}N$ values if it is not removed (Hussey et al. 2010, Shiffman et al. 2012). An additional problem related to urea retention is that any mathematical correction for lipids is based on the CN ratios which would also be influenced by the urea concentration. Urea concentration differs among species, individuals and between tissues (Hussey, Olin, et al. 2012). There is no one size fits all solution, and the best solution depends on the relative amounts of urea to lipid in the tissue. The standard method for removing urea from elasmobranch tissues is water-rinsing (Mathew et al. 2002). Any chemical treatment has the potential to alter the bulk isotope composition. So ideally controlled experiments applying multiple treatments must be carried out for each tissue type and species. A combination of (1) raw water washed and lipid extracted, (2) water washed and mathematically corrected, and (3) just mathematically corrected to determine the species- and tissue-specific effects on bulk $\delta^{15}N$.

Diet tissue discrimination factors (DTDF) are the change in isotopic signature between prey and predator, or between tissues within an individual (Peterson & Fry 1987, Kim et al. 2011). Using appropriate DTDF's are important to avoid inaccurate conclusions (Logan & Lutcavage 2010b). DTDF's are species-specific, and probably specific to individuals, but few controlled feeding studies have been done with elasmobranchs, mainly because of the logistical and ethical challenges (Olin et al. 2013). Therefore, researchers currently use a proxy from the most ecologically similar elasmobranch, but elasmobranchs are diverse, and using proxies incorrectly can alter interpretation of a species' ecological role (Olin et al.

2013). Currently the most frequently used DTDF for planktivorous elasmobranchs are from a controlled feeding study on three leopard sharks (*Triakis semifasciata*) (Kim et al. 2012), and a semi-controlled study on a large large lemon shark (*Negaprion brevirostris*) and sand tiger sharks (*Carcharias taurus*) (Hussey et al. 2010). The DTDF for leopard sharks has been used in isotope studies of whale sharks (Marcus 2017), and other large planktivorous elasmobrnachs such as manta rays (Cortes 1999, Borrell et al. 2011, Couturier, Rohner, Richardson, Marshall, et al. 2013, Burgess, Guerrero, Richardson, et al. 2018). However leopard sharks, lemon sharks, and sand tigers are all high trophic level, predatory sharks and do not reflect the feeding ecology or tropic position of large planktivores. As a lower tropic level species large pelagic planktivores are likely to have larger fractionation values with larger associated error (McMahon et al. 2010). By using the DTDF of a higher tropic level species tropic level estimates within food webs could be inflated and thus be misleading in tropic ecology studies.

Isotopic turnover rate is another parameter that needs to be carefully considered, as it will estimate the time frame over which assimilation has occurred. Isotopic turnover is defined as the time taken to incorporate newly assimilated dietary C and N into newly formed tissue, including the contribution of C and N from catabolism of existing tissue. Isotopic turnover rates are therefore shorter n juvenile fast growing animals where turnover is dominated by tissue growth, and slower in older animals where turnover rates may be dominated by catabolism and regeneration of tissue. The time scale of isotopic integration is set by the tissue type and the species-specific cell turnover rate (Matich et al. 2011, Trueman et al. 2012, Kim et al. 2012). For the same reasons as DTDF, few values have been estimated for elasmobranchs from controlled experiments, but generally metabolically active tissues like blood and liver have faster turnover rates than muscle and cartilage (MacNeil et al. 2005).

1.3.4 Fatty acids as a dietary tracers

Direct observation of feeding events of consumers, especially marine consumers, is rarely possible. Indirect methods are broadly used to reconstruct diet. As a complementary method to SIA, fatty acid (FA) signatures can also be used as an indirect method of assessing dietary preferences and trophic ecology. Fatty acids represent a large group of molecules that together comprise the majority of lipids found in all organisms. They are extremely diverse and their quantities and patterns in organisms are used in many areas of research, ranging from human health, nutrition and metabolism to ecosystem structure (Budge et al. 2006).

Much like SIA, the concept of fatty acid trophic markers is based on the observation of marine primary producers laying down recognisable fatty acid profiles that are transferred to – and conserved in – primary consumers with little modification (Iverson 2009). Most higher trophic-level marine animals lack the ability to synthesise some FA (hence termed essential fatty acids), especially the long-chain polyunsaturated fatty acids (PUFA). Therefore, the source of these FA in the tissues of the predator must have originated from, and thus reflects some aspects of, their diet. In marine systems the major primary producers at the base of the food web are microalgae, which support pelagic, offshore, and benthic foodwebs (Parsons 1963, Lalli & Parsons 1997) and have long been proven to synthesise PUFA *de novo* (Bell & Tocher 2009, Monroig et al. 2013). Hetrotrophic protists (Lee Chang et al. 2011), bacteria (Johns & Perry 1977, Russell 2013), and more recently invertebrates (Monroig et al. 2013) are also able to synthesise a specific array of PUFAs. The FA profile of the marine environment is driven by the biomass and species composition of the primary producers, which is in turn determined by larger ocean processes. The diversity and unique origins of these FA has allowed us to quantitatively look at marine ecosystem structure and diet composition (Budge et al. 2006, Iverson 2009). To reconstruct the likely long term diet and trophic ecology of the predator we can look at the types and quantities of essential fatty acids in their tissues and compare this to known prey items. Using FA profiles in conjunction with SI analysis we can start to tease apart the spatial and food source effects of wide ranging animals which are not easily accessible over most of their spatial and temporal distribution.

1.3.5 Lipid composition in marine fishes

Most marine fish store their lipids in the form of neutral lipids that generally reflect diet (Dalsgaard et al. 2003). Polar lipids often have a structural role in the cell membrane and are linked to physiological changes and requirements within the body (Ackman, 1998, Lands, 2009). As cell membrane structure is fairly specific, polar lipids are more stable within individuals and conserved relative to diet. Neutral lipids, of which the most common storage group in vertebrates are triacylglycerols (TAG), can be preferentially stored or routed to different tissues depending on requirements and external conditions. PUFA compositions in fish can change in response to environmental conditions such as salinity and temperature (Cordier et al. 2002), and dietary lipid FA composition (Copeman et al. 2002, Fountoulaki et al. 2003). The consumer tissues then reflect this and will show particular FA occurring in lower or higher proportions as a result (Copeman et al. 2002, Castell et al. 2003, Fountoulaki et al. 2003, Hessen & Leu, 2006).

Marine fish are generally known for their high levels of ω3 PUFA in the context of human nutrition (Tanakol et al. 1999). They have especially high proportions of docosahexanoic acid (DHA) and eicosapentaenoic acid (EPA) originating from marine phytoplankton (Kolakowska et al 2002). FA analysis has been used successfully to provide insight into food web structure and nutrient flow in marine environments (Iverson 2009), but like SIA, tissue type, sample handling and storage, and careful interpretation need to be planned (Budge et al. 2006)..

1.3.6 Applying biochemical analyses to study whale shark ecology

As discussed above, whale sharks are elusive animals and dierct observation of individuals can only provide limited insights into feeding and spatial ecology. Both FA and SIA analysis can be powerful tools to reconstruct the trophic and spatial ecology of large, highy mobile, species like the whale shark. Ideally this requires analysis of multiple individuals within several populations over time, coupled with individual data and movement behaviour. In the final section of this introduction I outline the geograhic context of my study sites and the whale shark aggregations found there.

1.4 Study sites

1.4.1 Tanzania

Mafia Island in Tanzania is a small island offshore from the mainland Rufiji river delta. It is roughly 50 km long and 15 km wide. The main town, Kilindoni, is on the southwestern side of the island. Kilindoni Bay is mostly shallow (<30 m deep) with a wide intertidal zone (~1 km) and mangroves line most of the bay (See Figures 2.3 & 4.1). Kilindoni Bay is home to a relatively small aggregation of whale sharks (Cagua et al. 2015, Rohner, Armstrong, et al. 2015a) with 189 sharks identified in Tanzania in the Wildbook for Whale Sharks online database (April 2018, S. Pierce pers. comm.) This aggregation is unique as at least some non-provisioned sharks are present year-round. Results from acoustic tagging and field survey work have shown a clear seasonality to their habitat use and movements within the bay (Cagua et al. 2015). During the ‘peak’ season from October to January whale sharks can be seen close inshore feeding on dense patches of large sergestid shrimp (*Belzebub hanseni*) (Rohner, Armstrong, et al. 2015a). Whale shark sightings here are closely tied to the presence of these dense zooplankton patches (Rohner et al. 2015a). The whale sharks share the bay with local fishermen who use ring nets to catch the small planktivorous fishes that also feed on these dense sergestid patches. Whale sharks regularly become encircled in these nets. Field observations indicate that fisher whale shark interactions are common and usually end without any harm to the sharks, although many whale sharks have injuries from the some of the few harmful interactions with the fishers (pers. obs.).

Figure 1.3 Aerial photograph of Killindoni Bay, Maifa Island. Credit: Dr Simon J Pierce.

1.4.2 Mozambique

Tofo Beach (Praia do Tofo) is a small coastal village in the Inhambane province of Mozambique (See figures 2.1 & 4.1). The continental shelf is narrow along this coast, exposing inshore waters to eddy-driven oceanographic processes (Rohner et al. 2018). To date, there are over 700 individual whale sharks identified in this aggregation (Wild Book www.whaleshark.org). Unlike other aggregations there is no distinct seasonality for sightings (Rohner et al. 2013). However, there has been a significant (79%) and continuing decline in sightings since monitoring work began in 2005 until 2011 (Rohner et al. 2013b), and to the present (Rohner unpubl. data). Aerial surveys and electronic tagging show whale sharks spend a lot of their time in shallow coastal waters where they are threatened by an increasing number of gill nets (Rohner et al. 2017). Movement patterns suggest that the predominant reason for remaining in coastal waters is to target high productivity areas, presumably for feeding (Rohner, Weeks, et al. 2018), but whale sharks are not often seen surface feeding in the area around Tofo Beach during the day (Haskell et al. 2014). Whale sharks have been a focal species for marine tourism in Praia do Tofo and adjacent areas for many years, and are an important source of income for the area (Pierce et al. 2010a, Tibiriçá et al. 2011, Haskell et al. 2014), yet the species remains unprotected in the country.

Figure 1.4 Aerial photograph of Tofo Point and Tofo Bay, Mozambique. Credit: Dr Simon J Pierce.

1.5 Thesis Aims and Objectives

Whale sharks are an enigmatic, iconic, and globally endangered species. The Indo-Pacific whale shark subpopulation, in particular, has shown worrying decreasing population trends (CMS, 2017, Pierce & Norman 2017). Effective management and conservation of such a highly mobile species requires a detailed understanding of their trophic and spatial ecology (Simpfendorfer et al. 2011).

Whale sharks are now listed on several international treaties, but several signatory countries do not have any in-country protective legislation in place. While this is not ideal, researchers have an opportunity to provide data to help develop effective legislation and conservation strategies. In countries where tourism has developed around the whale sharks, their economic value can also be used a leverage to engender legal protection. This is particularly pertinent for the Indian Ocean subpopulation which is most at risk. In this study the focus will mostly be on two aggregations in the Western Indian Ocean where whale sharks are currently not protected and where ecological data are most valuable and needed.

This project has been designed to examine the trophic and spatial ecology of whale sharks at two major aggregation sites in the Western Indian Ocean: Tofo Beach in Mozambique, and Mafia Island in Tanzania. Data from photo-ID and biochemical analysis of tissues will be applied in different ways to investigate habitat use and residency, and aim to identify appropriate management units over broader spatial scales. I will investigate whether the application of biochemical methods in addition to complementary techniques can bring new knowledge about the habitat use of specific aggregations of sub-equatorial African whale sharks. Specific aims are as follows:

- **Chapter 2**: I will use the latitudinal isotopic gradients present across the Indian Ocean and long-term photo-ID datasets to examine connectivity between aggregations and determine whether whale sharks in the southern and central areas of the Western Indian Ocean, in Mozambique and Tanzania, and those from the Arabian Gulf (Qatar), comprise a single functional subpopulation. I have submitted a revised manuscript (minor revisions) and expect publication of this chapter soon in *Marine Ecology Progress Series.*

- **Chapter 3**: I will use both stable isotope analysis and fatty acid analysis in conjunction to investigate the trophic ecology of whale sharks at Mafia Island, Tanzania. I will determine how much their seasonal inshore habitat use contributes to their overall dietary intake, in the context of the resident sharks' long-term site fidelity to this location, and what each method can tell us about their feeding ecology.

- **Chapter 4**: I will examine the trophic ecology of whale sharks in Tofo, Mozambique using stable isotope analysis and dietary mixing models to determine if whale sharks are specifically targeting coastal surface upwelling zones for feeding, as tagging data suggest, or if they are more likely to feed elsewhere, such as in deeper water or at night.

- **Chapter 5**: I will explore the global interactions of deep diving pelagic species with mesopelagic resources, with a view to highlighting the broad-scale ecosystem influence they are likely to have and the importance of their inclusion in future deep-sea research and legislation.

Chapters 2–5 have been prepared as manuscripts for journal submission so there will be some overlap between this introductory chapter and the respective introductions and methodologies.

Chapter 2 Limited latitudinal ranging of juvenile whale sharks in the Western Indian Ocean suggests the existence of regional management units

2.1 Abstract

Assessing the movements and connectivity of whale sharks *Rhincodon typus* through their range is difficult due to high individual mobility and limited knowledge of their behaviour following dispersal from coastal aggregation sites. Here, we use a large set of photo-identification and stable isotope data (δ^{15}N and δ ^{13}C) to test the assumption that sharks frequenting aggregation sites in Mozambique, Tanzania, and Qatar are a mixed stock, as inferred by genetic data. Photo-identification revealed negligible connectivity among aggregation sites, and none between the southern and central areas of the Western Indian Ocean (Mozambique and Tanzania) and the Arabian Gulf (Qatar). Sight-resight data indicated that shark movements at each site could be best represented by a model that included emigration, re-immigration and some mortality or permanent emigration. Although there was high individual variation in the isotope profiles of sharks from each location, comparison with latitudinal isotope data suggest that sharks had shown site fidelity to within a few hundred kilometres of each study area over the period of isotopic integration. Given the endangered status of whale sharks, and regional differences in anthropogenic threat profiles, further studies – and conservation assessment efforts – should consider the possibility that whale shark subpopulations exist over smaller geographical scales than previously documented.

2.2 Introduction

Successful species conservation requires an accurate assessment of ecological connectivity among geographically separated subpopulations (Worboys et al. 2010, Dubois et al. 2016). If a change in environmental or anthropogenic circumstances threatens a species in a particular location, this should be considered with reference to the entire geographical range inhabited by the species

(Juinio-Meñez 2015). If a subpopulation is truly isolated, any local threat may result in decline and local extinction (Johnson et al. 2015), while interconnectivity means that emigration and an eventual shift in area utilisation could occur instead (Lima et al. 1996). Temporal aspects to connectivity also exist. A subpopulation can still decline to local extinction, even if genetic connectivity exists on a long term, multi-generational timescale, in the face of a fast-acting threat (Ciach 2015). Hence it is important to examine both long-term (genetic) and short-term (ecological, subpopulation-level) connectivity. Investigating the spatial ecology of a species can indicate where functional population units and movement corridors may require specific management (de la Torre et al. 2016).

Marine populations are commonly structured as a ‘metapopulation’, where loosely-connected subpopulations are linked by the exchange of individuals (Dubois et al. 2016). Understanding this connectivity continues to be a major focus for highly mobile marine species, as large spatial scales often need to be considered for their management (Hays et al. 2016). The movement ecology of the whale shark *Rhincodon typus* (Smith 1828), the world’s largest fish, has proven to be a complex topic. Whale sharks are predictably observed in a small number of specific coastal areas and islands scattered through the tropics and subtropics (Rowat & Brooks 2012, Rohner et al. 2015a), where in many cases they are clearly targeting ephemeral bursts of productivity (Rowat & Brooks 2012). However, only certain life-stages tend to be present at these sites, with local population structure typically biased towards juvenile males (Rohner et al. 2015, Robinson et al. 2016)

Whale sharks have been targeted by fisheries in several countries (Alava et al 1997, Pravin, 2000, Anderson & Waheed, 2001, Hsu et al. 2012), which has led to significant population decline and a 2016 “Endangered” listing on the IUCN Red List for both the Indo-Pacific (IO) subpopulation and the global population, and listings on Appendix II of both the *Convention on International Trade of Endangered Species* and the *Convention on Migratory Species* (Pierce & Norman 2016). Whale sharks are long-lived, slow-growing, and late to mature (Hsu et al. 2014, Norman & Morgan 2016) and as such are particularly vulnerable to other human threats, such as boat strikes and non-targeted catches in gillnet and tuna purse-seine fisheries (Speed et al. 2008, Pierce & Norman 2016, Li et al. 2012). Though several countries offer national or territory level management measures for whale sharks (Pierce & Norman 2016), the species remains unprotected in most Western Indian Ocean (WIO) range states, including all three countries explicitly considered in this study. At the same time, however, swimming and diving with whale sharks is a multi-million dollar tourism industry, and is popular within several WIO countries (Jones et al. 2009, Pierce et al. 2010a, Cagua et al. 2014, Ziegler et al. 2015).

The Western Indian Ocean region is a global stronghold for whale sharks with several identified coastal and offshore aggregations (Davis et al. 1997, Pravin 2000, Cliff et al. 2007, Jonahson & Harding 2007, Riley et al. 2010, Rowat et al. 2011, Akhilesh et al. 2013, Robinson et al. 2013, 2016, Rohner et al 2015a, Cochran et al. 2016). Broadly speaking, WIO coastal sites are classically dominated by juvenile male whale sharks, with larger individuals assumed to favour more offshore habitats as seen in the Atlantic and Pacific Oceans (Borrell et al. 2011, Robinson et al. 2013, Clingham, Webb, et al. 2016, Ramírez-Macías et al. 2017). Large-scale population genetics studies on whale sharks have found no defined structure within the Indo-Pacific region, indicating that such aggregations are broadly connected over evolutionary time scales (Castro et al. 2007, Schmidt et al, 2007, Vignaud et al. 2014, Sigsgaard et al. 2017). However, over shorter periods, connectivity studies using photo-identification (Brooks et al. 2010, Andrzejaczek et al. 2016, Norman et al. 2017) and satellite tags (Rowat & Gore 2007, Brunnschweiler et al. 2009, Berumen et al. 2014, Robinson et al. 2017) have found minimal connectivity between these areas.

Photo-ID is routinely used for monitoring whale shark population structure, abundance and connectivity (Graham & Roberts, 2007, Holmberg et al. 2009, Brooks et al. 2010, Norman et al. 2017). The unique and stable skin colouration pattern of whale sharks (Arzoumanian et al. 2005, Marshall & Pierce 2012) allows individual sharks to be identified and re-identified over decadal time-scales (Norman & Morgan 2016, Norman et al. 2017). Photo-ID also represents a comparatively simple and inexpensive means of sampling a large number of individual sharks (Robinson et al. 2016, Norman, Holmberg, Arzoumanian, Reynolds, Wilson, Rob, Pierce, Gleiss, de la Parra, et al. 2017). However, there is significant spatial bias in most whale shark photo-ID datasets, as studies often focus on aggregation sites where whale sharks may be readily seen, but in which they may spend a relatively small proportion of their time (Rowat et al. 2009, 2011, Fox et al. 2013). Although maximum likelihood methods can be used to account for temporal variation of effort in the data (Whitehead 2001), whale sharks are typically not available for "visual recaptures" outside aggregation sites in which there is either dedicated research or citizen science activity (Cagua et al. 2015, Rohner et al. 2017). When considering population-level connectivity, then, it is prudent to combine these photo-ID data with a sightings-independent method.

Biochemical "tags" offer a cost-effective complement to other methodological approaches. The ratio of heavy and light stable isotopes of certain elements vary spatially within ecosystems (Hobson 1999). Stable isotope composition of nitrogen and carbon (expressed as δ^{15}N and δ^{13}C values, respectively) are typically used in the marine environment as they vary dynamically in space and time (Graham et al. 2010, Trueman et al. 2012). These isotopic gradients can be mapped over a range of geographic scales to produce "isoscapes" (Graham et al. 2010). The isotopic composition of baseline production is transferred through the food web in a predictable manner, allowing the retrospective assignment of consumers' feeding areas (McMahon et al. 2013, Trueman et al. 2017). Isotopes are therefore useful in residency and movement studies, as it is a reflection of the location of their food sources (Graham et al. 2010).

This study tests the hypothesis that predominantly juvenile and or male whale shark aggregations are localised and show low connectivity between three major whale shark aggregations in the Western Indian Ocean: Tofo Beach, Inhambane province, in southern Mozambique, ~1, 800 km north at Kilindoni Bay, Mafia Island, Tanzania: and ~4, 000 km north again to Al Shaheen oil field off Qatar in the Arabian Gulf (Figure 2.1). The population ecology of whale sharks in these three study areas has been previously documented. The Inhambane coast hosts 4-9 m total length (TL) sharks, biased towards males (74%) (Haskell et al 2014, Rohner et al. 2015). Whale sharks here appear to preferentially use productive coastal waters (Rohner et al. 2017). A significant decline in sightings occurred in the Tofo Beach area between 2005 and 2011 (Rohner et al. 2013) and appears to have continued until 2016 (Pierce & Norman 2016). Mafia Island is home to a smaller group of resident whale sharks (Cagua et al. 2015). Population structure here is similar to that in Mozambique, with a bias towards male sharks (89%) and a size range of 4-10 m TL (Rohner et al. 2015). The Qatar aggregation is around 90 km offshore (Robinson et al. 2013, 2016, 2017). Photo-ID and sat-tagging studies have concluded that these sharks are largely resident to the Arabian Gulf and Gulf of Oman and are predominantly mature males (Robinson et al. 2016, 2017). Median male TL at Al Shaheen is larger (8 m) than in Mozambique or Tanzania, with more mature individuals present, although the overall size range is similar at 4-10 m TL (Robinson et al. 2016).

Here, we use a large set of photo-ID and stable isotope data to test the assumption that the sharks frequenting these three separate aggregation sites are a separate management stock. We demonstrate that there are significant differences in stable isotope ratios and minimal connectivity among sites, with a pronounced differentiation between sharks in the southern and central areas of the WIO (Mozambique and Tanzania), and those from the Arabian Gulf (Qatar).

2.3 Materials & Methods

2.3.1 Study areas

This study was conducted in three whale shark aggregation sites: (1) off Praia do Tofo, Inhambane province (23° 52’S 35° 33’E), Mozambique (see Pierce et al. 2010, Rohner et al. 2013a,b, 2015a, 2017 in review, Haskell et al. 2014), (2) Kilindoni Bay, Mafia Island, Tanzania (7.29° S 39.65° E) (see Cagua et al. 2015, Rohner et al. 2015a,b), and (3) the Al Shaheen Oil field, 90 km off the coast of Qatar in the Arabian Gulf (26.6°N, 51.9°E) (Robinson et al. 2013, 2016) (Figure 2.1). Photo-ID and stable isotope data of whale shark dermal connective tissue were collected at all three sites. Stable isotope date were compared to known isotopic gradients in the Western Indian Ocean using data from goose barnacles (*Lepas anatifera*) (Lorrain et al. 2014) along with yellowfin (*Thunnus albacares*) and skipjack tuna (*Katsuwonus pelamis*) (Sardenne et al. 2016).

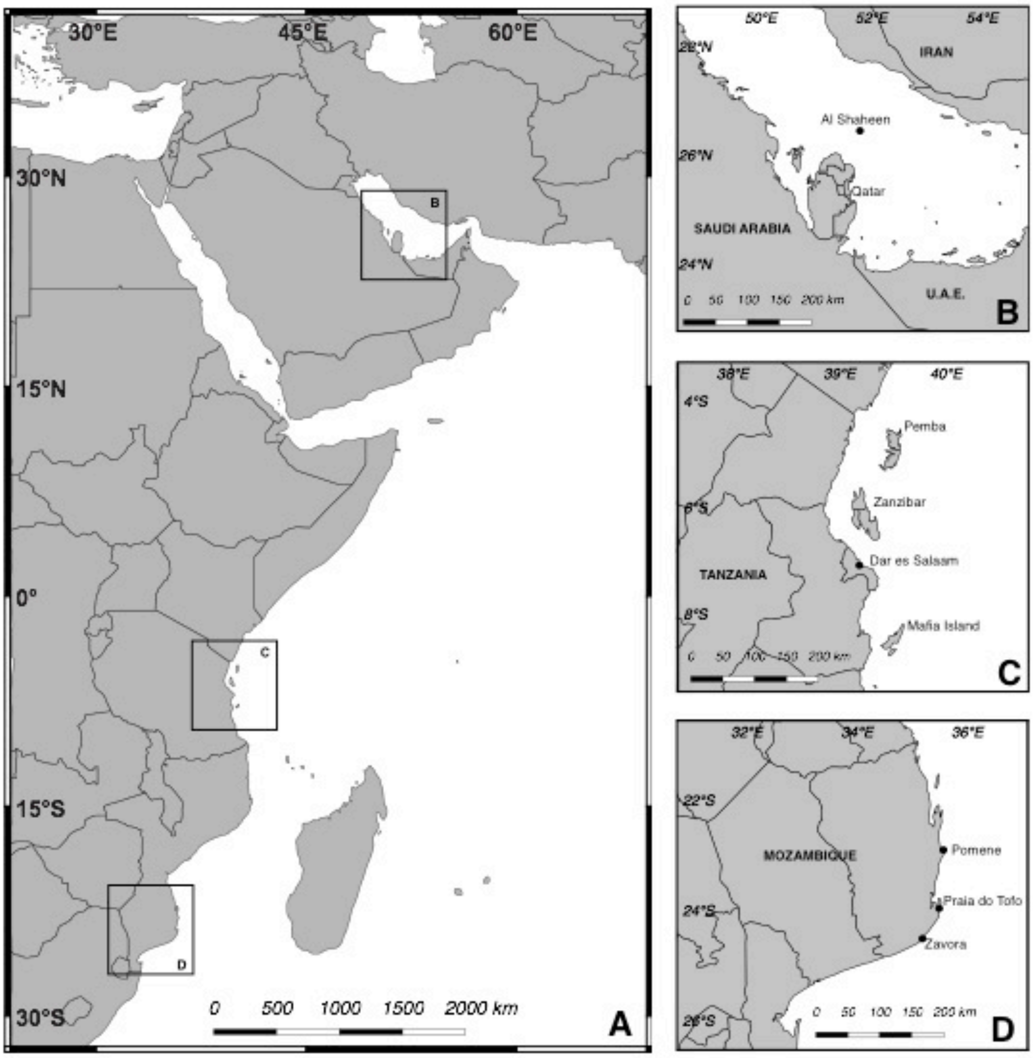

Figure 2.1 Map of the east coast of Africa and Arabian Sea. (A) The position of our three study sites in the western Indian Ocean, (B) the Al Shaheen whale shark aggregation in the Arabian Gulf, (C) Mafia Island off the coast of Tanzania: and (D) Praia do Tofo on the coast of southern Mozambique.

2.3.2 Ethics Statement

Work in Mozambique was carried out with the full knowledge and approval of the Maputo Natural History Museum. All samples from Mozambique were exported (CITES Export MZ0260/16, and permission from the Maputo Natural History Museum), transported (APB Ref: U1246053/APB/OTHER) and imported (CITES Import 550360/01, DEFRA Authorisation No: ITIMP16/1049) into the UK for analysis. In Qatar work was approved by, and carried out in conjunction with, Qatar Ministry of Municipality and Environment (QMMOE). Work in Tanzania was conducted with approval from the Tanzanian Commission for Science and Technology

(COSTECH) (#2015-165/6-NA-2015-161). All samples from Tanzania were exported (CITES Export #30015 / Special export licence Fisheries Development Division) and imported (CITES Import #552057/01) into the U.K. under the same APB/DEFRA licences as Mozambican samples. Project ethics approval came from the Research and Governance Department at the University of Southampton (#13918).

2.3.3 Photo-identification

Individual whale sharks were identified using underwater photographs of the body region immediately posterior to the gills (Arzoumanian et al. 2005). These images were uploaded, processed using a pattern matching algorithm to identify individual sharks, and stored on the online database Wildbook for Whale Sharks (www.whaleshark.org). Each 'encounter' is here defined as one sighting per identified shark per day. Estimated total length (TL) and sex were determined (based on the presence or absence of claspers, as per Rohner et al. 2015a) where possible. Data were from the earliest encounter record for each study site until the 31 December 2015. Neither standardised nor concurrent sampling across the sites were possible due to differences in the seasonality and accessibility of the aggregations.

The Wildbook for Whale Sharks database was used to obtain the total number of encounters and identified individuals for each study site along with sex and size metadata. Pairwise comparisons were made between each of the study sites to identify individuals that had been identified in both. Sighting data were used to assess the lagged identification rate within study sites (R_{LI}) (Whitehead 2001). Lagged identification rate represents the probability of re-sighting identified individuals over increasing time periods, here measured in days (Whitehead 2001). Eight models of lagged identification, each representing a hypothetical population with varying combinations of immigration, emigration, re-immigration and mortality, were fitted to the empirical data. The Akaike information criterion (AIC), or quasi-AIC (QAIC) for over-dispersed data, was used to compare these models to identify the best fit at each site (Whitehead 2007). Parameter estimates with 95% confidence intervals and standard error were generated for R_{LI} by bootstrapping data 100 times (Buckland & Garthwaite 1991). These analyses were conducted using the movement module in SOCPROG 2.6 (Whitehead 2009). These were the first calculations of this kind for the Tanzanian and Mozambican aggregations, and updated previous calculations from Qatar (Robinson et al. 2016) with 2015 sightings data (an additional 192 encounters).

2.3.4 Biopsy sample collection and chemical analysis

Dermal connective tissue biopsies of live, unrestrained whale sharks were taken in Mozambique (2011-2013), Tanzania (2012-2014), and Qatar (2012-2014) (Summary in Table 2.1). First, sharks were individually identified and sexed (as above). Samples were taken using a hand spear with a modified tip that extracted biopsies laterally from between the first and second dorsal fins. Samples were stored on ice in the field. Upon the recommendation of multiple elasmobranch isotope studies, including whale sharks (Kim & Koch 2012, Hussey, Olin, et al. 2012, Burgess & Bennett 2016, Li et al. 2016, Marcus et al. 2017), samples were rinsed in fresh water to remove any possible contaminants and excess urea. The upper dermal denticle layer was then separated from the white connective tissue layer in each sample. This deeper connective tissue layer was frozen onsite and used for all further analyses. We also collected red muscle samples from local, non-migratory, planktivorous fishes in Mozambique and Tanzania to represent isotopic conditions at the collection site. This was not possible in Qatar. All samples were from halfbeaks *Hemiramphus* spp. fishes. Ten samples were collected from Tanzania, and nine from Mozambique.

Site	Males	Females	Sex UK	ID UK	Repeats	Total	TL estimate
Mozambique	**40**	8	2	0	18	68	500-800 (608 ±79)
Tanzania	61	13	4	9	110	188	500-900 (712± 117)
Qatar	22	4	1	9	10	48	400-800 (597 ± 99)

Table 2.1 Number of whale shark samples collected, of each sex and unknown sex (Sex UK), at each study site, and the number of repeat samples taken from individuals and unknown individuals (ID UK), total length estimate (TL) range (mean ± S.D).

Samples were frozen and transported to the University of Southampton, UK, where they were freeze-dried and homogenised prior to analysis in an EA 1110 elemental analyser, linked to a Europa Scientific 2020 isotope ratio mass spectrometer at OEA Laboratories Cornwall. Raw data were corrected using the reference materials USGS40 and USGS41 (glutamic acid from USGS Reston USA). An internal QC material bovine liver standard (NIST 1477a) was used to monitor the precision of the instrument. Precision was on average 0.21 per mil for both C and N.

Isotope ratios are expressed per mille (‰) deviations from the reference materials VPDB and air for $\delta^{13}C$ and $\delta^{15}N$ values respectively.

Elemental C:N ratios for water washed whale shark connective tissue shark samples were similar and relatively low in each study site (Mozambique: 2.65± 0.24 S.D. Qatar: 2.94± 0.24 S.D. Tanzania: 2.89± 0.26 S.D.) and matched lipid extracted CN ratios for similar tissues from Australian whale sharks (Marcus et al. 2017). No chemical treatment was undertaken to remove lipids, and analytical results were not adjusted to correct for lipid contents. During the sampling period we collected more than one, and up to four samples, from 0 – 1063 days apart. The first sample of every individual was analysed. Then, assuming a half-life (isotope turnover rate) of 30 days, we also kept any samples from the same individuals that were taken greater than five half lives (> 150 days) after the initial sample. After five half- lives the sample would then contain only ~3% of the sample before and can thus be considered independent. Planktivore samples from Tanzania had sufficiently high C:N values to merit mathematical lipid correction (Post et al. 2007b) (3.52 ± 0.2), while Mozambican planktivore samples did not (3.1 ± 0.04). Lipid removal is a complex, species- and tissue-specific issue in stable isotope analysis (Post et al. 2007b). As it was not possible within this study to calculate pre and post lipid removal values for each sample type, we applied a lipid correction factor to the bulk Tanzania planktivore $\delta^{13}C$ data (Kiljunen et al. 2006). To assess within-sample variance in whale shark tissues, we ran five repeat samples from the same biopsy for three individuals, respectively, two from Mozambique (Wildbook IDs MZ-013 and MZ-607) and one from Qatar (Q-073).

2.3.5 Stable isotope analysis

Differences and patterns between study sites were investigated for $\delta^{15}N$ and $\delta^{13}C$ bulk values. The mean, standard deviation, and range of $\delta^{15}N$, $\delta^{13}C$, and estimated TL values for each study site were calculated. All data were tested for normality using a Shapiro-Wilk test. Bartlett's test was used to compare homogeneity of variance between the study sites. Within-sample variance of the three chosen samples was compared to overall variance at each site. Box, residuals, and Q-Q plots were created to visualise any outliers and patterns in variance. ANOVA (normally distributed data) or Kruskal-Wallis (non-normally distributed data) tests were then performed on data to examine between site, between sex, and TL differences. Any missing values for sex or estimated TL were not included in the analysis. Post-hoc Tukey HSD (normally distributed data) or Nemenyi (non-normally distributed data) tests were used to explore the pairwise comparisons with significant p-values. Linear regression models were applied to test the effects of estimated TL on $\delta^{15}N$ and $\delta^{13}C$ values.

Published and established isotopic gradients in the Western Indian Ocean were used to spatially assess the whale shark isotope data. $\delta^{15}N$ and $\delta^{13}C$ isotopic data from yellowfin tuna (*Thunnus albacares*) and skipjack tuna (*Katusuwonus pelamis*) were taken from Sardenne *et al* (2016). Barnacle $\delta^{15}N$ data from Lorrain *et al* 2014 were used as a proxy for $\delta^{15}N$ baseline values across latitude. For a generalised representation of baseline values of $\delta^{13}C$ across latitude, we extracted predicted bulk phytoplankton $\delta^{13}C$ values from an isotopic extension to the NEMO-medusa global biogeochemical model (Magozzi et al. 2017). Model data were averaged by latitude in 5° increments from -30° to 30° latitude in this region.

A tissue conversion factor (dermal connective-muscle) was applied to the bulk whale shark data to allow for direct comparisons to the tuna and barnacle isotope values. We used a figure of -2.5‰ for $\delta^{13}C$ values. This figure was arrived at after consideration of results from tissue comparisons of an ecologically similar species, the reef manta ray *Mobula alfredi* (Couturier, Rohner, Richardson, Marshall, et al. 2013), paired samples from the blue-spotted mask ray

Neotrygon kuhlii (Burgess & Bennett 2016) and results from this study for three whale shark samples that contained both muscle and dermal connective tissue (Table 2.2). As the sample sizes for these species were all small, we also considered published values for silky *Carcharhinus falciformis* and blue sharks *Prionace glauca* (Li et al. 2016) currently the only study that compares band muscle tissue in sharks.

	M. alfredi	***N. kuhlii***	***R. typus***
Muscle (n)	11	5	4
Skin (n)	6	5	4
δ^{13}C			
Muscle	-17.4 ± 0.49	-14.38 ± 1.13	-16.35 ± 1.47
Skin	-14.55 ± 0.81	-12.1 ± 1.38	-13.86 ± 1.52
Difference	-2.85	-2.28	-2.49
δ^{15}N			
Muscle	8.95 ± 1.1	12.34 ± 1.07	11.14 ± 1.14
Skin	8.89 ± 1.09	13.64 ± 1.07	9.73 ± 1.2
Difference	0.06 (-2.13 - 2.25)	-1.3 (-3.46 - 0.86)	1.42 (-0.91 - 3.75)

Table 2.2 Bulk isotope values for all muscle and dermal connective tissue per species, mean ± standard deviation. Difference between tissue types and the range given the calculated standard deviation. *Mobula alfredi* (Couturier et al. 2013), *Neotrygon kuhlii* Burgess *et al* (2016) and whale shark results from this study.

	M .alfredi		***R .typus***	
	ID	Difference	ID	Difference
$\delta^{13}C$	8SIA	-2.7	Q118	-3.1
	10SIA	-2.5	Q125	-2.65
	18SIA	-1.4	TZ-009	-2.33
	27SIA	-2.2		
	32SIA	-2.6		
		-2.28 ± 0.53		**-2.69 ± 0.39**
$\delta^{15}N$	8SIA	-1.2	Q118	2.33
	10SIA	-1.8	Q125	1.58
	18SIA	-1.5	TZ-009	1.58
	27SIA	-0.8		
	32SIA	-1.2		
		-1.3 ± 0.37		**1.83 ± 0.44**

Table 2.3 Difference between muscle and dermal connective tissue bulk isotope values for paired samples only. Values in bold are the mean ± standard deviation. Couturier et al (2013), this study.

Elasmobranch skin tissue is comprised of many layers of collagen fibre bundles, particularly in the dorsal region where they support the fins (Motta 1977, Meyer & Seegers 2012). The major amino acid in the type I collagens found in shark skin is glycine, a non-essential amino acid which is typically ^{13}C-enriched compared to bulk protein (McMahon et al. 2010). Consequently collagen is typically enriched in ^{13}C (i.e. shows more positive δ^{13}C values) than muscle protein by approximately 3-5 per mille (Satterfield & Finney 2002). The offset to connective tissue here is much smaller than pure collagen, roughly -2.5‰. Whale shark, manta, and masked ray dermal connective tissues either contain a lower percentage of collagen, or we are seeing some temporal effects where these tissues are integrating a change in diet at different rates. Even though the data is limited, and uncontrolled, the ranges of the offsets between individuals of δ^{13}C are still small (Table 2.3) suggesting the offset is remarkably consistent across individuals. δ^{15}N values in collagen are generally indistinguishable from muscle meaning any offset is likely to be because of temporal effects.

To visually compare the isotopic niche of the whale sharks, we calculated Bayesian ellipses and convex hulls for each study site (Jackson et al. 2011). We used small sample size corrected Bayesian ellipses within SIEBER package to account for potential bias between different sample sizes. Second order polynomial regression models were applied to all datasets to visualise, evaluate and compare latitudinal trends. All statistical calculations were conducted using the statistics platform R (R Development Core Team, 2013).

2.4 Results

2.4.1 Photo-identification

Most encounters (n = 2027) and individuals (n = 664) were recorded in Mozambique. Tanzania had the fewest identified individuals (n = 139), but a comparatively high number of encounters (n = 1282). Qatar had over double the number of identified individuals as Tanzania (n = 437), but with fewer encounters logged (n = 482). Tanzania had the highest re-sighting rate with 71% of individuals were seen on multiple sampling days, followed by Mozambique (53%), and Qatar (46%). Tanzania had the highest percentage observed in multiple years (55%), followed by Mozambique (44%), and Qatar (35%). All three aggregations were male-dominated, with similar size distributions in Mozambique and Tanzania, and larger more mature sharks in Qatar (Table 2.4). Qatar sharks had the largest mean TL (TL = 714 cm ± 116 S.D.), with and Tanzania (TL = 603 cm ± 94 S.D.) and Mozambique (TL = 605 cm ± 76) having smaller and similar mean TL's. Sharks from Qatar were significantly larger than individuals from Tanzania ($p < 0.001$) and Mozambique ($p < 0.001$) overall. There was no significant difference in TL between sharks from Mozambique and Tanzania ($p = 0.99$). Only two individual sharks were recorded in more than one aggregation site in this study, both moving between Mozambique and Tanzania, representing 0.25% of the total identified population of both sites. Individuals MZ-129 and MZ-136 were both first identified in Mozambique, yet both have more numerous and more recent encounters logged in Tanzania (Table 2.5). Neither shark has been seen in Mozambique following the first sighting in Tanzania. While not explicitly considered in this study, use of Wildbook for Whale Sharks meant that sharks in these three study areas were also available for matching with other countries in the WIO region from which sharks have been submitted. Matches were found between the Tofo area in Mozambique and South Africa (n = 24), representing 49% of all whale sharks identified in South Africa, and between Al Shaheen and Oman (n = 9), representing 10% of all sharks identified in Oman.

Site	Study period	Encounters	Identified	Male (%)	Female (%)	UK (%)	Size range (mean ± SD)		# Between	% Between
Moz	May 2005 : Dec 2015	2027	664	347 (52.3)	137 (20.6)	180 (27.1)	432-917cm (673 ±118.8) *(Rohner et al 2015)*	-	-	-
								Moz <-> Tanz	2	0.2491
Tanz	Dec 2006 – Dec 2015	1282	139	109 (78.4)	17 (12.2)	13 (9.4)	420-990cm (641 ±133) *(Rohner et al 2015)*	-	-	-
								Tanz <-> Qatar	0	0
Qatar	Aug 2007 – Dec 2015	870	437	244 (55.8)	110 (25.2)	83 (19)	400-900cm (690 ±124) *(Robinson et al 2016)*	-	-	-
								Qatar <-> Moz	0	0

Table 2.4 Number of identified individuals and total number of encounters at each study site over the study period. Data compared to establish number of identified individuals seen between study sites. All data from Wildbook for Whale Sharks (www.whaleshark.org).

	Year	**2006**	**2007**	**2012**	**2013**	**2014**	**2015**
Site	**Shark ID**						
Moz	MZ-129	1	1	3			
	MZ-136	1					
Tanz	MZ-129				3	5	4
	MZ-136			3	3	7	3

Table 2.5 Total sightings records per year of the only two individuals recorded in both Mozambique and Tanzania. Dotted line signifies the period between 2007-2012 during which neither shark were sighted at either study site.

Modelled lagged identification rate for sharks sighted in Tanzania steeply declined from ~1 to ~136 days, down to a LIR of 0, then jumped up again at ~256 days, followed by another gradual decline (Figure 2.2). The 0 value at ~188 days is an artefact of the seasonal sampling regime rather than an indication of periodic returns to the area. The best fit model in all cases included immigration, emigration, and mortality (Table 2.6), and for the Tanzanian data contained an estimated 34.78 sharks ± S.E. 3.62 (C.I. 26.5 – 39.7) within the Mafia aggregation on any given day. The estimated mean residency time in the area was 30.63 days ± S.E. 11.18 (C.I. 10.4 – 49.0), with a mean time out of the area of 23.9 days ± S.E. 8.3 (C.I. 10.6 – 44.7), and a mortality rate of 0.0003 ± S.E. 0.00009 (C.I. 0.0001 – 0.0004).

Modelled LIR for sharks sighted in Mozambique steeply declined from day ~1 to day ~16, then gradually declined to approach 0 at over ~4000 days (Figure 2.2). The best-fit model for Mozambique was based on QAIC as opposed to AIC values as there was over-dispersion in these data. The model produced an estimate of 50.6 sharks ± S.E. 11.8 (C.I. 30.6 – 68.5) within the study area on an average day. The mean residency time in the area was 9 days ± S.D. 5.03 (C.I. 3.21 -

20.9), with mean time out of the area of 29.9 days ± S.E. 10.1 (C.I. 15.3 – 48.7), and a mortality rate of 0.0006 ± S.E. 0.00009 (C.I. 0.0005 – 0.0008).

Modelled LIR for sharks sighted in Qatar steeply declined from day ~1 to day ~64, with a slight increase between day ~64 and day ~256, where there is a secondary peak, followed a gradual decline beyond day ~1025 (Figure 2.2). The best fit model contained an estimated 115.9 sharks ± S.D. 17.7 (C.I. 83.8 – 151) in the aggregation at any one time. The mean residency time in the area was 17.5 days ± S.D. 9.6 (C.I. 7.00 – 42.2), with a mean time away from the area of 37.54 days ± S.D. 15.7 (C.I. 19.3 – 78.3), and a mortality rate of 0.0004 ± S.E. 0.0001 (C.I. 0.0002 – 0.0007).

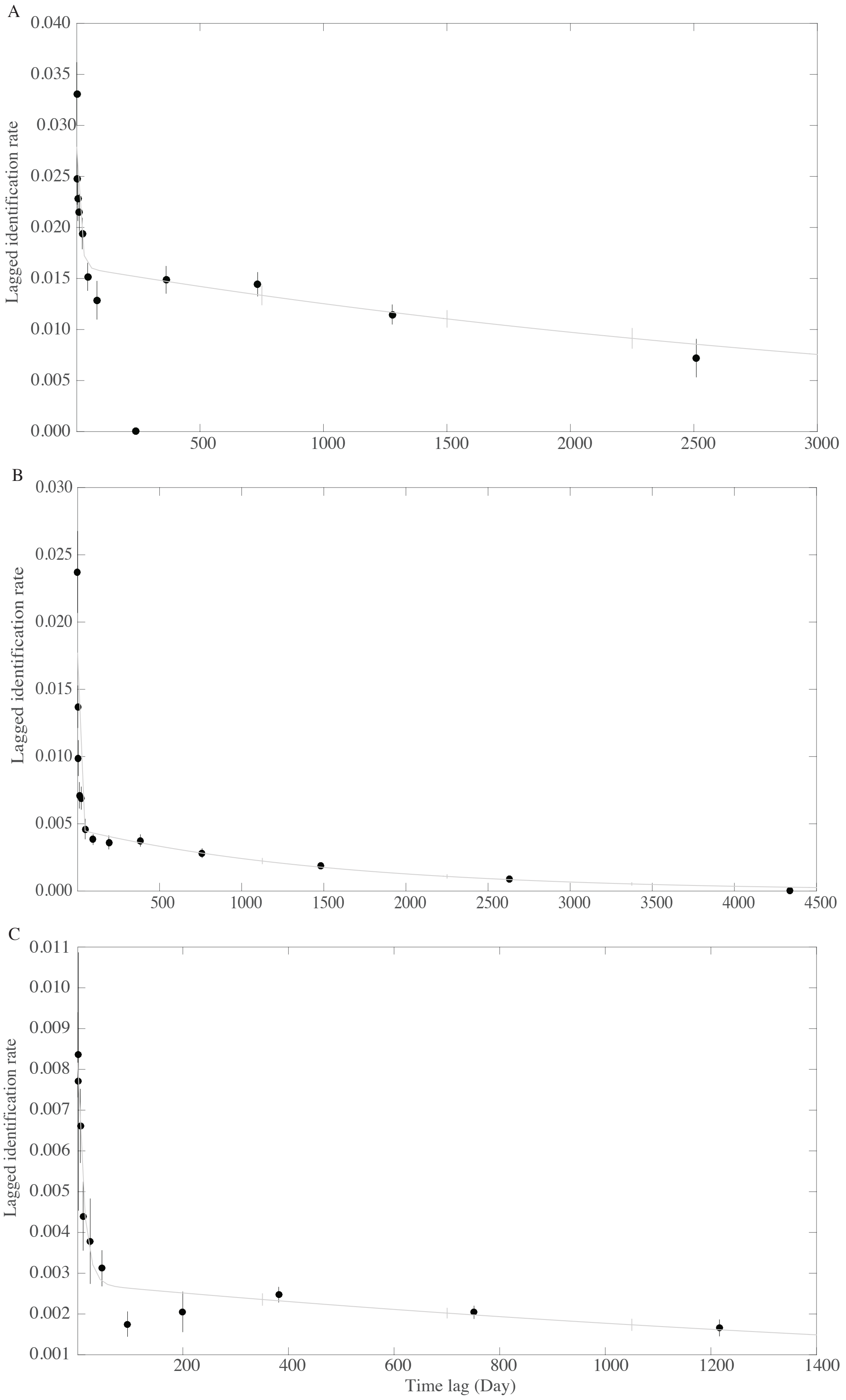

Figure 2.2 Lagged identification rate (LIR) for whale sharks in (A) Tanzania, (B) Mozambique, and (C) Qatar. Modelled from fitted emigration + remigration +mortality rate (mean ± S.E.).

2.4.2 Stable isotope analysis

The stable isotope compositions of all individuals are shown in Figure 2.4. Isotopic niche areas (Jackson et al. 2011) shows some separation by site although there is partial overlap of the Mozambique and Tanzania ellipses (Figure 2.4). Mean whale shark dermal connective tissue δ^{15}N values increased from Mozambique (8.1‰ ± 0.5 S.D.) to Tanzania (9.4‰ ± 0.8 S.D.), and Qatar (10.2‰ ± 0.8 S.D.), and the range in δ^{15}N values was similar between sites between 3-4.2‰. Mean δ^{13}C values in shark dermal connective tissues also increased with latitude Mozambique ($^{-}$15‰ ± 0.3), Tanzania ($^{-}$14.3% ± 0.7), and Qatar ($^{-}$12.6‰ ± 0.2). The range of δ^{13}C was similar for Mozambique (2.3‰, range = $^{-}$16 --13.7), and Qatar (1.8‰, range = $^{-}$13.7 - $^{-}$11.9), but sharks from Tanzania were more variable in δ^{13}C values (4%, range =$^{-}$16.4 - $^{-}$12.4).

All δ^{15}N data were normal (Mozambique Shapiro-Wilk p = 0.2, Tanzania p = 0.1, Qatar p = 0.07), with equal variance between sites (Bartlett p = 0.1). δ^{13}C data were normal for Tanzania (Shapiro-Wilk p = 0.9), but non-normal for Mozambique ($p < 0.05$) and Qatar (p = 0.02). Boxplots of δ^{13}C by study site identified three obvious outliers: two from Qatar (3 and 2.7 s.d. from the mean), and one from Mozambique (4.6 s.d. from the mean), driving the non-normal result (Figure 2.3). There were no patterns in residuals or variance. We tested the data using ANOVA including the outliers as δ^{13}C values also had equal variance between sites (Bartlett's p = 0.1) despite the violation of normality (Underwood 1997). We also tested the data omitting the outliers where δ^{13}C data were then normal for all sites: Mozambique (Shapiro-Wilk p = 0.2), Tanzania (Shapiro-Wilk p = 0.9), and Qatar (Shapiro-Wilk p = 0.7). ANOVA results were significant with and without these outliers, given this and the large deviations from the mean, these three points are therefore addressed separately, and omitted from further analysis to uphold the terms of normality.

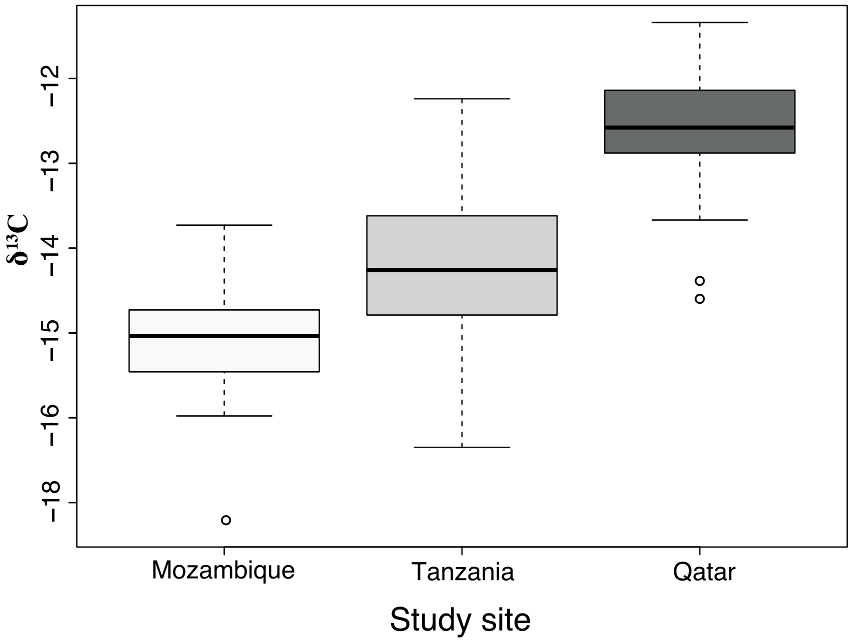

Figure 2.3 $\delta^{13}C$ separated by study site. The central box spans the interquartile range, the middle line denotes the median, and the whiskers above and below shoe the maximum and minimum values. Outliers are shown as circles.

Mean $\delta^{15}N$ and $\delta^{13}C$ (Figure 2.3) values were significantly different between sites (ANOVA: $F_{2,218} = 68$, $p<0.001$, ANOVA: $F_{2,218} = 121$, $p<0.001$) respectively. Tukey HSD test results for $\delta^{15}N$ and $\delta^{13}C$ values were significant for all pairwise comparisons, with $p<0.001$. There were no overall differences between the sexes for $\delta^{15}N$ (ANOVA: $F_{2,206}= 1.7$: $p = 0.2$) or $\delta^{13}C$ (ANOVA: $F_{2,206} = 3.7$: $p = 0.3$). Estimated total length had a significant effect on both $\delta^{15}N$ ($R^2 = 0.08$, 199 d.f. $P < 0.001$) and $\delta^{13}C$ values ($R^2 = 0.23$, 199 d.f. $P < 0.001$), with larger shark dermal connective tissue being more enriched in ^{15}N and ^{13}C. However, there was a low explanatory power for both models. There was also a significant effect of study site on estimated total length (Kruskal-Wallis: $\chi^2(25.9)$, $p < 0.001$), driven by Qatar which had the largest sharks, with no significant size difference between Mozambique and Tanzania (Nemenyi test Qatar-Mozambique: $p < 0.001$, Qatar-Tanzania: $p < 0.001$, Mozambique-Tanzania $p = 1$).

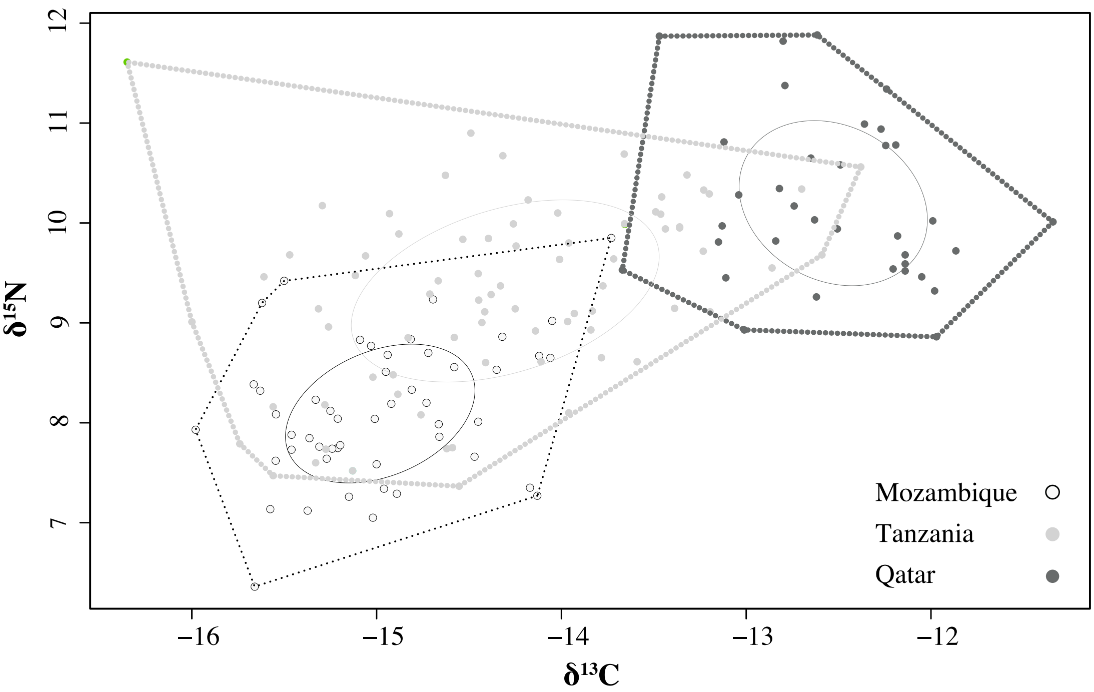

Figure 2.4 Biplot of δ^{15}N and δ^{13}C values. Mean values and standard deviations by study site. Ellipses show 40% standard ellipses, polygons show convex hulls.

The Mozambican outlier point (δ^{15}N = 7.29 and δ^{13}C = -18.5) was a juvenile male shark with whaleshark.org shark ID number MZ-587 with an estimated TL of 500cm. This individual has only one sample in this study. He has seven encounters logged on Wildbook between 2011 and the end of the study period spread evenly throughout this time. Interestingly he had been spotted ~200km north of Tofo in Vilankulos.

The two Qatari outliers are a female with an estimated TL of 700cm with whaleshark.org shark ID number Q-128 (δ^{15}N = 8.73 and δ^{13}C = -14.7) and a juvenile male with an estimated TL of 500cm (δ^{15}N = 11.17 and δ^{13}C = -14.47). The female has only been recorded once in Al Shaheen. The male has been recoded three times between 2011 - 2012. All the outlier sharks have δ^{15}N values within 2 standard deviations of the mean for their location.

2.4.3 Stable isotopes across latitude

δ^{15}N values generally increase with decreasing latitude in barnacles, tuna and sampled whale sharks. However, datasets are incomplete, and the apparent latitudinal trends could reflect a relatively abrupt transition to relatively high and invariant δ^{15}N values north of around 10°S (Figure 2.5).

δ13C values estimated from a isotope-enabled global biogeochemical model show a similar abrupt increase at around 10S, approximately indicating the transition from South Indian Ocean and Arabian Sea surface waters. Neither tuna nor whale shark data reflect the predicted changes in phytoplankton δ13C values (Figure 2.6), both fish groups showing relatively limited latitudinal variation in δ13C values.

There was a similar overall trend for gradual enrichment of δ15N and δ13C values with increasing latitude, with a less pronounced gradient for δ13C values (Figures 2.5 and 2.6).

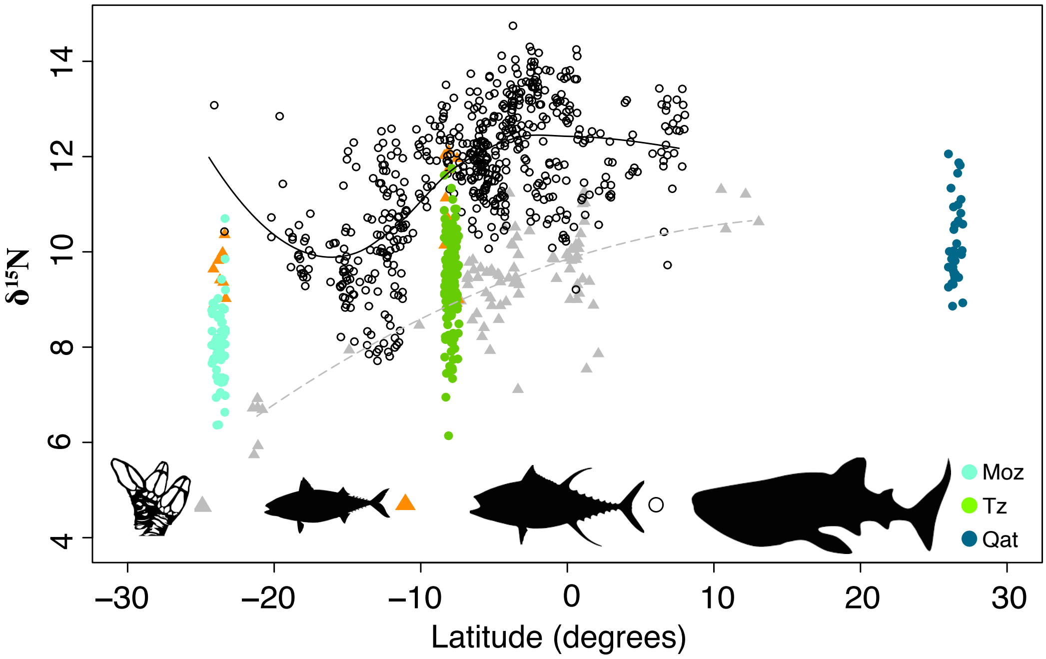

Figure 2.5 Plot of $\delta^{15}N$ values by latitude.

All whale shark (closed coloured circles) and planktivore (orange triangles) values are from this study, tuna data (black open circles) are from Sardenne *et al* (2016), and barnacle data (grey triangles) are from Lorrain *et al* (2015). 2nd order polynomial models are plotted through barnacle data and a loess smoother plotted through tuna data.

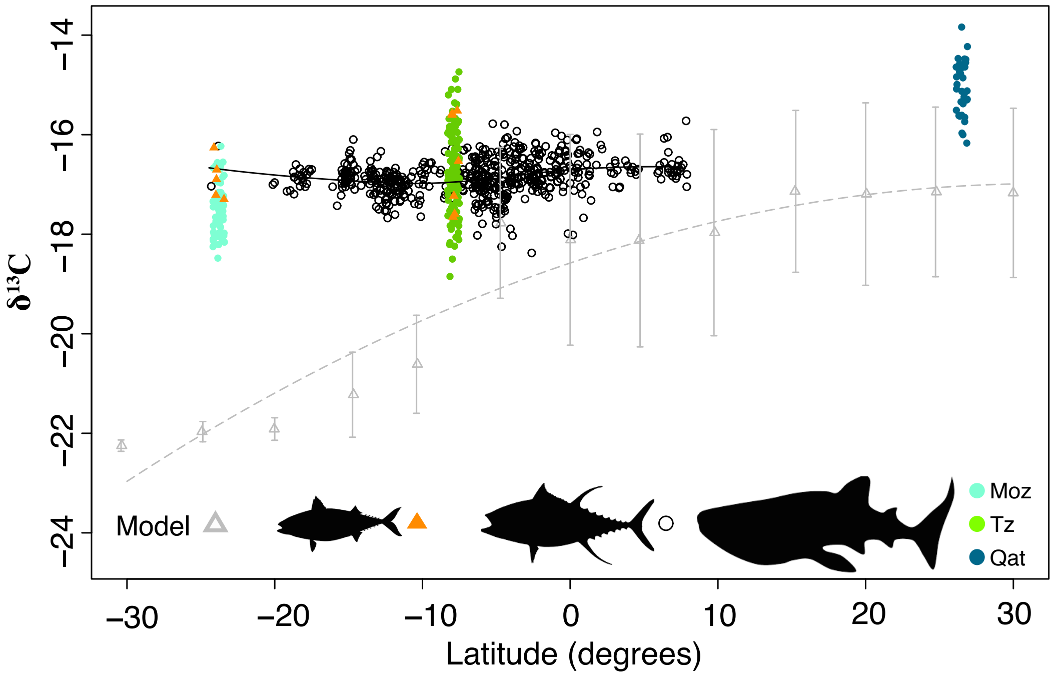

Figure 2.6 Plot of δ^{13}C values by latitude.

All whale shark (closed coloured circles) and planktivore (orange triangle) values are from this study, tuna data (black open circles) are from Sardenne *et al* (2016), and δ^{13}C averaged model output data (grey triangles) are from (Magozzi et al. 2017) ± S.D. 2nd order polynomial models are plotted through model δ^{13}C data and a loess smoother plotted through tuna data.

2.5 Discussion

Whale sharks are undoubtedly capable of making large ocean-scale movements (Hueter et al. 2013, Norman et al 2016). However, our results from the Western Indian Ocean are consistent with other whale shark aggregations dominated by juvenile and adult males showing little evidence of broad-scale dispersal or connectivity between distant feeding sites (Norman et al. 2017). Differentiation between study sites was shown over a 10-year time-frame for photo-ID, and 2-3 years for SIA. While modelled shark movement at all three sites was characterised by emigration and re-immigration, with some mortality or permanent emigration, a significant proportion of individual sharks displayed feeding site fidelity (Chapman et al. 2015, Robinson et al. 2017). Values for both δ15N and δ13C differentiated each study site, despite some individual variability within the results. Although both δ13C and δ15N enriched with larger estimated total length, as this parameter was not independent of study site, we do not have enough data to draw any inferences from this result. The observed ellipse overlap between sharks from Mozambique and Tanzania indicates that sharks are exposed to similar isotopic conditions, making it impossible to test for regional mixing in the vicinity of the Mozambique Channel (Jackson et al. 2011), although only two sharks were observed moving between these sites following extensive survey effort at both locations. The lack of overlap between Mozambique and Qatar convex hull and ellipses, coupled with a lack of photo-ID re-sightings over this area, indicates that they are experiencing different isotopic conditions and appear to represent different functional populations for management purposes. Photo-ID results here and previous tagging results (Robinson et al. 2017) show no evidence of connectivity between Tanzania and Qatar, this suggests that the hull overlap between them is unlikely to represent shared individuals or resources. More likely this is a result of individual diet choice and the isotopic signatures of available prey producing similar integrated results in both locations.

Sharks from Tanzania had the largest variability in both $\delta^{15}N$ and $\delta^{13}C$, and thus the broadest overall isotopic niche. Despite the relatively high site fidelity noted in the Tanzanian sharks, they still move into slightly deeper water in the 'off' season (Cagua et al. 2015). Thus some of this variability is likely to come from foraging in different locations. However the range of $\delta^{13}C$ isotope values seen in the Tanzanian sharks is as wide as the latitudinal changes predicted over the whole latitudinal range of the study (Magozzi et al. 2017). This suggests, based on our current knowledge of their movements, that the result could reflect a wider variety of isotopic feeding sources being available in the Mafia Island area, or more individual specialisation in prey types. While visual observations of feeding and surface sampling has documented that whale sharks feed on sergestiid shrimp (Rohner et al. 2015a or b) and small baitfish (pers. obs.), high-resolution tracking results suggest whale sharks switch to prey sources near the substrate at night (Paulsen et al unbubl. data).

Mozambican sharks were more mobile, with a residency time less than a third of that in Tanzania, double the emigration rate, and the steepest decline in LIR. Mozambique is one of the only large non-seasonal whale shark aggregations (Rohner et al. 2013), with local abundance at Praia do Tofo relating at least in part to productivity (Rohner et al. Submitted). Mozambican sharks had more enriched values for δ15N and δ13C than the baseline barnacle data, or the δ13C model predictions. This suggests that the sharks in Mozambique are feeding either in more isotopically-enriched areas, or on more enriched prey sources. While photo-ID results reported in this study clearly indicate some linkage with South African waters, latitudinal isotope model predictions indicate that northern South African waters will be less enriched than those further north in the Mozambican Channel (Magozzi et al. 2017). The northern Mozambican Channel, in particular, is a notable hotspot for whale sharks (Sequiera et al. 2012). Movement to this region could contribute to the ellipse overlap observed between sharks from Mozambique and Tanzania, supporting a hypothesis of some broad-scale resource-sharing. However, results from dietary fatty acid studies in Mozambican sharks, supported by tracking studies (Brunnschweiler et al. 2009, Rohner et al. Submitted), have indicated that they feed in deeper offshore waters (Rohner et al. 2013). This could result in a similar level of isotopic enrichment (Graham et al. 2010). Both theories could also explain the highly enriched carbon value of the outlier shark from Mozambique if he had recently arrived in the study site from extended deep sea or offshore feeding. A further, more detailed isotopic investigation of the local and mesopelagic food web in Mozambican waters would be needed to clarify this result.

Qatar is a highly seasonal feeding aggregation (Robinson et al. 2013) with sharks staying for several months during the peak season (Robinson et al. 2017), and a high mean re-sight rate (41%) of individuals among seasons (Robinson et al. 2016). The sharks disperse from the Al Shaheen area outside the tuna spawning season (Robinson et al. 2013, 2016, 2017). Movement model estimates and isotope values obtained in this study further support these previous results. Borrell *et al.* (2011) used muscle biopsies from whale sharks in Veraval, India, to investigate isotope ecology in northern Indian Ocean area. Using our tissue enrichment value (2.5‰ $\delta^{13}C$) the Borrell *et al.* (2011) values span a corrected range of -12.2‰ to -15.4‰, values similar to the raw bulk results for Qatar, which lies at a similar latitude to Veraval, India, and fall in line with the established $\delta^{13}C$ latitude gradient (Magozzi et al. 2017).

However, the Borrell *et al.* (2011) $\delta^{15}N$ values are more enriched than any found in this study. Borrell *et al.* (2011) observed that all species in their study had high $\delta^{15}N$, possibly due to high organic pollution, we suggest that the high denitrification which in the Arabian Sea (Sokoll et al. 2012, Gaye et al. 2013) could also result in the observed $\delta^{15}N$ enrichment. If the sharks seen in the Arabian Gulf did indeed make frequent feeding forays into the Arabian Sea, undetected by electronic tagging or photo-ID, we would expect them to have $\delta^{15}N$ values closer to those recorded from sharks captured in India. The large observed difference between $\delta^{15}N$ of the two locations suggests this is not occurring with any regularity.

The comparatively enriched $\delta^{13}C$ values of two Qatari outlier sharks suggest they had been feeding in different isotopic conditions. This raises the possibility that these sharks had recently come from outside the Arabian Gulf before sampling occurred. Electronic tagging shows sharks predominantly resident to the Arabian Gulf year-round yet some do venture through the Straight of Hormuz to the Gulf of Oman and beyond with one female travelling as far as Somalia (Robinson et al. 2017). Smaller (~5m) whale sharks are not common in Qatar and potentially have different habitats to the larger mature sharks as they are mostly seen in more coastal areas and into the Gulf of Oman (Robinson et al. 2016, 2017).

Determining the time frame over which stable isotopes are assimilated (the tissue turnover rate) is challenging. Turnover rate refers to the time taken to completely replace a specific tissue pool, in this case to replace connective tissue proteins. These rates are tissue-specific, species-specific and probably even individual-specific, so they need to be estimated in controlled feeding experiments (Wolf et al. 2009, Logan & Lutcavage 2010, Kim et al. 2011). The large size and Endangered conservation status of whale sharks (Pierce & Norman 2016) creates complex logistical, ethical and financial challenges for whale shark husbandry (Leu et al. 2015, Dove et al. 2011), meaning long-term controlled feeding studies for whale sharks are unlikely to take place. Few thorough controlled feeding studies have been conducted in elasmobranchs, with most focussing on muscle, liver and blood tissues rather than the dermal connective tissue we collected from whale shark biopsies (MacNeil et al. 2005, Logan & Lutcavage 2010, Hussey et al. 2010, Kim et al. 2012). The turnover rate of dermal connective tissue would be determined by the replacement of epithelial cells in the outer layers, and metabolic tissue replacement internally. In chondrichthyans the epidermis (including placoid scales or dentacles in sharks) is a continuously metabolically active layer that protects them from the environment, and cell turnover occurs through damage, cell shedding, and regrowth (Meyer & Seegers 2012). Consequently isotopic incorporation is likely relatively fast, perhaps a few weeks. In elasmobranchs deeper layers of the dermis also has strong mechanical capabilities and is composed of numerous collagen fibre bundles, only the deeper layers of the hypodermis are highly vascularised (Meyer & Seegers 2012), suggesting that the outer epithelial cells have slower cell turnover, replacement, and isotopic incorporation rates, perhaps a by few months (Martínez Del Rio et al. 2009).

Initial scarring studies support these inferred turnover rates, demonstrating whale sharks' maximum healing times from deep skin lacerations over a few months (Womersley et al. 2016). As we used tissue close to the outer epidermal layers in this study, we predict that the results here represent nearer a few weeks of integrated foraging.

The broadly enriching trend of all the groups in the $\delta^{15}N$ latitudinal plot suggests the differences in $\delta^{15}N$ values among study sites are at least in part driven by, and reflect, the baseline $\delta^{15}N$ in the local environment. However, some of the tuna caught in lower latitudes do not display the predicted baseline $\delta^{15}N$. This could partly be a result of the distribution of tuna data, as there were fewer samples from these latitudes included in the model. Sampling methodology may also affect the variation, as the coordinates assigned to each sample were the mean of up to a 5° square. In addition, the tuna are highly mobile. Though a tuna was caught in the latitudes below -15° does not preclude the possibility that it had recently been feeding at more isotopically enriched latitudes. Using a fourth order polynomial to fit these data is not ideal, and reduces the analytical power of the model applied to the data. However, it does correctly represent the discrepancy observed at the lower latitudes. While the tuna data are, therefore, not a perfect proxy dataset for latitudinal variation in the isoscapes, this is the most complete dataset available for this large region. Tuna are a highly mobile group. As a relative measure the comparison with whale shark data indicates that whale sharks, which show less variation, are less mobile than the tuna.

While this study only considers three of the several known aggregations in the Indian Ocean, broader photo-ID studies (Brooks et al. 2010, Andrzejaczek et al. 2016, Norman et al. 2017) have similarly found minimal evidence for connectivity of juvenile and sub-adult whale sharks among coastal aggregations in the region (although Andrzejaczek et al. (2016) noted the high sampling effort required to state this with confidence). Sequeira et al. (2013) also postulated that separate whale shark subpopulations, respectively, may exist in (1) the southern and central Western Indian

Ocean, and (2) the northern Western Indian Ocean and Arabian Sea region. Low connectivity has also been identified in other large marine species in the region. Indian Ocean humpback dolphin (formerly *Sousa chinensis*, now *S. plumbea* (Jefferson & Rosenbaum 2014) populations in Oman, Tanzania and Mozambique showed significant differences in mitochondrial DNA, with this divergence hypothesised to be a consequence of partial oceanographic isolation (Mendez et al. 2011). The South Equatorial Current tracks westwards across the Indian Ocean, splitting into northbound and southbound coastal flows when it hits northern Madagascar and then the African continent at approximately 10°S, creating environmental differences between marine habitats off Mozambique and Tanzania (Mendez et al. 2011). (This is a likely driver of the model $\delta^{13}C$ differences around 10°S) Resolution of whale shark population structure in this region would be facilitated by sampling adult sharks, which have been previously tagged in offshore waters (Sequeira et al. 2012, Escalle et al. 2016) and higher-resolution genetic or genomic studies. Until then, the results of this study imply that the dispersal of juvenile whale sharks from coastal feeding areas is limited by oceanographic boundaries in the Western Indian Ocean.

2.5.1 Conservation and management implications

Whale sharks were reclassified as globally Endangered by the IUCN in 2016 (Pierce & Norman 2016), with the Indian Ocean subpopulation also being Endangered. A regional IUCN Red List assessment for whale sharks in the Arabian Sea region also classified the species as Endangered in that area in 2017 (Pierce & Norman 2016, Jabado et al. 2017). Whale sharks in the Arabian Gulf and the Gulf of Oman also face threats from busy shipping lanes (Reynolds 1993) and several other anthropogenic threats (Robinson 2016), including a small opportunistic fishery active in Oman (Robinson unpubl. data). Small-scale harpoon and entanglement fisheries for whale sharks have taken place in several other countries, such as Iran and Pakistan (Rowat & Brooks 2012). As even the larger, adult male sharks show some residency or site fidelity to the Arabian Gulf and Gulf of Oman (Robinson et al. 2016, 2017), these impacts will have a disproportionate effect on what may be a small shark population (Robinson et al. 2016, Pierce & Norman 2016).

Whale sharks in Tanzania and Mozambique, respectively, also face differing anthropogenic threats. A high proportion of Mafia Island sharks bear scars from interactions with fisheries (Rohner unpubl. data). While no population trend data are available from East African waters, further south in the northern Mozambique Channel there was an approximate 50% decline in peak monthly whale shark sightings from tuna observers between 1991 and 2007 (Sequeira et al. 2014). In Inhambane, Mozambique, in the southern Mozambique Channel, sightings declined 79% between 2005 and 2011 (Rohner et al. 2013), and increasing gillnet use along this coast is thought to be having a significant negative impact on megafauna sightings (Rohner et al. 2017, Rohner et al. 2017)

Fatty acid	**Whale shark**				**Zooplankton (n=17)**	**Planktivores (n=12)**	**Piscivores (n=7)**	**Absolute difference in % TFA within individual**	**% variation of %TFA within individual**
	Season1 (n=16)	**Season2 (n=16)**	**Season3 (n=33)**	**Season4 (n=15)**					
14:0	3.1 ± 0.2	2.7 ± 0.2	2.7 ± 0.1	2.2 ± 0.2	7.6 ± 1.0	3.8 ± 0.4	3.0 ± 0.6	0.0052 ± 0.0014	0.17
i15:0	1.3 ± 0.3	1.1 ± 0.1	0.8 ± 0.0	0.5 ± 0.1	0.2 ± 0.1	0.1 ± 0.0	0.1 ± 0.0	0.0017 ± 0.0007	0.13
15:0	0.7 ± 0.1	0.6 ± 0.1	0.6 ± 0.0	0.6 ± 0.1	1.3 ± 0.1	1.2 ± 0.1	0.9 ± 0.1	0.0014 ± 0.0004	0.20
16:0	24.4 ± 0.9	20.9 ± 0.9	21.4 ± 1.0	21.9 ± 1.8	29.4 ± 1.1	26.6 ± 0.8	23.9 ± 1.0	0.0491 ± 0.0123	0.20
17:0a	1.3 ± 0.1	1.4 ± 0.1	1.3 ± 0.1	1.2 ± 0.1	0.2 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.0033 ± 0.0008	0.24
i17:0	0.2 ± 0.1	0.1 ± 0.0	0.3 ± 0.2	0.0 ± 0.0	0.4 ± 0.0	0.4 ± 0.1	0.5 ± 0.1	0.0006 ± 0.0003	0.20
17:0	1.6 ± 0.1	1.3 ± 0.1	1.5 ± 0.1	1.3 ± 0.1	2.1 ± 0.2	1.9 ± 0.1	1.6 ± 0.1	0.0032 ± 0.0011	0.20
18:0	31.1 ± 1.6	25.4 ± 1.3	25.3 ± 1.5	29.8 ± 1.3	9.9 ± 0.9	12.4 ± 0.8	11.4 ± 0.3	0.0621 ± 0.0200	0.20
20:0	0.3 ± 0.1	0.2 ± 0.1	0.1 ± 0.0	0.0 ± 0.0	0.5 ± 0.1	0.4 ± 0.1	0.5 ± 0.1	0.0009 ± 0.0003	0.30
22:0	1.2 ± 0.1	1.0 ± 0.1	0.8 ± 0.1	0.5 ± 0.1	1.1 ± 0.2	0.4 ± 0.1	0.4 ± 0.1	0.0020 ± 0.0006	0.17
24:0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	1.0 ± 0.4	0.6 ± 0.1	0.5 ± 0.0	0.0 ± 0.0	0.00
Total SFA	**65.1 ± 2.8**	**54.6 ± 1.4**	**54.8 ± 2.0**	**58.1 ± 2.9**	**54.5 ± 2.6**	**48.9 ± 1.7**	**43.4 ± 1.7**	**0.1302 ± 0.0362**	**0.20**
16:1ω7	1.3 ± 0.3	2.4 ± 0.3	1.2 ± 0.1	1.0 ± 0.1	5.5 ± 0.7	3.5 ± 0.3	3.5 ± 0.6	0.0039 ± 0.0011	0.16
18:1ω9	11.8 ± 1.7	17.0 ± 1.1	14.2 ± 0.7	13.3 ± 1.2	4.4 ± 0.4	6.4 ± 0.7	7.5 ± 0.8	0.0438 ± 0.0174	0.26
18:1ω7	2.8 ± 0.5	2.9 ± 0.3	3.0 ± 0.2	2.8 ± 0.3	4.3 ± 0.4	3.4 ± 0.3	3.0 ± 0.3	0.0102 ± 0.0033	0.34
20:1ω9	0.1 ± 0.0	0.4 ± 0.3	0.5 ± 0.2	0.1 ± 0.1	0.2 ± 0.1	0.2 ± 0.1	0.3 ± 0.1	0.0018 ± 0.0008	0.36
22:1ω9	1.4 ± 0.8	0.5 ± 0.4	0.0 ± 0.0	0.2 ± 0.1	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0000 ± 0.0000	0.00
Total MUFA	**18.2 ± 2.1**	**24.6 ± 1.7**	**19.7 ± 1.2**	**17.5 ± 1.6**	**15.2 ± 0.7**	**15.1 ± 1.3**	**16.2 ±1.8**	**0.0599 ± 0.0225**	**0.24**
18:2ω6	0.2 ± 0.1	0.4 ± 0.1	0.4 ± 0.1	0.4 ± 0.1	1.3 ± 0.2	1.1 ± 0.1	1.2 ± 0.1	0.0017 ± 0.0007	0.43
18:4ω3	0.8 ± 0.1	0.7 ± 0.3	0.1 ± 0.0	0.0 ± 0.0	0.2 ± 0.2	0.0 ± 0.0	0.0 ± 0.0	0.0015 ± 0.0007	0.19
20:4ω6 (ARA)	5.6 ± 1.1	6.9 ± 0.8	12.3 ± 0.9	12.9 ± 1.1	2.5 ± 0.5	3.5 ± 0.4	3.9 ± 0.3	0.0273 ± 0.0106	0.21
20:5ω3 (EPA)	0.1 ± 0.1	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	8.4 ± 1.5	4.6 ± 0.6	4.8 ± 0.5	0.0000 ± 0.0000	0.00
22:4ω6	2.9 ± 0.5	3.6 ± 0.4	5.8 ± 0.4	6.1 ± 0.6	0.3 ± 0.1	0.4 ± 0.1	0.6 ± 0.1	0.0136 ± 0.0050	0.22
22:5ω6	4.1 ± 1.0	4.2 ± 1.0	2.6 ± 0.5	1.7 ± 0.6	1.4 ± 0.3	1.9 ± 0.1	2.3 ± 0.2	0.0083 ± 0.0032	0.20
22:5ω3	1.7 ± 0.2	1.8 ± 0.2	1.1 ± 0.1	1.0 ± 0.3	0.9 ± 0.2	1.6 ± 0.2	1.5 ± 0.2	0.0026 ± 0.0008	0.14
22:6ω3 (DHA)	1.3 ± 0.3	2.5 ± 0.3	2.8 ± 0.2	2.3 ± 0.4	13.2 ± 1.7	22.0 ± 2.5	24.7 ± 3.1	0.0072 ± 0.0023	0.26
Total PUFA	**16.7 ± 1.2**	**20.8 ± 1.1**	**25.5 ± 1.1**	**24.4 ± 1.6**	**30.2 ± 0.7**	**36.1 ± 2.9**	**40.4 ± 3.3**	**0.0613 ± 0.0197**	**0.25**
ω3/ω6	**0.3 ± 0.1**	**0.4 ± 0.1**	**0.2 ± 0.0**	**0.1 ± 0.0**	**4.6 ± 1.1**	**4.1 ± 0.4**	**3.8 ± 3.0**	**0.01346 ± 0.0499**	**3.37**
Others	0.8 ± 0.3	2.0 ± 0.7	1.2 ± 0.5	0.0 ± 0.0	3.7 ± 0.6	3.6 ± 0.5	3.8 ± 0.5	-	-

Table 3.5 The mean fatty acid (FA) profile (% TFA) of functional groups (whale sharks, plankton, planktivores, and piscivores) , grouping all FA <0.2% as others. Other FA for all samples included: SFA: a15:0, i16:0, 19:0, 21:0, MUFA: 16:1ω9, 16:1ω5, 17:1ω8, 17:1ω7, 18:1ω11, 18:1ω5, 20:1ω11, 20:1ω7, 22:1ω11, 24:1ω9, PUFA: 18:2ω4, 18:3ω6, 18:3ω4, 18:3ω3, 18:4ω6, 20:2ω6, 20:3ω6.

The current view of whale shark population structure in the Indo-Pacific is that the area can be regarded as a single panmictic management unit. Here we have shown that the range of juvenile sharks is more locally restricted, with oceanographic barriers having a significant influence on dispersal. Relatively localised human threats may have a more pronounced impact on whale sharks than was previously recognised.

2.6 Supplementary Table

	Model	**Model Description**	
Tanzania			**ΔAIC**
	A	Closed ($1/a1=N$)	547.0283
	B	Closed ($a1=N$)	547.0283
	C	Emigration/mortality($a1$=emigration rate; $1/a2=N$)	159.46917
	D	Emigration/mortality ($a1$=N; $a2$=Mean residence time)	159.4691
	E	Emigration + reimmigration	105.7028
	F	Emigration + reimmigration ($a1=N$; $a2$=Mean time in study area; $a3$=Mean time out of study area)	105.7026
	G	Emigration + reimmigration + Mortality	71.9039
	H	Emigration + reimmigration + Mortality	0
Mozambique			**ΔQAIC**
	A	Closed ($1/a1=N$)	80748.064
	B	Closed ($a1=N$)	1040.9435
	C	Emigration/mortality($a1$=emigration rate; $1/a2=N$)	154.1138
	D	Emigration/mortality ($a1$=N; $a2$=Mean residence time)	155.428
	E	Emigration + reimmigration	155.4281
	F	Emigration + reimmigration ($a1=N$; $a2$=Mean time in study area; $a3$=Mean time out of study area)	155.428
	G	Emigration + reimmigration + Mortality	158.1141
	H	Emigration + reimmigration + Mortality	0
Qatar			**ΔQAIC**
	A	Closed ($1/a1=N$)	9742.2858
	B	Closed ($a1=N$)	52.2323
	C	Emigration/mortality($a1$=emigration rate; $1/a2=N$)	22.4372
	D	Emigration/mortality ($a1$=N; $a2$=Mean residence time)	22.4372
	E	Emigration + reimmigration	32.4377
	F	Emigration + reimmigration ($a1=N$; $a2$=Mean time in study area; $a3$=Mean time out of study area)	22.4368
	G	Emigration + reimmigration + Mortality	26.443
	H	Emigration + reimmigration + Mortality	0

Table 2.6 Model descriptions, and relative QAIC/AIC values for all models for each site.

Chapter 3 Trophic and feeding ecology of whale sharks at Mafia Island, Tanzania

3.1 Abstract

Despite their size, relatively passive behaviour and commercial significance, knowledge of the behavioural ecology of whale sharks is limited. The difficulty of tracking individual animals at sea encourages indirect biochemical approaches, such as stable isotope (SIA) and fatty acid (FAA) analysis, but the indirect nature of these biochemical methods coupled with a lack of experimental validation, and incomplete sampling of the local environment can lead to conflicting interpretations of dietary ecology.

Whale sharks at Mafia island in Tanzania form a comparatively small and resident aggregation that have been monitored for several successive years. As such, they provide a rare opportunity to study biochemical changes over time at the individual and population level. Here we sampled 53% of the identified individuals over a multi-year period, and uniquely undertook pairwise and multi-year SIA and FAA of the same individuals. Stable isotope data suggest foraging within the local food web. By contrast, FAA shows highly distinctive lipid class compositions for whale sharks, but also indicates a reliance on epipelagic prey. Previously, anomalous FAA concentrations in whale sharks have been interpreted as representing a major contribution of an unrecognised and unknown diet source, we propose an additional explanation of preferential dietary routing. As such, we strongly suggest that in future both methods be used in conjunction to more fully understand the feeding ecology of this, and other marine species. We also call for future research into biological lipid pathways and isotope tissue fractionation in whale sharks, and highlight the importance of sampling different tissues from opportunistic strandings to more accurately understand the long-term feeding ecology of this endangered and charismatic megaplanktivore.

3.2 Introduction

Whale sharks are the largest fish in the world, and the one of only three planktivorous shark species. Whale sharks have a circumtropical distribution but are not distributed evenly through their range (Rowat & Brooks 2012). Most direct observations of juvenile whale sharks occur at coastal aggregation sites where the majority of the sharks seem to be present predominantly to target ephemeral bursts in productivity (Meekan et al. 2009, Robinson et al. 2013, Rohner et al. 2015b). ‘Whale shark season’ often only lasts a for a few months a year at each site, and on average individuals are only present from a few days, up to a few weeks at a time (Araujo et al. 2014, Sigsgaard et al. 2016). The dominant food source can often be quickly identified at each location (Duffy 2002, Nelson & Eckert 2007, Meekan et al. 2009, Robinson et al. 2013, Rohner et al. 2015a) and have even been quantified at times of peak productivity (Motta & Wilga 2001, Rohner et al. 2015a, Armstrong et al. 2016).

It is unclear whether these bursts in local productivity represent a major component of the diet for each individual shark, or form a small part of their overall feeding strategy.

Intrinsic biochemical markers tracking the proportional assimilation of diet items, such as stable isotope analysis and fatty acid analysis, enable researchers to examine time-integrated results to give a retrospective, longer-term picture of feeding ecology (Layman et al. 2012). Stable isotope analysis (SIA) is now a routine tool in trophic and spatial ecology, and has been used extensively to study elasmobranch ecology (Best & Schell 1996, Wallace et al. 2006, Ménard et al. 2007, Reich et al. 2007, Cherel et al. 2008, Jaeger et al. 2010, Hussey et al. 2011, Kiszka et al. 2011, Borrell et al. 2011, Speed et al. 2011, Hussey, MacNeil, et al. 2012). As a complimentary method to SIA, fatty acid analysis (FAA) signatures can also be used as an indirect method of assessing dietary preferences and trophic ecology. Much like SIA the concept of fatty acid trophic markers is based on marine primary producers laying down recognisable fatty acid profiles that are transferred to, and conserved in primary consumers with little modification (Iverson 2009). This process is possible as most higher trophic level marine animals lack the ability to synthesise some fatty acids (termed essential fatty acids), especially the long-chain polyunsaturated fatty acids (PUFA), therefore the presence and relative abundances of these fatty acids in the tissues of the predator indicates aspects of their diet (Budge et al. 2006, Iverson 2009).

Whale sharks resident at Mafia Island, Tanzania offer a rare opportunity to resolve diet preferences in coastal whale sharks, as this aggregation has been reliably monitored for several successive years, allowing analysis of biochemical variation over time at the individual and aggregation level. Though the whale sharks are present in Mafia all year round, acoustic tagging work does show seasonality to their habitat use and movements within the bay (Cagua et al. 2015), which may help to remove some of the spatial variability from biochemical data, providing a clearer picture of their habitat use. Additionally, during the ‘peak’ season from October to January their target prey is easily identifiable as whale sharks can be seen very close inshore feeding on dense patches of large sergestid shrimp

(*Belzebub hanseni*) (Rohner et al. 2015a). From February to September some sharks are still present in the bay, but are in lower numbers and are predominantly found further away from Mafia Island, giving them opportunity to target other prey items. Early in the season the sharks have been observed also feeding on small baitfish at the surface and displaying vertical feeding (Personal observation, observations by local fishers).

The aim of the current study is to use combined stable isotope and fatty acid analysis to determine the proportional contribution of onshore and offshore feeding to overall diet within a relatively resident shark population, and therefore to determine the importance of the Mafia Island feeding site habitat to the local whale shark aggregation.

3.3 Study Area

Killindoni bay off Mafia Island, Tanzania (7.9° S, 39.6° E) (Rohner et al. 2015a) is a shallow bay not exceeding 30 m depth (Figure 3.1). The intertidal zone is up to ~1 km wide and mangroves line most of the bay. Sand is the dominant substrate type, with patches of sea grass and muddy areas next to the mangroves, and dispersed coral bommies and rubble throughout the bay. The whale shark demographic structure is biased towards males (89%) and their size range is 4-10 m TL (Rohner et al. 2015a). Fieldwork was carried out at Mafia Island between Oct 2011 - Dec 2016. Effort was focused during the 'peak season' in each year (Cagua et al. 2015). Season 1: 31st Oct 2012 – 20th Feb 2013, Season 2: 7th Nov 2013 – 8th Jan 2014, Season 3: 6th Nov 2014 – 18th Dec 2014, Season 4: 23rd Oct 2015 – 23rd Dec 2015, and Season 5: 10th Nov - 16th Dec 2016. All surveys began and ended from shore just north of Kilindoni town and were designed to find whale sharks in the bay from shallow water close to shore (~2 – 15 m depth) to deeper water ~10 km west of Kilindoni (30 m depth).

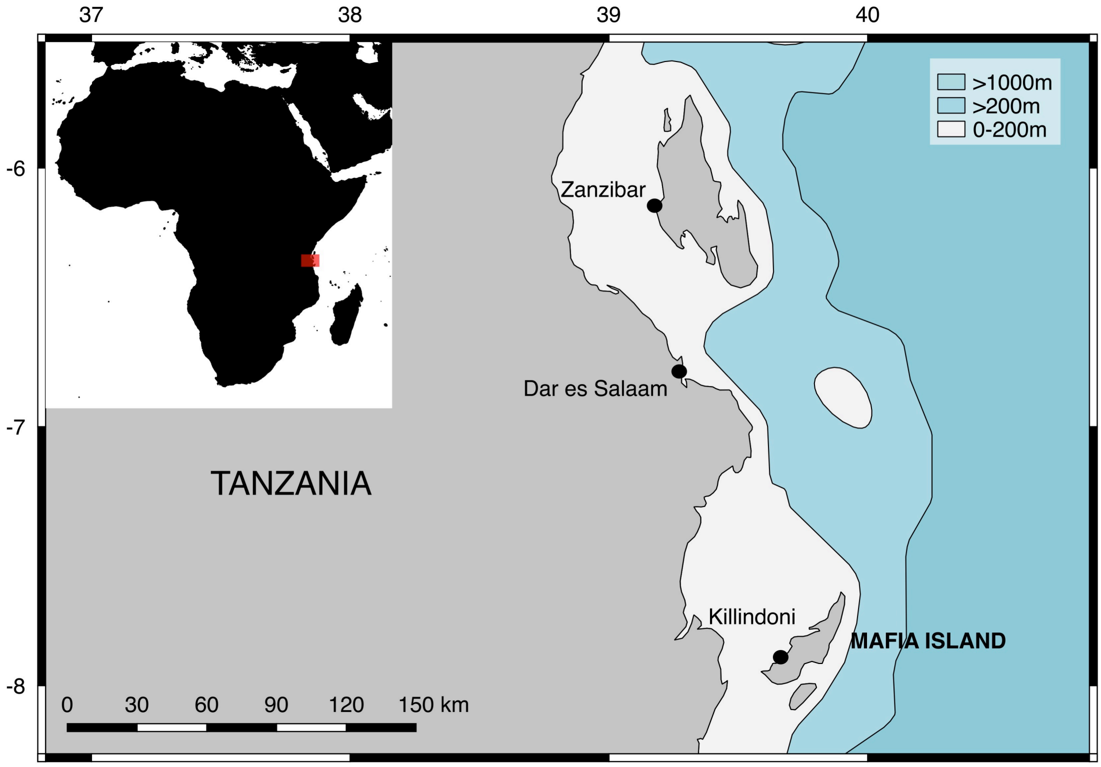

Figure 3.1 Mafia Island in Tanzania within Africa, and the location of Killindoni town on Mafia Island.

3.4 Ethics Statement

Work in Mafia was conducted with approval from the Tanzanian Commission for Science and Technology. All samples were legally exported, transported and imported into the U.K. with appropriate permits. Project ethics approval came from the Research and Governance Department at the University of Southampton(#13918).

3.5 Sample Collection

Whale sharks were individually identified using underwater photographs of the body region immediately posterior to the gills (Arzoumanian et al. 2005). These images were processed using a pattern matching algorithm (Arzoumania wt al. 2005) to identify individual sharks, and stored on the online database, Wildbook for whale sharks (www.whaleshark.org). Estimated total length (TL) and sex were determined (based on the presence or absence of claspers (as per Rohner et al. 2015a) where possible.

Tissue samples were taken using a hand spear with a modified tip to extract biopsies laterally from between the first and second dorsal fins. Samples were stored on ice in the field, then rinsed in fresh water upon return to remove any possible contaminants and excess urea. The upper dermal denticle layer was then separated from the white connective tissue layer in each sample. This deeper tissue layer, dermal connective tissue henceforth, was frozen onsite and used for all further analyses.

Potential diet items were sampled in local and distant waters of Kilindoni Bay. We collected the dominant local food source in peak season, the sergestid shrimp *Belzebub hanseni*, with a 10 cm diameter 100 µm mesh towed by a swimmer near feeding whale sharks. We collected night surface plankton with a larger, 50 cm diameter 200 µm mesh net towed behind a boat, and emergent zooplankton were collected with a weighted 200 µm mesh net overnight. Local plankton samples were filtered, washed with fresh water, and kept on ice until returning to shore where they were immediately frozen. We also collected bell tissue from local jellyfishes and muscle tissue from non-migratory, planktivorous and larger predatory piscavorous fishes to represent the biochemical conditions at the collection site for context and comparison.

Deep-water plankton were collected offshore as part of the Aghulas II cruise in October-November 2017. Hauls were conducted during daylight hours at eight locations between the latitudes -20.68 in Southern Mozambique, and -6.09 in Northern Tanzania. Plankton samples were collected with a five net Vertical Multinet (Type Midi $0.25 m^2$ mouth area). Hauls were of max depth 1,482 m with integrated samples between depths of 0 m, 200 m, 400 m, 600 m, 800 m, 1000 m and 1,482 m.

3.6 Chemical Analysis

3.6.1 Stable isotope analysis

Samples were kept frozen and transported to the University of Southampton, UK, where they were freeze-dried and homogenised prior to analysis in an EA 1110 elemental analyser, linked to a Europa Scientific 2020 isotope ratio mass spectrometer at OEA Laboratories Cornwall. Raw data were corrected using the reference materials USGS40 and USGS41 (glutamic acid from USGS Reston USA). An internal QC material bovine liver standard (NIST 1477a) was used to monitor the precision of the instrument.

Mean elemental C:N ratios for water washed whale shark dermal connective tissue samples (2.6 ±0.4 S.D.) were similar to C:N ratios from lipid-free (lipid-extracted) dermal connective tissues from Australian whale sharks (Marcus et al. 2017), and below a suggested threshold indicating problematic contamination of protein by lipid (Post et al. 2007a). Consequently, no chemical treatment was undertaken to remove lipids, and analytical results were not adjusted to correct for lipid content. Some samples of potential prey items did exhibit high (>3.5) molecular C:N ratios, including muscle tissue from the bullet tuna (5.6± 2.1 S.D.), kingfish (3.6± 0.3 S.D.), mackerel (4.3± 1.4 S.D.), and all zooplankton samples, including emergent (6.6), feeding (6.2± 1.5 S.D.), night (6.3± 1.3 S.D.) and deep-water (4.4± 0.6 S.D.). We therefore applied a mathematical lipid correction factor (Kiljunen et al. 2006) to $\delta^{13}C$ values from all diet source samples.

Dermal collagen is frequently enriched in amino acids with relatively high $\delta^{13}C$ values compared to muscle, and a tissue conversion factor (skin-muscle) was applied to the bulk whale shark data to allow for direct comparisons to fish muscle samples and zooplankton in this study. We used a figure of -2.5‰ for $\delta^{13}C$ as in Prebble et al. 2018 (*in review*) (Chapter 2).

3.6.2 Fatty acid analysis

Lipid extraction

Lipid extraction was performed using a modified Folch method (Folch et al. 1957). Wet weight of each sample was determined prior to analysis. Fresh tissue samples were placed in a chloroform:methanol 2:1 solution, vortexed, sonicated in an ultrasonic bath for 10 minutes, then left to soak at -20°C for a minimum of 12 hrs.

20 μl of tricosanoic acid (C23:0) standard was added to an aliquot of lipid extract to allow fatty acid content to be quantified. Extracts were then evaporated and trans-esterified with 800 μl of methanolic sulphuric acid (MeOH-H_2SO_4 3.4% v/v), before being placed in a heating block at 100°C for 10 minutes. After cooling, formed fatty acid methyl esters (FAME) were extracted by adding hexane. Three washings were carried out by adding 1.5 ml of hexane-saturated distilled water, by shaking and by centrifuging for 1 minute. Each time, the lower phase was removed. After the last washing, samples were placed at -20°C. Once the lower phase had frozen, the upper organic phase was transferred to a 2 ml vial for further analysis.

Lipid class

A subset of 53 samples were available for lipid class analysis, including whale shark, zooplankton, and tuna muscle samples. Zooplankton and tuna samples were chosen randomly. Whale shark samples with clear gas chromatograph results (see next section) demonstrating a reasonable concentration of lipids in the extraction were chosen for lipid class analysis. High performance thin layer chromatography (HPTLC) analyses were performed to separate and identify classes of neutral lipids (e.g. free fatty acids and triacylglycerol (TAG)) and polar lipids (phospholipids). Silica gel plates (20 x 10 cm) were washed in 20 ml of standard solvent solution with known quantities of common lipid classes. Plates were activated by heating at 120°C for 20 minutes. Pre-run solutions for neutral lipid separation contained 250 ml of hexane and 8 ml of diethyl ether. Pre-run solutions for polar lipid separation consisted of 70 ml of methyl acetate, 70 ml of isopropyl alcohol, 70 ml of chloroform, 28 ml of methanol and 25 ml of potassium chloride 25%.

Sample lipid extracts were laid down on activated plates (between 1 and 10 μl for neutral lipids and 20 μl for polar lipids) using of a CAMAG automatic TLC sampler ATS4. Standards were deposited on each plate every three samples for calibration. Lipid separation was performed in a closed saturated tank containing a mobile phase. Polar lipid migration used the same solution as the pre-run. Two successive migrations were performed for neutral lipids, the first solution consisted of 200 ml of hexane, 50 ml of diethyl ether and 5 ml of acetic acid, the second migration solution was the same as the pre-run. Plates were then dipped into an aqueous solution of 8% phosphoric acid and 3% copper acetate then immediately charred at 180°C for 30 minutes. Plates were then scanned using a densitometry CAMAG TLC scanner 3 at 370 nm. Concentrations of separated lipid classes were determined from the intensity of the absorption via peaks areas using visionCATS v2.3 (CAMAG).

Fatty acid profiles

FAME were analysed using a gas chromatograph (Varian CP3800) with an auto-sampler equipped with both polar and apolar capillary columns (ZB-WAX, 30 m length × 0.25 mm i.d. × 0.25 µm film thickness and ZB-5 30 m length × 0.25 mm i.d. × 0.25 µm film thickness), a splitless injector and a flame ionization detector. The carrier gas was H_2, at a constant flow of 2 ml/min. The identification of FAME was carried out by comparison of retention times with a commercially available standard 37-component FAME mix. The relative abundance for each FA was determined from the area of chromatogram peaks and all FA were expressed as percentage of total FA.

3.7 Statistical analysis

3.7.1 Stable Isotope analysis

We calculated the mean and standard deviations (SD) of $\delta^{13}C$, $\delta^{15}N$ and C:N for each species collected. We then categorised the samples into functional group and calculated the mean and SD for each group. We calculated seasonal mean and SD for all whale sharks as well as mean differences within individuals overall and within season. For those individuals with more than one biopsy within a season, to avoid replication we calculated mean $\delta^{13}C$ and $\delta^{15}N$ values within each season, this value was then used in further calculations. We produced biplots of $\delta^{13}C$ and $\delta^{15}N$ values to visualise and compare the isotope results of all samples. We used mixed effects linear models to identify any significant factors affecting $\delta^{13}C$ and $\delta^{15}N$ within groups. Whale shark identity and the species within groups were set as random effects. Pairwise adjusted Tukey contrasts were performed to identify the level of significance among pairs within factors.

3.7.2 Trophic position

We estimated the trophic position (TP) of whale sharks using the equation $TP = \lambda + (\delta^{15}N_{Consumer} - \delta^{15}N_{Base}) / \Delta_n$,where λ is the trophic position of the consumer used as a baseline, $\delta^{15}N_{Base}$ is the mean $\delta^{15}N$ of the baseline, $\delta^{15}N_{Consumer}$ is the $\delta^{15}N$ value of the whale sharks and Δ_n is the fractionation value of the consumer. Fractionation values are species specific, and accurate estimation needs to be determined in controlled feeding studies. To date, there are no experimental fractionation values for whale sharks or any other planktivorous elasmobranchs. Currently, the closest proxies are from a long term, controlled feeding study on leopard sharks (*Triakis semifasciata*) (Kim et al. 2012) and semi-controlled study on larger predatory sharks (Hussey et al. 2010). As lower trophic level species, whale sharks are likely to have larger fractionation values, with a larger associated error (McMahon et al. 2010). Therefore, a range of probable trophic enrichment factors (2.8-4% enrichment per trophic level) was used to estimate a distribution of likely trophic levels. If available, standard deviations of all other values in the trophic level calculation were also included to account for error. The value chosen for the base was the mean and standard deviation $\delta^{15}N$ zooplankton feeding value, as samples were made up predominantly of sergestid shrimp, the observed target species for whale sharks foraging in Mafia (Rohner et al. 2015a). The λ value used was the mean and standard error of calculated trophic position from zooplankton and euphausiids (Hobson & Welch 1992, Cortes 1999). The consumer value used was the $\delta^{15}N$ mean and standard deviation of the whale sharks. Trophic level sampling values were restricted to a biologically meaningful range of 0-6, with 0 representing primary producers, and 6 representing very high trophic level consumers. The dataset was resampled 1,000 times to produce a trophic level likelihood distribution curve.

3.7.3 Fatty acids

Lipid classes

Lipid classes were expressed as percentages of total lipid, of polar lipids (PL) and neutral lipids (NL). Neutral lipids measured were free sterols (FS), glyceride ethers (GE), free fatty acids (FFA), triacylglycerols (TAG), alcohols, and steryl esters (SE). Polar lipids measured were glycerophosphoserines (Ps), glycerophospohocholines (Pc, Lpc – lyso species), glycerophosphoinositols (Pi), glycerophosphoethanolomines (Pe), cardiolipins (Cl), and sphingomyelins (Sm).

Fatty acid profiles

Fatty acids were coded as A:B ωD. Where A is the number of carbon atoms, B is the number of double bonds in the carbon chain and ωD is the position of the first double bond from the terminal methyl end of the molecule. Fatty acids were categorised as saturated (SFA), monounsaturated (MUFA) and polyunsaturated (PUFA), and each FA expressed as a percentage of the total FA (%TFA).

All FA detected above trace levels >0.2% were used for within-category comparison, while all FA >1% for all categories were used for among-categories comparison. Data were not transformed to avoid giving more weight to FA present in small quantities. Data are shown as mean ± standard error %TFA.

When calculating seasonal means for whale sharks, to avoid replication those individuals with multiple samples within the same season, only one sample was chosen to calculate fatty acid means between years, the rest were discarded. Chosen samples had the collection data nearest the mid-date of the season, and where samples were collected on the same day, the sample with the largest sample weight was chosen. Zooplankton samples (e.g. feeding tow, non-feeding tow, night tow and emergent zooplankton) were pooled between seasons due to lower sampling size.

All % TFA detected above trace levels (>1%) were used to create non-metric multi-dimensional scaling (nMDS) plots to visualise groupings within and among the functional groups. We produced an nMDS dissimilarity matrix using Bray-Curtis distance. We used PERMSDIP to compare homogeneity and dispersion between groups, and PERMANOVA (based on 999 permutations, k=3) to test for factorial differences between groups. We used SIMPER to identify which FA contributed most to any dissimilarities within and among groups. We used mixed effects linear models to identify any significant factors affecting the percentage TFA and the % TFA ratios within groups. Pairwise adjusted Tukey contrasts were performed to identify the level of significance among pairs within factors.

3.7.4 Fatty acid profiles vs isotopes

Paired samples that were chemically analysed for both stable isotopes and fatty acids (n=80) were compared with a Mantel test of their respective dissimilarity matrix using Bray-Curtis distance. All δ^{13}C data we multiplied by -1 to remove negative values before generating the dissimilarity matrix. We used PERMAOVA to compare the whale shark FA dissimilarity matrix to δ^{15}N and δ^{13}C real values separately, followed by pairwise second order polynomial models run between each major (>0.2%) FA %TFA values separately for both δ^{15}N and δ^{13}C.

All statistical tests and plots were generated using the statistical platform R (R Core Team 2013).

3.8 Results

3.8.1 Stable isotopes

Throughout the sampling period we collected 324 dermal connective tissue biopsies from 98 individual sharks, 74% of which were male. Forty-six of these individuals were sampled in more than one, and up to five field seasons. The estimated total length of sampled sharks ranged from 450 – 900 cm (mean 612± 103 S.D.). Estimated trophic level for whale sharks in this ecosystem was 2.8 (2.33 - 3.32 quartile spread). Whale sharks had a broad range of both δ^{13}C and δ^{15}N values (Table 3.1). δ^{13}C values for whale shark dermal connective tissue corrected to be comparable to fish muscle tissue samples ranged from -14.7 to -18.9‰, while uncorrected values ranged from -12.2 to -16.4‰. δ^{15}N values ranged from 6.4 to 10.7.

One hundred and thirty three samples of zooplankton and other local species were collected (Table 3.1). Whale sharks occupied a similar isotopic space to the other planktivorous fishes sampled. Piscivorous fishes had the highest δ^{15}N values, and zooplankton and jellyfish had the lowest values. A few species had a wide range of δ^{13}C values, more pronounced in the lower tropic level species.

The *Mastigias* jellyfish are an inshore mangrove-associated species, and the *Cassiopeia* jellyfish are a benthic and sea-grass associated species. Both had the highest δ^{13}C values of all species sampled. The more pelagic species of jellyfish (the *Aurelia* and *Rhopilema* jellyfish) had a wide range of δ^{13}C values. The moon jellyfish had a δ^{13}C range of 6.6‰, which was principally due to the difference between sampling seasons 4 and 5, where season 5 samples had higher δ^{13}C values (mean = -13.6‰ ± 0.8 S.D.) than those from season 4 (mean = -18.1 ± 0.4 S.D.). Zooplankton collected at night also had a large $\delta^{13}C$ range of 6.6‰. Some samples contained pieces of moulted amphipod exoskeleton, which were not acidified, and this could account for the higher $\delta^{13}C$ values seen here.

Deep-water zooplankton had the lowest $\delta^{13}C$ values overall, with sampling latitude having no significant effect ($X^2 = 1$, $p = 0.3$), and a weak relationship with sampling depth ($X^2 = 9.6$, $p = 0.09$), with $\delta^{13}C$ increasing slightly with increasing depth. Deep-water zooplankton had similar $\delta^{15}N$ values to whale sharks and planktivorous fishes at Mafia Island. As with $\delta^{13}C$, there was no significant relationship between $\delta^{15}N$ and sampling latitude ($X^2 = 2.2$, $p = 0.1$), and a weak relationship with depth ($X^2 = 11$, $p = 0.05$), where $\delta^{15}N$ increased with depth.

Species	Functional Group	Tissue type	n	δ^{13}C	Range	δ^{15}N	Range	C:N
Whale Shark Rhincodon typus	Whale Shark	Subdermal	202	-16.8 (±0.8)	4.19	9.3 (±0.9)	5.47	2.6 (±0.4)
Bullet tuna **Auxix rochei**	Piscivore	Muscle	2	-17.3 (±0.4)	0.71	13.1 (±0.8)	1.6	5.6 (±2.1)
Kingfish **Scomberomorus guttatus**	Piscivore	Muscle	2	-15.8 (±1.0)	1.4	11.8 (±0.9)	1.3	3.6 (±0.3)
Indian Ocean Mackerel **Rastrelliger kanagurta**	Planktivore	Muscle	28	-16.4 (±0.7)	3	10.8 (±1.7)	8.9	4.3 (±1.4)
Baitfish **Unknown**	Planktivore	Muscle	4	-14.5 (±0.7)	1.61	8.9 (±0.3)	0.8	3.3 (±0.1)
Night baitfish **Unknown**	Planktivore	Muscle	2	-17.6 (±0.0)	0.05	9.1 (±0.2)	0.3	3.2 (±0.0)
Half-beak Hemiramphus spp.	Planktivore	Muscle	9	-16.7 (±0.9)	2.13	10.8 (±1.2)	3.2	3.5 (±0.2)
Zooplankton feeding Lucifer hansenii	Zooplankton Feeding	Mixed	13	-16.1 (±1.1)	4.1	7.0 (±2.1)	7.2	6.2 (±1.5)
Zooplankton Emergent Mixed	Zooplankton Emergent	Mixed	1	-13.5	-	8.3	-	6.6
Zooplankton Night Mixed	Zooplankton Night	Mixed	6	-13.5 (±2.5)	6.6	5.4 (±1.4)	3.5	6.3 (±1.3)
Moon jellyfish Aurelia aurita	Jellyfish	Bell	14	-15.6 (±2.4)	6.6	4.5 (±1.0)	3.7	3.9 (±0.4)
Nomad jellyfish Rhopilema nomadica	Jellyfish	Bell	8	-14.0 (±1.3)	4.4	6.4 (±2.2)	6.7	3.8 (±0.9)
Spotted jellyfish Mastigias papua	Jellyfish	Bell	1	-12.6	-	8.75	-	4.8
Upside-down jellyfish Cassiopea ornata	Jellyfish	Bell	1	-10.9	-	3.47	-	7.4 (±0)
Zooplankton 0 - 200 m	Zooplankton Offshore	Mixed	7	-18.8 (±0.3)	0.7	8.8 (±0.9)	2.3	4.1 (±0.5)
Zooplankton 200 - 400 m	Zooplankton Deep	Mixed	6	-18.9 (±0.4)	1	9.6 (±1.2)	3.6	4.2 (±0.4)
Zooplankton 400 - 600 m	Zooplankton Deep	Mixed	8	-18.5 (±0.8)	2.59	10.4(±1.3)	3.7	4.2 (±0.4)
Zooplankton 600 - 800 m	Zooplankton Deep	Mixed	5	-17.5 (±1.7)	4	10.3(±1.0)	2.8	5.0 (±0.5)
Zooplankton 800 - 1000 m	Zooplankton Deep	Mixed	7	-18.1 (±1.0)	2.7	10.4(±1.0)	2.7	4.2 (±0.5)
Zooplankton 1000 - 1500 m	Zooplankton Deep	Mixed	3	-18.1 (±1.0)	1.8	9.5(±1.6)	3.2	5.5 (±0.8)

Table 3.1 Species sample list and mean bulk δ^{15}N and δ^{13}C values ± standard deviation.

Functional Group	n	$\delta^{13}C$	$\delta^{15}N$	C:N
Piscivore	5	-16.7(±1.0)	12.6(±1.1)	4.8(±1.8)
Planktivore	44	-16.4(±1.0)	10.6(±1.6)	4.0(±1.2)
Jellyfish	24	-14.7(±2.3)	5.26 (±1.9)	4.0(±0.9)
Deep-water Zooplankton	29	-18.3 (±1.0)	10.1 (±1.1)	4.4 (±0.7)

Table 3.2 Mean bulk $\delta^{15}N$ and $\delta^{13}C$ values ± standard deviation of functional groups where multiple species or sample types were combined within each group

Functional Group	$\delta^{15}N$	$\delta^{13}C$
Piscivore	0.21	1
Planktivore	0.99	1
Feeding Zooplankton	0.72	1
Emergent Zooplnkton	1	0.7
Night Zooplankton	0.1	0.5
Jellyfish	0.08	0.22
Offshore zooplankton	1	1
Deep-water zooplankton	1	1

Table 3.3 Results (P-values) from pairwise adjusted Tukey tests between whale sharks and other functional groups for $\delta^{15}N$ and $\delta^{13}C$.

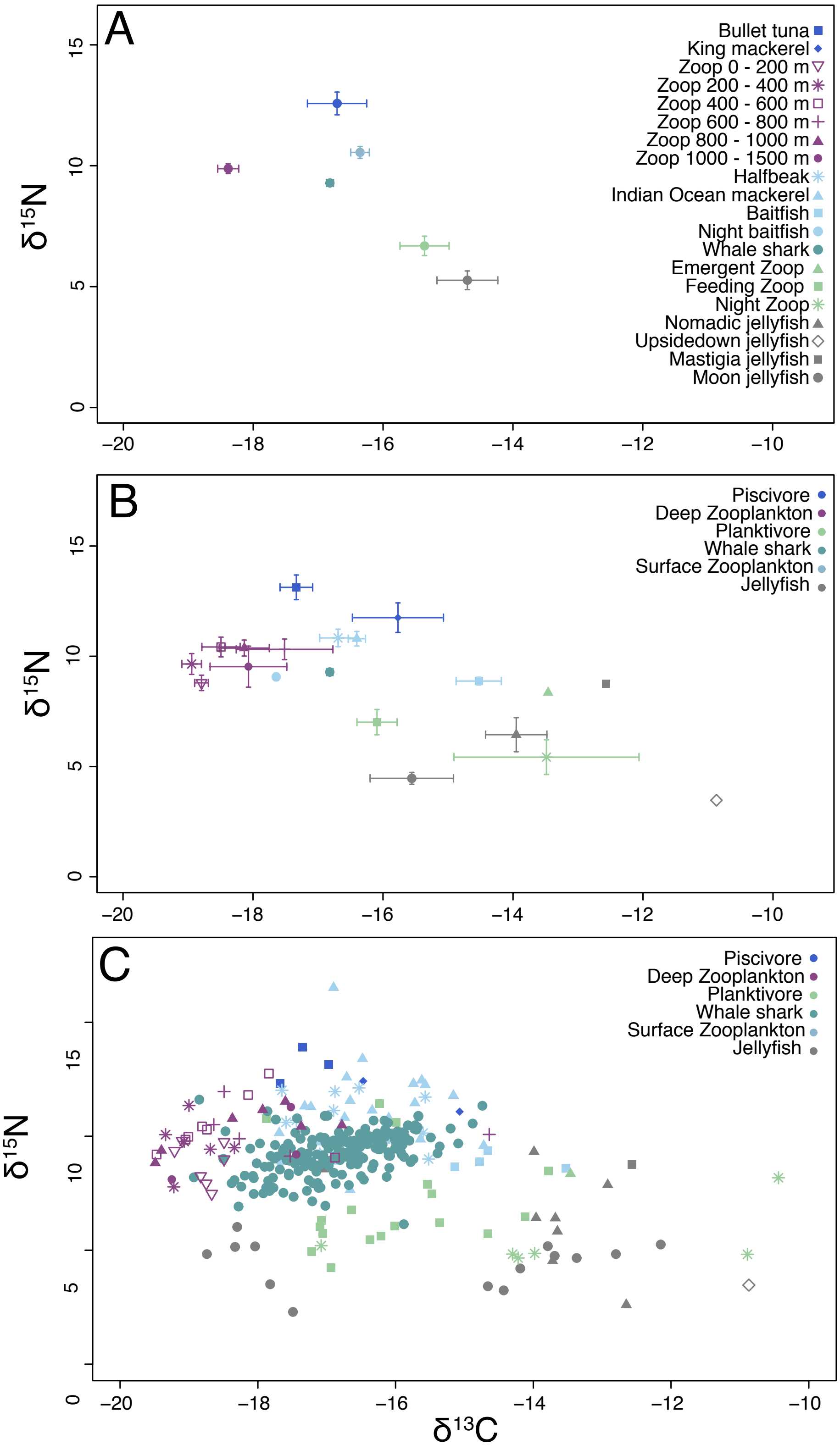

Figure 3.2 Biplots of (A) mean isotopic values (‰± standard error) of each functional group, (B) mean isotopic values (‰± standard error) of each species collected from this study, (C) isotopic values of all samples collected in this study.

For whale sharks, estimated total length was a significant predictor for $\delta^{13}C$ values ($X^2 = 20.2$, $p<0.001$), with the interaction between season and sex also significant, but explaining a smaller amount of the variance ($X^2 = 8.1$, $p = 0.004$). $\delta^{13}C$ values increase with increasing estimated shark size. For $\delta^{15}N$ values, season and sex were the most significant predictors ($X^2 = 10.3$, $p = 0.001$). Size explained more of the variance, but was less significant ($X^2 = 10.4$, $p = 0.03$). $\delta^{15}N$ values also increased with increasing estimated size. However, boxplots show a lot of variation of $\delta^{13}C$ and $\delta^{15}N$ around each size estimate and substantial overlap of both $\delta^{13}C$ and $\delta^{15}N$ values between the sexes (Figure 3.3, Panels C &D). Males had higher mean $\delta^{13}C$ (Male = -16.8 ± 0.8 S.D. Female = -17.1 ± 0.6 S.D.) and $\delta^{15}N$ values (Male = 9.4 ± 0.9 S.D. Female = 9 ± 1 S.D.), but the standard deviation in all cases was larger than the difference between the means.

Pairwise comparisons of $\delta^{15}N$ and $\delta^{13}C$ between functional groups showed that whale sharks were not significantly different from any other groups, but were most dissimilar to the jellyfish piscivores and night zooplankton. Among the zooplankton groups, whale sharks were most similar to the feeding, deep-water and offshore zooplankton. Emergent and night zooplankton were the least similar (Table 3.3).

Yearly variation

Mixed models suggest season could explain some of the variation of $\delta^{13}C$ and $\delta^{15}N$ values for whale sharks, with a decreasing range of $\delta^{13}C$ and $\delta^{15}N$ values across seasons one to four (Figure 3.3). For plankton, season came out as a marginally significant predictor of $\delta^{13}C$ ($X^2 = 10.5$, $p = 0.01$), with a near significant difference between season 2 and season 4 ($p = 0.03$). Planktivores showed no seasonality for $\delta^{13}C$ ($p = 0.3$), but did show some seasonality for $\delta^{15}N$ ($X^2 = 13.1$, $p = 0.004$), with near significant differences between seasons 1&2 ($p = 0.09$) and seasons 3&4 ($p = 0.08$). Plankton also showed seasonality of $\delta^{15}N$ ($X^2 = 14.7$, $p=0.002$) between seasons 2&3 ($p=0.01$) and seasons 2&4 ($p = 0.03$).

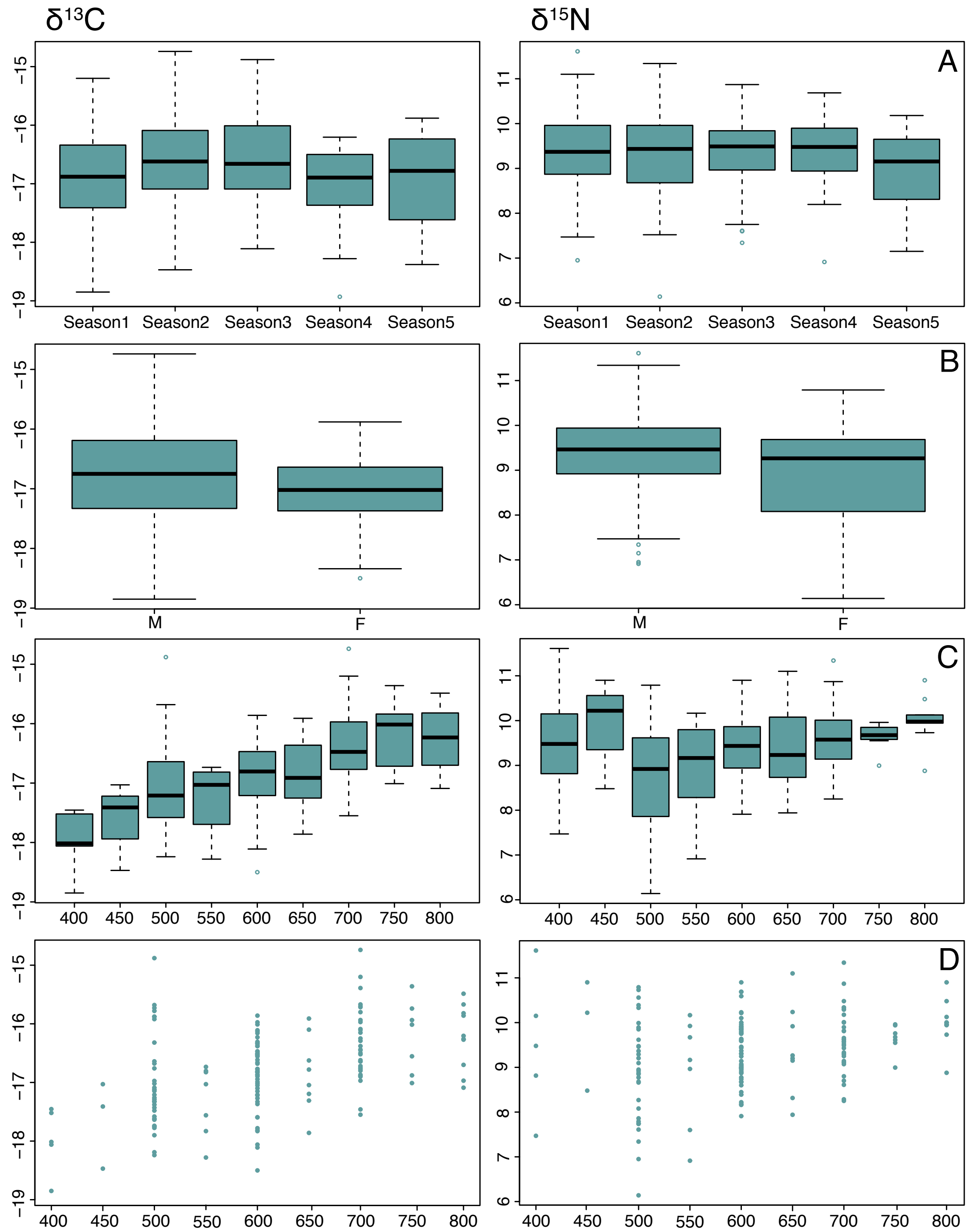

Figure 3.3 Whale shark δ^{15}N and δ^{13}C data displayed as (A) boxplots by season, (B) boxplots by sex with M = male and F = female, (C) boxplots by size estimate measured in cm, and (D) scatterplots by size estimate measured in cm.

3.8.2 Fatty acids

Lipid classes

Fatty acids of whale sharks were mostly derived from phospholipids (~71%), of which the dominant class was glycerophosphoethanolomines (Pe) (Table 3.4).

	Lipd Class	Whale sharks % TLE ± SE
NL	FFA	10.0 ± 1.6
	FS	15.7 ± 1.5
	GE	0.0 ± 0.0
	TAG	1.2 ±0.8
	SE	1.9 ±1.3
PL	Ps	3.5 ± 0.6
	Pc	6.4 ± 1.5
	Pi	3.4 ± 0.5
	Sm	21.2 ± 3.7
	Cl	0.9 ± 0.3
	Pe	21.8 ± 2.3
	Lpc	14.1 ± 3.4
Lipid content mg g-1 wm		5.1 ± 0.8

Table 3.4 Summary of lipid classes for whale sharks showing each class of neutral lipid (NL) and polar lipid (PL) measured, and the mean wet weight lipid content of whale shark connective tissue.

Fatty acid	**Zooplankton**		
	Feeding tow (n=12)	**Night tow (n=3)**	**Emergent (n=2)**
14:0	5.2 ± 0.3	10.4 ± 2.1	10.6 ± 0.1
i15:0	0.1 ± 0.1	0.3 ± 0.1	0.4 ± 0.1
15:0	1.2 ± 0.0	1.6 ± 0.2	1.3 ± 0.1
16:0	28.2 ± 0.6	32.3 ± 3.7	28.4 ± 1.0
17:0a	0.3 ± 0.0	0.1 ± 0.1	0.2 ± 0.0
i17:0	0.5 ± 0.0	0.3 ± 0.2	0.5 ± 0.1
17:0	2.0 ± 0.1	2.4 ± 0.5	2.0 ± 0.2
18:0	8.6 ± 0.2	12.0 ± 2.9	10.5 ± 1.5
20:0	0.4 ± 0.1	0.4 ± 0.2	0.7 ± 0.3
22:0	1.0 ± 0.2	1.2 ± 0.3	1.0 ± 0.3
24:0	0.5 ± 0.1	1.9 ± 1.3	1.0 ± 0.4
Total SFA	49.3 ± 0.8	63.2 ± 7.0	57.2 ± 3.7
16:1ω7	4.0 ± 0.2	6.9 ± 1.9	7.7 ± 1.2
18:1ω9	4.6 ± 0.6	4.1 ± 0.3	4.5 ± 0.8
18:1ω7	4.8 ± 0.4	4.0 ± 0.6	3.2 ± 0.5
20:1ω9	0.1 ± 0.1	0.2 ± 0.2	0.7 ± 0.7
22:1ω9	0.0 ± 0.0	0.0 ± 0.0	0.2 ± 0.2
Total MUFA	14.0 ± 0.4	**16.1 ± 1.9**	17.6 ± 0.4
18:2ω6	1.3 ± 0.2	1.4 ± 0.7	1.4 ± 0.1
18:4ω3	0.3 ± 0.2	0.0 ± 0.0	0.0 ± 0.0
20:4ω6 (ARA)	2.7 ± 0.7	2.1 ± 0.2	2.4 ± 0.2
20:5ω3 (EPA)	11.2 ± 1.3	5.1 ± 3.3	5.2 ± 0.5
22:4ω6	0.3 ± 0.1	0.3 ± 0.2	0.4 ± 0.1
22:5ω6	1.2 ± 0.1	1.9 ± 1.0	0.1 ± 0.1
22:5ω3	0.7 ± 0.1	1.2 ± 0.6	1.1 ± 0.1
22:6ω3 (DHA)	16.7 ± 1.1	7.1 ± 2.9	11.7 ± 2.6
Total PUFA	**36.6 ± 0.9**	20.7 ± 6.0	25.2 ± 3.3
ω3/ω6	5.9 ± 1.2	2.5 ± 0.3	3.6 ± 0.8
Others	4.2 ± 0.6	2.8 ± 0.9	3.8 ± 1.5

Table 3.6 The mean fatty acid (FA) profile (% TFA) of zooplankton samples grouping all FA <0.2% as others. Other FA as in table 3.5.

Fatty acid profiles – between groups

Overall whale sharks contained saturated FA (SFA: 57.3% TFA) as the major FA group, followed by PUFA (23.4%) and MUFA (19.3%) (Table 3.5.). Within-individual variation was extremely low for whale sharks and contributed little to the %TFA variance.

The grouped zooplankton had slightly lower SFA (54.5%) and MUFA (15.2%) overall, and higher PUFA (30.2%). Planktivores had lower SFA (48.9%) to both the whale sharks and grouped zooplankton, and similar MUFA (15.1%) and PUFA (36.1%) to the zooplankton. Piscivores had lowest proportion of SFA (43.4%) of all groups, and the highest PUFA (40.4%), with similar MUFA to grouped zooplankton (16.2%).

Within the zooplankton group (Table 3.6), feeding tows predominantly made up of sergestid shrimp had lower SFA (49.3%) and MUFA (14%) and higher PUFA (36.6%) than whale sharks. Night time and emergent plankton had similar SFA (63.2%, 57.2%) to the whale sharks, intermediate PUFA (20.7%, 25.2%) and MUFA (16.1%, 17.6%) between the whale sharks and feeding tows respectively. The emergent zooplankton did not have larger proportions of 15:0 and 17:0 branched bacterial fatty acids (including –iso and –antiso) (Budge et al. 2002) compared to the other zooplankton, as might be expected.

Major FA for all groups were 14:0, 16:0, and 18:0. Major FA specifically for whale sharks were 18:1ω9, and 20:4ω6 arachidonic acid (ARA). In contrast, major FA for zooplankton, planktivores and piscivores were 20:5ω3 pentaenoic acid (EPA), and 22:6ω3 docosahexaenoic acid (DHA). Whale sharks had ~3 times as much ARA as all other groups, ~60 times less EPA, and ~9 times less DHA than all other groups. As a result, the ω3:ω6 ratios of whale sharks were relatively low (<1) compared to all other groups. Whale sharks also had twice as much oleic acid (18:1ω9) and its precursor stearic acid (18:0) than all other functional groups.

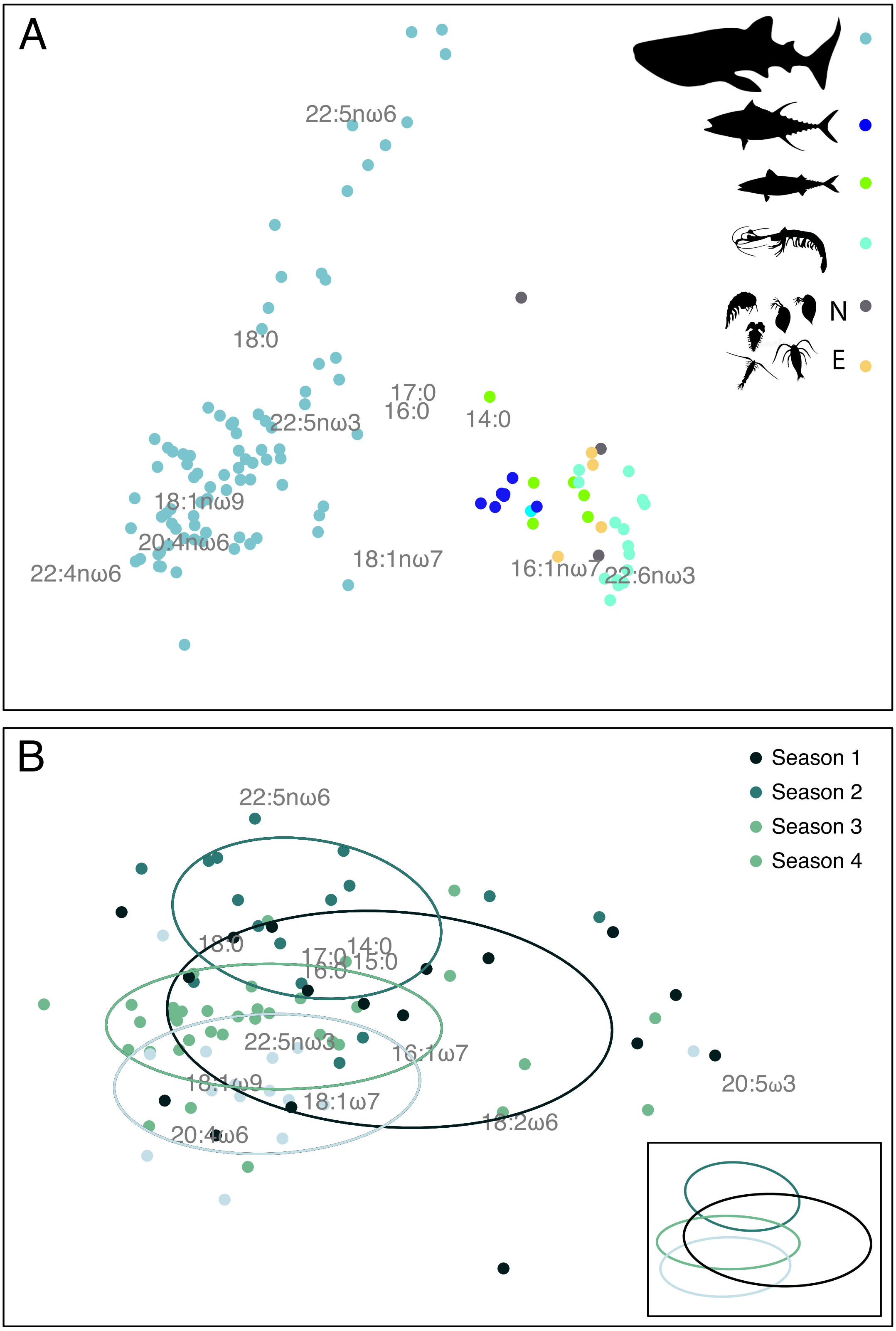

Figure 3.4 Multi-dimensional scaling ordinations with fatty acid labels showing the main coefficients contributing to each axis. (A) Whale shark, piscivore, planktivore, 'feeding' zooplankton, N night zooplankton, and E emergent zooplankton (<1% TFA) with black open circle marking the centroid of the ordination. (B) Whale shark (<0.2% TFA) with 0.5 confidence ellipses by season. Insert shows season ellipses without data points.

The multidimensional plot shows whale shark connective tissue contains distinct fatty acid compositions compared to all other functional groups. Whale shark FA profiles appear equally dissimilar to similar to all types of plankton samples (Figure 3.3 Panel A). Functional group is responsible for a lot of the dissimilarity between the samples (PERMANOVA pseudo-F = 53.9, p = 0.001). Functional groups did not show any heterogeneity in multivariate dispersion (PERMDISP F = 1.6, p = 0.19), indicating that the dissimilarity is not due to a dispersion effect. This is corroborated by SIMPER results showing that whale sharks are over twice as different to each other functional group than they are to each other (Table 3.7). SIMPER analysis supports the aforementioned observations of the %TFA table showing that 18:0, 18:1ω9, ARA, EPA and DHA are the dominant FA contributing to dissimilarities between groups.

	14:0	**16:0**	**18:0**	**18:1ω9**	**20:4ω6 (ARA)**	**20:5ω3 (EPA)**	**22:6ω3 (DHA)**	**Average Dissimilarity**
WS vs PL		8.6	21.9	12.5	8.5	12.7	13.5	46.1
WS vs PLA		7.4	21.5	10.9	9.0		27.1	43.6
WS vs PI			22.0	9.9	9.0		29.5	40.7
PL vs PLA	8.4	7.6	7.6			16	31.6	21.6
PL vs PI		11.6	9.2	10.9		7.7	34.1	23.5
PLA vs PI	9.1	11		9.2		16	26.6	15.5

Table 3.7 SIMPER results. Main FA and (%) contribution to the differences in each pairwise comparison of functional groups, with average % difference between groups. WS – whale shark, PLA – Planktivore, PL – Plankton, PI – Piscivore.

Fatty acid profiles – seasonality

For whale sharks, season was as a significant predictor for FA 14:0 (X^2 = 115, p<0.001), 16:0 (X^2 = 68, p<0.001), 18:0 (X^2 = 60, p<0.001), 18:1ω9 (X^2 = 67, p<0.001), ARA (X^2 = 69, p<0.001), DHA (X^2 = 86, p<0.001) (Figure 3.5). Season was also a significant predictor for 22:5ω6 (X^2 = 6.23, p = 0.013), but to a lesser degree. EPA values were too low to run a reliable model. Sex was also a significant predictor in all of these FA, but only accounted for a very small amount of the variance. Adjusted Tukey tests revealed that most of the difference within season was explained by comparisons between seasons 2 & 3 and 2 & 4 (Table 3.7). Season was also a strong predictor of the absolute amount (μg) of ARA (X^2 = 16.8, p<0.001). Analysis of all grouped zooplankton samples mirrored this seasonal change and showed a significant effect of season on ω3:ω6 values between season 2 & 3 (X^2 = 16.8, p<0.001, S2-S3 p=0.08), which was driven by ARA (p=0.01) (Figure 3.5). No other zooplankton FA showed significant differences between seasons. Global variance of the main (>1%) FA in whale sharks decreased across seasons (season 1 = 2.85, season 2 = 2.17, season 3 = 1.37, season 4 = 0.92).

Season Pairs	**14:0**	**16:0**	**18:0**	**18:1ω9**	**20:4ω6 (ARA)**	**22:5ω6**	**22:6ω3 (DHA)**	**22:6ω3 (DHA)**
S1 - S2	0.2267	0.3213	0.5026	0.0518	0.0518	0.9479	0.1535	0.1535
S1 -S3	0.27	0.1162	0.0918	0.6572	0.6572	0.9996	1	1
S1 - S4	0.1457	0.2528	0.3241	0.6604	0.6604	0.1913	0.9809	0.9809
S2 - S3	**0.001**	**0.0005**	**0.0012**	**0.0006**	**0.0006**	0.8788	0.0852	0.0852
S2 - S4	**0.0009**	**0.0041**	**0.0162**	**0.0026**	**0.0026**	**0.0555**	0.0783	0.0783
S3 - S4	0.8935	1	0.9888	0.997	0.997	0.1216	0.9676	0.9676

Table 3.8 Results for data post-hoc adjusted Tukey pairwise tests for each season of data collection. Whale shark fatty acid data only. Significant (p<0.01) results highlighted in blue.

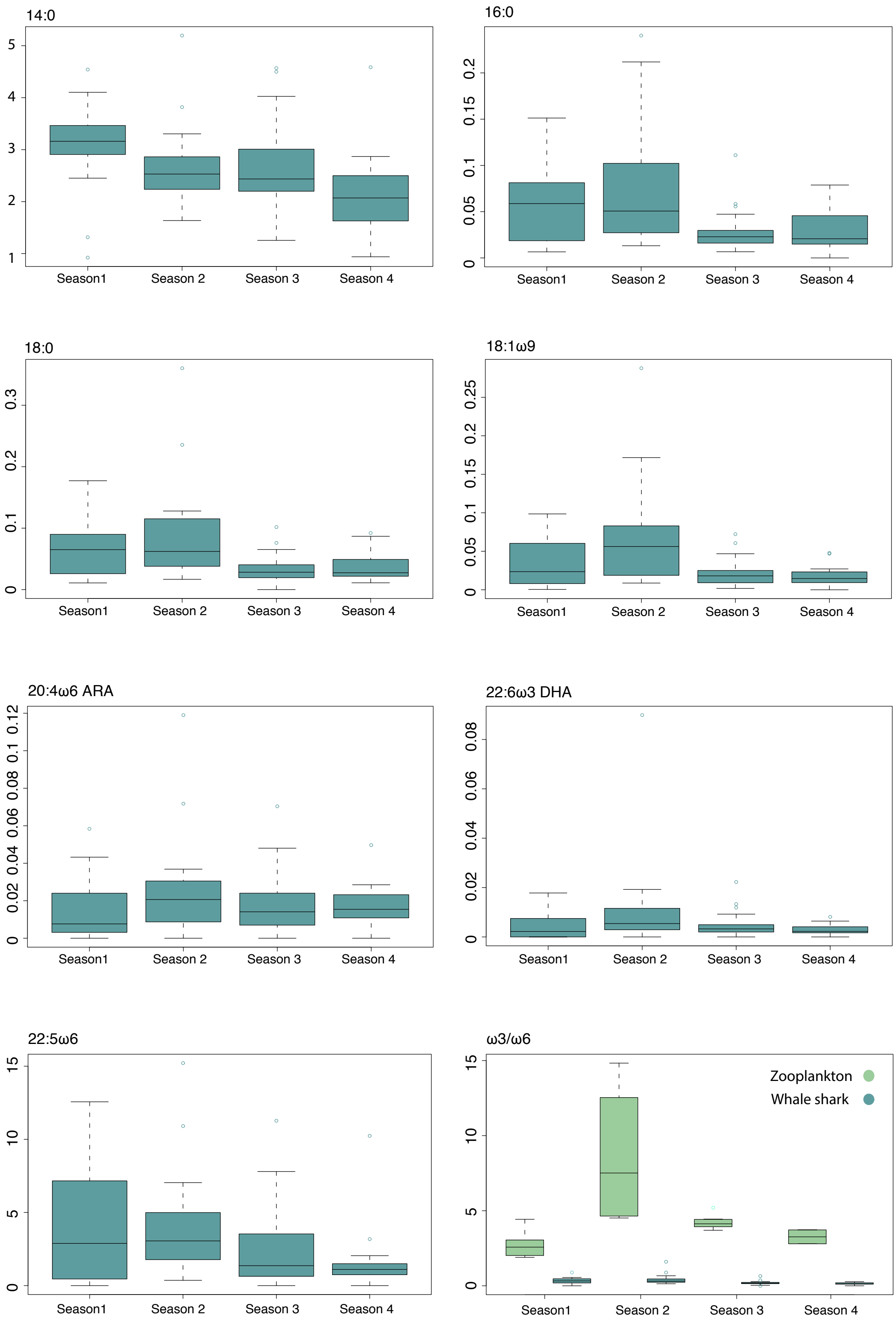

Figure 3.5 Boxplots of whale shark %TFA by season for 14:0, 16:0, 18:0, 18:1ω9, 20:4ω6 ARA, 22:6ω3 DHA, 22:5ω6 and a plot of ω3/ω6 by season for whale sharks and zooplankton.

Fatty acid profiles – individual differences

Data exploration of outlier data points revealed some individual whale sharks with relatively large ω3:ω6 ratios, here defined as values more than one standard error away from the mean. These sharks were of varying sex and size, and samples were collected in different seasons. The driver behind this higher than average ω3:ω6 was lower proportions of ARA in the samples. The three individuals with the largest ω3:ω6 ratios in their biopsies were two juvenile males and a female.

Most interesting was individual MZ-136, a juvenile 700 cm long male with a ω3:ω6 ratio of 1.6, which had been sampled twice over the sampling period in seasons two and four. In season two there was no ARA detected in the sample at all and 10%TFA of the FA 20:3ω3, and in season 4 there was a higher than average proportion of ARA 15.5% TFA with no 20:3ω3 detected. Fatty acids 22:1ω9, 18:2ω6, 22:5ω6 also had minor differences between seasons but all other FA were similar.

The sample from season one of individual TZ-070, a 500 cm long juvenile male, also had a higher than average ω3:ω6 ratio of 0.9. TZ-070 was sampled in seasons one, three and four with the ω3:ω6 ratio decreasing across time driven by a increase in the %TFA of ARA and an overall decrease in 22:5ω3 over time.

Shark TZ-063, a 600 cm long female, had samples collected in seasons two, three and four. The season two sample had a higher than average ω3:ω6 value of 0.9 driven by a high % TFA of DHA.

Fatty acid profiles – literature comparison

Fatty acid profiles of all whale shark connective tissues are consistently similar to each other regardless of the country of origin, year of collection, and what other species profiles were included in the ordination (Figure 3.6). Profiles of all species are generally more similar to others that inhabit the same ocean layer or depth regardless of functional group (e.g. elasmobranch, teleost fish, zooplankton), feeding mode (e.g. planktivore, piscivore, apex predator), or year of sampling. Whale shark profiles are more similar to other epipelagic species and surface zooplankton (Fig 3.6 panel A). Whale shark profiles are not particularly similar to either species of manta ray, which are actually more similar to the other mesopelagic elasmobranchs. Whale sharks more closely match the profiles of surface zooplankton than most of the other elasmobranchs (Fig. 3.6 panel B). When compared to epipelagic species alone, whale sharks are more like epipelagic sharks than surface zooplankton (Fig 3.6 panel C).

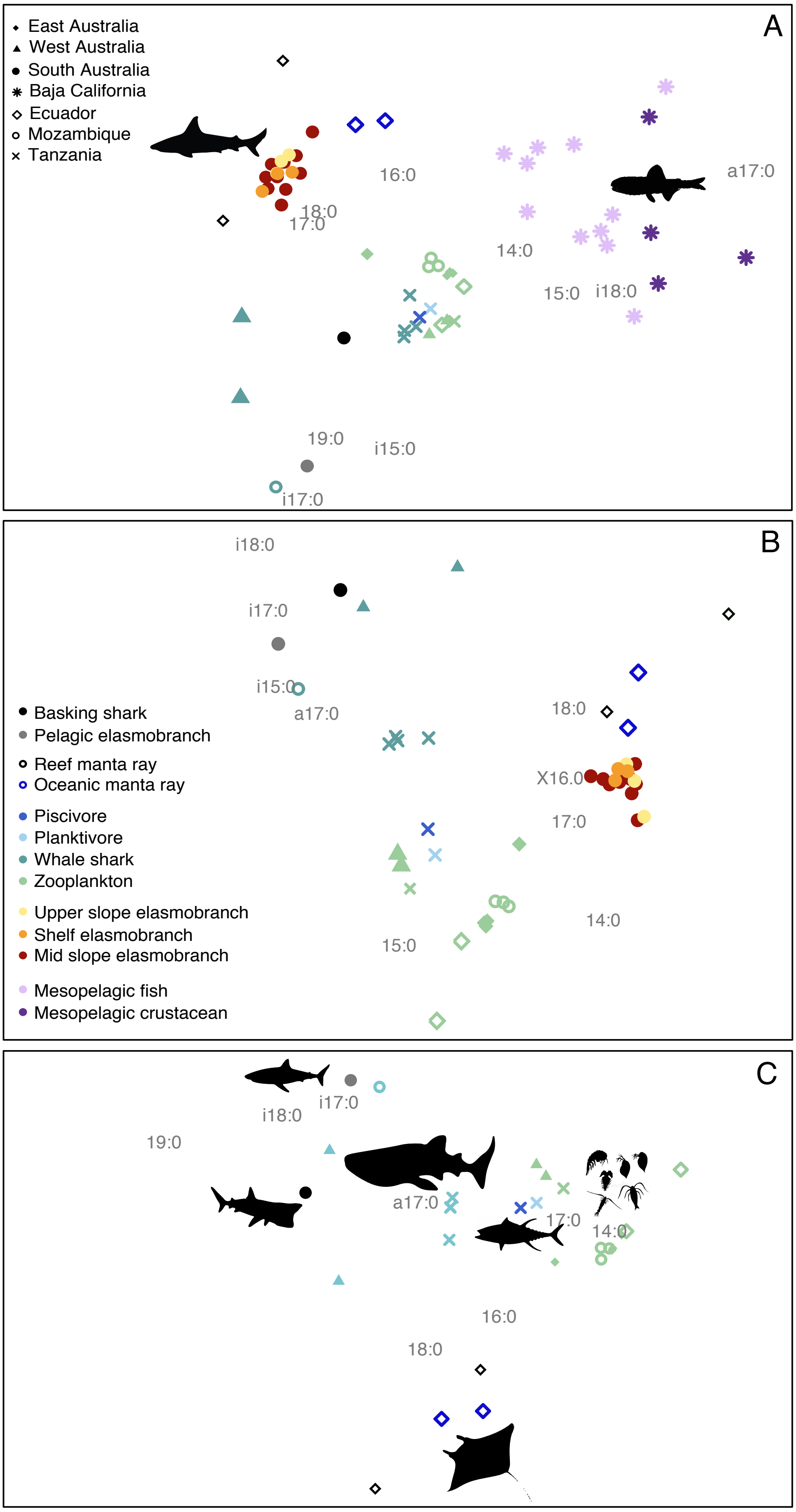

Figure 3.6 Multi-dimensional scaling ordinations with FA profiles from this study and profiles from literature.

Fatty acid labels showing the main coefficients contributing to each axis. FA with >1% TFA included in each ordination respectively. Colours represent different functional groups, point shapes represent country of sample origin. Data from this study plotted with (A) 'All' - Epipelagic, mesopelagic, and bathypelagic species including elasmobranchs, teleost fish. (B) 'Elasmobranchs' – FA profiles of epipelagic to mesopelagic elasmobranchs. (C) 'Epipelagic' – FA profiles of predominantly epipelagic elasmobranchs.

Fatty acid profiles vs isotopes

There was no evidence of correlation between individual whale sharks with very different (large Bray-Curtis distance from centroid) fatty acid profiles (with FA >0.2%) and individuals with very different isotope values (Mantel p = 0.41). Almost all fatty acid distances were spread between 0-0.8, with most SI distances between 0-0.6 (Figure 3.7).

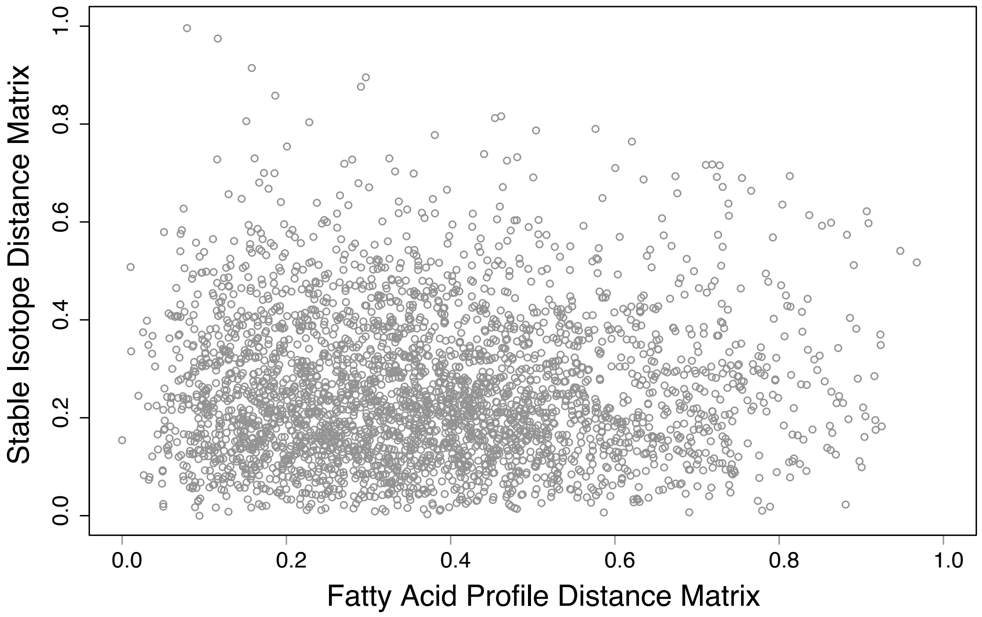

Figure 3.7 Plot showing the paired similarity matrices of stable isotope values ($\delta^{13}C$ and $\delta^{15}N$) and fatty acid profiles. Fatty acid profile matrix include fatty acids with >0.2% total fatty acids.

3.9 Discussion

3.9.1 Stable isotopes

The number of individuals sampled here represents 53% of the identified aggregation at Mafia Island (as of April 2018), which provides an opportunity to assess diet across the majority of the sharks that are routinely present.

Yearly variation

Mean stable isotope compositions of whale shark tissues did not vary between seasons over the five-year study period. Thus, the broad range of $\delta^{13}C$ and $\delta^{15}N$ values seen within the sampled population indicates isotopic variation in diets of individuals. Sergestid shrimp and the other low trophic level species show similar broad isotopic ranges, suggesting isotopically distinct nutrient pathways, rather than isotopically distinct prey species. Mafia Island has several distinct topographical features that could account for this. Around Killindoni bay there are terrestrial carbon inputs via freshwater runoff from the island, and detrital carbon inputs from the seagrass and mangrove system lining the bay. Both would enrich the $\delta^{13}C$ signature close to shore, which is evident here in the $\delta^{13}C$ enriched *Cassiopea* and *Mastiagias* jellyfish signatures. Anthropogenic nitrogen sources flow into the bay via Killindoni town, which includes a large fish factory, likely causing localised enrichment of $\delta^{15}N$. Additionally from the mainland across from Mafia, the plume of terrestrial output from the Rufiji River can often extend half-way to the island. This is a large terrestrial output that would certainly contribute to a $\delta^{13}C$ rich nutrient pathway when the whale sharks are further away from Mafia outside of peak season.

While the average isotope values for whale sharks at Mafia Island change little across the seasons, the range of values does. So although there is no indication that feeding preferences have changed dramatically, this does point to a reduction in the range of available prey items, and an aggregation level response to the changing conditions. This same range reduction is reflected in the fatty acid results (discussed latterly).

Size, and sex

Previous studies on whale sharks have observed higher $\delta^{13}C$ and $\delta^{15}N$ values in males, but here any sex-based effect was marginal and potentially influenced by the strong male bias in the population and we are reluctant to draw any conclusions from this result. Enrichment of both $\delta^{13}C$ and $\delta^{15}N$ for larger whale sharks indicates there may be an size related diet shift, also consistent with previous studies on whale sharks (Borrell et al. 2011, Marcus 2017). However, we did not sample very young (<4 m long) or adults (>9 m long) sharks in this study and thus miss the samples that are more likely to reveal any marked diet changes between the different life stages. Relatively high $\delta^{13}C$ and $\delta^{15}N$ values in larger sharks could reflect a larger proportion of diet derived from inshore feeding, deep water feeding or feeding at lower latitudes (Hobson 1999, Trueman et al. 2014). Observational work at Mafia Island indicates that it is unlikely that larger sharks show a preference for inshore feeding (Cagua et al. 2015, Prebble et al. 2018 *in review*). Feeding at lower latitudes is possible, with some evidence that Tanzanian sharks are sharing resources with Mozambican sharks at lower latitudes (Prebble et al. *in revision*).

Changes in feeding preference could also explain observed ontogenetic isotope changes, with higher trophic level feeding and an increase in deep-water feeding both potential causes (Hobson 1999, Davenport & Bax 2002, Trueman et al. 2014). Whale sharks at Mafia Island have been observed vertically feeding on small planktivorous fishes further offshore outside Killindoni Bay during periods where the dense patches of sergestid shrimp were unavailable (Pers obs). Based on the respective isotope values of whale sharks and planktivorous fishes measured here, if the larger sharks are targeting these fishes more frequently, this could result in isotopic enrichment of their tissues.

Generally, stable isotope enrichment is assumed to increase with depth (Trueman et al. 2014). However, there was a much stronger 'offshore' $\delta^{13}C$ depleted signal here than there were isotopic changes with depth. As such, where it is isotopically possible that the whale sharks are feeding offshore and on deep-water zooplankton. However it's less likely the behaviour driving the size related differences we see here.

For both $\delta^{13}C$ and $\delta^{15}N$, given the low sampling numbers at both ends of the size range, and the wide variability of isotope values for each size, we interpret both results with caution.

Trophic position

Given the uncertainties and assumptions surrounding TP calculations (Hussey et al. 2013b) the median TP value for whale sharks in this study (TP = 2.83, ± 0.7 S.D.) was similar to previous estimates for juvenile whale sharks in other locations (South Africa TP = 3.6 (Cortes 1999), Arabian Sea TP = 3.3 (Borrell et al. 2011), Australia TP = 2.6 (Marcus 2017). Their position as secondary consumers in the local food web was as expected, considering observations from feeding events and tracking results that show they spend a lot of time at this site (Cagua et al. 2015, Prebble et al *in revision*).

Whale sharks have also been tied to deep water feeding (Rohner, Couturier, et al. 2013, Couturier, Rohner, Richardson, Marshall, et al. 2013, Stewart et al. 2014, Burgess et al. 2016), which could inflate the TP estimate (Trueman et al. 2014). Two other megaplanktivores *Mobula alfredi* TP ~3 (Couturier et al. 2013) *Mobula birostris* TP = 3.5 (Burgess et al. 2016) also have similar TP to juvenile whale sharks and have also been suggested to feed in deeper water.

3.9.2 Fatty acid profiles

The fatty acid profiles of whale sharks at Mafia Island show individual variation but are still much more similar to each other than anything else we measured within the study site. The key difference in FA between whale sharks and other functional groups were similar to the two previous whale shark FA studies (Rohner et al. 2013, Marcus et al. 2016), namely high proportions of ARA, low proportions of EPA and DHA, and a low ω3:ω6 ratio overall in whale shark dermal connective tissue.

The diet and trophic ecology of whale sharks from Mozambique and Western Australia has also been investigated using FAA in dermal connective tissues (Rohner et al. 2013, Marcus et al. 2016). Both studies also found FAA profiles distinct from those seen in local marine fauna, and the authors inferred that whale shark diets were predominantly supported by unrecognised diet items from allochthoous or ephemeral diet sources, with unusually high proportions of ARA and low proportions of EPA and DHA. Deep sea and emergent zooplankton were identified as possible diet sources based on incomplete matches in the fatty acid literature and tracking data showing extensive vertical movements (Hueter et al. 2013). Corresponding conclusions were drawn from FA profiles of muscle tissue of the ecologically similar reef (*Mobula alfredi*) and giant manta rays (*Mobula birostris*) (Couturier et al. 2013, Burgess et al. 2018), suggesting these shared unique FA profiles are tied to their shared feeding ecology and perhaps shared dietary sources.

As the whale sharks in these studies can seasonally travel up to thousands of kilometres from their coastal aggregations in various directions (Wilson et al. 2005, Sleeman et al. 2010a, Rohner et al. 2018) there is a vast area to consider, with many potential prey items that could not possibly be sampled and thus there is a strong chance that important dietary items have been left un-sampled. This makes it entirely possible that these un-sampled items may be responsible for the whale sharks' unusual FA profiles. Unlike previous studies, sharks at Mafia Island are somewhat more resident than at other aggregations (Cagua et al. 2015), specifically targeting sergestid shrimp over multiple seasons (Rohner et al. 2015b), and this study shows stable isotope values consistent with the assumed diet of whale sharks in Mafia. This perhaps removes some of the spatial uncertainty within this system, however, the whale sharks would still have ample time and opportunity in the 'off-season' to target other food sources not apparent during active fieldwork (Cagua et al. 2015). It is therefore still possible that major (principle) diet items are missing from the reference diet samples, however, it is now less likely.

Where previous studies have more than adequately discussed the potential dietary items and pathways that could lead to the FA profiles we see in the whale sharks, it is still difficult to test this hypothesis. Published research has yet to identify any marine or freshwater species that is close to the FA proportions observed in whale shark connective tissues. Generally, only trace amounts of ARA are reported in marine fish (Pethybridge et al. 2010). Some algae (Bhaskar et al. 2004, Illijas et al. 2009), herbivorous gastropods (Saito & Aono 2014), herbivorous fishes (Jiarpinijnun et al. 2017) and smaller carnivorous fishes (Dunstan et al. 1989) have reportedly high proportions of ARA, but none of the described taxa are present in the Mafia Island food webs, and still contain less extreme fatty acid compositions than seen in whale shark connective tissues.

So, while we agree that deep-water and night-time emergent zooplankton feeding likely make up some proportion of whale sharks' overall diet, and that researchers are unlikely to have exhaustively sampled all potential whale shark diet items, here we discuss an additional and alternative, more easily testable hypothesis. The ubiquitous uniqueness of whale shark FA profiles suggests that there could be other physiological explanations for the distinct FA profile observed in whale shark connective tissues. Comparing proportions of fatty acids between proposed predators and prey sources relies on the assumption that different fatty acids are transferred between tissues in equal proportions. This assumption may be relatively robust when comparing lipid classes among physiologically equivalent tissues (Couturier et al. 2013, Pethybridge et al. 2014, Jiarpinijnun et al. 2017), but it is at least theoretically possible that some lipid classes are preferentially routed to connective tissues.

There is biological precedent for fishes to preferentially route different FA to tissues. Most evidence comes from controlled aquaculture research and studies show that differential fatty acid requirements in fish reflect different dietary and metabolic adaptations to different habitats (Sargent et al. 1999). Most relevant to the unique FA levels we see in the whale sharks are changes in ARA levels in tissues in response to increased growth rates and survival and environmental stress (Bell & Sargent 2003). Fishes also often have higher ARA levels in their eggs compared to parent tissues, and this is thought to be ubiquitous among fish species (Bell & Sargent 2003, Emata et al. 2003).

There are currently no experimental or controlled studies that we are aware of testing for preferential routing of fatty acids between connective and other tissues in whale sharks or other mega-planktivores. Given the similarity and consistently anomalous FA profiles observed in whale shark and manta ray connective and muscle tissues, we suggest that these results can also consistent with ecologically linked physiological routing as well as a dietary explanation.

Season

Temporal changes in composition and variance of whale shark fatty acid profiles overshadowed individual variations suggesting a strong population level response to changing conditions. The reduction in variance of whale shark FA profiles across each field season points to a reduction in the different types of FA available in their diet from prey items, or differences in physiological routing. Fatty acid profiles of zooplankton do mirror this seasonal change, although to a lesser degree, suggesting that temporal differences reflect changes in local conditions rather than whale shark physiology. This happened concurrently with a decrease in some major SFA in their tissue, and an overall decrease in the ω3:ω6 value. Although not conclusive, we can say there exists some link between the two. Where less varied prey items are available to the whale sharks, there is a population level response whereby higher ARA, lower ω3/ω6prey prey types are targeted. Or alternatively these FA's are preferentially channel these into their connective tissue.

3.9.3 Stable isotopes vs fatty acid analysis

Stable isotope analysis in this case appears less sensitive to projecting the variability in whale shark diets than FAA. Those individuals that are very different in terms of FA profiles are not necessarily very different isotopically, and vice versa. However, both methods picked up seasonal changes in the range of prey availability.

If there is a major unmeasured dietary item(s) responsible for the unique FA profiles, it is not particularly isotopically distinct to anything in the system we have measured, and thus would have to be reasonably locally available, also making it less likely to have been missed.

3.9.4 Conclusions and future work

While there is a wide range of individual diets amongst the whale sharks at Mafia Island, they respond together to environmental changes in prey availability suggesting they have a common feeding strategy at an aggregation level.

Stable isotope data on resident whale sharks in Mafia Island confirms the expected low trophic level common to other whale shark aggregations. There are close relationships between the stable isotope values of the whale sharks and surface zooplankton at this site. There is also some evidence of ontogenetic changes in their diet and habitat use.

Fatty acid profiles by contrast imply that whale sharks have a highly distinct diet to the local food web. This is inconsistent with the known behaviour of whale sharks at this site. We therefore conclude that it is more likely that the fatty acids are differently routed in dermal tissues of whale sharks, and perhaps other elasmobranch planktivores. We therefore caution against interpretation of fatty acid profiles as an unbiased proxy for diet fatty acid compositions in this tissue for whale sharks.

We highlight the importance of concurrent multiple tissue sampling and pairwise SIA and FA analysis to more accurately assess the diet and tropic ecology of whale sharks. We suggest that the swift sampling and preservation of different tissue types from opportunistic stranding’s will become a very important resource to investigate any differential partitioning of lipids and isotope fractionation.

Chapter 4 Trophic ecology of juvenile whale sharks in southern Mozambique

4.1 Abstract

Most coastal whale shark feeding aggregations are seasonal but, in Mozambique, whale sharks are present year-round. Previous work indicates that whale sharks target Tofo Beach as an important feeding area, but they are rarely seen feeding at the surface when they are present. In this study we use stable isotope analysis to investigate the trophic ecology of whale sharks in Tofo Beach within the context of the local food web. We found evidence of seasonal dietary changes between months, which may be linked to changing ocean-scale dynamics influencing whale shark behaviour and abundance along the Inhambane coast. There is weak evidence supporting sex- and size-based dietary shifts as seen in other coastal whale shark aggregations. Isotopic data suggest that whale sharks at this site feed primarily on epipelagic zooplankton both inshore and offshore, with a possible contribution from emergent and deep-water zooplankton. There is also evidence of an un-sampled dietary item, potentially from offshore, oligotrophic waters.

4.2 Introduction

Whale sharks (*Rhincodon typus*) are well-known as the world's largest fish, and as filter-feeding planktivores, but details of their trophic ecology remain elusive (Rowat & Brooks 2012, Pierce & Norman 2016). Sightings at most seasonal whale shark aggregations coincide with easily identifiable biological events, such as fish spawning (Heyman et al. 2010, Robinson et al. 2013), that increase the availability of suitable food on a predictable schedule (Meekan et al. 2009, Robinson et al. 2013, Rohner, Armstrong, et al. 2015b).

Tofo Beach in Mozambique hosts a uniquely aseasonal aggregation of whale sharks (Rohner et al. 2013) and, with over 700 sharks identified, the Tofo aggregation is likely to play an important role in regional whale shark ecology. Whale sharks are preferentially found in cooler upwelling areas along the Inhambane coast and, though they may travel long distances (Brunnschweiler et al. 2009), many demonstrate a high level of site fidelity (Rohner et al. 2017). Sightings here have been linked to larger-scale oceanographic influences that create sporadic upwelling events and year-round enhanced productivity (Rohner et al. 2018).

Though whale sharks may be present off Praia do Tofo in any month, a relatively low proportion of sightings are of surface-feeding sharks (Haskell et al. 2014). This raises questions about the nature of diet sustaining the population. Provisional fatty acid studies have indicated that the sharks may also be feeding on emergent and deep-water zooplankton and fishes (Rohner et al. 2013). Here, I use a large sample size of stable isotope samples to investigate population-level foraging and movement ecology of whale sharks at this globally-important aggregation.

4.3 Study Area

Praia do Tofo (Tofo Beach, 23.85 S, 35.56 E) is in the Inhambane province of southern Mozambique. Similar to other coastal whale shark aggregations, the aggregation off Praia do Tofo is dominated by juvenile males from 300–950 cm total length (Rohner et al. 2015a). The continental shelf is narrow along this coast, with a steep shelf between the Vilanculos area and Praia do Tofo (Figure 4.1). To the south of Tofo the shelf broadens again, in the lee of a major coastline inflection, causing several types of eddies and upwellings (Rohner et al. 2018).

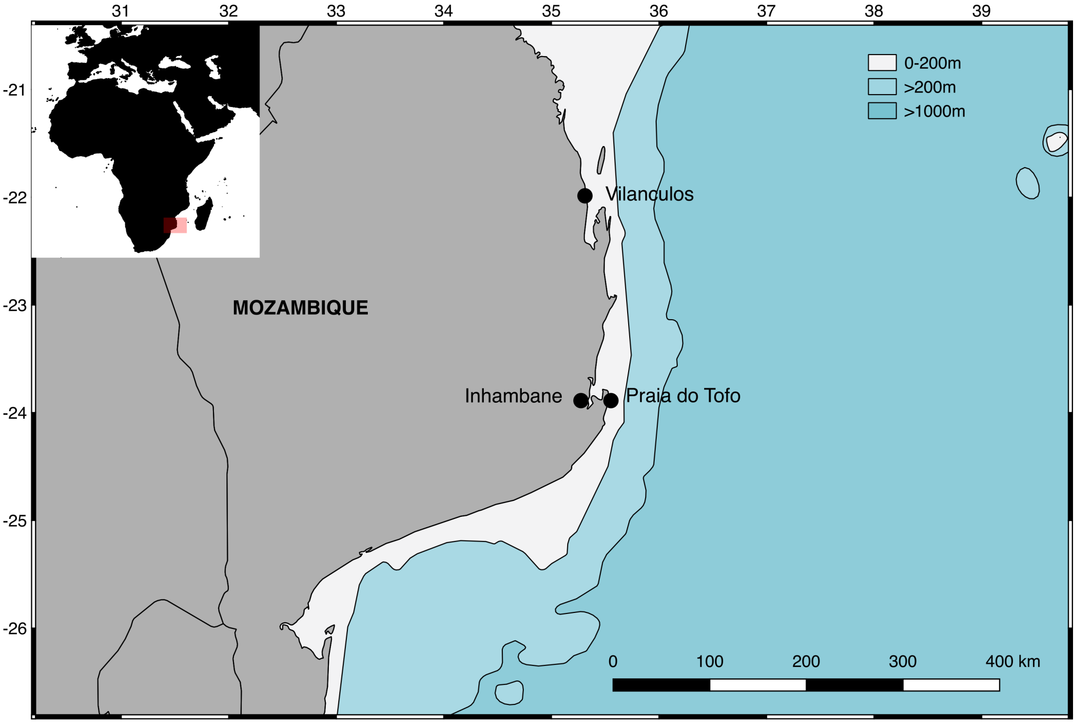

Figure 4.1 Map showing location of southern Mozambique within Africa and the location of Praia do Tofo.

4.4 Ethics Statement

Work in Mozambique was carried out with the full knowledge and approval of the Maputo Natural History Museum. All samples from Mozambique were exported and imported with the appropriate paperwork, and with permission from the Maputo Natural History Museum. Project ethics approval came from the Research and Governance Department at the University of Southampton (#13918).

4.5 Sample Collection

Whale shark dermal tissue samples were collected between August 2011 – December 2013. Boat surveys were conducted from rigid-hull inflatable snorkelling vessels, as outlined in Haskell et al. (2014). Whale sharks were individually identified using underwater photographs of the body region immediately posterior to the gills (Arzoumanian et al. 2005). These images were later uploaded, processed using a pattern-matching algorithm to identify individual sharks, and stored on the online database, Wildbook for Whale Sharks (www.whaleshark.org). Estimated total length (TL) and sex was determined – based on the presence or absence of claspers, as per Rohner et al. (2015a) – for each individual where possible.

Tissue samples were taken using a pole with a modified tip to extract biopsies laterally from between the first and second dorsal fins. Samples were stored on ice in the field, then rinsed in fresh water upon return to remove any possible contaminants and excess urea. The upper dermal denticle layer was then separated from the white connective tissue layer in each sample. This deeper layer of connective tissue was frozen on-site and used for all further analyses. I also collected mesogleal tissue from local jellyfish, and white muscle tissue from a range of fishes present at Praia do Tofo, including non-migratory planktivores, larger predatory piscivores, and mesopelagic fishes, to represent the biochemical conditions near the collection site for context and comparison. These samples were collected in June 2016 (Table 4.1)

Deep-water plankton were collected offshore as part of the Aguhlas II cruise in October-November 2017. Hauls were conducted during daylight hours at eight locations between the latitudes -6.09 in southern Mozambique and -20.68 in northern Tanzania). Plankton samples were collected with a five net Vertical Multinet (Type Midi 0.25m^2 mouth area). Hauls were of max depth 1, 482 m with integrated samples between depths of 0 m, 200 m, 400 m, 600 m, 800 m, 1, 000 m, and 1, 482 m.

4.6 Chemical Analysis

Samples were kept frozen and transported to the University of Southampton, UK, where they were freeze-dried and homogenised prior to analysis in an EA 1110 elemental analyser linked to a Europa Scientific 2020 isotope ratio mass spectrometer at OEA Laboratories Cornwall. Raw data were corrected using the reference materials USGS40 and USGS41 (glutamic acid from USGS Reston USA). An internal QC material bovine liver standard (NIST 1477a) was used to monitor the precision of the instrument. Isotope ratios are expressed per mille (‰) deviations from the reference materials VPDB and air for $\delta^{13}C$ and $\delta^{15}N$ values respectively.

Elemental C:N ratios for water-washed whale shark connective tissue shark samples were below the 3.5 threshold suggested by Post (2.6 ± 0.3 S.D.) and matched lipid extracted CN ratios for similar tissues from Australian whale sharks (Marcus et al. 2017). No chemical treatment was used to remove lipids, so analytical results were not adjusted to correct for lipid contents. Connective tissue proteins are largely collagenous, containing a high proportion of glycine with relatively heavy $\delta^{13}C$ values compared to muscle proteins, consequently a skin-muscle tissue conversion factor was estimated and applied to the bulk whale shark data to allow for direct comparisons to fish muscle samples and zooplankton analysed in this study. We used a figure of -2.5‰ for $\delta^{13}C$ following Prebble et al. (*in revision*). Deep-water zooplankton samples exhibited high C:N ratios (>3.5). We therefore applied a mathematical lipid correction (Kiljunen et al. 2006) to the bulk results to correct the $\delta^{13}C$ values.

4.7 Statistical Analysis

4.7.1 Bulk analysis

We calculated the mean and standard deviations (SD) of $\delta^{13}C$, $\delta^{15}N$ and C:N for each species collected. I then categorised the samples into functional group and calculated the mean and SD for each group. Biplots and boxplots of $\delta^{13}C$ and $\delta^{15}N$ values were used for graphical analysis. I estimated whale shark trophic position (TP) relative to the mean of the TP distribution curve previously calculated for whale sharks in Tanzania (Chapter 2) and the isotopic spacing ($\Delta^{15}N$) between whale shark and the planktivorous fish, half-beak (*Hemiramphus* sp.) in both Mozambique and Tanzania. The TP curve was calculated using the equation $TP = \lambda + (\delta^{15}N_{Consumer} - \delta^{15}N_{Base}) / \Delta_n$ where λ is the trophic position of the consumer used as a baseline, $\delta^{15}N_{Base}$ is the mean $\delta^{15}N$ of the baseline, $\delta^{15}N_{Consumer}$ is the $\delta^{15}N$ value of the whale sharks and Δ_n is the fractionation value of the consumer. As the diet tissue discrimination factor (DTDF) of whale sharks is unknown, a range of probable trophic enrichment factors (2.8-4% enrichment per trophic level) was used to estimate a distribution of likely trophic levels. The value chosen for the base was the mean and standard deviation of $\delta^{15}N$ for zooplankton prey. The λ value used was the mean and standard error of calculated trophic position from zooplankton and euphausiids (Hobson & Welch 1992, Cortes 1999). The consumer value used was the $\delta^{15}N$ mean and standard deviation of the whale sharks in Tanzania (Chapter 2). Trophic level sampling values were restricted to a biologically meaningful range of 0-6, with 0 representing primary producers and 6 representing high trophic level consumers. The dataset was resampled 1, 000 times to produce a trophic level likelihood distribution curve.

We used mixed effects linear models to test whether factors significantly influenced $\delta^{13}C$ and $\delta^{15}N$ values within whale shark data. Initial mixed effects model results indicated there was no effect of year of sampling on whale shark $\delta^{13}C$ or $\delta^{15}N$ values so ‘year’ was included as a random effect to allow a more robust analysis of any temporal patterns across months of sampling. Whale shark identity and the year of sampling were then set as random effects in subsequent models to control for repeated sampling and temporal variation.

4.7.2 Mixing models

We used Bayesian mass-balance mixing model (MixSIAR package in R) to explore possible prey contributions to whale shark diets and to identify the likely isotopic composition of potential missing diet items.

The isotopic compositions of prey sources, consumer tissues and trophic enrichment factors were allowed to vary to represent uncertainty in the model. Sources included in the model were deep-offshore zooplankton (200–1500 m), the mesopelagic fish species shortnose greeneye (*Chloropthalmus agassizi*), and nomadic jellyfish (*Rhopilema nomadica*). (Table 4.1). Deep-water zooplankton values for all samples (200–1500 m) were aggregated to represent deep-water zooplankton feeding. The shortnose greeneye, chosen to represent mesopelagic feeding on deep-water fishes, is a circumglobal bathydemersal species that typically occurs at 300-600 m. We sampled individuals with an estimated TL of 10-15 cm, which are the size of pelagic juveniles in this species (Merrett 1990, Russell et al. 2015). Nomadic jellyfish are a large epipelagic planktotroph (Galil 2006). Here, they have not been considered as a prey item in these models, but rather as a proxy for animals feeding exclusively on inshore epipelagic zooplankton.

There are no experimentally determined diet tissue discrimination factors (DTDF) available for whale sharks or other planktivorous elasmobranch species. Therefore, to account for trophic fractionation in the model we assumed that, based on their known diet, the deep-water fishes and jellyfish would be at a similar trophic level to the whale sharks. A DTDF of 0‰ was then applied to δ^{13}C and δ^{15}N values for both these sources. Large possible contributions of deep-water fishes or jellyfishes in model solutions therefore implies an isotopically-similar diet between whale sharks these end member groups. Deep-water zooplankton were assumed to be one trophic level lower than the whale sharks. DTDF corrections were set at 3.4‰ for δ^{15}N and 0.4‰ for δ^{13}C. To account for the uncertainty of this assumptions, we applied a large standard deviation of 2‰ to all DTDF corrections.

To investigate the uncertainty in the estimated tissue conversion factor between connective dermal tissue and muscle (Chapter 2, Prebble et al. *in revision*), we ran two separate models to investigate how the likely diet proportions would change based on different assumptions of this parameter. Model 1 assumed an initial correction factor of -2.5‰ from skin to muscle for δ^{13}C derived from paired tissue samples (Prebble et al. *in revision*), Model 2 assumed a correction factor of -4.6‰, similar to the isotopic difference between pure muscle and pure collagen (Satterfield & Finney 2002).

4.8 Results

4.8.1 Bulk Isotopes

Throughout the sampling period we collected 68 samples from 51 identified individuals, 84% of which were males (43 male, 7 female and one of unknown sex). Estimated TL ranged from 500–850 cm (mean 610 ± 88). Corrected $\delta^{13}C$ values for whale sharks ranged from -16.2 to -20.7‰, and $\delta^{15}N$ values from 6.4 to 10.7. Estimated trophic position (TP) for whale sharks in this food web was 2.7.

A total of one hundred and thirteen samples were collected from local coastal marine species and deep-water zooplankton. Bulk isotope values are summarised in Table 4.1. Whale sharks occupied a similar isotopic niche to other planktivorous fishes sampled here (Figure 4.2). Though there is some overlap, mean isotope values for whale shark tissues fell between those of the local planktivores and jellyfish species. Whale sharks had higher $\delta^{15}N$ values than the jellyfish, but lower $\delta^{13}C$ values. The sharks had higher $\delta^{13}C$ values than the planktivores, but lower $\delta^{15}N$ values. Piscivorous fishes (tuna and bonito) and the masked ray had the highest $\delta^{15}N$ values of the epipelagic and benthic species. However, the mesopelagic fish species, the shortnose greeneye, had the highest $\delta^{15}N$ values of all species sampled. The inshore benthic karanteen had the lowest $\delta^{15}N$ values, with a wide range of $\delta^{13}C$ values, and the highest $\delta^{13}C$ values of all species.

When comparing functional groups, the mean $\delta^{15}N$ values of the benthic karanteen were significantly different to the deep-water zooplankton ($p = 0.01$), the rays ($p = 0.01$), and the piscivores ($p = 0.01$). The karanteen mean $\delta^{13}C$ values were also significantly different to these groups as well as the mesopelagic fishes ($p < 0.01$) and planktivores ($p < 0.01$). (Table 4.2 / Figure 4.2). Whale shark mean isotope values were not significantly different to any of the other sampled groups.

Species	Functional Group	Tissue type	n	δ^{13}C	Range	δ^{15}N	Range	C:N
Whale Shark Rhincodon typus	Whale Shark	subdermal	68	-17.6 (±0.6)		8.0 (±0.8)		2.6 (±0.3)
Leopard ray Himantura uarnak	Ray	muscle	1	-17.4		9.6		3
Mask ray Neotrogon kuhlii	Ray	muscle	2	-16 (±0.2)		11.4 (±1.0)		2.7 (±0.2)
Tuna Thunnus albacares	Piscivore	muscle	3	-17.2 (±0.2)		11.3 (±0.5)		3.0 (±0.0)
Bonito Katsuwonus pelamis	Piscivore	muscle	2	-17.6 (±0.3)		11.5 (± 0.4)		3.0 (±0.0)
Karanteen Crenidens crenidens	Benthic	muscle	9	-10.3 (±3.10)		5.9 (±0.7)		3.1 (±0.1)
Carapau' Unknown baitfish	Planktivore	muscle	22	-18.7 (±0.3)		8.9 (±0.4)		3.2 (±0.1)
Half-beak Hemiramphus sp.	Planktivore	-	9	-17.1 (±0.4)		9.6 (±0.5)		3.1 (±0.0)
Shortnose greeneye Chloropthalmus agassizi	Mesopelagic	muscle	9	-18.6 (±0.3)		12.0 (±0.3)		3.2 (±0.1)
Jellyfish Rhopilema nomadica	Jellyfish	bell	20	-16.8 (±1.1)		7.6 (±0.6)		3.4 (±0.3)
Zooplankton 0 - 200 m	Zooplankton Deep	Mixed	7	-18.8 (±0.3)	0.7	8.8 (±0.9)	2.3	4.1 (±0.5)
Zooplankton 200 - 400 m	Zooplankton Deep	Mixed	6	-18.9 (±0.4)	1	9.6 (±1.2)	3.6	4.2 (±0.4)
Zooplankton 400 - 600 m	Zooplankton Deep	Mixed	8	-18.5 (±0.8)	2.59	10.4(±1.3)	3.7	4.2 (±0.4)
Zooplankton 600 - 800 m	Zooplankton Deep	Mixed	5	-17.5 (±1.7)	4	10.3(±1.0)	2.8	5.0 (±0.5)
Zooplankton 800 - 1000 m	Zooplankton Deep	Mixed	7	-18.1 (±1.0)	2.7	10.4(±1.0)	2.7	4.2 (±0.5)
Zooplankton 1000 - 1500 m	Zooplankton Deep	Mixed	3	-18.1 (±1.0)	1.8	9.5(±1.6)	3.2	5.5 (±0.8)

Table 4.1 The mean isotopic values for corrected δ^{13}C and δ^{15}N values (‰ ± standard error) of each sampled species.

Functional Group	$\delta^{13}C$	$\delta^{15}N$	C:N
Whale Shark	-17.6 (±0.6)	8.0 (±0.8)	2.6 (±0.3)
Ray	-16.8 (±0.9)	10.9 (±1.8)	2.8 (±0.3)
Piscivore	-17.3 (±0.3)	11.4 (±0.4)	3.0 (±0.0)
Planktivore	-18.2 (±0.8)	9.1 (±0.5)	3.1 (±0.1)
Benthic	-10.3 (±3.10)	5.9 (±0.7)	3.1 (±0.1)
Mesopelagic	-18.6 (±0.3)	12.0 (±0.3)	3.2 (±0.1)
Jellyfish	-16.8 (±1.1)	7.6 (±0.6)	3.4 (±0.3)
Deep-water Zooplankton	-18.4 (±1.0)	9.9 (±1.2)	4.4 (±0.6)

Table 4.2 The mean isotopic values for corrected $\delta^{13}C$ and $\delta^{15}N$ values (‰ ± standard error) of each functional group.

Whale shark total length was a significant predictor for $\delta^{13}C$ ($X^2 = 2.3$, $p < 0.001$), and a model that included both the month of sampling and TL as non-interacting terms ($X^2 = 1.78$, $p < 0.001$). However, the month of sampling alone, although less significant, explained more $\delta^{13}C$ variance ($X^2 = 12.3$, $p = 0.05$). The month and TL model came out as the most significant predictor for whale shark $\delta^{15}N$ values ($X^2 = 3.3$, $p < 0.001$). Using month alone explained more variation, but was less significant ($X^2 = 12.4$, $p = 0.05$).

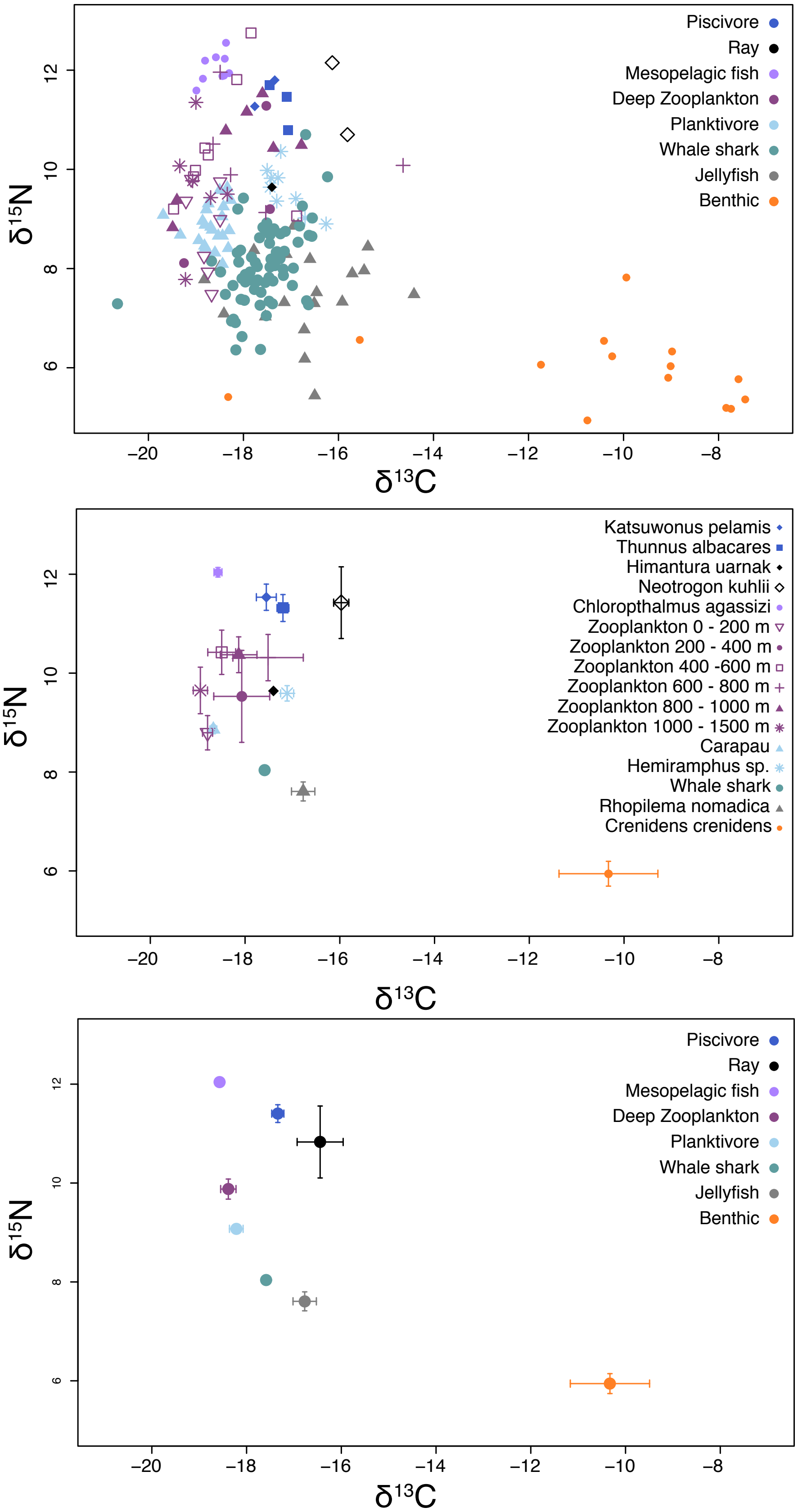

Figure 4.2 Biplots of (A) isotopic values of all samples collected in this study. (B) mean isotopic values (‰± standard error) of each species collected from this study, and (C) mean isotopic values (‰± standard error) of each functional group.

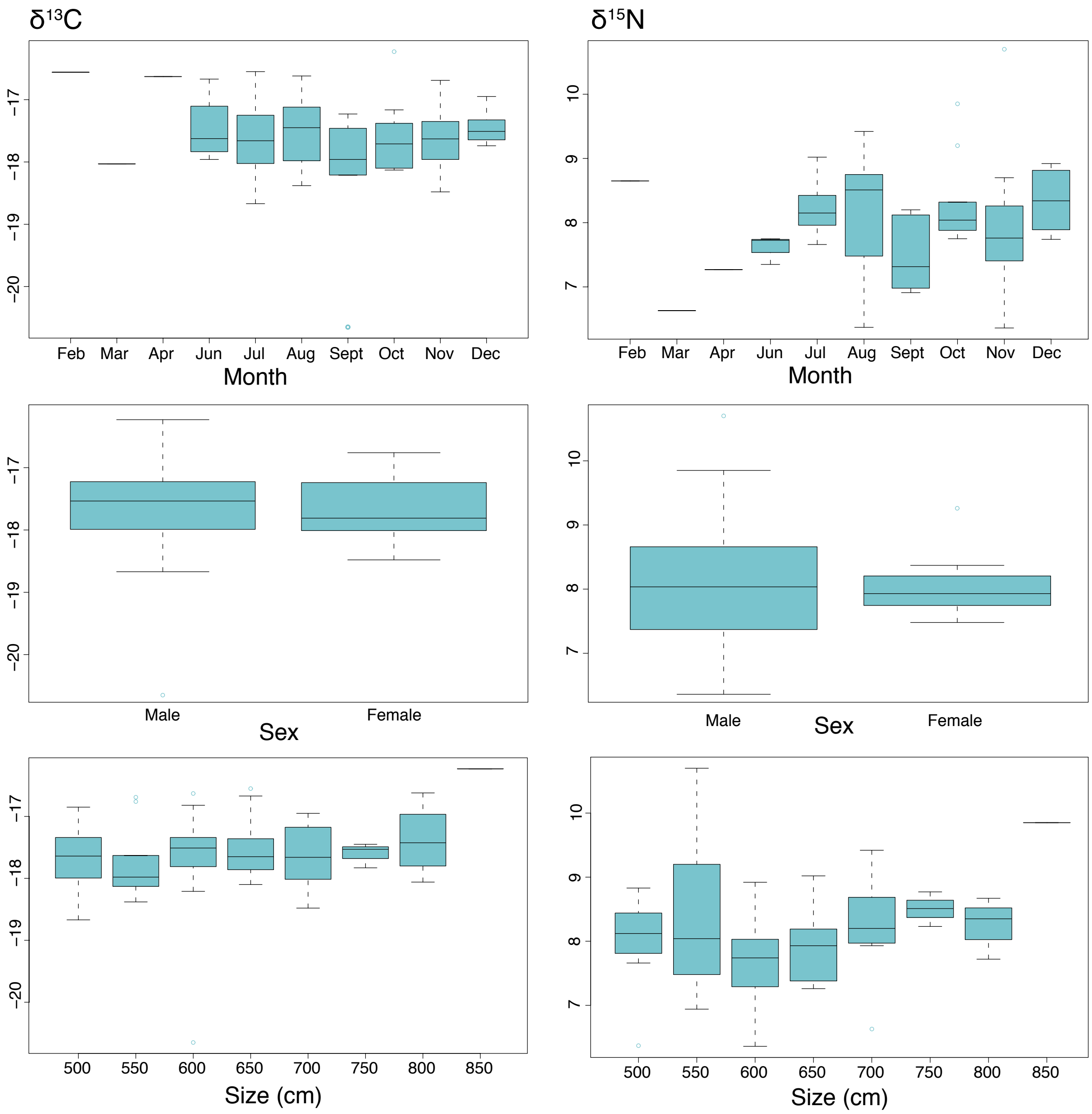

Figure 4.3 Boxplots of whale shark $\delta^{13}C$ and $\delta^{15}N$ corrected bulk values and month of sample collection, sex of shark, and estimated total length of shark.

Group	Whale shark	Ray	Piscivore	Planktivore	Benthic	Mesopelagic	Jellyfish	Deep-water Zoop
Whale Shark	-	0.92	1	1	0.01	0.9	0.94	0.88
Ray	0.2	-	0.99	0.69	0	0.47	1	0.35
Piscivore	0.06	0.98	-	0.99	0	0.85	1	0.81
Planktivore	0.87	0.5	0.15	-	0	0.99	0.72	0.99
Benthic	0.45	0.01	0.01	0.09	-	0	0.02	0
Mesopelagic	0.05	0.74	0.99	0.13	0.02	-	0.54	1
Jellyfish	1	0.09	0.04	0.48	0.57	0.05	-	0.42
Deep-water Zoop	0.33	0.96	0.49	0.99	0.01	0.25	0.24	-

Table 4.3 Results (P-values) from pairwise adjusted Tukey tests between functional groups for δ^{15}N (top) and δ^{13}C (bottom). Significant values in blue

4.8.2 Mixing Models

The plots showing the model data in isotopic space after applying the corrections and variance both imply that whale shark occupy a similar trophic level to the nomadic jellyfish, as expected (Figure 4.5 panels A &C). The -4.6‰ tissue-corrected whale shark data (panel A) are more depleted in ^{13}C than the deep-water zooplankton and mesopelagic fish, but if a -2.5‰ correction is used whale shark dermal tissues are enriched in ^{13}C compared to both potential prey sources. From the two mixing models generated, the nomadic jellyfish source, here interpreted as reflecting an epipelagic zooplankton diet, was inferred as representing around 85% of the diet in each case (Figure 4.5 panels B & D). For our assumed tissue conversion factor of -4.6‰, deep-water zooplankton were most likely to be absent from the whale sharks' diet, and contribute up to 20% in rare cases. Mesopelagic fish would contribute slightly more in this case, most probably around 15% and up to 25% in rare cases. If we assume a lower tissue correction factor of -2.5‰ for the whale sharks, consumption of deep-water zooplankton is more likely contributing between 5% and 25% of diet. Mesopelagic fishes had a similar contribution to the -4.6‰ model. The width of the scaled posterior density curves were wider for all dietary sources in the -2.5‰ model, suggesting a greater range of possible credible solutions surrounding the model predictions compared to the -4.6‰ model.

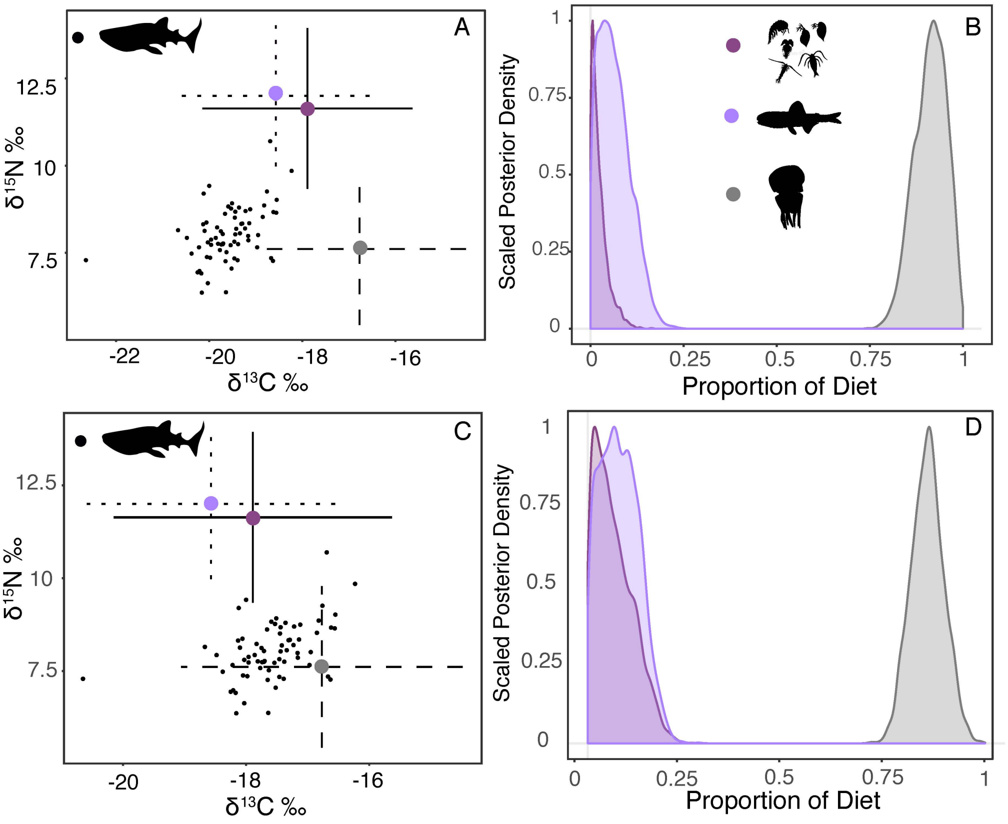

Figure 4.4 Figure showing corrected data and mixing model diet proportion results for the whale shark dermal connective tissue (black) as the consumer, and prey sources including deep zooplankton (purple), shortnose greeneye (pink), and nomadic jellyfish (grey). (A & C) Shows the corrected source and consumer data assuming a skin-muscle tissue correction factor of -4.6‰ (A) and -2.5‰ (C). (B & D) Scaled posterior density plots showing the likelihood of diet proportions for each source applying -4.6‰ (B) and -2.5‰ (D) consumer tissue corrections respectively.

4.9 Discussion

Stable isotope data imply that whale sharks in Tofo feed primarily on epipelagic zooplankton, both inshore and in offshore upwelling areas, with possible contributions from emergent and deep-water zooplankton (Figs 4.2, 4.5). Seasonal population level changes between suggest ocean-scale dynamics influence on whale shark abundance and behaviour along the Inhambane coast, rather than a specific seasonal event as is seen in some other locations (i.e. Mafia, Qatar etc).

4.9.1 Trophic position

As trophic discrimination factors and species-specific tissue discrimination factors for whale sharks are poorly-known, trophic positions should be used as relative indices rather than directly compared between studies and locations. Based on the previous literature on whale shark ecology, a trophic position between 2-4 would be predicted. The median TP value for whale sharks in this study (TP = 2.7) was, however, similar to previous estimates for whale sharks in Tanzania (TP = 2.8 (Prebble et al. *in revision*), South Africa (TP = 3.6, (Cortes 1999), the Arabian Sea (TP = 3.3, (Borrell et al. 2011), and Australia (TP = 2.6, Marcus 2017). Small differences in TP estimates may represent differences in the trophic position of whale sharks at each location, or the different methods and parameters used to calculate TP in each study.

It is more useful to evaluate the relative trophic position of whale sharks within the local food web. Here, the isotopic compositions of whale sharks were consistent with other planktivorous and low trophic-level epipelagic species sampled in Mozambican waters. While there was some individual variation in diet, the Tofo whale sharks appear to be experiencing similar isotopic conditions to resident local fauna. The sharks are therefore likely to be reasonably resident to this region over the timescale of isotopic turnover. The whale sharks were most similar, isotopically, to the nomadic jellyfish, an epipelagic planktotroph native to East Africa which are generally recorded 2-4 km offshore and are distributed predominantly by ocean currents (Galil 2006). While not conclusive, their isotopic similarity, and high $\delta^{13}C$ values relative to the resident planktivores, suggests that an aspect of the Mozambican whale shark foraging strategy might similarly involve following ocean currents and targeting epipelagic zooplankton (McMahon et al. 2013). Another interpretation could be that the whale sharks in Mozambique are consuming the nomadic jellyfish, though this is unlikely to be the case, as fully grown nomadic jellyfish are larger than whale sharks could physically swallow (commonly 40 – 60 cm diameter) (Motta et al. 2010). While the smaller medusa stages can be around 10 cm and could potentially, therefore, be caught in the whale sharks filtering pads and swallowed

(Heyman et al. 2001, Motta et al. 2010), this has not been recorded. Large numbers of pelagic medusas can quickly form in warm (>24°) water (Galil 2006), perhaps enough biomass if consumed to influence the isotopic values of the whale shark tissues. Leatherback turtles (*Dermochelys coriacea*), which exclusively target jellyfish and gelatinous zooplankton, are spotted consistently (albeit at low densities) at the surface near Praia do Tofo (Williams 2017). There are literature records of some shark species ingesting gelatinous zooplankton (Koen Alonso et al. 2002) so too do marine mammals (Childerhouse et al. 2001) and some fish (Williams et al. 2015), although we are not aware of any direct observations of whale sharks doing so.

Initial evidence from fatty acid and stomach contents analysis on Mozambican sharks suggested that their diet may include emergent zooplankton which they feed on at night, and deep-water zooplankton and fishes (Couturier et al. 2013, Rohner et al. 2013). From the data presented here it is isotopically possible that some of the whale sharks had been foraging on offshore and deep-water zooplankton, particularly those sharks with more relatively high $\delta^{15}N$ values. However, the whale sharks generally plot closer to the epipelagic (0–400 m) zooplankton trawls than the deeper-water (400–1500 m). The whale sharks were also isotopically distinct to the mesopelagic fish species sampled in this study. While we only sampled one species of mesopelagic fish, the juvenile sizes that were sampled (~15-20 cm) are predominantly pelagic feeders at 300-600 m (Merrett 1990, Russell et al. 2015), so are likely to represent the isotopic conditions of the mesopelagic fish community (Merrett 1971). If mesopelagic fish are a part of the whale sharks' diet, it is likely a small proportion. This aligns with the observed diving behaviour of a female whale shark tagged slightly south of Praia do Tofo. A large proportion of her time was spent in the top 100 m, for most of which she was shallower than 10 m, suggesting a predominantly epipelagic lifestyle (Brunnschweiler et al. 2009). While in coastal waters she stayed significantly deeper at night, perhaps to target emergent zooplankton, which were found in stomach contents analysis of Mozambican sharks by Rohner et al. (2013). Larger proportions of night-time feeding on emergent zooplankton may also be reflected in those individuals with higher $\delta^{13}C$ values (Abrantes & Barnett 2011). However, while offshore, her diving behaviour changed to more frequent epipelagic diving punctuated by deep dives, a pattern also observed in other areas such as Mexico (Graham et al. 2006)

4.9.2 Size, and sex

There was some, albeit weak, evidence for isotopically distinct foraging habits between sexes, with relatively low $\delta^{13}C$ and $\delta^{15}N$ values recorded in female whale sharks. The relatively high $\delta^{13}C$ and $\delta^{15}N$ levels seen in males were consistent with previous studies (Borrell et al. 2011, Marcus 2017). The difference observed is marginal, and we are cautious of drawing strong conclusions with the low numbers of females sampled. However, the low proportion of identified females in Mozambique, and many other aggregations, points to clear spatial segregation between males and females (Rohner et al.

2015a). The absence of females at coastal aggregations suggests they prefer offshore, and or deep-water habitats, which is given some tentative support from these results.

Equally, while estimated total length was a significant predictor of tissue isotope composition, larger mature individuals are rarely sighted in Mozambique (Rohner et al. 2013, Rohner et al. 2015a). As such, the size range sampled here is relatively small, with particularly low numbers sampled at the extremities. As with the females, the absence of larger mature individuals from the aggregation in itself indicates a behavioural shift over ontogeny. Again, perhaps what we are detecting here is the start of a gradual behaviour shift where increasing $\delta^{13}C$ values could indicate larger sharks feed more often in upwelling areas, more often at night on emergent zooplankton, or more frequently in deeper water. Increasing $\delta^{15}N$ values could indicate that larger sharks feed at higher trophic levels or, again, in upwelling areas.

4.9.3 Seasonality

There is some evidence of changes in feeding behaviours between months across the population, which does not extend to the inter-annual scale. We have observed seasonal fluctuations in the isotopic values of the whale shark tissues potentially tied to the inconsistent variation in oceanographic conditions in the Mozambique Channel (Kolasinski et al. 2012,). Upwelling cells are potentially what draw the whale sharks to the area and likely sustain the year-round aggregation (Rohner et al. 2018) (Figure 4.5). More consistent sampling of whale shark tissues across time, in conjunction with surface plankton samples in future, would help clarify this further.

4.9.4 Mixing models

The potential diet sources included in mixing model analyses cannot fully explain the whale shark isotopic compositions (Figure 4.4), requiring either contributions of un-sampled prey and / or differences in tissue conversion or trophic enrichment factors. Notably, whale shark tissues show relatively low $\delta^{13}C$ and $\delta^{15}N$ values compared to potential prey items. Low $\delta^{13}C$ values could be explained either by an un-sampled $\delta^{13}C$ source that is depleted in ^{13}C, or the preferential routing of ^{13}C depleted lipids from zooplankton into the connective dermal tissue proteins.

If we assume there is an un-sampled $\delta^{13}C$ source, there could be a small contribution from a very ^{13}C deplete source, or a larger contribution of a less ^{13}C deplete source. The potential $\delta^{13}C$ value of any un-sampled source also depends on the tissue correction value used, but whale sharks would have to travel to much higher latitudes from 28°S to perhaps beyond 40°S to sample diets plankton diets with low $\delta^{13}C$ values of around -20 to -22 (Rau et al. 1982, Goericke & Fry 1994, Magozzi et al 2017), 28°S latitude is approximately the same latitude as the province of KwaZulu-Natal in South Africa. Whale sharks have been observed at these latitudes from aerial surveys and by divers (Beckley et al. 1997, Cliff et al. 2007). Sightings in this area, and particularly further south, are often stranding events as it is thought that the whale sharks succumb to the colder waters around the Western Cape (Beckley et al. 1997). As no photo ID data were collected we cannot know if any of these sharks have been sighted in Mozambique aggregation. For the $\delta^{13}C$ values found at the latitudes around South Africa, the whale sharks would have to be sourcing a large proportion of their overall diet there to explain the $\delta^{13}C$ deplete values predicted in Figure 4.5. So although we cannot rule it out, there is no real data that supports whale sharks routinely feeding in lower latitudes.

It seems more likely that we are missing a low $\delta^{13}C$ nutrient pathway or dietary item, rather than a large latitudinal spatial effect. The southwestern Indian Ocean is a dynamic oceanographic system. The bathymetry and mesoscale circulation features come together to stimulate upwelling of deep nutrient-rich waters (Raj et al. 2010, Rohner et al. 2018). As a result some of the largest phytoplankton blooms in the worlds ocean develop in the southwest Indian Ocean during the austral summer (Raj et al. 2010). On a large scale, these blooms occur in the Madagascar Basin, the Mozambique Basin and the Mozambique channel. Cyclonic eddies moving down the Mozambique channel also cause more localised upwellings and play a role in transporting coastal production offshore (Kolasinski et al. 2012). These conditions of high productivity can result in areas with more negative $\delta^{13}C$ values, as ^{12}C is preferentially taken up by phytoplankton (Kolasinski et al. 2012).

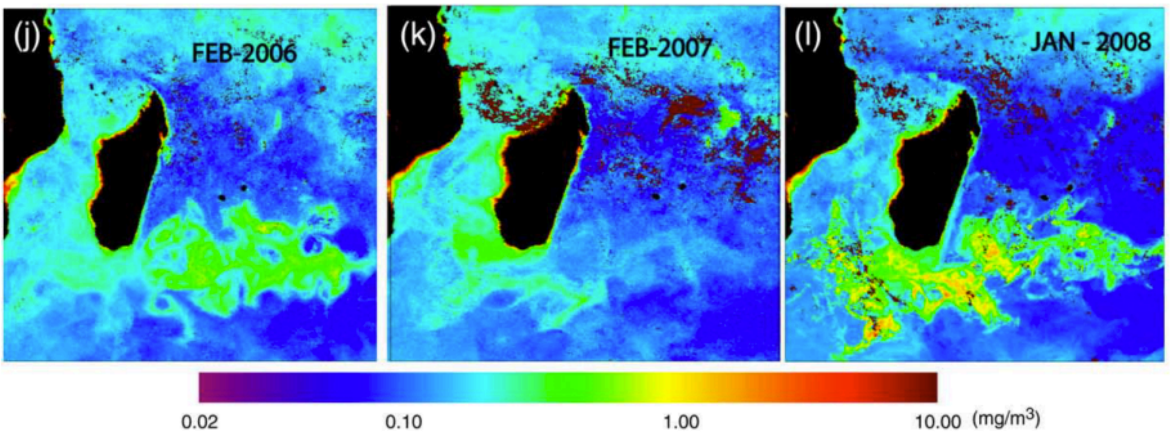

Figure 4.5 Spatial distribution of monthly averaged Chl-a concentration in the southwest Indian Ocean during the peak periods of the austral summer plankton bloom. Adapted from Raj et al. 2010.

In oligotrophic conditions high bacterial growth and nitrogen fixation can also lead to POM with low $\delta^{15}N$ values (up to 4‰), which could also explain some of the comparatively low $\delta^{15}N$ whale shark values after DTDF corrections (Minagawa & Wada 1986, Karl et al. 1997). In the marine environment, in oligotrophic conditions, often a build up of a filamentous blue-green cyanobacteria, tricodesmium, forms in surface waters (Capone 1997). While the whale sharks do not have fine enough sieve plates to capture the bacteria directly (Motta et al. 2001), zooplankton species such as copepods are known to target tricodesmium as a food source (O'Neil & Roman 1992). Any tricodesmium grazers would uptake carbon and nitrogen with low δ^{15}N and δ^{13}C values, which would be passed up the food chain. Tricodesmium has consistent seasonality in Tanzania with the highest biomasses recorded during from December to April (Lugomela et al. 2002). A small number of whale sharks originally identified in the Mozambique aggregation have been sighted in Tanzania at this time, (Prebble et al. *in revision* Chapter 2) and have been observed feeding amongst Tricodesmium slicks (pers obs, Figure 4.6), although they have not subsequently been resighted in Mozambique.

Figure 4.6 Whale shark in Mafia Island Tanzania feeding on dense clouds of *Belzebub hanseni* shrimp amongst bacterial *Tricodesmium* spp. blooms.

Credit: Dr Simon J Pierce.

Low δ^{13}C values, compared to measured prey items, could also be indicative of a novel biological pathway. In most stable isotope dietary studies it is assumed that the amino acids in a consumers' tissues are completely routed from dietary protein, and no other macromolecules like lipids or carbohydrates significantly contribute to the biosynthesis of tissues. By contrast, in dietary mixing models it is assumed that dietary macromolecules are broken into their elemental constituents and are resynthesized to build tissues. The reality is likely to be somewhere between and influenced by the macromolecular diet composition. Evidence of flexible diet-related protein routing has been observed in a few species, even in fish (Podlesak & McWilliams 2006, Voigt et al. 2008, Kelly & Martínez del Rio 2010). It has been demonstrated in the common house mouse that dietary lipids can also be used as a source of elemental carbon to build tissues (Newsome et al. 2014, Wolf et al. 2015). Certain amino acids were particularly sensitive to diet composition, and the routing of proteins and lipids affected the tissue-specific isotopic discrimination in the mouse tissues. In this study, high lipid diets resulted in an increase of $\delta^{13}C$ in tissues by 2-6 ‰. There is also evidence of isotopic routing in Nile tilapia (*Oreochromis niloticus*) (Kelly & Martínez del Rio 2010). Carbon structures from carbohydrates and lipids were preferentially routed to build essential amino acid, especially where they were fed protein deficient diets. Lipids are ~3-8 ‰ more depleted in ^{13}C than the associated proteins. It is theoretically possible that whale sharks could be routing more of the ^{13}C-depleted lipid carbon molecules from the zooplankton they are eating to the protein structures in the dermal connective tissue. A metabolic pathway coupled with a low protein high lipid diet such could feasibly account for the results observed here. Indeed, zooplankton from upwelling and coastal biomes are characterised by large lipid stores (Lee et al. 2006), which feeds back into our previous conclusions.

We could be seeing a mixture of both the missing ^{13}C deplete nutrient pathway, and the dietary routing. Or indeed, another process that we have not measured or considered in this study. The next steps would require resolving any dietary routing that may be occurring. Analysis of multiple whale shark tissue types simultaneously would go some way towards this, and should be a priority going forward.

4.9.5 Conclusions

While the sample numbers here were larger than previous SIA studies on whale sharks (Borrell et al. 2011, Marcus 2017), we still only sampled 7% of the identified population in Mozambique. We were also unable to sample the whale sharks in the same years as the other species used to build a food web model. Although there is no evidence of annual differences in the whale shark isotopic profiles, results here must still be interpreted with caution, as the Mozambican Channel is an oceanographically dynamic system (Rohner et al. 2018) and baseline isotope values are subject to change as a result (Kolasinski et al. 2012).

In addition to this, there are significant unknowns relating to elasmobranch physiology and metabolism, especially in whale sharks that limit the strength of conclusions that can currently be drawn from biochemical analysis.

The biochemical evidence in this study supports the hypothesis that the juvenile whale sharks found at Praia do Tofo are indeed using the site as a feeding area, targeting coastal epipelagic zooplankton and opportunistically taking advantage of offshore upwellings in the Mozambique Channel. It is likely that the boat-based visual surveys are missing some cryptic feeding behaviour, likely because the whale sharks are feeding sub-surface during the day, or feeding on emergent zooplankton at night. To address this, future efforts should focus on additional sampling of deep-water zooplankton and fish communities in the Mozambique channel, as well as emergent zooplankton at Praia do Tofo. Ideally these samples should be collected concurrently with whale shark dermal connective tissue across all months of the year to capture any isotopic seasonality, and allow direct comparisons. With improved knowledge of the isotopic variability and isotopic space that emergent and deep water communities occupy, we will be able to more confidently assess the contribution of each feeding strategy to the whale sharks diet. Electronic tags fitted with 3-axis accelerometers to reveal detailed short term behaviours short

Chapter 5 Dinner in the twilight zone: epipelagic species use deep ocean resources

Results from the biochemical analysis of whale shark tissues has shown that while it may not be a large part of their diet, deep-water plankton and fishes certainly play a role in whale shark feeding a movement behaviour. This is also the case for a plethora of other pelagic species across the globe. In this chapter I discuss how and why these species interact with potential food sources at depth, and the importance of such a resource both ecologically, and commercially.

5.1 Abstract

Research and commercial interest in the deep ocean has exploded over the past two decades. The deep scattering layer, often situated in the mesopelagic zone, is already recognised as an economically and ecologically important fish resource. These mesopelagic species are preyed upon by a diverse range of epipelagic predators around the world. Current knowledge likely underestimates the number of deep-diving pelagic species, and the extent to which they use mesopelagic food resources. Here, we review recent insights into the mesopelagic layer and summarise the diverse range of species that exploit this resource. We argue that these deep-diving pelagic species should be included in future studies of mesopelagic food web biomass and structure and considered in legislation pertaining to deep-water resources.

5.2 The Mesopelagic layer

The marine environment provides 99.83% of the habitable volume on this planet. The deep ocean is the world's largest ecosystem (Dawson 2012). The deep ocean system was historically characterised as devoid of life (Sutton 2013), but modern sampling techniques, particularly combinations of acoustic and optical sensing, intensive net-sampling and computer modelling (Sutton 2013, Irigoien et al. 2014) have radically changed this view. It is now well established that water habitats can host relatively high levels of biodiversity (Kendall & Haedrich 2006, Danovaro et al. 2010, Campell et al. 2011), high biomasses (Irigoien et al. 2014), and provide globally important ecosystem services (Robison 2009).

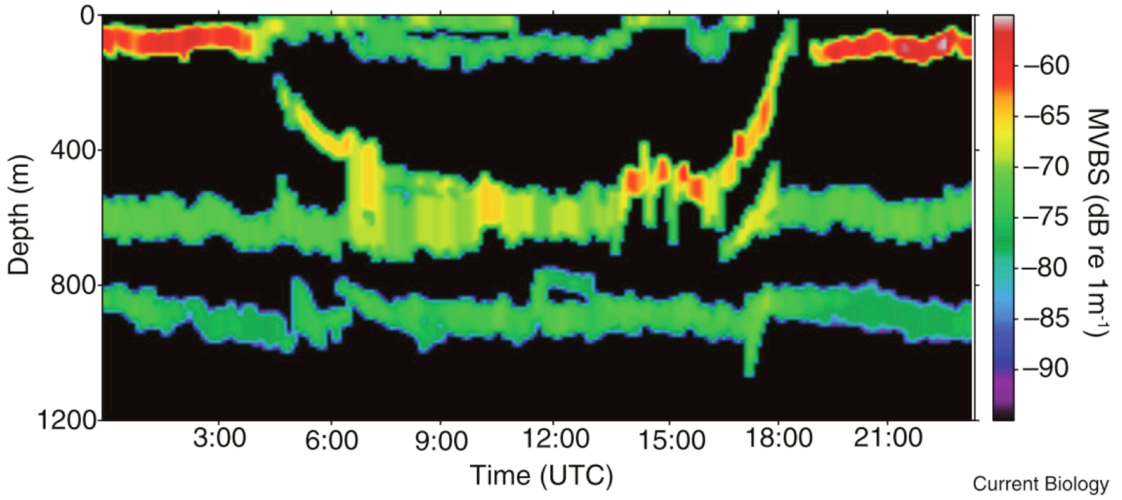

Table 5.1 Figure adapted from Brierley (2014). Diel vertical migration in the open ocean. An echogram from data in the southwest Indian Ocean. The colour scale represents echo intensity, with stronger echoes from denser aggregations of organisms. Figure demonstrates that some organisms 'sit and wait' where layers remain constant over time, while one layer shows classic DVM as organisms downwardly migrate at dawn and return to the surface at dusk.

The deep pelagic is typically defined as the part of the water column deeper than 200 m. The mesopelagic zone specifically refers to ocean waters between 200–1000 m depth. The mesopelagic zone often contains a deep scattering layer (DSL), named after horizontal zones of living organisms, usually schools of fishes and shrimp, that scatter or reflect sound waves leaving distinct echoes in depth sounders (Figure 5.1). Although the mesopelagic layer(s) do not receive enough sunlight to support photosynthesis, the fauna that inhabit these depths are tightly coupled to the diel cycle. This regular change in light levels drives a characteristic feature of the DSL: diel vertical migration (DVM) (van Haren & Compton 2013). Herbivorous and omnivorous mesozooplankton feed mainly on phytoplankton and microzooplankton. Phytoplankton and microzooplankton are at their maximum densities in well-lit surface waters, where the mesozooplankton also face the threat of predation. To minimise predation risk a large volume of the mesozooplankton use deeper water as a dark refuge, moving synchronously towards the surface at dusk to feed, then returning to depth and safety at dawn. This phenomenon is one of the largest daily movements of biomass on the planet (Brierley 2014). The DVM has a profound effect on the daily movements of predators following the migrators of the DSLs including micronektonic squids, crustaceans, fishes, marine mammals and birds (Hays 2003, Potier et al. 2013), among other animals, all of which adapt their behaviour to take advantage of migrating prey (Hays 2003, Anderson et al. 2018). Among the most important groups in the DSL are the myctophid fishes. These fishes are the most common vertebrate group on earth, comprising an enormous global biomass and creating a major tropic link between low and high level predators as well as playing an important role in the active transport of carbon from productive surface layers to the deep sea (Irigoien et al. 2014, Davison et al. 2015).

Mesopelagic fishes have also been highlighted as an under-exploited commercial resource that could be used in production of fish meal and omega-3 dietary supplements for humans and aquaculture (St. John et al. 2016, Anderson et al. 2018). Evaluating the sustainability of such enterprise requires an understanding of the potential biological repercussions. Currently, there are no accurate broad-scale biomass estimates, which are fundamental data for effective decision-making. Manual sampling methods originally estimated the mesopelagic fish biomass in the open ocean at ~1 gigatonne (Gt) (Gjøsaeter et al. 1980, Lam et al. 2005), which were followed by acoustic and modelling techniques that increased this estimate 10-fold to ~11-15 Gt (Irigoien et al. 2014) (Figure 5.1). Manual methods are susceptible to underestimates, as some fishes can sense the pressure of an approaching net and will avoid capture (Kaartvedt et al. 2012). Acoustic estimates are more precise than trawls, but difficulties interpreting the resonance and scattering response of gas-filled swim bladders still lead to substantial uncertainties in density and biomass metrics (Kaartvedt et al. 2012, Sutton 2013, Davison et al. 2015, Anderson et al. 2018). More recent assessments, using carbon flux food web models, have put the figure at a lower ~2.4 Gt (Anderson et al. 2018b). Though biomass estimation techniques have advanced, none of the published estimates to date have included latitudes above 40°N and below 40°S, nor have they incorporated spatiotemporal variability in fish biomass. Mesopelagic fish research remains in its infancy.

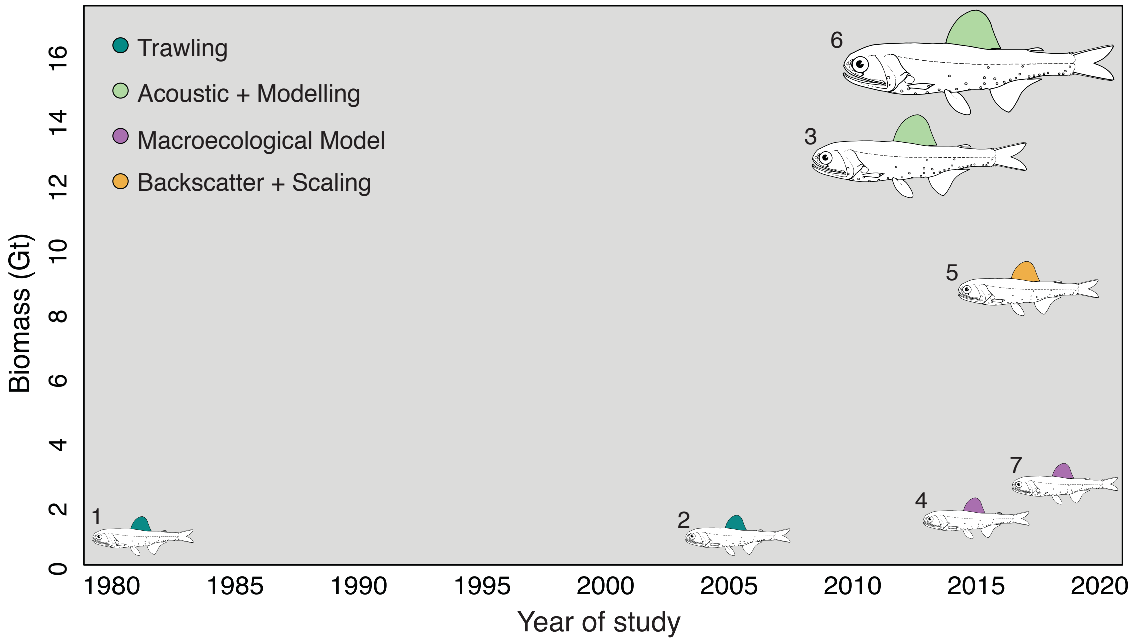

Figure 5.1 Figure showing the range of mesopelagic biomass estimates and the methods used.

Numbers refer to paper references. (1) Gjøsaeter et al 1980, (2) Lam & Pauly 2005, (3) Irigoien et al 2014, (4) Jennings & Collingridge 2015, (5) Proud et al 2016, (6) Proud et al 2018, (7) Anderson et al 2018.

The role of pelagic species in cycling nutrients between the surface and the mesopelagic is greater than previously thought (Buesseler et al. 2007, Roman & McCarthy 2010, Saba & Steinberg 2012). The DSL layer represents a substantial food and energy source for many vertebrate predators (Pethybridge et al. 2011). Such predators include highly mobile pelagic species that move long distances between patches of ephemeral productivity in the euphotic zone (Lalli & Parsons 1997, Walli et al. 2009, Bauer et al. 2017), or between breeding and feeding grounds (Costa et al. 2011). The results of large-scale tagging studies (Block et al. 2011) have shown that the extensive horizontal and vertical movements of these predators are likely to influence the marine system through energy transport and storage (Brierley 2014, Estes et al. 2016). These effects are strongest in areas that are resource-limited. Energy transfer of key limiting nutrients encourages phytoplankton growth, which is then perpetuated up the food chain to create a positive feedback system that enhances biodiversity and biomass (Estes et al. 2016). As more pelagic predators are tracked with depth sensors, the extent to which they utilise the mesopelagic zone is beginning to be discovered. Here, we will explore how pelagic divers use and access mesopelagic resources, the cumulative ecological effects of such behaviour, and where we believe the crucial omissions in research currently exist.

5.3 Deep diving pelagic species

5.3.1 Barriers to access & adaptations

The relative proportion of an epipelagic species' time spent in the mesopelagic zone foraging, and the importance of this resource to each, varies greatly. This is principally due to the different environmental conditions a predator encounters at these depths compared to its epipelagic habitat. A dive from the surface to the mesopelagic zone is coupled with a decrease in water temperature, less natural light, increased hydrostatic pressure, and less dissolved oxygen (Sepulveda et al. 2010). The mesopelagic layer often contains the oxygen minimum zone (OMZ), or zones where animal respiration result in low oxygen saturation levels (below the normal 4-6 mg l^{-1} to below 2 mg l^{-1}) even approaching anoxic conditions in some areas. Some species, such as the 'vampire squid' (*Vampyroteuthis infernalis*) have adapted to live permanently within low-oxygen zones, but many migrate in and out (Lalli & Parsons 2013). Another feature often found in the mesopelagic layer is the permanent thermocline, the water layer where the temperature gradient is at its steepest and temperatures can drop by as much as 20°C. There are also seasonal and annual changes in the thermocline, prey movement (Gleiss et al. 2013), and dynamic oceanographic conditions (Fuller et al 2015). For many epipelagic species, these inhospitable conditions mean that access to these depths is prohibited altogether or severely limited through reduced dive times or dive frequencies. That said, many have developed specialised behavioural and physiological adaptations (Kooyman & Ponganis 1998, Thorrold et al. 2014a) to facilitate foraging access. However, each species still has to balance the energy costs and gains (Thygesen et al. 2016) if they are to be effectively exploit deep-water resources.

5.3.2 Mammals and birds

The major adaptations deep diving species have developed are tied closely to their phylogeny. Air-breathing marine mammals and birds maximise their aerobic dive limit and breath hold-capacity to extend dive time. This is achieved physiologically by maximising oxygen storage with increased lung capacity, blood volume, haemoglobin concentration, myoglobin concentration, and muscle mass (Kooyman & Ponganis 1998). To reduce oxygen consumption, the mammalian dive response is triggered when submerged: bradycardia (slowed heart rate) sets in to reduce the cardiac output, blood pressure decreases as a result and vasoconstriction augments blood flow to the central nervous system and heart (Panneton 2013). To deal with the effects of pressure on internal air spaces, diving birds and mammals have flexible bones and tissue in their chest and structural modifications in their lungs to allow compression collapse of the air spaces at depth. This also helps to avoid N_2 absorption and subsequent gas bubble formation in blood and tissues (Kooyman & Ponganis 1998).

There are also thermoregulatory costs involved for mammals and birds diving into colder waters at depth. The animals have to balance metabolic rate, thickness of the lipid insulation layer, buoyancy, and body temperature (Butler 2004, Rosen et al. 2007). Tied to thermoregulation and diving capabilities is body size (Mori 2002). Animals with a larger mass have relatively lower mass-specific metabolic rates, larger thermal inertia and greater oxygen storage capabilities (Noren & Williams 2000). A relationship between deep diving and body sizes is apparent in intra- as well as inter-specific comparisons (Irvine et al. 2000, Fowler et al. 2007). An animal's adaption to dealing with the constraints of the mesopelagic zone is not the only factor influencing their diving behaviour. If comparable prey resources can be accessed for less energy output, for example in warmer water or at shallower depths, epipelagic predators may not have to go to their physiological limits (Schreer & Kovacs 1997, Croll et al. 2001, Carlson et al. 2014).

5.3.3 Fishes

Body size also influences diving capabilities in fishes, including dive frequency and duration (Schaefer et al. 2011). Diving duration is often closely tied to thermal inertia in ectothermic animals (Fuller et al. 2015), as bodily heat is lost quickly to water. During a dive to cooler water, fishes need to both conserve heat acquired in surface waters and ensure that vital organs (brain, eyes, and muscles) maintain functionality. Larger individuals lose heat slower, and can thus stay longer in cooler water. Apart from the general concept of thermal inertia, fishes also employ both physiological and behavioural strategies to handle low water temperatures at depth. Endothermy, the ability to maintain an elevated body temperature through internally, muscle-generated heat, is an unusual occurrence in teleost fishes. So far it has only been documented in tunas, mackerels (*Scombroidei*), billfishes (*Istiophoriformes*) and some elasmobranchs, all of which all have oxidative muscle tissue (heat producing red muscle) and achieve reductions in heat loss (Block & Finnerty 1994). Tunas warm their muscles, brain and viscera using counter-current heat exchangers in the circulation (Thygesen et al. 2016), while billfishes and mackerel have a thermogenic organ situated beneath the brain which heat their brain and retina (Block & Finnerty 1994, Schaefer et al. 2011, Chiang et al. 2014). Several families of elasmobranchs also employ analogous systems (Carlisle et al. 2011, Braun et al. 2014a, Thorrold et al. 2014), including lamnid (porbeagle, mackerel, salmon, and white sharks) and alopiid (thresher sharks) (Block & Finnerty 1994). Fishes also behaviourally thermoregulate to maintain body temperature. This includes basking behaviour in warmer surface water after deep dives (Dewar et al. 2011, Howey et al. 2016) and diving behaviour to cool themselves after feeding in warmer water

(Robinson et al. 2017). Additional physiological limitations imposed by hydrostatic pressure at depth can also restrict the vertical habitat range of fishes (Graham 2004, Howey et al. 2016). Teleost fish species with uncompressible gas-filled swim bladders save energy by maintaining neutral buoyancy but are markedly more affected by pressure changes than elasmobranchs which control their buoyancy instead with their fatty livers (Harden-Jones 1951, Denton & Marshall 1958). Tuna's have either small or no gas bladders which allows easier diving, but does require them to swim relatively rapidly to generate enough hydrodynamic lift to avoid sinking (Graham 2004). However for all fishes, increased hydrostatic pressure at depth can have significant affects on proteins and lipoprotein membranes causing reduced or loss of functionality which can lead to motor coordination impairment, paralysis and can affect cardiac function (Lalli & Parsons 2013, Brown 2014). As with decreased temperature, this increased stress to the cells increases metabolic demand (Brown & Thatje 2015), and the thermoregulatory adaptations mentioned above along with relatively large hearts and gill area help to meet high metabolic demands (Randall 1970, Brill 1996, Graham 2004). Intertwined with lower water temperatures are low oxygen conditions. In upwelling areas, at depth, and in the OMZ hypoxic conditions restrict the vertical distribution of fishes as the partial pressure of oxygen in the water decreases it is less readily taken up by the gills making it harder to meet the physiological demands which leads to either changes in activity, vertical or horizontal habitat changes, and if inescapable suffocation and death (Kramer 1987, Prince & Goodyear 2006, Chan et al. 2008). To counter this, the deepest diving tuna have blood that has a significantly higher affinity for oxygen than other tunas allowing them to be more tolerant of lower ambient oxygen conditions (Randall 1970, Lowe et al. 2000).

5.3.4 Reptiles

Reptiles have not been studied as much as diving birds and mammals, but they face the same challenges during deep dives: low temperatures, low dissolved oxygen concentrations, low light levels, and high pressures. Marine turtles spend as little as 3-6% of their life at the surface and thus have been referred to as 'surfacers' rather than 'divers' (Hochscheid et al. 1999, Lutz & Musick 2002). Marine reptiles overcome the challenges associated with deep diving in much the same way that marine mammals do, with efficient oxygen transport systems, tolerance of hypoxia, adjustable metabolism, and important respiratory adaptations (Andersen 1966, Lutz & Musick 2002). Their body plan also helps maximise their underwater efficiency, and the deepest diving marine turtles, leatherbacks (*Dermochelys coriacea*), can conserve body heat to allow them to access colder waters. It is their distinct morphology and physiology that allows leatherback turtles to dive uniquely deep for a marine reptile (Fossette et al. 2012). Their flexible carapace and plastron, and compressible trachea and lungs help to prevent nitrogen saturation (Fossette et al. 2010) similar to marine mammals. Aside from a counter-current heat exchanger in their flippers (Greer et al. 1973), leatherbacks have a mostly unexplained ability to thermoregulate (Bostrom et al. 2010). As a result they have very low thermal limit and consequently are the only marine turtle species to be found in cooler, temperate waters (Witt et al. 2007). There is a wide variation in diving behaviour within and across species, but aside from the unique leatherback, marine turtles generally use their adaptations for prolonging dive time (up to 5 hrs recorded), rather than dive depth, with most species remaining in the upper 20-50 m, which is congruent with their broad but predominantly epipelagic diets (Lutz & Musick 2002).

5.4 Classification

Observations of deep diving pelagic species have been heavily biased towards cetaceans and pinnipeds, that are relatively easy to tag when they surface or haul out (Watwood et al. 2006, Spitz et al. 2011, Woodworth et al. 2012, Filous et al. 2017), and commercially important fish species such as tuna (Walli et al. 2009, Goñi et al. 2011, Parrish et al. 2014, Pethybridge et al. 2014), billfishes (Dewar et al. 2011), other teleosts (Sepulveda et al. 2011, Merten et al. 2014, Hedger et al. 2017) and some elasmobranchs (Stevens et al. 2010, Cartamil et al. 2011). Many other sharks (Bonfil et al. 2005, Stevens et al. 2010), rays (Canese et al. 2011, Braun et al. 2014, Stewart et al. 2016), some sea birds (Pütz & Cherel 2005, Sleeman et al. 2010), and the occasional reptile (Okuyama et al. 2016) also enter the mesopelagic zone, but are less represented in studies to date.

Deep-diving species have global impact. Their habitats extend from the poles (Raclot & Groscolas 1998, Gallon et al. 2013) to temperate (Spitz et al. 2011) and tropical seas (Walli et al. 2009). They extend through the food web, and include apex predators (Bonfil et al. 2005) large planktivorous filter feeders (Brunnschweiler et al. 2009), and exploit a broad range of dietary resources (Chilvers et al. 2006, Williams et al. 2015). Body size ranges from the diminutive 5 kg Adélie penguin (Cottin et al. 2014), to the largest animal on the planet, the 122, 000 kg blue whale (Noren & Williams 2000, Croll et al. 2001). Many deep-diving species also have broad distributions, and are highly mobile both horizontally across ocean basins and vertically through the water column (Bonfil et al. 2005, Perrin 2009, Orlov & Baitalyuk 2014, Blanchet et al. 2015, Bauer et al. 2017).

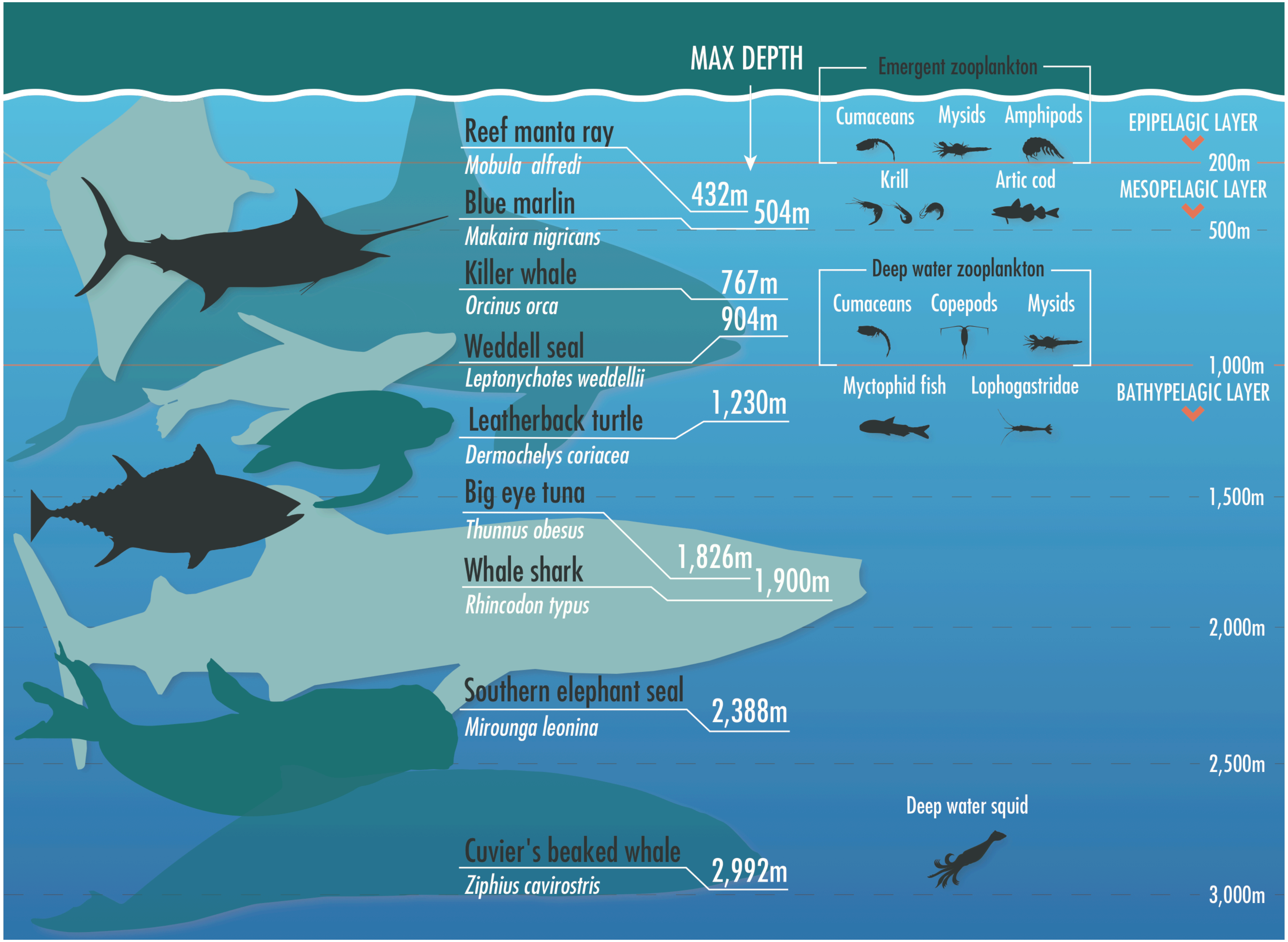

Figure 5.2 Infographic showing the maximum dive depth of some pelagic divers and prey items relative to the mesopelagic and bathypelagic layers.

We will survey epipelagic species, here defined as those species that must default back to waters shallower than 200m, the surface, on indeed the shore, for critical behaviours such as breathing air, reproduction, behavioural thermoregulation, and rest and recovery. Here ‘mesopelagic resources’ are defined as organisms that are part of the DSL, and or are predominantly considered resident to the mesopelagic layer (200 m – 1000 m), including those that consistently remain at depth, and those that undergo DVM. While this is a fluid concept, based on their usage of the mesopelagic resource, epipelagic species can be broadly categorised into one of four groups. (1) **Obligates**, to whom the resources in the mesopelagic layer are integral to their survival. (2) **Opportunists**, to whom the resources in the mesopelagic layer are somewhat important, but not an essential part of their survival. (3) **Transients**, which have been recorded in this layer, but infrequently or only for short amounts of time. (4) **Epipelagics**, who rarely dive below 200 m, but show evidence of tracking, and taking advantage of the DSL when it moves into the epipelagic zone.

Obligates are dominated by marine mammals and include some of the most well-known deep divers, such as elephant seals (*Mirounga leoninaare*) (Jouma’a et al. 2016), Cuvier’s beaked whales (*Ziphius cavirostris*) (Schorr et al. 2014), and sperm whales (*Physeter macrocephalus*) that extensively feed on deep-sea squid (Guerra et al. 2017). Obligates also include a few large fish species, including swordfish (Sepulveda et al. 2010), and the largest of the penguins (Kooyman & Kooyman 1995, Pütz & Cherel 2005). In these species, a large proportion of their dives enter the mesopelagic layer and are associated with foraging based on known diet (Dewar et al. 2011) and behavioural proxies that correlate with feeding behaviour (Gallon et al. 2013, Naito et al. 2013).

Southern elephant seals in the Southern Ocean, one of the most well-known deep-diving pinnipeds, feed on deep water squid and fishes and spend almost all their time whilst in the water diving, with up to half their diving time foraging in the DSL (Robinson et al. 2012). Southern elephant seals are dependent on small mesopelagic prey species (Naito et al. 2013). Swordfish (*Xiphias galdius*) are one of the few epipelagicteleosts that depend on mesopelagic resources and spend up to 85% of their deeper than 200 m in offshore waters in the Eastern Pacific (Dewar et al. 2011), though their diving behaviour varies temporally and spatially. Tracks of both species show minimal surface intervals or basking time between dives, leading to very distinctive dive profiles that indicate a heavy reliance on DSL resources. Emperor and king penguins (*Aptenodytes forsteri* and *A. patagonicusi*) are the largest of the penguin species, and the deepest diving birds (Kooyman & Kooyman 1995, Pütz & Cherel 2005). Although most of the time their dives do not extend beyond 200 m, their heavy dependence on myctophid fishes, squid, and deep water amphipods in their diet means they straddle the obligate and epipelagic category (Cherel & Kooyman 1998, Cherel et al. 2002).

Opportunists encompass many more pelagic species than obligates. There are several large elasmobranch species in this category (Gore et al. 2008, Brunnschweiler et al. 2009, Braun et al. 2014a), large endothermic teleosts (Walli et al. 2009, Gushchin & Corten 2017), some cetacean species of varying size (Ohizumi et al. 2003, Baumgartner et al. 2017) and pinnipeds (Villegas-Amtmann et al. 2008, Peterson et al. 2015). The dive profiles of these species show that most dives are restricted to the euphotic zone with occasional deep dives to, or through, the mesopelagic layer. Deep dives are followed by long intervals in surface waters, and bouts of deep dives often coincide with specific seasons or environmental conditions.

Whale sharks (*Rhincodon typus*) and large-bodied mobulid rays (*Mobula spp.*) dive into the mesopelagic layer to forage on mesopelagic fishes, shrimps, and zooplankton (Rohner et al. 2013, Thorrold et al. 2014, Tyminski et al. 2015, Marcus et al. 2016). Dive profiles of whale sharks indicate that they might, in addition to accessing the DSL in shallow water at night, also forage in the mesopelagic zone opportunistically while on longer movements where they increase their dive frequency and depth (Brunnschweiler et al. 2009). As well as the largest fish (whale shark), and the largest rays (*Mobula* spp.) on earth, the 'opportunist' category also includes the largest teleost, the ocean sunfish (*Mola mola*). Ocean sunfish target gelatinous zooplankton track seasonal upwelling fronts where their prey are most concentrated (Sims et al. 2009a, Dewar et al. 2010, Thys et al. 2015). Ocean sunfish dive deeper and more frequently during the day where they can engage in basking behaviour to thermoregulate (Dewar et al. 2010) and target the DSL for foraging.

Tuna and mackerel species are a uniquely endothermic group of fish species which target a wide variety of prey items in the DSL. As with other endothermic pelagic predators they need to thermoregulate and maintain high muscle temperatures. Thus they adapt the frequency, depth and daily and seasonal timing of their diving behaviour to optimise their energy gains (Bauer et al. 2017, Gushchin & Corten 2017). Atlantic bluefin tuna (*Thunnus thynnus*) dive more frequently, deeper, and more often during the daytime in the winter months, where the reverse is true in the summer. Their increased diving behaviour coincides with reduced water stratification and greater biological productivity, making diving less energetically costly and with a higher chance of foraging success (Bauer et al. 2017).

Bigeye tuna (*T. obesus*) diving and foraging strategy is likewise geared towards maximising energy harvest rate. As well as diel changes in diving behaviour, their diving strategy also changes as they grow and their foraging efficiency increases. Models predict that the optimal foraging strategy for larger bigeye tuna is to target the DSL during the day, where prey are abundant, and warm up at the surface between dives. Smaller tuna should adopt more constant depth strategies. These behaviours are mirrored in observed patterns (Thygesen et al. 2016).

Dall's porpoises (*Phocoenoides dalli*) switch their foraging strategy from epipelagic to mesopelagic resources depending on prey availability (Ohizumi et al. 2003). This seasonal, opportunistic behaviour represents many of the opportunistic marine mammal species (Burns et al. 2004, Villegas-Amtmann et al. 2008, Blanchet et al. 2015, Hedger et al. 2017). Opportunistic sharks also show seasonal patterns in diving, but are more influenced by water temperature and stratification than prey availability (Shepard et al. 2006, Carlisle et al. 2011, Carlson et al. 2014).

Conversely, mesopelagic resources play an important role for bluefin tuna during times of low productivity, increasing deep-diving behaviour while in areas where prey is scarce at the surface (Bauer et al. 2017). Bluefin tuna show increased dive depth during longer migrations to key feeding grounds, and opportunistically forage at depth on their way (Walli et al. 2009). Opportunistic use of mesopelagic resources in this way is also important to several other species (Bonfil et al. 2005, Campana et al. 2011), including the only true deep-diving reptile, the leatherback turtle (Fossette et al. 2012). Leatherbacks' deep dives occur predominantly while they target gelatinous zooplankton in the DSL during transit to foraging grounds, where they stay shallow and feed on scyphozooan jellyfish (Houghton et al. 2008, Fossette et al. 2010).

Transient species are a mixture of animal classes and sizes. Grey seals (*Halichoerus grypuin)* in Scotland occasionally dive into the mesopelagic zone, mostly during daylight hours when the DSL is at depth (Beck et al. 2003), but as their main fish and cephalopod prey items (Hammond & Wilson 2016) can all be found mostly in epipelagic waters (Bauchot 1987, Cohen et al. 1990) it is likely they only occasionally need to dive deeper to find other prey.

Black marlin (*Istiompax indica*), blue marlin (*Makaira nigricans*), and white marlin (*Kajikia albiida*) spend most of their time in the upper 100 m of the water column, with infrequent deep excursions (Braun et al. 2015, Carlisle et al. 2017, Vaudo et al. 2017). Although most billfish possess physical thermoregulatory abilities that would facilitate feeding in cooler waters, they show a strong thermal preference for water >22°C, and actively avoid regions with low dissolved oxygen. Both istiophorids and swordfish are visual predators, and while their characteristic large eyes may provide an advantage during low light level feeding, their deepest dives are predominantly during the day where they feed in the DSL. Their narrow thermal preference range restricts foraging dives into cooler deeper waters to being short and infrequent (Braun et al. 2015, Williams et al. 2017).

Oceanic whitetip sharks (*Carcharhinus longimanus*) also spend a small proportion of their dive time deeper than 200 m (Howey-Jordan et al. 2013b). They have unusual V-shaped dives but their slow ascent rates still suggest that one explanation for their deeper dives are might be foraging (Howey-Jordan et al. 2013). Unlike other large deep diving species, like whale sharks, Chilean devil rays, and tunas, their diving and swimming behaviour is wholly geared towards maximum energy efficiency (Papastamatiou et al. 2018). Oceanic whitetips are ectothermic, and predominantly use behavioural thermoregulation to manage their body temperature and so forays below the thermocline might be considered 'high risk' behaviour and are thus infrequent (Papastamatiou et al. 2018).

Epipelagics represent most of the diving bird species, smaller cetaceans and pinnipeds, and lunge-feeding baleen whales. Several of these species are concentrated at the poles. Antarctic penguin species (African, adéle, gentoo, chinstrap) rely on Antarctic krill (*Euphausia superba*) as the most important food resource, but as visual predators their foraging is governed by ambient light levels (Wilson et al. 1993). Although krill swim deeper during the day than at night, the penguins have greater foraging success during the day (Wilson et al. 1993), perhaps because the krill tend to form more dense schools at depth during daylight hours and twilight (Demar & Hewitt 1995). Antarctic krill are only occasionally found below 200 m depth (Siegel 2005). Penguins, along with other epipelagic species that rely on krill, such as some baleen whales (fin, minke, humpback) thus do not need to be deep divers to access their major prey resource. While most of their ecological impact is expected to be in the epipelagic zone, these species still play a role in the deep sea ecosystem as krill are important in the coupling of benthic and pelagic food webs (Schmidt et al. 2011) and baleen whales have been tied to the critical storage and cycling of iron in the Southern Ocean (Nicol et al. 2010).

Several fur seal species have the opposite diving strategy and concentrate their diving and foraging effort at night and target vertically migrating prey (Horning & Trillmich 1997, Francis & Boness 1998, Gallo-Reynoso et al. 2008). In the Galápagos, the DSL remains below 200 m during the day where it cannot be accessed by the Galápagos fur seals (*Arctocephalus galapagoensis*) (Horning & Trillmich 1997). At night however, the DSL is in shallower water when the fur seals dive up to a maximum of ~100m and use their exceptional eyesight and sensitive whiskers to target their prey (Horning & Trillmich 1999, Ulrike Griebel 2002). Even though Galápagos fur seals rarely dive below 200 m, their primary prey are myctophid and bathylaglid fishes and cephalopods. Their dive patterns are strongly affected by the seasonal lowering of the thermocline and lunar cycle decreasing food availability (Horning & Trillmich 1997, 1999).

5.5 Ecosystem effects

Deep-diving pelagics have multiple effects on mesopelagic ecosystems, and vice versa. By acting as vectors, deep-divers will play a role in active biological nutrient cycling connecting the surface to deeper water. Through their uptake of biomass at depth they transfer energy and materials into the euphotic zone from the mesopelagic (Estes et al. 2016). They will also transport this energy horizontally through the oceans, potentially across large distances (Estes et al. 2016), enhancing primary productivity (Roman & McCarthy 2010). Concurrently, deep-divers likely play a role in the biological active transport of carbon from surface waters to the mesopelagic for deep-storage. An important process balancing the biogeochemical carbon budgets, whilst simultaneously supporting the nutritional needs of a large demersal fish biomass (Trueman et al. 2014).

Predator-prey interactions of species in the mesopelagic with deep-divers will have a direct and indirect trophic effects, including consumption, risk avoidance behaviour, and competition (Estes et al. 2016). Myctophid fishes and deep-water cephalopods are the dominant prey choices for many deep-divers. Changes in the distribution and population numbers of either prey or predator will affect the other. Fisheries or conservation management will have to consider these interactions.

The extent and type of ecosystem influence will vary between the deep-diving categories presented here. Obligates make up a small proportion of all deep diving pelagics. However, given their intense diving and feeding regimes (Bailleul et al. 2010, Schorr et al. 2014, Guerra et al. 2017), they would have a disproportionately large effect on local nutrient cycling, and direct predation on mesopelagic species where they are present (Estes et al. 2016). They will also be the first and most acutely affected group by any environmental or anthropogenic changes in mesopelagic food abundance or location (Rodhouse 1990, Hindell et al. 2003).

Opportunists make up a larger proportion of deep diving species. While they might have a less acute influence on the mesopelagic layer locally, the variety of species, target prey, ecosystems, and seasonal timing of deeper diving in this group (Table 5.1) mean they would be expected to have a widespread effect on the mesopelagic. This would mostly include predation of mesopelagic resources in particular environmental conditions that promote energetically efficient deep-water feeding (Ohizumi et al. 2003, Bauer et al. 2017).

Transient species, though capable of accessing deep water resources, choose to limit the time they spend at depth as it is often not energetically possible, or efficient to do so. While they will have a smaller impact on nutrient cycling and trophic ecology at depth, they are at risk of being by-catch in mesopelagic fishing efforts and will be affected by future management of commercial deep-water fisheries (Dewar et al, 2011, Bianchi et al. 2013, Braun et al. 2015). Already in New Zealand sea lions (*Phocarctos hookeri*), resource competition with fisheries could be pushing the species into transient mesopelagic diving behaviour, beyond their aerobic limits, in marginal habitat (Chilvers et al. 2006, Leung et al. 2013, Meynier et al. 2014).

Some of the epipelagic species are visual hunters and diurnal divers and some are nocturnal divers and taking advantage of DVM to access prey. As they rarely enter the mesopelagic layer, if ever, their ecological impact is perhaps more indirect but disproportionately large, because of the sheer numbers of some epipelagic species (Wilson et al. 1991, Heise 1996) and the broad extent of other species (Jefferson & Curry 1994, Heise 1996, Perrin 2009).

5.6 Observations and key research questions

It is clear that mesopelagic resources play an important global role to many epipelagic species. Quantifying the importance of the mesopelagic layer to mobile pelagic species is challenging. This in part due to the fact that our current understanding of energy flux, biomass dynamics and trophic relationships within the mesopelagic is in it's infancy (Anderson et al. 2018). One method is to attach electronic sensors to individual animals and assess their behaviour and how much time they spend in the mesopelagic zone. Such tagging studies are often limited by low sample sizes due to high costs of tags (Canese et al. 2011), although the number of tagged animals is rising steeply. Equally, although accelerometers have improved interpretations of diving behaviour (Watwood et al. 2006, Gallon et al. 2013), without complementary diet studies these data are sometimes cautiously interpreted as proxy behaviours are used to assume foraging behaviour where perhaps energy conservation, efficient digestive absorption, or navigation may be the true motivations (Watwood et al. 2006, Walli et al. 2009, Gallon et al. 2013, Tyminski et al. 2015, Howey et al. 2016). Stomach content analysis leaves less room for interpretation, but is a snapshot of the most recent meal and has a tendency to overestimate the importance of prey with more indigestible body parts, such as fish bones, over soft-bodied prey such as jellyfishes (Goñi et al. 2011). Multi-disciplinary approaches (Raclot & Groscolas 1998, Banks et al. 2014) have provided more detailed and conclusive results over space and time (Potier et al. 2013) but it remains likely that the scale of interactions between deep divers and mesopelagic resources has so far been underestimated. Species that are logistically easier to tag and commercially important species have received a disproportionate amount of research efforts - many more species are likely to access and influence deep-water resources. Pelagic deep divers are therefore an underestimated and unquantified link between the mesopelagic and epipelagic layers.

We also advocate approaching this area of research another angle, where deep-diving animals need not be simply passive subjects of deep-sea research, but appreciate that their extensive physiological adaptations and behaviours mean they can be complicit in data collection (Lydersen et al. 2002, Costa et al. 2008, Bailleul et al. 2015). The continued use of diving predators as oceanographic sentinels, and use of autonomous 'drone' CTD's (Conductivity-Temperature Depth tags) has generated great swathes of data already (Treasure et al. 2017). In particular deep-divers can access remote areas and provide in-situ data, for example elephant seals fitted with Argos CTD-SRDL tags have successfully collected oxygen profiles in the Southern Ocean helping to monitor the decrease in oxygen concentrations, a major global concern, and provide essential information about the biological status of a remote area which would be near-impossible with research vessel surveys (Bailleul et al. 2015).

It is clear that mesopelagic resources can play an important role in the vertical dividing behaviour of many pelagic species, yet there is little available information on how this might affect their horizontal distribution and abundance.

The daytime depth of the DVM layer varies systematically over space and time reflecting variables like oxygen concentration and SST (Bianchi et al. 2013, Klevjer et al. 2016). Variability in daytime depth would also alter the proportional use of deep-diving resources for pelagic species, especially those with more limited access. For many opportunists mesopelagic resources are potentially important resources for long distance migrations, particularly when surface resources are scarce. During these longer movements then, access to mesopelagic resources could shape or influence migration patterns in pelagic opportunists. Whale sharks are found in relatively unproductive tropical and subtropical waters where their food is scarce and patchily distributed, so access to deep-water resources while searching for dense food patches is likely to be important. Their diving behaviour has been explored as a proportion of their overall behaviour, and spatially (ref). Separate to this their dietary input with regards to deep-water resources has been investigated. However this has occurred as point estimates – the two are likely to influence each other and this dimensional disconnect needs to be investigated. Therefore it would be prudent to explore the spatial distribution of deep-water resource acquisition.

There is already some evidence that deep-water (>200m) temperatures can affect the distribution and abundance of deep-diving fishes (Kleisner et al. 2010). Basking sharks, another opportunistic deep-diver and megaplanktivore also show variable diving profiles depending on their migration patterns. Those sharks that spent proportionally more time at depth were on longer seasonal migrations from Scotland to the warmer waters of the Iberian peninsula and North Africa (Doherty et al. 2017).

With the increasing interest in the deep-sea, suggest pelagic species should be considered and included in future research on mesopelagic food web biomass and structure, legislation of deep-water resources, and nutrient cycling.

There is a need for more research to be conducted on mesopelagic and DSL biomass estimates, particularly in temperate and polar seas where estimates are completely lacking at the moment (Webb et al. 2010). The role of pelagic species in the nutrient cycling between the surface and the mesopelagic appears to be much greater than previously thought (Roman & McCarthy 2010, Saba & Steinberg 2012) and is essential information for accurate assessments of how mesopelagic resources might be sustainably harvested (Anderson et al. 2018).

Deep-sea cephalopods play an important role in marine food webs, and are an important food source to many pelagic species (See Table 5.2 for refs). They are often poorly known as they have lower value in commercial fisheries but should be considered in future biomass and food web assessments (Spitz et al. 2011).

Few studies have looked into food web linkages within the mesopelagic zone (Petursdottir et al. 2008) and applied this to management of commercial species (Parrish et al. 2014). Information is lacking on how removal of pelagic species by overfishing might have top down effects on the mesopelagic food web, and indeed how removal of harvested mesopelagic species may affect the distribution and survival of pelagic species which rely on them (Robinson et al. 2010). This becomes particularly pertinent when considering threatened species which exploit the DSL (Rohner (Rohner et al. 2013).

Many of the species listed are endangered (Barceló et al. 2013, Francis et al. 2015, Giménez et al. 2018), are sensitive to climate change affecting their diving behaviour and subsequent survival and exposure to fisheries by catch (Dewar et al. 2011, Bianchi et al. 2013, Howey-Jordan et al. 2013b, Parrish et al. 2014, Braun et al. 2015, Brown & Thatje 2015) and some already use mesopelagic resources as a lifeline in scarce times (Merrick & Loughlin 1997). As such, pelagic deep divers should certainly be considered in future as more regulations of deep sea resources are debated and implemented (Clarke et al. 2015).

Common name	Species name	Class	Max (m)	Mean (m)	Motivation	Feeding Category	DVM?	Prey	Electro	Bio	Stomach	Location	Paper refs
King_penguins	*Aptenodyte_patagonicus*	Bird	343	55	Foraging	Obligate	Y	Myctophid fishes	Y	N	Y	Southern Ocean	Putz et al et al 2005; Bost et al 2015: Cherel 2002
Emperor_penguin	*Aptenodytes_forsteri*	Bird	534		Foraging	Obligate	Y	Antarctic silverfish, squid, krill	Y		Y	Antarctic	Kooyman & Kooyman 1995; Cherel & Kooyman 1998
Bigeye_thresher	*Alopias_superciliosus*	Elasmobranch	600			Obligate	Y		Y	N		Southeast Indian Ocean	Stevens et al 2010; Cartamil 2010
Pacific_sleeper_shark	*Somniosus pacificus*	Elasmobranch	2008	100-300	Foraging	Obligate		Squid, crustaceans, fishes			Y	North Pacific Ocean	Orlov & Baitalyuk 2014
Short_beaked_common_dolphin	*Delphinus_delphis*	Mammal	280	30-60	Foraging	Obligate		Myctophid fishes			Y	Alboran Sea	Gimenez et al 2018; Ponganis 2015
Harp_seal	*Phoca_groenlandica*	Mammal	568	50-300	Foraging	Obligate	Y	Polar cod, capelin, crustaceans	Y			Greenland Sea, Denmark Straight, Barents Sea	Folklow et al 2004; Ponganis 2015
Beluga_whale	*Delphinapterus_leucas*	Mammal	647	50-350	Foraging	Obligate		Capelin, arctic cod	Y	Y		Candian High Arctic	Martin & Smith 1999; Watt et al 2016; Citta et al 2013
Long_finned_pilot_whales	*Globicephala_melas*	Mammal	648		Foraging	Obligate	Y	Ceplopods	Y		Y	Norwegian Sea, Northeast Atlantic	Spitz et al 2011: Aoki et al 2013; Baird et al 2002
False_killer_whale	*Pseudorca_crassidens*	Mammal	650	50-650	Foraging	Obligate	Y	Mesopelagic fishes, squid	Y			Sea of Japan	Minamikawa et al., 2013
Killer_whale	*Orcinus _orca*	Mammal	767.5		Foraging	Obligate	Y	Cephalopods, patagonian toothfish	Y	N		Prince Edwards Islands in the Southern Ocean	Reisinger et al 2015
Ross_seal	*Ommatophoca_rossii*	Mammal	792	100–300	Foraging	Obligate	Y	Antarctis silverfish, squid, krill,myctophids	Y			Queen Maud Land, Antarctica	Blix et al 2007
Blainville's_beaked_whales	*Mesoplodon_densirostris*	Mammal	1599	835-1099	Foraging	Obligate	N	Squid	Y				Johnson et al 2004; Baird et al 2008
Sperm_whale	*Physeter_macrocephalus*	Mammal	2250	400-900	Foraging	Obligate	Y		Y	N		Atlantic Ocean; Tasman Sea	Guerra et al 2017; Watwood et al 2006; Jaquet et al 2000
Southern_Elephant_seal	*Mirounga_leonina*	Mammal	2388	269-552	Foraging	Obligate	Y		Y	Y		Southern Indian Ocean; Southern Ocean	Bailleul et al 2010; Jouma'a et al 2015
Cuvier's_beaked_whale	*Ziphius_cavirostris*	Mammal	2992	1070-1334	Foraging	Obligate	Y	Cephlopods	Y			Northeast Pacific, Mediterranean, Hawaii	Schorr et al 2014; Johnson et al 2004; Baird et al 2008
Weddell_seal	*Leptonychotes_weddellii*	Mammal	904	150-400	Foraging	Obligate	Y	Antarctic silverfish				Antarctic	Heerah et al 2013; Castellini et al 1992a;
Pilot_whale	*Globicephala sp.*	Mammal	1019	100-800	Foraging	Obligate	Y	Squid	Y			Mediterranean, Norewegian Sea	Baird et al 2002; Heide-jørgensen et al 2002
Baird's_beaked_whale	*Berardius_bairdii*	Mammal	1777	100-1500	Foraging	Obligate		Cephlopods, hake, rat-tails	Y		Y	Antarctic, Brazil, Japan	Ponganis 2015; MacLeod et al 2003; Minamikawa et al 2007
Hooded_seal	*Cystophora_cristata*	Mammal	>1016	100-600	Foraging	Obligate	Y	Squid, polar cod, capelin	Y		Y	Norwegian Sea, Greenland Sea	Folkow & Blix 1999; Haug et al
Pigmy sperm whale	*Kogia_breviceps*	Mammal	-	-	Foraging	Obligate	Y	Cephlopods, fishes, crustaceans					Scott et al 2001; Mcalpine 2009
Risso's_dolphin	*Grampus_griseus*	Mammal	-	-	Foraging	Obligate		Squid				North Pacific, South Pacific, North Atlantic	Wells et al 2009
True's_beaked_whale	*Mesoplodon_mirus*	Mammal	>700	-	Foraging	Obligate		Squid, mesopelagic fishes				Macaronesia, New Zealand, South Africa, Ireland	Hernandez-Milian et al 2017
Swordfish	*Xiphias_gladius*	Teleost_Fish	673	31-273	Foraging	Obligate	Y	Cephlopods, mesopelagic fishes	Y			Eastern North Pacific, Southern California Bight	Sepulveda et al 2010b
Bigeye_tuna	*Thunnus_obesus*	Teleost_Fish	1826		Foraging	Obligate	Y	Crustaceans	Y			Western Pacific, Eastern Pacific	Thygsen et al 2016; Fuller et al 2015; Musyl et al 2003
Northern _bottlenose_whale	*Hyperoodon_ampullatus*	Mammal	1483	800	Foraging	Obligate						Nova Scotia	Hooker & baird 1999; Ponganis 2015
Narwhal	*Monodon_monocerus*	Mammal	>1400		Foraging	Obligate		Polar cod, squid, shrimp, halibut	Y		Y	Candian High Arctic	Heide-Jørgensen et al 2003; Laodre et al 2003
Sowerby's_beaked_whale	*Mesoplodon_bidens*	Mammal	~1000		Foraging	Obligate		Mesopelagic & benthopelagic fishes, gadiformes			Y	North Atlantic	Spitz et al 2011; Wenzel et al 2013
Yellowfin_tuna	*Thunnus_albacares*	Teleost_Fish	1423		Foraging	Obligate	Y	Epipelagic fish, crustaceans, cephlopods	Y		Y	Baja California, Seychelles	Schaefer et al 2011; Dagorn et al 2006
Bowhead_whales	*Balaena_mysticetus*	Mammal	582	36-115	Foraging	Opportunistic		Calanoid copepods	Y		Y	Canadian High Arctic	
Caribbean_reef_shark	*Carcharhinus_perezi*	Elasmobranch	356		Foraging	Opportunistic	Y/N	Snapper, grouper	Y			West Caribbean Sea	Chapman et al 2007
Reef_manta_ray	*Mobula_alfredi*	Elasmobranch	432		Foraging	Opportunistic	Y	Plankton	Y	Y		Red Sea	Braun et al 2015;
Spiny_dogfish	*Squalus_acanthias*	Elasmobranch	481.5	25-100	Foraging	Opportunistic	Y	Squid, hake, ctenophores	Y			Northwestern Atlantic, Gulf of Maine, North Carolina	Carlson et al 2014
White_shark	*Carcharodon_carcharias*	Elasmobranch	980		Navigation/Foragir	Opportunistic		Fishes, marine mammals	Y			South Africa	Bonfil et al 2005
Porbeagle_shark	*Lamna_nasus*	Elasmobranch	1024	766.4	Foraging	Opportunistic	Y	Myctophids, cephlopods, flatfish	Y	N		New Zealand	Francis et al 2015; Joyce et al 2002
Salmon_shark	*Lamna_ditropis*	Elasmobranch	364) >700		Foraging	Opportunistic	Y	Salmon, herring	Y			Gulf of Alaska	Weng et al 2005; Carlisle et al 2011

Table 5.2 Table showing deep-pelagic dicing species. Maximum recorded dive depth (Max (m)), mean recorded diving depth (Mean(m)), their feeding category as described here, evidence of diel vertical migration diving behaviour, their recorded target prey types, they types of studies on each species including electronic tagging (Electro), biochemical analysis (Bio), and stomach contents analysis (Stomach), and the location and references of studies published.

Scalloped_hammerhead_shark	*Sphyrna_leweini*	Elasmobranch	>980		Foraging	Opportunistic		Squid, mesopelagic fishes	Y		Y	Gulf of California	Jorgensen 2009
Basking_shark	*Cetorhunus_maximus*	Elasmobranch	750-1000		Foraging	Opportunistic	Y	Zooplankton	Y			English Chennel	Sims et al 2003; Shepard et al 2006
Giant_devil_ray	*Mobula_mobular*	Elasmobranch	700		Foraging	Opportunistic	N	Plankton	Y			Mediterranean	Canese et al 2011;
Chilean_devil_ray	*Mobula tarapacana*	Elasmobranch	1896		Foraging	Opportunistic	Y	Plankton	Y	N		Central North Atlantic Ocean	Alexander et al 1996; Thorrold et al 2014
Short_fin_mako_shark	*Isurus _oxyrinchus*	Elasmobranch	620			Opportunistic	Y		Y	N		Southeast Indian Ocea, California Bight	Stevens et al 2010; Holts et al 1993
Thresher_shark	*Alopias _vulpinus*	Elasmobranch	640			Opportunistic	Y		Y	N		Southeast Indian Ocean	Stevens et al 2010; Cartamil 2011
Blue_shark	*Prionace _glauca*	Elasmobranch	1008	412	Foraging	Opportunistic	Y	squid and fish	Y	N		Southeast Indian Ocean, Northeast Atlantic Canadian High Arctic	Stevens et al 2010; Quieroz et al 2010; Campana et al 2011
Whale_shark	*Rhincodon_typus*	Elasmobranch	1900		Foraging	Opportunistic	Y	Plankton, mesopelagic fish	Y	Y	Y	Western Indian Ocean,	Brunnschweiler et al 2009; Tyminski et al 2015; Rohner et al 2013
Oceanic_manta_ray	*Mobula_birostris*	Elasmobranch	>750		Foraging	Opportunistic	Y		Y	Y	Y	Northeast Pacific; West Pacific	Stewart et al 2016; Burgess et al 2017
Harbour_seal	*Phoca_vitulina*	Mammal	481	5-100	Foraging	Opportunistic	Y	Cod, herring, sand lance	Y	N		Svalbard Archipelago,	Blanchet et al 2015
Dusky_dolphin	*Lagenorhynchus_obscurus*	Mammal	130	50-65	Foraging	Opportunistic		Myctophid fishes, small squid				Kaikoura Canyon, New Zealand	Benoit-Bird et al 2004,
Northern_right_whale	*Eubalaena_glacialis*	Mammal	174	121	Foraging	Opportunistic		Calanoid copepods; euphausiids	Y			Northwest Atlantic	Baumgartner & Mate 2003; Baumgartner et al 2017
Bryde's_whale	*Balaenoptera_edeni*	Mammal	292	40-200	Foraging	Opportunistic		Zooplankton	y			Gulf of Mexico	Soldevilla et al 2017; Alves et al 2010
Galapagos_sea_lion	*Zalophus_wollebaeki*	Mammal	387	50-150	Foraging	Opportunistic	Y	Myctophid fish	Y			Galapagos	Villegas-Amtmann et al 2008, 2010
Crabeater_seal	*Lobodon_carcinophagus*	Mammal	664	90	Foraging	Opportunistic	Y	Antarctic krill	Y			Marguerite Bay. Southern Ocean	Burns et al 2004
Northern_Elephant_seal	*Mirounga_angustirostris*	Mammal	1735	497	Foraging	Opportunistic	Y	myctophid and a bathylagid	Y	N		Southern Ocean; Northeast Pacific Ocean	Naito et al 2013; Peterson et al 2015; Robinson et al 2 Gallon et al 2013; Ferraro at al 2017
Dall's_porpoises	*Phocoenoides_dalli*	Mammal	-		Foraging	Opportunistic	Y	Myctophid fishes, gonatid squid			Y	Subarctic North Pacific, Bering Sea	Ohizumi et al 2003
Fin_whale	*Balaenoptera_physalus*	Mammal	>470	<100	Foraging	Opportunistic	Y	Euphausiids	Y			Mediterranean Sea; Gulf of California	Panigada et al 1999; Mate et al 2016; Croll et al 2001
Bottlenose_dolphins	*Tursiops_truncatus*	Mammal	>500	20	Foraging	Opportunistic	Y	Mesopelagic fish, squid					Corkeron & Martin 2004, Klatsky et al 2007
Short_finned_pilot_whale	*Globicephala_macrorhynchus*	Mammal	~800	685	Foraging	Opportunistic	Y		Y	N		Tenerife, Canary Islands	Jensen et al 2011
New_Zealand_sea_lion	*Phocarctos_hookeri*	Mammal	597	123	Foraging	Opportunistic	N	Benthic fishes, cephlopods, crustaceans,	Y		Y	Auckland Islands New Zealand	Chilvers et al 2006; Gales & Mattlin 1997
California_sea_lion	*Zalophus_californianus*	Mammal	>482	20-280	Foraging	Opportunistic	N	Squid, anchovy, epipelagic fishes	Y		Y	Baja California	Feldcamp et a 1989, Melin et al 2008
Hawiian_monk_seal	*Monachus_schauinslandi*	Mammal	>500	20-450	Foraging	Opportunistic	Y	Reef fishes, cephlopods, crustaceans	Y		Y	Hawaii	Parrish et al 2002; Goodman-Lowe 1998
Ribbon_seal	*Histriophoca_faciata*	Mammal	>600	200-600	Foraging	Opportunistic		Epipelagic and benthic fishes, cephlopods, crustaceans				Alaska, Bearing Sea, North Pacific Ocean	Ponganis 2015;
Leatherback_turtle	*Dermochelys_coriacea*	Reptile	1230	10 to 75m	Thermoregulate	Opportunistic	Y	Gelatinous plankton				Northwest Atlantic	Dodge et al 2014; James et al 2006; Okuyama et al 2016; Hays et al 2004
Skipjack_tuna	*Katsuwonus_pelamis*	Teleost_Fish	596	44	Foraging	Opportunistic	Y					Equatorial Pacific	Schaefer & Fuller 2007
Atlantic_salmon	*Salmo_salar*	Teleost_Fish	707	<10	Foraging	Opportunistic	Y		Y			Norwegian Sea	Hedger et al 2017
Bluefin_tuna	*Thunnus_thynnus*	Teleost_Fish	1217	32.5	Foraging	Opportunistic	Y	fish, squid crustaceans	Y	N		North Atlantic, Northwest Mediterranean	Walli et al 2009; Bauer et al 2017
Atlantic_horse_mackerel	*Trachurus_trachurus*	Teleost_Fish	-		Foraging	Opportunistic		Myctophid fishes			Y	Southeastern North Atlantic, Mauritania	Gushchin & Corten 2017
Cunene_horse_mackerel	*Trachurus_trecae*	Teleost_Fish	-		Foraging	Opportunistic		Myctophid fishes			Y	Southeastern North Atlantic, Mauritania	Gushchin & Corten 2017
Albacore_tuna	*Thunnus_alalunga*	Teleost_Fish	>1150	40-100	Foraging	Opportunistic		Crustaceans, fishes, molluscs, zoop, myctophids	Y	N		Northeast Pacific, South Pacific	Williams et al 2015; Childers et al 2011
Olive_ridley	*Lepidochelys_olivacea*	Reptile	290	20-50		Opportunistic							Lutz & Musick 2002
Hawksbill_turtle	*Eretmochelys_imbricata*	Reptile	<50	20-50		Opportunistic							Lutz & Musick 2002
Green_turtle	*Chelonia_mydas*	Reptile	110	20-50		Opportunistic							Lutz & Musick 2002
Ocean _sunfish	*Mola_mola*	Teleost_Fish	644	50	Foraging	Opportunistic	Y	Gelatinous zooplankton	Y			North east Atlantic, California bight, Japan	Simset al 2009, Dewar et al 2010, Thys et al 2015
Megamouth_shark	*Megachasma_pelagios*	Elasmobranch	166	20-140	Foraging	Transient	Y	Euphausiid shrimp	Y	Y	Y	Northwest Pacific	Nelson et al 1997; Compagno et al 2001; Moura et al 2015
Oceanic_whitetip shark	*Carcharhinus_longimanus*	Elasmobranch	1082	0-200	Foraging?	Transient	N					Bahamas Western North Atlantic	Howey 2016; Howey-Jordan et al 2013
Australian_fur_seal	*Arctocephalus_pusillus_doriferus*	Mammal	164	65-85	Foraging	Transient	Y		Y			Bass Strait, Australia	Arnould & Hindell 2001
Australian_sea_lion	*Neophoca_cinerea*	Mammal	200	60	Foraging	Transient			Y			Great Australian Bight, Australia	Costa et al 2003; Costa et al 2001; Fowler et al 2006

Antarctic_fur_seal	*Arctocephalus_gazella*	Mammal	240	30	Foraging	Transient	Y	Krill (Arctocephalus gazella)				South Georgia, Antarctic	Boyd & Croxall 1992
Grey_seal	*Halichoerus_grypus*	Mammal	436	10-120	Foraging	Transient		Sandeel, cod, ling	Y			Northern North Atlantic, Nova Scotia	Hammond & Wilson 2016; Beck et al 2003, Goulet et al 2001
Stellar_sea_lion	*Eumetopias_jubata*	Mammal	452	9-24	Foraging	Transient		Pollock, mackerel, cod, cephalopods				Alaska	Merrick & Loughlin 1997
Bearded_seal	*Erignathus_barbatus*	Mammal	480	10-60	Foraging	Transient		Cod, flatfish, invertebrates				Svalbard Arctic	Gjertz et al 2000; Kraftt et al 2000; Crawford et al 2015
Ringed_seal	*Phoca_hispida*	Mammal	500	20-100	Foraging	Transient		Polar cod, cepahlopods	Y			Baffin Bay; Greenland Sea	Born et al 2004; Gjertz et al 2000
Loggerhead_turtle	*Caretta_caretta*	Reptile	>340	10-100	Foraging?	Transient		Gelatinous zooplankton	Y			Southwestern Atlantic, Brazil, Uruguay, Japan	Barceló et al 2013
Wahoo	*Acanthocybium_solandri*	Teleost_Fish	253	18	Foraging	Transient	Y		Y			Eastern North Pacific, Baja California Sur	Sepulveda et al 2010a
Black_marlin	*Istiompax_indica*	Teleost_Fish	700	50-100	Stress	Transient	N		Y			Coral Sea Australia, Northwest Pacific	Williams 2017; Braun et al 2015; Chiang et al 2014; Gunn et al 2003
Dolphinfish	*Coryphaena_hippurus*	Teleost_Fish	255	<30	Foraging	Transient	Y	Mesopelagic fishes, invertebrates	Y			Western Central Atlantic	Merten et al 2014
Blue_marlin	*Makaira_nigricans*	Teleost_Fish	504	<100	Foraging	Transient	N	Squid, small pelagic fishes	Y			Hawaii, North Pacific Ocean	Carlisle et al 2017; Kerstetter et al 2003; Kraus & Rooker 2007; Block et al 1992
White_marlin	*Kajikia_albida*	Teleost_Fish	456	<100	Foraging	Transient	N	Dolphinfish, herring, scombrids				Atlantic Ocean	Vaudo et al 2017; Braun et al 2015
Tiger_shark	*Galeocerdo_cuvier*	Elasmobranch	920	<20	Foraging?	Transient	Y	Seabirds, cetaceans	Y		Y	East Australia	Holmes 2014
Little_auk	*Alle_alle*	Bird	59.5		Foraging	Epipelagic	Y	Zooplankton, copepods	y	N		East Greenland	Amélineau et al 2016; Harding et al 2009
Adélie_penguin	*Pygoscelis_adeliae*	Bird	111		Foraging	Epipelagic	Y	Antarctic krill	Y	Y		Dumont d'Urville in Adélie Land	Cottin et al 2014; Wilson et al 1993; Wilson et al 1991
African penguin	*Spheniscus_demersus*	Bird	130	0-30	Foraging	Epipelagic		Myctophid fishes, cape anchovy	Y		Y	Antarctica	Wilson et al 1993: Wilson et al 1985
Gentoo penguin	*Pygoscelis_papua*	Bird	212	20-30	Foraging	Epipelagic		Antarctic krill	Y		Y	Antarctica	Wilson et al 1993; Robinson & Hindell 1996
Chinstrap penguin	*Pyoscelis_antartica*	Bird	>240	10-45	Foraging	Epipelagic		Antarctic krill	Y		Y	Antarctica	Wilson et al 1993
Sei_whale	*Balaenoptera_borealis*	Mammal	57	17.9	Foraging	Epipelagic	Y	Copepods (Neocalanus spp)	Y			Japan North Pacific	Ishii et al 2017
Northern_Right_whale	*Eubalaena_ glacialis*	Mammal	175	120	Foraging	Epipelagic	Y	Copepods (Calanus finmarchicus)	Y			Nova Scotia	Baumgartner & Mate 2003; Nowacek et al 2001
Juan_Fernandez_fur_seal	*Arctocephalus_philippii*	Mammal	90	<10	Foraging	Epipelagic	Y	Myctophid fishes, cephalopods	Y		Y	Chile	Francis et al 1998
Minke whale	*Balaenoptera_acutorostrata*	Mammal	105	<50	Foraging	Epipelagic	Y	Krill	Y			Southern Ocean	Friedlaender et al 2014
Galapagos_fur_seal	*Arctocephalus_galapagoensis*	Mammal	115	26	Foraging	Epipelagic	Y	Myctophid fishes, cephalopods	Y			Galapagos	Horning & Trillmich 1997; Horning & Trillmich 1999
Guadalupe_fur_seal	*Arctocephalus_townsendi*	Mammal	130	10-20	Foraging	Epipelagic	Y	Squid, myctophid fish, epipelagic mackerel	Y		Y	Guadalupe Baja California	Gallo-Reynoso et al 2008; Lander et al 2000
South_American_fur_seal	*Arctocephalus_australis*	Mammal	170	20-60	Foraging	Epipelagic	Y	Cephlopods, various teleosts				Uruguay	Ponganis 2015; Naya 2002
Blue_whale	*Balaena_musculus*	Mammal	204	140	Foraging	Epipelagic	Y	Euphausiids	Y			Gulf of California	Croll et al 2001; Lagerquist et al 2000
South_African_fur_seal	*Arctocephalus_pusillus_pusillus*	Mammal	204	45	Foraging	Epipelagic						Soutern Africa	Ponganis 2015;
Sub-Antarctic_fur_seal	*Arctocephalus_tropicalis*	Mammal	208	10-30	Foraging	Epipelagic	Y	Myctophid fishes	Y			Amseterdam Island, Indian Ocean	Georges et al 2000a. 2000b; Beauplet et al 2004
Spotted_dolphin	*Stenella_attenuata*	Mammal	213	20-60	Foraging	Epipelagic	Y	Epipelagic teleosts, squid, crustaceans, mesopelagic fishes	Y			Hawaii	Baird et al 2001; Perrin et al 2009
Pacific_white_sided_dolphin	*Lagenorhynchus_obliquidens*	Mammal	215		Foraging	Epipelagic		Fishes, squid, crustaceans, mesopelagic fishes				Southern California, British Columbia	Ponganis 2015; Heise 1996
Harbor_porpoise	*Phocoena_phocoena*	Mammal	226	14-40	Foraging	Epipelagic	Y	Fishes, squid, crustaceans, mesopelagic fishes				Japan, Nova Scotia	Ponganis 2015; Otani et al 1998; Westgate et al 1995; Smith & Gaskin 1974; Jefferson & Curry 1994
Southern_sea_lion	*Otaria_flavescens*	Mammal	243	20-40	Foraging	Epipelagic	Y	Cephlopods				Falkland Islands	Thompson 1998
Northern_fur_seal	*Callorhinus_ursinus*	Mammal	256	65	Foraging	Epipelagic		Pollock, squid				Alaska	Sterling & Ream 2004; Gudmundson et al 2006; Ponganis 2015
New_Zealand_fur_seal	*Arctocephalus_forsteri*	Mammal	274	30-75	Foraging	Epipelagic	Y	Myctophids, red cod, squid				New Zealand	Mattlin et al 1998; Harcourt et al 2001
Leopard_seal	*Hydrurga_leptonyx*	Mammal	304	10-50	Foraging?	Epipelagic		Krill, crabeater seals, penguins				Antacrctica	Nordøy & Blix 2009; Krause 2016
Humpback whales	*Megaptera novaengliae*	Mammal	156	23-118	Foraging	Epipelagic	Y	Zooplankton (Krill)	Y	N		Central coast of California	Goldbogen et al 2008; Witteveen et al 2008

Chapter 6 Summary Conclusions

6.1 Summary of Findings

Whale sharks are the largest extant fish. They are an iconic, docile, and charismatic megafauna species. Yet whale sharks still face multiple threats worldwide, and the global population has been halved by over-exploitation in recent years (Pierce & Norman 2016). Many fundamental aspects of their biology and life-history remain poorly-known (Rowat & Brooks 2012, Pierce & Norman 2016). The identification of coastal aggregation sites for the species has provided an excellent opportunity for researchers to study whale sharks. This has led to thorough characterisation of aggregation demographics and visual observations of diurnal feeding behaviour at many of these sites (Wilson et al. 2005, Meekan et al. 2006, Rohner et al. 2011, 2015, Rowat et al. 2011, Robinson et al. 2013, Hueter et al. 2013). However, these sites are almost all biased towards juvenile male sharks, and their movements and habitat use when they are absent from these areas have received less attention. In this project the overarching aim was to integrate modern biochemical techniques with longer-term individual photo-identification data to provide a more holistic, time integrated insight of the habitat use and feeding habits of whale sharks – although similarly constrained to juvenile shark aggregations.

Chapter 2: I aimed to use the natural latitudinal isotopic gradients present across the Indian Ocean in conjunction with long-term photo-ID datasets to assess the connectivity between whale shark coastal aggregations sites. I set out to test the assumption that the whale shark aggregations in Tanzania, Mozambique, and Qatar comprised a single functional subpopulation as genetic data suggests.

I have demonstrated that we can use existing isotopic latitudinal gradients and bulk $\delta^{15}N$ and $\delta^{13}C$ values to provide new information about connectivity between whale shark aggregations. Data show that although there was high individual variation in the isotope profiles of sharks from each location, comparison with latitudinal isotope data suggest that sharks had shown site fidelity to each study area over the period of isotopic integration. I have also identified a potential area of resource sharing for Tanzanian and Mozambican sharks. Comparing whale shark data to existing $\delta^{13}C$ spatial models I have surmised that this is most likely in the productive upwelling areas in northern Mozambique. These data have led to the conclusion that the subpopulations addressed here exist over smaller geographical scales than previously thought. Conservation initiatives can therefore target local aggregations for regional results. This means that threat profiles and population trends should be assessed on a more local and / or regional basis, rather than ocean-basic scale.

Chapter 3: Following on from Chapter 2, I aimed to use both stable isotope analysis and fatty acid analysis simultaneously to take a more detailed look at the trophic ecology of whale sharks at Mafia Island. I aimed to explore what these methods could tell us about the importance of the whale sharks' seasonal inshore feeding to their overall dietary intake, and their true feeding ecology within in the context of the Mafia Island food web.

In this chapter I have demonstrated that both stable isotope and fatty acid analysis can be useful and informative tools for investigating whale shark trophic ecology. Each method revealing slightly different aspects of whale shark feeding behaviour. Stable isotope data suggest the sharks are foraging within the local food web where a wide range of isotopically different nutrient pathways available to them, corroborating results from Chapter 2.

By contrast, fatty acid analysis shows highly distinctive lipid class compositions for whale sharks, but also indicates a reliance on epipelagic prey. Fatty acid analysis was more sensitive to temporal dietary shifts than stable isotopes, and showed seasonal population-level responses to changing environmental conditions. Previously, anomalous fatty acid profiles in whale sharks have been interpreted as representing a major contribution of an unrecognised and unknown diet source. Here I proposed an alternative hypothesis, preferential dietary routing, which would suggest the fatty acid analysis results need to be interpreted with caution as they may not be a direct reflection of diet. Based on these results, I suggest that in future both methods be used in conjunction to more fully understand the feeding ecology of both whale sharks and other marine species.

Chapter 4: I aimed to use stable isotope analysis to more closely examine the trophic ecology of whale sharks within the food web in Tofo Beach, Mozambique. I set out to investigate whether the sharks were predominantly targeting coastal upwelling zones, or if there is any evidence of them feeding elsewhere.

Here I used stable isotope analysis and dietary mixing models to demonstrate that the whale sharks at Tofo Beach, Mozambique seem to be feeding predominantly on surface zooplankton both inshore and offshore. Isotope results do not preclude the possibility of contributions to diet from emergent and deep-water zooplankton and fishes. Whale sharks show a degree of feeding site fidelity to the area. This is consistent with previous tagging studies, and results from Chapter 2. Within year temporal variations at the population level suggests that ocean-scale dynamics are likely to play an important role in the whale shark abundance and sightings along the Inhambane coast.

The potential diet sources included in the mixing model analyses could not fully explain the observed whale shark isotopic compositions. This could be explained either by an un-sampled $\delta^{13}C$ source that is depleted in ^{13}C potentially offshore or oligotropic in nature, or the preferential routing of ^{13}C depleted lipids from zooplankton into the connective dermal tissue proteins.

Chapter 5: In this chapter I set out to explore and critically assess the current literature on the interactions of deep-diving pelagic species with mesopelagic resources.

Research and commercial interest in the deep ocean has exponentially grown over the past two decades. The deep scattering layer is already considered an economically and ecologically important resource. A diverse range of pelagic animals already exploit and rely on this resource, with species spanning multiple classes within the animal kingdom. Adaptations, usually congruent within each class, determine the potential diving capabilities and access each species has to the deep-water resources. These species have global reach and have a wide range of resultant ecosystem effects. Current knowledge likely underestimates the number of deep-diving pelagic species, and the extent to which they use mesopelagic food resources. I have argued that these deep-diving pelagic species should be included in future studies of mesopelagic food web biomass and structure and considered in legislation pertaining to deep-water resources.

With the threat of overfishing, increase deep-water fishing, climate change, and increasing oxygen minimum zones, many pelagic species are under threat. An increased understanding of pelagic species interaction with the deep scattering layer is critical information for and predicting their responses to change, and planning conservation strategies. Emerging technology for tracking and remote data collection and biochemical techniques will play an important role in improving this knowledge in the future.

6.2 Method Assessment and Work Going Forward

Whilst beyond the scope of this thesis, there are several areas of research that could be pursued in order to improve and develop the work presented here.

Currently one of the major barriers to further, more insightful isotope studies includes the lack of data and understanding of isoscapes in the Western Indian Ocean. Better characterisation of $\delta^{15}N$ $\delta^{13}C$ and also $\delta^{34}S$ surface isoscapes, isotopic depth gradients, and temporal changes in isotope values would allow more detailed and conclusive studies on whale shark movement and residency. These isoscapes could then be applied to any highly mobile marine species in the region. These data would help tackle outstanding information about pelagic species' behaviour as outlined in Chapter 5. While this is a huge undertaking, currently there is scarce isotopic data from this region on this spatial scale. Ideally 'on the ground' sampling from multiple trophic level species should be carried out to characterise the baseline isotopic conditions. There is potential in utilising future cruises running from ports in South Africa and Madagascar through Mozambique Channel up to Tanzania to sample multiple species, and perhaps build up a temporal dataset

A more inclusive latitudinal stable isotope studies should be undertaken with all the known aggregation sites along the east coast of Africa and the Arabian region. This would allow further the understanding of connectivity between all the major whale shark sites, and add to the current knowledge about their connectivity from long-term global photo-ID datasets. This would provide a more explicit and larger scale picture of the interactions and potential resource sharing of whale sharks in this ocean basin.

There was some isotopic evidence of sex-and size-related dietary shifts in whale sharks throughout this study (Chapters 2-4). As previously discussed here (Chapters 2-4), this could be the result of either a shift in target, or a spatial shift. Results presented here suggest that larger sharks are feeding at higher trophic levels, and / or deeper in the water column, and potentially a lower latitudes (Borrell et al. 2011, Marcus et al. 2016). Results from females suggests they are likely feeding more often offshore, and potentially a lower trophic levels. The results presented here were inconclusive. Although, given the absence of larger sharks and females from coastal feeding areas it seems likely that such a difference exists.

One of the main limitations throughout this study has been the assumptions and lack of knowledge about whale shark specific tissue fractionation and lipid pathways, and the mechanisms of preferential routing as outlined in Chapter 3. A future focus of the global whale shark community should be to prioritise what are historically quite rare stranding events to sample multiple tissue types within individuals. Calculating specific tissue offsets and comparing lipid profiles of multiple sharks will provide much needed clarification about biochemical pathways in this species, and may shed light on the similarly unique species of manta rays.

Chapter 5: highlights the need for a greater understanding of pelagic species' feeding and movement ecology. Especially those that are wide ranging and deep-diving, like whale sharks, as they can have broad ecological impacts.

Overall I have demonstrated that stable isotope analysis is a useful and flexible tool that can be used in multiple ways to investigate the trophic ecology of a mobile marine animal. In this context stable isotope analysis is best used in conjunction with other methods (Chapters 2 & 3) and prior knowledge to infer more conclusive and meaningful insights. Here I have presented novel understanding of both the feeding ecology, and the movement and residency of whale sharks in the western Indian Ocean and Arabian Gulf (Chapters 2-4). I have applied this across large spatial and temporal scales (Chapter 2), and within local food webs (Chapters 2 & 4).

When used as a complementary technique stable isotopes stable isotopes provide researchers with a useful tool for spatial and trophic ecology studies. I believe with additional isoscape data and more a refined understanding of biochemical pathways stable isotope analysis will be able to provide clearer, more detailed insights.

Bibliography

Abrantes K, Barnett a (2011) Intrapopulation variations in diet and habitat use in a marine apex predator, the broadnose sevengill shark Notorynchus cepedianus. Mar Ecol Prog Ser 442:133–148

Aca EQ, Schmidt J V (2011) Revised size limit for viability in the wild : Neonatal and young of the year whale sharks identified in the Philippines. 20:361–367

Acuña-Marrero D, Jiménez J, Smith F, Doherty PF, Hearn A, Green JR, Paredes-Jarrín J, Salinas-de-León P (2014) Whale shark (Rhincodon typus) seasonal presence, residence time and habitat use at darwin island, galapagos marine reserve. PLoS One 9:e115946

Akhilesh K V. Shanis CPR, White WT, Manjebrayakath H, Bineesh KK, Ganga U, Abdussamad EM, Gopalakrishnan A, Pillai NGK (2013) Landings of whale sharks Rhincodon typus Smith, 1828 in Indian waters since protection in 2001 through the Indian Wildlife (Protection) Act, 1972. Environ Biol Fishes 96:713–722

Amélineau F, Grémillet D, Bonnet D, Bot T Le, Fort J (2016) Where to Forage in the Absence of Sea Ice? Bathymetry As a Key Factor for an Arctic Seabird. PLoS One 11:1–19

Andersen HT (1966) Physiological adaptations in diving vertebrates. Physiol Rev 46:212–243

Anderson TR, Martin AP, Lampitt RS, Trueman CN, Henson SA, Mayor DJ (2018a) Quantifying carbon fluxes from primary production to mesopelagic fish using a simple food web model Thomas. ICES J Mar Sci

Anderson TR, Martin AP, Lampitt RS, Trueman CN, Henson SA, Mayor DJ (2018b) Quantifying carbon fluxes from primary production to mesopelagic fish using a simple food web model. ICES J Mar Sci fsx234

Anderson C, Waheed A (2001) The economics of shark and ray watching in the Maldives. Shark News 13:1–3

Andrzejaczek S, Meeuwig J, Pierce S, Davies T, Fisher R, Meekan M (2016) The ecological connectivity of whale shark aggregations in the Indian Ocean : a photo-identification approach. R Soc Open Sci 3:160455

Aoki K, Sakai M, Miller PJO, Visser F, Sato K (2013) Body contact and synchronous diving in long-finned pilot whales. Behav Processes 99:12–20

Araujo G, Lucey A, Labaja J, So CL, Snow S, Ponzo A (2014) Population structure and residency patterns of whale sharks, *Rhincodon typus*, at a provisioning site in Cebu, Philippines. PeerJ 2:e543

Armstrong AO, Armstrong AJ, Jaine FRA, Couturier LIE, Fiora K, Uribe-Palomino J, Weeks SJ, Townsend KA, Bennett MB, Richardson AJ (2016) Prey Density Threshold and Tidal Influence on Reef Manta Ray Foraging at an Aggregation Site on the Great Barrier Reef. PLoS One 11:e0153393

Arnould JPY, Hindell M a. (2001) Dive behaviour, foraging locations, and maternal-attendance patterns of Australian fur seals (Arctocephalus pusillus doriferus). Can J Zool 79:35–48

Arzoumanian Z, Holmberg J, Norman B (2005) An astronomical pattern-matching algorithm for computer-aided identification of whale sharks *Rhincodon typus*. J Appl Ecol 42:999–1011

Bailleul F, Cotté C, Guinet C (2010) Mesoscale eddies as foraging area of a deep-diving predator, the southern elephant seal. Mar Ecol Prog Ser 408:251–264

Bailleul F, Vacquie-Garcia J, Guinet C (2015) Dissolved oxygen sensor in animal-borne instruments: An innovation for monitoring the health of oceans and investigating the functioning of marine ecosystems. PLoS One 10:1–14

Baird RW, Borsani JF, Hanson MB, Tyack PL (2002) Diving and night-time behavior of long-finned pilot whales in the Ligurian sea. Mar Ecol Prog Ser 237:301–305

Baird RW, Ligon AD, Hooker SK, Gorgone AM (2001) Subsurface and nighttime behaviour of pantropical spotted dolphins in Hawai'i. Can J Zool 79:988–996

Banks J, Lea M-A, Wall S, McMahon CR, Hindell M a. (2014) Combining bio-logging and fatty acid signature analysis indicates spatio-temporal variation in the diet of the southern elephant seal, *Mirounga leonina*. J Exp Mar Bio Ecol 450:79–90

Barceló C, Domingo A, Miller P, Ortega L, Giffoni B, Sales G, McNaughton L, Marcovaldi M, Heppell SS, Swimmer Y (2013) High-use areas, seasonal movements and dive patterns of juvenile loggerhead sea turtles in the Southwestern Atlantic Ocean. Mar Ecol Prog Ser 479:235–250

Bauer RK, Fromentin J-M, Demarcq H, Bonhommeau S (2017) Habitat use, vertical and horizontal behaviour of Atlantic bluefin tuna (*Thunnus thynnus*) in the Northwestern Mediterranean Sea in relation to oceanographic conditions. Deep Sea Res Part II Top Stud Oceanogr 141:248–261

Baumgartner MF, Mate BR (2003) Summertime foraging ecology of North Atlantic right whales. Mar Ecol Prog Ser 264:123–135

Baumgartner MF, Wenzel FW, Lysiak NSJ, Patrician MR (2017) North Atlantic right whale foraging ecology and its role in human-caused mortality. Mar Ecol Prog Ser 581:165–181

Beauplet G, Dubroca L, Guinet C, Cherel Y, Dabin W, Gagne C, Hindell M (2004) Foraging ecology of subantarctic fur seals *Arctocephalus tropicalis* breeding on Amsterdam Island: seasonal changes in relation to maternal charcteristics and pup growth. Mar Ecol Progr Ser 273:211–225

Beck CA, Don Bowen W, McMillan JI, Iverson SJ (2003a) Sex differences in the diving behaviour of a size-dimorphic breeder: the grey seal. Anim Behav 66:777–789

Beck CA, Don Bowen W, McMillan JI, Iverson SJ (2003b) Sex differences in diving at multiple temporal scales in a size-dimoprhic capital breeeder. J Anim Ecol 72:979–993

Beckley LE, Cliff G, Smale MJ, Compagno LJV (1997) Recent strandings and sightings of whale sharks in South Africa. Environ Biol Fishes 50:343–348

Bell JG, Sargent JR (2003) Arachidonic acid in aquaculture feeds: current status and future opportunities. Aquaculture 218:491–499

Bell M, Tocher D (2009) Biosynthesis of polyunsaturated fatty acids in aquatic ecosystems: General pathways and new directions (M Arts, M.T. Brett, M. Kainz, Ed.). Springer-Verlag

Benoit-Bird KJ, Würsig B, McFadden CJ (2004) Dusky Dolphin (*Lagenorhynchus obscurus*) Foraging in Two Different Habitats: Active Acoustic Detection of Dolphins and Their Prey. Mar Mammal Sci 20:215–231

Berumen ML, Braun CD, Cochran JEM, Skomal GB, Thorrold SR (2014) Movement patterns of juvenile whale sharks tagged at an aggregation site in the Red Sea. PLoS One 9

Best PB, Schell DM (1996) Stable isotopes in southern right whale (*Eubalaena australis*) baleen as indicators of seasonal movements, feeding and growth. Mar Biol 124:483–494

Bhaskar N, Kazuo M, Masashi H (2004) Comparative Evaluation of Fatty Acid Composition of Different Sargassum (*Fucales, Phaeophyta*) Species Harvested from Temperate and Tropical Waters. J Aquat Food Prod Technol 13:53–70

Bianchi D, Galbraith ED, Carozza D a. Mislan K a. S, Stock C a. (2013) Intensification of open-ocean oxygen depletion by vertically migrating animals. Nat Geosci 6:545–548

Blanchet MA, Lydersen C, Ims RA, Kovacs KM (2015) Seasonal, oceanographic and atmospheric drivers of diving behaviour in a temperate seal species living in the high arctic. PLoS One 10:1–28

Blix a S, Folkow B (1983) Cardiovascular adjustments to diving in mammals ans birds. Handb Physiol Cardiovasc Syst Peripher Circ organ blood flow Bethesda, MD Am Physiol Soc, sect 2, vol 3, part 2:917–945

Block BA, Booth DT, Carey FG (1992) Depth and temperature of the blue marlin, *Makaira nigricans*, observed by acoustic telemetry. Mar Biol 114:175–183

Block BA, Finnerty JR (1994) Endothermy in fishes: a phylogenetic analysis of constraints, predispositions, and selection pressures. Environ Biol Fishes 40:283–302

Block B a, Jonsen ID, Jorgensen SJ, Winship a J, Shaffer S a, Bograd SJ, Hazen EL, Foley DG, Breed G a, Harrison a-L, Ganong JE, Swithenbank a, Castleton M, Dewar H, Mate BR, Shillinger GL, Schaefer KM, Benson SR, Weise MJ, Henry RW, Costa DP (2011) Tracking apex marine predator movements in a dynamic ocean. Nature 475:86–90

Bonfil R, Meyer M, Scholl MC, Johnson R, O'Brien S, Oosthuizen H, Swanson S, Kotze D, Paterson M (2005) Transoceanic migration, spatial dynamics, and population linkages of white sharks. Science (80-) 310:100–103

Born EW, Teilmann J, Acquarone M, Riget FF (2004) Habitat use of ringed seals (*Phoca hispida*) in the north water area (North Baffin Bay). Arctic 57:129–142

Borrell A, Aguilar A, Gazo M, Kumarran RP, Cardona L (2011) Stable isotope profiles in whale shark (*Rhincodon typus*) suggest segregation and dissimilarities in the diet depending on sex and size. Environ Biol Fishes 92:559–567

Borrell A,. Cardona L, Kumarran RP, Aguilar a. (2010) Trophic ecology of elasmobranchs caught off Gujarat, India, as inferred from stable isotopes. ICES J Mar Sci 68:547–554

Bost CA, Cotté C, Terray P, Barbraud C, Bon C, Delord K, Gimenez O, Handrich Y, Naito Y, Guinet C, Weimerskirch H (2015) Large-scale climatic anomalies affect marine predator foraging behaviour and demography. Nat Commun 6

Bostrom BL, Jones TT, Hastings M, Jones DR (2010) Behaviour and physiology: The thermal strategy of leatherback turtles. PLoS One 5:1–9

Boyd IL, Croxall JP (1992) Diving behaviour of lactating Antarctic fur seals. Can J Zool 70:919–928

Braun CD, Kaplan MB, Horodysky AZ, Llopiz JK (2015) Satellite telemetry reveals physical processes driving billfish behavior. Anim Biotelemetry 3

Braun CD, Skomal GB, Thorrold SR, Berumen ML (2014a) Diving behavior of the reef manta ray links coral reefs with adjacent deep pelagic habitats. PLoS One 9:1–8

Braun CD, Skomal GB, Thorrold SR, Berumen ML (2014b) Diving behavior of the reef manta ray links coral reefs with adjacent deep pelagic habitats. PLoS One 9:e88170

Brierley AS (2014) Quick guide Diel vertical migration. Curr Biol 24:1074–1076

Brill RW (1996) Selective Advantages Confered by the hight performance physiology of tunas, buillfish and dolphin fish Brill.pdf. Comp Biochem Physiol 113A:3–15

Brooks K, Rowat D, Pierce SJ, Jouannet D, Vely M, Al KBET (2010) Seeing Spots : Photo-identification as a Regional Tool for whale shark Identification Study area. 9:185–194

Brown A. (2014) The role of hydrostatic pressure in constraining the bathymetric distribution of marine ectotherms. University of Southampton.

Brown A, Thatje S (2015) The effects of changing climate on faunal depth distributions determine winners and losers. Glob Chang Biol 21:173–180

Brunnschweiler JM, Baensch H, Pierce SJ, Sims DW (2009) Deep-diving behaviour of a whale shark Rhincodon typus during long-distance movement in the western Indian Ocean. J Fish Biol 74:706–14

Brunnschweiler JM, Pratt HL (2008) Putative Male – Male Agonistic Behaviour in Free-Living Zebra Sharks , *Stegostoma fasciatum*. Science (80-):23–27

Buckland ST, Garthwaite PH (1991) Quantifying Precision of Mark-Recapture Estimates Using the Bootstrap and Related Methods. Biometrics 47:255–268

Budge SM, Iverson SJ, Bowen WD, Ackman RG (2002) Among- and within-species variability in fatty acid signatures of marine fish and invertebrates on the Scotian Shelf , Georges Bank , and southern Gulf of St . Lawrence. 898:886–898

Budge SM, Iverson SJ, Koopman HN (2006) Studying Trophic Ecology in Marine Ecosystems Using Fatty Acids: a Primer on Analysis and Interpretation. Mar Mammal Sci 22:759–801

Buesseler KO, Lamborg CH, Boyd PW, Lam PJ, Trull TW, Bidigare RR, Bishop JKB, Casciotti KL, Dehairs F, Elskens M, Honda M, Karl DM, Siegel D A, Silver MW, Steinberg DK, Valdes J, Mooy B Van, Wilson S (2007) Revisiting Carbon Flux Through the Ocean ' s

Twilight Zone. Science (80-) 316:567–570

Burgess KB, Bennett MB (2016) Effects of ethanol storage and lipid and urea extraction on δ15N and δ13C isotope ratios in a benthic elasmobranch, the bluespotted maskray *Neotrygon kuhlii*. J Fish Biol:1–7

Burgess KB, Couturier LIE, Marshall AD, Richardson AJ, Weeks S, Bennett MB (2016) *Manta birostris*, predator of the deep? Insight into the diet of the giant manta ray through stable isotope analysis. R Soc Open Sci

Burgess KB, Guerrero M, Marshall AD, Richardson J, Bennett MB, Couturier LIE (2018) Novel signature fatty acid profile of the giant manta ray suggests reliance on an uncharacterised mesopelagic food source low in polyunsaturated fatty acids. 13

Burgess KB, Guerrero M, Richardson AJ, Bennett MB, Marshall AD (2018) Use of epidermal mucus in elasmobranch stable isotope studies: A pilot study using the giant manta ray (*Manta birostris*). Mar Freshw Res 69:336–342

Burns JM, Costa DP, Fedak MA, Hindell MA, Bradshaw CJA, Gales NJ, McDonald B, Trumble SJ, Crocker DE (2004) Winter habitat use and foraging behavior of crabeater seals along the Western Antarctic Peninsula. Deep Res Part II Top Stud Oceanogr 51:2279–2303

Butler PJ (2004) Metabolic regulation in diving birds and mammals. Respir Physiol Neurobiol 141:297–315

Cagua EF, Cochran JEM, Rohner CA, Prebble CEM, Sinclair-taylor TH, Pierce SJ, Berumen ML (2015) Acoustic telemetry reveals cryptic residency of whale sharks. Biol Lett 11:20150092

Cagua EF, Collins N, Hancock J, Rees R (2014) Whale shark economics: a valuation of wildlife tourism in South Ari Atoll, Maldives. PeerJ 2:e515

Campana SE, Dorey A, Fowler M, Joyce W, Wang Z, Wright D, Yashayaev I (2011) Migration pathways, behavioural thermoregulation and overwintering grounds of blue sharks in the Northwest Atlantic. PLoS One 6

Campell N, Neat F, Burns F, Kunzlik P (2011) Species richness, taxonomic diversity, and taxonomic distinctness of the deep-water demersal fish community on the Northeast Atlantic continental slope ICES Subdivision Vla. ICES J Mar Sci 68:365–376

Canese S, Cardinali a, Romeo T, Giusti M, Salvati E, Angiolillo M, Greco S (2011) Diving behavior of the giant devil ray in the Mediterranean Sea. Endanger Species Res 14:171–176

Capone DG (1997) Trichodesmium, a globally significant marine cyanobacterium. Science (80-)

276:1221–1229

Carlisle AB, Kim SL, Semmens BX, Madigan DJ, Jorgensen SJ, Perle CR, Anderson SD, Chapple TK, Kanive PE, Block B a (2012) Using Stable Isotope Analysis to Understand the Migration and Trophic Ecology of Northeastern Pacific White Sharks (*Carcharodon carcharias*). PLoS One 7:e30492

Carlisle AB, Kochevar RE, Arostegui MC, Ganong JE, Castleton M, Schratwieser J, Block BA (2017) Influence of temperature and oxygen on the distribution of blue marlin (*Makaira nigricans*) in the Central Pacific. Fish Oceanogr 26:34–48

Carlisle AB, Perle CR, Goldman KJ, Block BA, Jech JM (2011) Seasonal changes in depth distribution of salmon sharks (*Lamna ditropis*) in Alaskan waters: implications for foraging ecology. Can J Fish Aquat Sci 68:1905–1921

Carlson AE, Hoffmayer ER, Tribuzio CA, Sulikowski JA (2014) The use of satellite tags to redefine movement patterns of spiny dogfish (*Squalus acanthias*) along the U.S. east coast: Implications for fisheries management. PLoS One 9

Cartamil DP, Sepulveda CA, Wegner NC, Aalbers SA, Baquero A, Graham JB (2011) Archival tagging of subadult and adult common thresher sharks (*Alopias vulpinus*) off the coast of southern California. Mar Biol 158:935–944

Castell J, Blair T, Neil S, Howes K, Mercer S, Reid J, Young-Lai W, Gullison B, Dhert P, Sorgeloos P (2003) The effect of different HUFA enrichment emulsions on the nutritional value of rotifers (*Brachionus plicatilis*) fed to larval haddock (*Melanogrammus aeglefinus*). Aquac Int 11:109–117

Castellini MA, Kooyman GL, Ponganis PJ (1992) Metabolic Rates of Freely Diving Weddell Seals - Correlations with Oxygen Stores, Swim Velocity and Diving Duration. J Exp Biol 165:181–194

Castro a LF, Stewart BS, Wilson SG, Hueter RE, Meekan MG, Motta PJ, Bowen BW, Karl S a (2007) Population genetic structure of Earth's largest fish, the whale shark (*Rhincodon typus*). Mol Ecol 16:5183–92

Caut S, Angulo E, Courchamp F (2009) Variation in discrimination factors (δ15N and δ13C): The effect of diet isotopic values and applications for diet reconstruction. J Appl Ecol 46:443–453

Chan F, Barth JA, Lubchenco J, Kirincich A, Weeks H, Peterson WT, Menge BA (2008) Emergence of anoxia in the California current large marine ecosystem. Science (80-) 319:920

Chapman DD, Feldheim KA, Papastamatiou YP, Hueter RE (2015) There and Back Again: A review of residency and return migrations in sharks, with implications for population structure and management. Ann Rev Mar Sci 7:547–570

Chapman DD, Pikitch EK, Babcock EA, Shivji MS (2007) Deep-diving and diel changes in vertical habitat use by Caribbean reef sharks *Carcharhinus perezi*. Mar Ecol Prog Ser 344:271–275

Che-tsung C, Kwang-Ming L, Shoon-Jeng J (1997) Preliminary report in Taiwan's whale shark fishery. TRAFFIC Bull 17:53–57

Cherel Y, Corre M, Jaquemet S, Ménard F, Richard P, Weimerskirch H (2008) Resource partitioning within a tropical seabird community: new information from stable isotopes. Mar Ecol Prog Ser 366:281–291

Cherel Y, Hobson K (2007) Geographical variation in carbon stable isotope signatures of marine predators: a tool to investigate their foraging areas in the Southern Ocean. Mar Ecol Prog Ser 329:281–287

Cherel Y, Kooyman GL (1998) Food of emperor penguins (*Aptenodytes forsteri*) in the western Ross Sea, Antarctica. Mar Biol 130:335–344

Cherel Y, Putz K, Hobson KA (2002) Summer diet of king penguins (*Aptenodytes patagonicus*) at the Falkland Islands, southern Atlantic Ocean. Polar Biol 25:898–906

Chiang W-C, Musyl MK, Sun C-L, DiNardo G, Hung H-M, Lin H-C, Chen S-C, Yeh S-Z, Chen W-Y, Kuo C-L (2014) Seasonal movements and diving behaviour of black marlin (*Istiompax indica*) in the northwestern Pacific Ocean. Fish Res

Childerhouse S, Dix B, Gales N (2001) Diet of New Zealand sea lions (*Phocarctos hookeri*) at the Auckland Islands. Wildl Res 28:291–298

Chilvers BL, Wilkinson IS, Duignan PJ, Gemmell NJ (2006) Diving to extremes: Are New Zealand sea lions (*Phocarctos hookeri*) pushing their limits in a marginal habitat? J Zool 269:233–240

Christiansen H, Fisk A, Hussey N (2015) Incorporating stable isotopes into a multidisciplinary framework to improve data inference and their conservation and management application. African J Mar Sci 37:189–197

Ciach M (2015) Rapid decline of an isolated population of the black grouse *Tetrao tetrix*: the crisis at the southern limit of the range. Eur J Wildl Res 61:623–627

Citta JJ, Suydam RS, Quakenbush LT, Frost KJ, O'Corry Crowe GM (2013) Dive behaviour of eastern Chukchi beluga whales (*Delphinapterus leucas*) 1998-2008. Arctic 66:389–406

Clarke J, Milligan RJ, Bailey DM, Neat FC (2015) A Scientific Basis for Regulating Deep-Sea Fishing by Depth. Curr Biol 25:2425–2429

Cliff G, Anderson-Reade MD, Aitken AP, Charter GE, Peddemors VM (2007) Aerial census of whale sharks (*Rhincodon typus*) on the northern KwaZulu-Natal coast, South Africa. Fish Res 84:41–46

Clingham E, Brown J, Henry L, Beard A, Dove AD (2016) Evidence that St. Helena island is an important multi-use habitat for whale sharks, *Rhincodon typus*, with the first description of putative mating in this species. PeerJ Prepr 4:e1885v1:1–21

Clingham E, Webb HD, De R, Venegas P, Schreiber C, Reid J, Pierce S, Hueter R, Hindle K, Henry L, Beard A, Dove ADM (2016) Further evidence of the importance of St . Helena as habitat for whale sharks. Q Sci:5339

CMS (2017) Convention on migratory species: proposal for inclusion of the whale shark (*Rhincodon typus*) on Appendix I of the convention. In: 12th Meeting of the conference of the parties Manila, Philippines, 23 - 28 October 2017.p UNEP/CMS/COP12/Doc.25.1.20

Cochran JEM, Hardenstine RS, Braun CD, Skomal GB, Thorrold SR, Xu K, Genton MG, Berumen ML (2016) Population structure of a whale shark *Rhincodon typus* aggregation in the Red Sea. J Fish Biol 89:1570–1582

Colman J (1997) A review of the biology and ecology of the whale shark. J Fish Biol:1219–1234

Copeman LA, Parrish CC, Brown JA, Harel M (2002) Effects of docosahexaenoic, eicosapentaenoic, and arachidonic acids on the early growth, survival, lipid composition and pigmentation of yellowtail flounder (*Limanda ferruginea*): A live food enrichment experiment. Aquaculture 210:285–304

Copping JP, Stewart BD, McClean CJ, Hancock J, Rees R (2018) Does bathymetry drive coastal whale shark (*Rhincodon typus)* aggregations? PeerJ 6:e4904

Cordier M, Brichon G, Weber J-M, Zwingelstein G (2002) Changes in the fatty acid composition of phospholipids in tissues of farmed sea bass (*Dicentrarchus labrax*) during an annual cycle. Roles of environmental temperature and salinity. Comp Biochem Physiol Part B Biochem Mol Biol 133:281–288

Corkeron PJ, Martin AR (2004) Ranging and diving behaviour of two offshore bottlenose dolphins, Tursiops sp. off eastern Australia. J Mar Biol Assoc UK 84:465–468

Cortes E (1999) Standardized diet compositions and trophic levels of sharks. ICES J Mar Sci 56:707–717

Costa DP, Breed G a. Robinson PW (2011) New Insights into Pelagic Migrations: Implications for Ecology and Conservation. Annu Rev Ecol Evol Syst 43:120830113150004

Costa DP, Gales NJ, Goebel ME (2001) Aerobic dive limit: How often does it occur in nature? Comp Biochem Physiol - A Mol Integr Physiol 129:771–783

Costa DP, Klinck JM, Hofmann EE, Dinniman MS, Burns JM (2008) Upper ocean variability in west Antarctic Peninsula continental shelf waters as measured using instrumented seals. Deep Res Part II Top Stud Oceanogr 55:323–337

Cottin M, MacIntosh AJJ, Kato A, Takahashi A, Debin M, Raclot T, Ropert-Coudert Y (2014) Corticosterone administration leads to a transient alteration of foraging behaviour and complexity in a diving seabird. Mar Ecol Prog Ser 496:249–262

Couturier LIE, Rohner C a, Richardson AJ, Marshall AD, Jaine FR a, Bennett MB, Townsend K a, Weeks SJ, Nichols PD (2013) Stable isotope and signature Fatty Acid analyses suggest reef manta rays feed on demersal zooplankton. PLoS One 8:e77152

Couturier LIE, Rohner CA, Richardson AJ, Pierce SJ, Marshall AD, Jaine FRA, Townsend KA, Bennett MB, Weeks SJ, Nichols PD (2013) Unusually high levels of n-6 polyunsaturated fatty acids in whale sharks and reef manta rays. Lipids 48:1029–1034

Croll DA, Acevedo-Gutiérrez A, Tershy BR, Urbán-Ramírez J (2001) The diving behavior of blue and fin whales: Is dive duration shorter than expected based on oxygen stores? Comp Biochem Physiol - A Mol Integr Physiol 129:797–809

Dagorn L, Holland K, Hallier J-P, Taquet M, Moreno G, Sancho G, Itano D, Aumeeruddy R, Girard C, Million J, Fonteneau A (2006) Deep Diving Behavior Observed in Yellowfin Tuna (*Thunnus Albacares*). Aquat Living Resour 19:85–88

Dalsgaard J, John M St. Kattner G, Müller-Navarra D, Hagen W (2003) Fatty acid trophic markers in the pelagic marine environment. Adv Mar Biol 46:225–340

Dalsgaard J, St John M, Kattner G, Müller-Navarra D, Hagen W (2003) Fatty acid trophic markers in the pelagic marine environment. Adv Mar Biol 46:225–340

Danovaro R, Company JB, Corinaldesi C, D'Onghia G, Galil B, Gambi C, Gooday AJ, Lampadariou N, Luna GM, Morigi C, Olu K, Polymenakou P, Ramirez-Llodra E, Sabbatini A, Sardá F, Sibuet M, Tselepides A (2010) Deep-sea biodiversity in the Mediterranean Sea:

The known, the unknown, and the unknowable. PLoS One 5

Davenport SR, Bax NJ (2002) A trophic study of a marine ecosystem off southeastern Australia using stable isotopes of carbon and nitrogen. Can J Fish Aquat Sci 59:514–530

Davis D, Banks S, Birtles A, Valentine P, Cuthill M (1997) Whale sharks in Ningaloo Marine Park: managing tourism in an Australian marine protected area. Tour Manag 18:259–271

Davison P, Lara-Lopez A, Anthony Koslow J (2015) Mesopelagic fish biomass in the southern California current ecosystem. Deep Res Part II Top Stud Oceanogr 112:129–142

Dawson MN (2012) Species richness, habitable volume, and species densities in freshwater, the sea, and on land. Front Biogeogr 4:105–116

Demar D, Hewitt R (1995) Bias in acoustic biomass estimates of deep scatterign layer southern ocean diel vertical migration. Deep Res Part I 42:455–475

Denton EJ, Marshall NB (1958) The buoyancy of bathypelagic fishes without a gas-filled swimbladder. J Mar Biol Assoc United Kingdom 37:753–767

Dewar H, Prince ED, Musyl MK, Brill RW, Sepulveda C, Luo J, Foley D, Orbesen ES, Domeier ML, Nasby-Lucas N, Snodgrass D, Michael Laurs R, Hoolihan JP, Block B A,. Mcnaughton LM (2011) Movements and behaviors of swordfish in the Atlantic and Pacific Oceans examined using pop-up satellite archival tags. Fish Oceanogr 20:219–241

Dewar H, Thys T, Teo SLH, Farwell C, O'Sullivan J, Tobayama T, Soichi M, Nakatsubo T, Kondo Y, Okada Y, Lindsay DJ, Hays GC, Walli A, Weng K, Streelman JT, Karl SA (2010) Satellite tracking the world's largest jelly predator, the ocean sunfish, Mola mola, in the Western Pacific. J Exp Mar Bio Ecol 393:32–42

Dodge KL, Galuardi B, Miller TJ, Lutcavage ME (2014) Leatherback turtle movements, dive behavior, and habitat characteristics in ecoregions of the Northwest Atlantic Ocean. PLoS One 9

Doherty PD, Baxter JM, Gell FR, Godley BJ, Graham RT, Hall G, Hall J, Hawkes LA, Henderson SM, Johnson L, Speedie C, Witt MJ (2017) Long-term satellite tracking reveals variable seasonal migration strategies of basking sharks in the north-east Atlantic. Sci Rep 7:1–10

Dubois M, Rossi V, Ser-Giacomi E, Arnaud-Haond S, López C, Hernández-García E (2016) Linking basin-scale connectivity, oceanography and population dynamics for the conservation and management of marine ecosystems. Glob Ecol Biogeogr 25:503–515

Duffy C (2002) Distribution, seasonality, lengths, and feeding behaviour of whale sharks

(*Rhincodon typus*) observed in New Zealand waters. New Zeal J Mar Freshw Res 36:565–570

Eckert SA, Stewart BS (2001) Telemetry and satellite tracking of whale sharks, *Rhincodon Typus*, in the Sea of Cortez , Mexico , and the North Pacific Ocean. Environ Biol Fishes

Emata AC, Ogata HY, Garibay ES, Furuita H (2003) Advanced broodstock diets for the mangrove red snapper and a potential importance of arachidonic acid in eggs and fry. Fish Physiol Biochem 28:489–491

Escalle L, Pennino MG, Gaertner D, Chavance P, Delgado de Molina A, Demarcq H, Romanov E, Merigot B (2016) Environmental factors and megafauna spatio-temporal co-occurrence with purse-seine fisheries. Fish Oceanogr 25:433–447

Estes JA, Heithaus M, McCauley DJ, Rasher DB, Worm B (2016) Megafaunal Impacts on Structure and Function of Ocean Ecosystems.

Feldkamp SD, Delong RL, Antonelis CA (1989) Diving patterns of California sea lions, Zalophus californianus. Can J Zool 67:872–883

Ferraro MS, Decker RR, Costa DP, Robinson PW, Houser DS, Crocker DE (2017) Evaluating gain functions in foraging bouts using vertical excursions in northern elephant seals. Anim Behav 129:15–24

Filous A, Friedlander A, Wolfe B, Stamoulis K, Scherrer S, Wong A, Stone K, Sparks R (2017) Movement patterns of reef predators in a small isolated marine protected area with implications for resource management. Mar Biol 164

Folch J, Lees M, Sloane-Stanley GH (1957) A simple method for the isolation and purification of total lipids from animal tissues. J Biol Chem 226:497–509

Folkow LP, Blix AS (1999) Diving behaviour of hooded seals (*Cystophora cristata*) in the Greenland and Norwegian Seas. Polar Biol 22:61–74

Folkow LP, Nordøy ES, Blix AS (2004) Distribution and diving behaviour of harp seals (*Pagophilus groenlandicus*) from the Greenland Sea stock. Polar Biol 27:281–298

Fossette S, Gleiss AC, Myers AE, Garner S, Liebsch N, Whitney NM, Hays GC, Wilson RP, Lutcavage ME (2010) Behaviour and buoyancy regulation in the deepest-diving reptile: the leatherback turtle. J Exp Biol 213:4074–4083

Fossette S, Putman N, Lohmann K, Marsh R, Hays G (2012) A biologist's guide to assessing ocean currents: a review. Mar Ecol Prog Ser 457:285–301

Fowler SL, Costa DP, Arnould JPY, Gales NJ, Burns JM (2007) Ontogeny of oxygen stores and physiological diving capability in Australian sea lions. Funct Ecol 21:922–935

Fox S, Foisy I, La Parra Venegas R De, Galván Pastoriza BE, Graham RT, Hoffmayer ER, Holmberg J, Pierce SJ (2013) Population structure and residency of whale sharks *Rhincodon typus* at Utila, Bay Islands, Honduras. J Fish Biol 83:574–87

Francis J, Boness D (1998) A protracted foraging and attendance cycle in female Juan Fernandez fur seals. Soc Mar Mammal 14:552–574

Francis MP, Holdsworth JC, Block BA (2015) Life in the open ocean: seasonal migration and diel diving behaviour of Southern Hemisphere porbeagle sharks (*Lamna nasus*). Mar Biol 162:2305–2323

Friedlaender AS, Goldbogen JA, Nowacek DP, Read AJ, Johnston D, Gales N (2014) Feeding rates and under-ice foraging strategies of the smallest lunge filter feeder, the Antarctic minke whale (*Balaenoptera bonaerensis*). J Exp Biol 217:2851–2854

Fuller DW, Schaefer KM, Hampton J, Caillot S, Leroy BM, Itano DG (2015) Vertical movements, behavior, and habitat of bigeye tuna (*Thunnus obesus*) in the equatorial central Pacific Ocean. Fish Res 172:57–70

Gales NJ, Mattlin RH (1997) Summer diving behaviour of lactating New Zealand sea lions, *Phocarctos hookeri*. Can J Zool 75:1695–1706

Galil BS (2006) Rhopilema nomadica. Delivering Alien Invasive Species Inventories Europe.

Gallo-Reynoso JP, Figueroa-Carranza AL, LeBoeuf BJ (2008) Foraging behavior of lactating Guadalupe fur seal females. Av en el Estud los Mamíferos México 2:595–614

Gallon S, Bailleul F, Charrassin JB, Guinet C, Bost CA. Handrich Y, Hindell M (2013) Identifying foraging events in deep diving southern elephant seals, *Mirounga leonina*, using acceleration data loggers. Deep Sea Res Part II Top Stud Oceanogr 88–89:14–22

Gaye B, Nagel B, Dähnke K, Rixen T, Emeis KC (2013) Evidence of parallel denitrification and nitrite oxidation in the ODZ of the Arabian Sea from paired stable isotopes of nitrate and nitrite. Global Biogeochem Cycles 27:1059–1071

Georges JY, Bonadonna F, Guinet C (2000) Foraging habitat and diving activity of lactating subantarctic fur seals in relation to sea-surface temperatures at Amsterdam Island. Mar Ecol Prog Ser 196:291–304

Georges J-Y, Tremblay Y, Guinet C (2000) Seasonal diving behaviour in lactating subantarctic fur seals on Amsterdam Island. Polar Biol 23:59–69

Giménez J, Marçalo A, García-Polo M, García-Barón I, Castillo JJ, Fernández-Maldonado C, Saavedra C, Santos MB, Stephanis R de (2018) Feeding ecology of Mediterranean common dolphins: The importance of mesopelagic fish in the diet of an endangered subpopulation. Mar Mammal Sci 34:136–154

Gjertz I, Kovacs KM, Lydersen C, Wiig (2000) Movements and diving of bearded seal (*Erignathus barbatus*) mothers and pups during lactation and post-weaning. Polar Biol 23:559–566

Gleiss AC, Norman B, Wilson RP (2011) Moved by that sinking feeling: variable diving geometry underlies movement strategies in whale sharks. Funct Ecol 25:595–607

Goericke R, Fry B (1994) Variations of marine plankton δ^{13} C with latitude, temperature, and dissolved CO_2 in the world ocean. Global Biogeochem Cycles 8:85–90

Goldbogen JA, Calambokidis J, Croll DA, Harvey JT, Newton KM, Oleson EM, Schorr G, Shadwick RE (2008) Foraging behavior of humpback whales: kinematic and respiratory patterns suggest a high cost for a lunge. J Exp Biol 211:3712–3719

Goñi N, Logan J, Arrizabalaga H, Jarry M, Lutcavage M (2011) Variability of albacore (*Thunnus alalunga*) diet in the Northeast Atlantic and Mediterranean Sea. Mar Biol 158:1057–1073

Goodman-Lowe GD (1998) Diet of the Hawaiian monk seal (*Monachus schauinslandi*) from the Northwestern Hawaiian Islands during 1991 to 1994. Mar Biol 132:535–546

Gore M a, Rowat D, Hall J, Gell FR, Ormond RF (2008) Transatlantic migration and deep mid-ocean diving by basking shark. Biol Lett 4:395–8

Goulet AM, Hammill MO, Barrette C (2001) Movements and diving of grey seal females (*Halichoerus grypus*) in the Gulf of St. Lawrence, Canada. Polar Biol 24:432–439

Graham JB (2004) Tuna comparative physiology. J Exp Biol 207:4015–4024

Graham BS, Koch PL, Newsome SD, Mcmahon KW, Aurioles D, Wunder MB (2010) Using isoscapes to trace the movements and foraging behavior of top predators in oceanic ecosystems. In: West JB, Bowen GJ, Dawson TE, Tu KP (eds) Springer Netherlands, Dordrecht, p 299–318

Graham RT, Roberts CM (2007) Assessing the size, growth rate and structure of a seasonal population of whale sharks (*Rhincodon typus* Smith 1828) using conventional tagging and photo identification. Fish Res 84:71–80

Graham RT, Roberts CM, Smart JCR (2006) Diving behaviour of whale sharks in relation to a predictable food pulse. J R Soc Interface 3:109–16

Greer AE, Lazell JD, Wright RM (1973) Anatomical evidence for a countercurrent heat exchanger in the leatherback turtle, (*Dermochelys coriacea*). Nature 244:181

Gudmundson C, Zeppelin T, Ream R (2006) Application of two methods for determining diet of northern fur seals (*Callorhinus ursinus*). Fish Bull 104:445–455

Guerra M, Hickmott L, Hoop J van der, Rayment W, Leunissen E, Slooten E, Moore M (2017) Diverse foraging strategies by a marine top predator: Sperm whales exploit pelagic and demersal habitats in the Kaikōura submarine canyon. Deep Res Part I Oceanogr Res Pap 128:98–108

Gunn JS, Patterson TA, Pepperell JG (2003) Short-term movement and behaviour of black marlin Makaira indica in the Coral Sea as determined through a pop-up satellite archival tagging experiment. Mar Freshw Res 54:515–525

Gunn JS, Stevens JD, Davis TLO, Norman BM (1999) Observations on the short-term movements and behaviour of whale sharks (*Rhincodon typus*) at Ningaloo Reef, Western Australia. Mar Biol 135:553–559

Gushchin A V. Corten A (2017) Feeding of pelagic fish in waters of Mauritania: 3.—Atlantic Chub mackerel Scomber colias, Atlantic horse mackerel *Trachurus trachurus*, Cunene horse mackerel *Trachurus trecae*. J Ichthyol 57:410–423

Hammerschlag N, Gallagher AJ, Lazarre DM (2011) A review of shark satellite tagging studies. J Exp Mar Bio Ecol 398:1–8

Hammond PS, Wilson LJ (2016) Grey Seal Diet Composition and Prey Consumption. Scottish Mar Freshw Sci 7:1–28

Harcourt RG, Bradshaw CJA, Davis LS (2001) Summer foraging behaviour of a generalist predator, the New Zealand fur seal (*Arctocephalus forsteri*). Wildl Res 28:599–606

Harden-Jones BYFR (1951) THE SWIMBLADDER AND THE VERTICAL MOVEMENTS OF TELEOSTEAN FISHES I. Physical Factors. Biol Rev:553–566

Harding AMA, Egevang C, Walkusz W, Merkel F, Blanc S, Grémillet D (2009) Estimating prey capture rates of a planktivorous seabird, the little auk (*Alle alle*), using diet, diving behaviour, and energy consumption. Polar Biol 32:785–796

Haren H van, Compton TJ (2013) Diel vertical migration in deep sea plankton is finely tuned to latitudinal and seasonal day length. PLoS One 8:e64435

Haskell PJ, McGowan A, Westling A, Méndez-Jiménez A, Rohner C a. Collins K, Rosero-Caicedo M, Salmond J, Monadjem A, Marshall AD, Pierce SJ (2014) Monitoring the effects of tourism on whale shark Rhincodon typus behaviour in Mozambique. Oryx:1–8

Haug T, Nilssen KT, Lindblom L, Lindstrøm U (2007) Diets of hooded seals (*Cystophora cristata*) in coastal waters and drift ice waters along the east coast of Greenland. Mar Biol Res 3:123–133

Hays GC (2003) A review of the adaptive significance and ecosystem consequences of zooplankton diel vertical migrations. Hydrobiologia 503:163–170

Hays GC, Bradshaw CJ a. James MC, Lovell P, Sims DW (2007) Why do Argos satellite tags deployed on marine animals stop transmitting? J Exp Mar Bio Ecol 349:52–60

Hays GC, Ferreira LC, Sequeira AMM, Meekan MG, Duarte CM, Bailey H, Bailleul F, Bowen WD, Caley MJ, Costa DP, Eguíluz VM, Fossette S, Friedlaender AS, Gales N, Gleiss AC, Gunn J, Harcourt R, Hazen EL, Heithaus MR, Heupel M, Holland K, Horning M, Jonsen I, Kooyman GL, Lowe CG, Madsen PT, Marsh H, Phillips RA, Righton D, Ropert-Coudert Y, Sato K, Shaffer SA, Simpfendorfer CA, Sims DW, Skomal G, Takahashi A, Trathan PN, Wikelski M, Womble JN, Thums M (2016) Key Questions in Marine Megafauna Movement Ecology. Trends Ecol Evol 31:463–475

Hays GC, Houghton JDR, Myers AE (2004) Pan-Atlantic leatherback turtle movements. Nature 429:522

Hearn AR, Román JGMH, Marrero DA (2016) Adult female whale sharks make long - distance movements past Darwin Island (Galapagos, Ecuador) in the Eastern Tropical Pacific. Mar Biol

Hedger RD, Rikardsen AH, Strøm JF, Righton DA, Thorstad EB, Næsje TF (2017) Diving behaviour of Atlantic salmon at sea: Effects of light regimes and temperature stratification. Mar Ecol Prog Ser 574:127–140

Heerah K, Andrews-Goff V, Williams G, Sultan E, Hindell M, Patterson T, Charrassin JB (2013) Ecology of Weddell seals during winter: Influence of environmental parameters on their foraging behaviour. Deep Res Part II Top Stud Oceanogr 88–89:23–33

Heide-jørgensen MP, Bloch D, Stefansson E, Mikkelsen B, Ofstad LH (2002) Diving behaviour of long-finned pilot whales *Globicephala melas* around the Faroe Islands. Wildl Biol 8:307–313

Heide-Jørgensen MP, Dietz R, Laidre KL, Richard P, Orr J, Schmidt HC (2003) The migratory behaviour of narwhals (*Monodon monoceros*). Can J Zool 81:1298–1305

Heise K. (1996) Life history parmeters of the pacific white-sided dolphin. The University of Guelph

Hernandez-Milian G, Lusher A, O'Brian J, Fernandez A, O'Connor I, Berrow S, Rogan E (2017) New information on the diet of True's beaked whale (*Mesoplodon mirus*, Gray 1850), with insights into foraging ecology on mesopelagic prey. Mar Mammal Sci 33:1245–1254

Hessen DO, Leu E (2006) Trophic transfer and trophic modification of fatty acids in high Arctic lakes. Freshw Biol 51:1987–1998

Heyman WD, Carr LM, Lobel PS (2010) Diver ecotourism and disturbance to reef fish spawning aggregations: It is better to be disturbed than to be dead. Mar Ecol Prog Ser 419:201–210

Heyman W, Graham R, Kjerfve B, Johannes R (2001) Whale sharks *Rhincodon typus* aggregate to feed on fish spawn in Belize. Mar Ecol Prog Ser 215:275–282

Higgs ND, Gates AR, Jones DOB (2014) Fish food in the deep sea: revisiting the role of large food-falls. PLoS One 9:e96016

Hindell MA, Bradshaw CJA, Sumner MD, Michael KJ, Burton HR (2003) Dispersal of female southern elephant seals and their prey consumption during the austral summer: Relevance to management and oceanographic zones. J Appl Ecol 40:703–715

Hobson K a. (1999) Tracing origins and migration of wildlife using stable isotopes: a review. Oecologia 120:314–326

Hobson K, Piatt J, Pitocchelli J (1994) Using stable iotopes to determine seabird trophic relationships. :786–798

Hobson K a, Welch HE (1992) Determination of trophic relationships within a high Arctic marine food web using $\delta^{13}C$ and $\delta^{15}N$ analysis. Mar Ecol Prog Ser 84:9–18

Hochscheid S, Godley BJ, Broderick AC, Wilson RP (1999) Reptilian diving: Highly variable dive patterns in the green turtle *Chelonia mydas*. Mar Ecol Prog Ser 185:101–112

Holmberg J, Norman B, Arzoumanian Z (2009) Estimating population size, structure, and residency time for whale sharks *Rhincodon typus* through collaborative photo-identification. Endanger Species Res 7:39–53

Holmes BJ, Pepperell JG, Griffiths SP, Jaine FRA, Tibbetts IR, Bennett MB (2014) Tiger shark

(*Galeocerdo cuvier*) movement patterns and habitat use determined by satellite tagging in eastern Australian waters. Mar Biol 161:2645–2658

Holtsa DB, Bedfordb DW (1993) Horizontal and Vertical Movements of the Shortfin Mako Shark , *Isurus oxyrinchus*, in the Southern California Bight. Aust J Mar Freshw Res 44:901–909

Hooker SK, Baird RW (1999) Deep-diving behaviour of the northern bottlenose whale, *Hyperoodon ampullatus* (*Cetacea: Ziphiidae*). Proc R Soc B Biol Sci 266:671–676

Horning M, Trillmich F (1997) Ontogeny of diving behaviour in the Galápagos fur seal. Behaviour 134:1211–1257

Horning M, Trillmich F (1999) Lunar cycles in diel prey migrations exert a stronger effect on the diving of juveniles than adult Galápagos fur seals. Proc Biol Sci 266:1127–1132

Houghton JDR, Doyle TK, Davenport J, Wilson RP, Hays GC (2008) The role of infrequent and extraordinary deep dives in leatherback turtles (*Dermochelys coriacea*). J Exp Biol 211:2566–2575

Howey-Jordan LA, Brooks EJ, Abercrombie DL, Jordan LKB, Brooks A, Williams S, Gospodarczyk E, Chapman DD (2013a) Complex Movements, Philopatry and Expanded Depth Range of a Severely Threatened Pelagic Shark, the Oceanic Whitetip (*Carcharhinus longimanus*) in the Western North Atlantic. PLoS One 8

Howey-Jordan L a, Brooks EJ, Abercrombie DL, Jordan LKB, Brooks A, Williams S, Gospodarczyk E, Chapman DD (2013b) Complex movements, philopatry and expanded depth range of a severely threatened pelagic shark, the oceanic whitetip (*Carcharhinus longimanus*) in the western North Atlantic. PLoS One 8:e56588

Howey LA, Tolentino ER, Papastamatiou YP, Brooks EJ, Abercrombie DL, Watanabe YY, Williams S, Brooks A, Chapman DD, Jordan LKB (2016a) Into the deep: the functionality of mesopelagic excursions by an oceanic apex predator. Ecol Evol 6:5290–5304

Howey LA, Tolentino ER, Papastamatiou YP, Brooks EJ, Abercrombie DL, Watanabe YY, Williams S, Brooks A, Chapman DD, Jordan LKB (2016b) Into the deep: the functionality of mesopelagic excursions by an oceanic apex predator. Ecol Evol 6:5290–5304

Hsu HH, Joung SJ, Liu KM (2012) Fisheries, management and conservation of the whale shark *Rhincodon typus* in Taiwan. J Fish Biol:no-no

Hueter RE, Tyminski JP, la Parra R de (2013) Horizontal movements, migration patterns, and population structure of whale sharks in the Gulf of Mexico and northwestern Caribbean sea.

PLoS One 8:e71883

Hueter RE, Tyminski JP, Parra R De, la Parra R de (2013) Horizontal movements, migration patterns, and population structure of whale sharks in the Gulf of Mexico and northwestern Caribbean sea. PLoS One 8:e71883

Hussey NE, Brush J, McCarthy ID, Fisk AT (2010) δ^{15}N and δ^{13}C diet-tissue discrimination factors for large sharks under semi-controlled conditions. Comp Biochem Physiol A Mol Integr Physiol 155:445–53

Hussey NE, Dudley SFJ, Mccarthy ID, Cliff G, Fisk AT (2011) Stable isotope profiles of large marine predators : viable indicators of trophic position, diet, and movement in sharks ? 2045:2029–2045

Hussey NE, Macneil MA, McMeans BC, Olin J a, Dudley SFJ, Cliff G, Wintner SP, Fennessy ST, Fisk AT (2013a) Rescaling the trophic structure of marine food webs. Ecol Lett

Hussey NE, MacNeil M a. Olin J a. McMeans BC, Kinney MJ, Chapman DD, Fisk a. T (2012) Stable isotopes and elasmobranchs: tissue types, methods, applications and assumptions. J Fish Biol:

Hussey NE, Olin J a. Kinney MJ, McMeans BC, Fisk AT (2012) Lipid extraction effects on stable isotope values (δ13C and δ15N) of elasmobranch muscle tissue. J Exp Mar Bio Ecol 434–435:7–15

Illijas MI, Indy JR, Yasui H, Itabashi Y (2009) Lipid Class and Fatty Acid Composition of a Little-known and Rarely Collected Alga *Exophyllum wentii* Weber-van Bosse from Bali Island, Indonesia. J Oleo Sci 58:103–110

Irigoien X, Klevjer TA, Røstad A, Martinez U, Boyra G, Acuña JL, Bode A, Echevarria F, Gonzalez-Gordillo JI, Hernandez-Leon S, Agusti S, Aksnes DL, Duarte CM, Kaartvedt S (2014) Large mesopelagic fishes biomass and trophic efficiency in the open ocean. Nat Commun 5:3271

Irvine LG, Hindell MA, vandenHoff J, Burton HR (2000) The influence of body size on dive duration of underyearling southern elephant seals (*Mirounga leonina*). 251:463–471

Ishii M, Murase H, Fukuda Y, Sawada K, Sasakura T, Tamura T, Bando T, Matsuoka K, Shinohara A, Nakatsuka S, Okazaki M, Miyashita K, Mitani Y, Ishii M, Murase H, Fukuda Y, Sawada K, Sasakura T (2017) Diving behavior of sei whales *Balaenoptera borealis* relative to the vertical distribution of their potential prey.

Iverson SJ (2009) Lipids in Aquatic Ecosystems (M Kainz, MT Brett, and MT Arts, Eds.).

Jackson AL, Inger R, Parnell AC, Bearhop S (2011) Comparing isotopic niche widths among and within communities: SIBER - Stable Isotope Bayesian Ellipses in R. J Anim Ecol 80:595–602

Jaeger A, Lecomte VJ, Weimerskirch H, Richard P, Cherel Y (2010) Seabird satellite tracking validates the use of latitudinal isoscapes to depict predators' foraging areas in the Southern Ocean. Rapid Commun Mass Spectrom 24:3456–3460

James MC, Ottensmeyer CA, Eckert SA, Myers RA (2006) Changes in diel diving patterns accompany shifts between northern foraging and southward migration in leatherback turtles. Can J Zool 84:754–765

Jaquet N, Dawson S, Slooten E (2000) Seasonal distribution and diving behaviour of male sperm whales off Kaikoura: foraging implications. Can J Zool 78:407–419

Jefferson TA, Curry BE (1994) A global review of porpoise (*Cetacea: Phocoenidae*) mortality in gillnets. Biol Conserv 67:167–183

Jefferson TA, Rosenbaum HC (2014) Taxonomic revision of the humpback dolphins (*Sousa spp.*), and description of a new species from Australia. Mar Mammal Sci 30:1494–1541

Jensen FH, Perez JM, Johnson M, Soto NA, Madsen PT (2011) Calling under pressure: short-finned pilot whales make social calls during deep foraging dives. Proc R Soc B Biol Sci 278:3017–3025

Jiarpinijnun A, Benjakul S, Pornphatdetaudom A, Shibata J, Okazaki E, Osako K (2017) High Arachidonic Acid Levels in the Tissues of Herbivorous Fish Species (*Siganus fuscescens, Calotomus japonicus* and *Kyphosus bigibbus*). Lipids 52:363–373

John MA St. Borja A, Chust G, Heath M, Grigorov I, Mariani P, Martin AP, Santos RS (2016) A Dark Hole in Our Understanding of Marine Ecosystems and Their Services: Perspectives from the Mesopelagic Community. Front Mar Sci 3

Johns RB, Perry GJ (1977) Lipids of the marine bacterium *Flexibacter polymorphus*. Arch Microbiol 114:267–271

Johnson CJ, Ehlers LPW, Seip DR (2015) Witnessing extinction - Cumulative impacts across landscapes and the future loss of an evolutionarily significant unit of woodland caribou in Canada. Biol Conserv 186:176–186

Johnson M, Madsen PT, Zimmer WMX, Aguilar de Soto N, Tyack PL (2004) Beaked whales echolocate on prey. Proc R Soc London Ser B Biol Sci 271:S383–S386

Jonahson M, Harding S (2007) Occurrence of whale sharks (*Rhincodon typus)* in Madagascar. Fish Res 84:132–135

Jones T, Wood D, Catlin J, Norman B (2009) Expenditure and ecotourism: predictors of expenditure for whale shark tour participants. J Ecotourism 8:32–50

Jorgensen SJ, Klimley AP, Muhlia-Melo AF (2009) Scalloped hammerhead shark *Sphyrna lewini*, utilizes deep-water, hypoxic zone in the gulf of California. J Fish Biol 74:1682–1687

Joshua A, Edgar DS, Kumli KR, Rubin RD (2016) Deep-water feeding and behavioral plasticity in Manta birostris revealed by archival tags and submersible observations. Zoology

Jouma'a J, Bras Y Le, Richard G, Vacquié-Garcia J, Picard B, Ksabi N El, Guinet C (2016) Adjustment of diving behaviour with prey encounters and body condition in a deep diving predator: The Southern Elephant Seal. Funct Ecol 30:636–648

Joung S-J, Chen C-T, Clark E, Uchida S, Huang WYP (1996) The whale shark, *Rhincodon typus*, is a livebearer: 300 embryos found in one "megamamma" supreme. Biol Fishes 46:219–223

Joyce WN, Campana SE, Natanson LJ, Kohler NE, Pratt HL, Jensen CF (2002) Analysis of stomach contents of the porbeagle shark (*Lamna nasus Bonnaterre*) in the northwest Atlantic. ICES J Mar Sci 59:1263–1269

Juinio-Meñez MA (2015) Biophysical and Genetic Connectivity Considerations in Marine Biodiversity Conservation and Management in the South China Sea. J Int Wildl Law Policy 18:110–119

Kaartvedt S, Staby a, Aksnes D (2012) Efficient trawl avoidance by mesopelagic fishes causes large underestimation of their biomass. Mar Ecol Prog Ser 456:1–6

Karl D, Letelier R, Tupas L, Dore J, Christian J, Hebel D (1997) The role of nitrogen fixation in biogeochemical cycling in the subtropical North Pacific Ocean. Nature 388:533–538

Kelly LJ, Martínez del Rio C (2010) The Fate of Carbon in Growing Fish: An Experimental Study of Isotopic Routing. Physiol Biochem Zool 83:473–480

Kendall VJ, Haedrich RL (2006) Species richness in Atlantic deep-sea fishes assessed in terms of the mid-domain effect and Rapoport's rule. Deep Res Part I Oceanogr Res Pap 53:506–515

Kerstetter DW, Luckhurst BE, Prince ED, Graves JE (2003) Use of pop-up satellite archival tags to demonstrate survival of blue marlin (*Makaira nigricans*) released from pelagic longline gear. Fish Bull 101:939–948

Ketchum JT, Galván-Magaña F, Klimley a. P (2012) Segregation and foraging ecology of whale sharks, *Rhincodon typus*, in the southwestern Gulf of California. Environ Biol Fishes

Kiljunen M, Grey J, Sinisalo T, Harrod C, Immonen H, Jones RI (2006) A revised model for lipid-normalizing δ13C values from aquatic organisms, with implications for isotope mixing models. J Appl Ecol 43:1213–1222

Kim SL, Casper DR, Galván-Magaña F, Ochoa-Díaz R, Hernández-Aguilar SB, Koch PL (2011) Carbon and nitrogen discrimination factors for elasmobranch soft tissues based on a long-term controlled feeding study. Environ Biol Fishes 95:37–52

Kim SL, Koch PL (2012) Methods to collect, preserve, and prepare elasmobranch tissues for stable isotope analysis. Environ Biol Fishes 95:53–63

Kim SL, Rio CM Del, Casper D, Koch PL (2012) Isotopic incorporation rates for shark tissues from a long-term captive feeding study. J Exp Biol 215:2495–500

Kiszka J, Simon-Bouhet B, Martinez L, Pusineri C, Richard P, Ridoux V (2011) Ecological niche segregation within a community of sympatric dolphins around a tropical island. Mar Ecol Prog Ser:273–288

Klatsky LJ, Wells RS, Sweeney JC (2007) Offshore Bottlenose Dolphins (*Tursiops Truncatus*): Movement and Dive Behavior Near the Bermuda Pedestal. J Mammal 88:59–66

Kleisner K, Walter J, Diamond S, Die D (2010) Modeling the spatial autocorrelation of pelagic fish abundance. Mar Ecol Prog Ser 411:203–213

Klevjer TA, Irigoien X, Røstad A, Fraile-Nuez E, Benítez-Barrios VM, Kaartvedt. S (2016) Large scale patterns in vertical distribution and behaviour of mesopelagic scattering layers. Sci Rep 6:1–11

Koen Alonso M, Alberto Crespo E, Aníbal García N, Noemí Pedraza S, Ariel Mariotti P, Judith Mora N (2002) Fishery and ontogenetic driven changes in the diet of the spiny dogfish, *Squalus acanthias*, in patagonian waters, Argentina. Environ Biol Fishes 63:193–202

Kolasinski J, Kaehler S, Jaquemet S (2012) Distribution and sources of particulate organic matter in a mesoscale eddy dipole in the Mozambique Channel (south-western Indian Ocean): Insight from C and N stable isotopes. J Mar Syst 96–97:122–131

Kooyman GL, Kooyman TG (1995) Diving Behavior of Emperor Penguins Nurturing Chicks at Coulman Island, Antarctica. Condor 97:536–549

Kooyman GL, Ponganis PJ (1998) The physiological basis of diving to depth: Birds and Mammals.

Kramer DL (1987) Dissolved oxygen and fish behavior. Environ Biol Fishes 18:81–92

Kraus RT, Rooker JR (2007) Patterns of Vertical Habitat Use By Atlantic Blue Marlin (*Makaira Nigricans*) in the Gulf of Mexico. Gulf Caribb Res 19:89–97

Krause D (2016) The foraging ecology, diet, and mass estimation of an apex predator, the leopard seal (*Hydrurga leptonyx*), at Livingston Island, Antarctic Peninsula. The University of California, San Diego.

la Parra Venegas R de, Hueter R, Gonz√°lez Cano J, Tyminski J, Gregorio Remolina J, Maslanka M, Ormos A, Weigt L, Carlson B, Dove A (2011) An unprecedented aggregation of whale sharks, *Rhincodon typus*, in Mexican coastal waters of the Caribbean Sea. PLoS One 6:e18994

la Torre JA de, Nuñez JM, Medellín RA (2016) Habitat availability and connectivity for jaguars (*Panthera onca*) in the Southern Mayan Forest: Conservation priorities for a fragmented landscape. Biol Conserv 206:270–282

Lagerquist B a, Stafford KM, Mate BR (2000) Satellite-Monitored Blue Whales (Balaenoptera Musculus) Off the Central California Coast. Mar Mammal Sci 16:375–391

Laidre KL, Heide-Jørgensen MP, Dietz R, Hobbs RC, Jørgensen OA (2003) Deep-diving by narwhals Monodon monoceros: Differences in foraging behavior between wintering areas? Mar Ecol Prog Ser 261:269–281

Lalli CM, Parsons T (1997) Phytoplankton and primary production. In: Biological oceanography: an introduction, 2nd edn. Elsevier, Oxford.

Lalli CM, Parsons TR (2013) Biological Oceanography an Introduction. J Chem Inf Model 53:1689–1699

Lander M (2000) Satellite tracking a rehabilitated Guadalupe fur seal (*Arctocephalus townsendi*). Aquat Mamm 26:137–142

Layman, C.A., M.S. Araujo, R. Boucek, E. Harrison, Z.R. Jud, P. Matich, C.M. Hammerschlag-Peyer, A.E. Rosenblatt, J.J. Vaudo, L.A. Yeager, D. Post, S. Bearhop. 2012. Applying Stable Isotopes to Examine Food Web Structure: An Overview of Analytical Tools. Biological Reviews 87: 542-562.

Lee Chang KJ, Mansour MP, Dunstan GA, Blackburn SI, Koutoulis A, Nichols PD (2011) Odd-chain polyunsaturated fatty acids in thraustochytrids. Phytochemistry 72:1460–1465

Lee RF, Hagen W, Kattner G (2006) Lipid storage in marine zooplankton. Mar Ecol Prog Ser

307:273–306

Leu M, Li J, Ju Y, Hsiao C, Chang C, Meng P, Tew KS, Wang W (2015) Transportation, husbandry, and release of a whale shark (*Rhincodon typus*). J Mar Sci Technol 23:814–818

Leung ES, Augé AA, Chilvers BL, Moore AB, Robertson BC (2013) Foraging Behaviour of Juvenile Female New Zealand Sea Lions (*Phocarctos hookeri*) in Contrasting Environments. PLoS One 8:1–10

Li Y, Hussey NE, Zhang Y (2016) Quantifying ontogenetic stable isotope variation between dermis and muscle tissue of two pelagic sharks. 25:53–60

Lima M, Marquet PA, Jaksic FM, M. L, A. MP, M. JF (1996) Extinction and colonization processes in subpopulations of five neotropical small mammal species. Oecologia 107:197–203

Logan JM, Lutcavage ME (2010a) Stable isotope dynamics in elasmobranch fishes. Hydrobiologia 644:231–244

Logan JM, Lutcavage ME (2010b) Reply to Hussey et al.: The requirement for accurate diet-tissue discrimination factors for interpreting stable isotopes in sharks. Hydrobiologia 654:7–12

Lorrain A, Graham BS, Popp BN, Allain V, Olson RJ, Hunt BP V, Potier M, Fry B, Galván-Magaña F, Menkes CER, Kaehler S, Ménard F (2014) Nitrogen isotopic baselines and implications for estimating foraging habitat and trophic position of yellowfin tuna in the Indian and Pacific Oceans. Deep Res Part II Top Stud Oceanogr 113:188–198

Lowe TE, Brill RW, Cousins KL (2000) Blood oxygen-binding characteristics of bigeye tuna (*Thunnus obesus*), a high-energy-demand teleost that is tolerant of low ambient oxygen. Mar Biol 136:1087–1098

Lugomela C, Lyimo TJ, Bryceson I, Semesi AK, Bergman B (2002) Trichodesmium in coastal waters of Tanzania: Diversity, seasonality, nitrogen and carbon fixation. Hydrobiologia 477:1–13

Lutz PL, Musick JA (2002) The Biology of Sea Turtles.

Lydersen C, Nøst OA, Lovell P, McConnell BJ, Gammelsrød T, Hunter C, Fedak MA, Kovacs KM (2002) Salinity and temperature structure of a freezing Arctic fjord-monitored by white whales (*Delphinapterus leucas*). Geophys Res Lett 29:34-1-34–4

MacLeod CD, Santos MB, Pierce GJ (2003) Review of data on diets of beaked whales: Evidence of niche separation and geographic segregation. J Mar Biol Assoc United Kingdom 83:651–665

MacNeil M, Skomal G, Fisk A (2005) Stable isotopes from multiple tissues reveal diet switching in sharks. Mar Ecol Prog Ser 302:199–206

Magozzi S, Yool A, Zanden HB Vander, Wunder MB, Trueman CN (2017) Using ocean models to predict spatial and temporal variation in marine carbon isotopes. Ecosphere 8

Marcus L, Virtue P, Nichols PD, Meekan MG, Pethybridge H (2017) Effects of sample treatment on the analysis of stable isotopes of carbon and nitrogen in zooplankton, micronekton and a filter-feeding shark. Mar Biol 164:124

Marcus L, Virtue P, Pethybridge HR, Meekan MG, Thums M, Nichols PD (2016) Intraspecific variability in diet and implied foraging ranges of whale sharks at Ningaloo Reef, Western Australia, from signature fatty acid analysis Intraspecific variability in diet and implied foraging ranges of whale sharks at Ningaloo Reef , Wester. Mar Ecol 554:115–128

Marshall AD, Pierce SJ (2012) The use and abuse of photographic identification in sharks and rays. J Fish Biol 80:1361–1379

Martin RA (2007) A review of behavioural ecology of whale sharks (*Rhincodon typus*). Fish Res 84:10–16

Martin AP, Naylor GJP (1997) Independent Origins of Filter-Feeding in Megamouth and Basking Sharks (Order *Lamniformes*) Inferred from Phylogenetic Analysis of Cytochrome b Gene Sequences. :39–50

Martin AR, Smith TG (1999) Strategy and capability of wild belugas, *Delphinapterus leucas*, during deep, benthic diving. Can J Zool 77:1783–1793

Martínez Del Rio C, Wolf N, Carleton SA, Gannes LZ (2009) Isotopic ecology ten years after a call for more laboratory experiments. Biol Rev 84:91–111

Mate BR, Irvine LM, Palacios DM (2016) The development of an intermediate-duration tag to characterize the diving behavior of large whales. Ecol Evol 7:585–595

Mathew S, Shamasundar MK and BA (2002) Effect of water washing of shark (*Scoliodon laticaudus*) meat on the properties of proteins with special reference\nto gelation. Nahrung/Food 46:78 ± 82

Matich P, Heithaus MR, Layman CA (2011) Contrasting patterns of individual specialization and trophic coupling in two marine apex predators. J Anim Ecol 80:294–305

Mattlin RH, Gales NJ, Costa DP (1998) Seasonal dive behaviour of lactating New Zealand fur seals (*Arctocephalus forsteri*). Can J Zool 76:350–360

McKinney JA, Hoffmayer ER, Holmberg J, Graham RT, Driggers WB, la Parra-Venegas R de, Galván-Pastoriza BE, Fox S, Pierce SJ, Dove ADM (2017) Long-term assessment of whale shark population demography and connectivity using photo-identification in the Western Atlantic Ocean. PLoS One 12:1–18

McKinney J, Hoffmayer E, Wu W, Fulford R, Hendon J (2012) Feeding habitat of the whale shark Rhincodon typus in the northern Gulf of Mexico determined using species distribution modelling. Mar Ecol Prog Ser 458:199–211

McMahon KW, Fogel ML, Elsdon TS, Thorrold SR (2010) Carbon isotope fractionation of amino acids in fish muscle reflects biosynthesis and isotopic routing from dietary protein. J Anim Ecol 79:1132–1141

McMahon KW, Hamady LL, Thorrold SR (2013) A review of ecogeochemistry approaches to estimating movements of marine animals. Limnol Oceanogr 58:697–714

Meekan MG, Bradshaw CJA, Press M, McLean C, Richards A, Quasnichka S, Taylor JG (2006) Population size and structure of whale sharks Rhincodon typus at Ningaloo Reef, Western Australia. Mar Ecol Prog Ser 319:275–285

Meekan M, Jarman S, McLean C, Schultz M (2009) DNA evidence of whale sharks (*Rhincodon typus*) feeding on red crab (*Gecarcoidea natalis*) larvae at Christmas Island, Australia. Mar Freshw Res 60:607–609

Melin SR, DeLong RL, Siniff DB (2008) The effects of El Niño on the foraging behavior of lactating California sea lions (*Zalophus californianus californianus*) during the nonbreeding season. Can J Zool 86:192–206

Ménard F, Lorrain A, Potier M, Marsac F (2007) Isotopic evidence of distinct feeding ecologies and movement patterns in two migratory predators (yellowfin tuna and swordfish) of the western Indian Ocean. Mar Biol 153:141–152

Mendez M, Subramaniam A, Collins T, Minton G, Baldwin R, Berggren P, Särnblad A, Amir OA, Peddemors VM, Karczmarski L, Guissamulo A, Rosenbaum HC (2011) Molecular ecology meets remote sensing: environmental drivers to population structure of humpback dolphins in the Western Indian Ocean. Heredity (Edinb) 107:349–361

Merrett NR (1971) Aspects of the biology of billfish (Istiophoridae) from the equatorial western Indian Ocean. J Zool London 163:351–395

Merrick RL, Loughlin TR (1997) Foraging behavior of adult female and young-of-the-year Steller sea lions in Alaskan waters. Can J Zool 75:776–786

Merten W, Appeldoorn R, Rivera R, Hammond D (2014) Diel vertical movements of adult male dolphinfish (*Coryphaena hippurus*) in the western central Atlantic as determined by use of pop-up satellite archival transmitters. Mar Biol 161:1823–1834

Meyer W, Seegers U (2012) Basics of skin structure and function in elasmobranchs: a review. J Fish Biol 80:1940–67

Meynier L, Morel PCH, Chilvers BL, Mackenzie DDS, Duignan PJ, MacLatchey D (2014) Foraging diversity in lactating New Zealand sea lions: insights from qualitative and quantitative fatty acid analysis. Can J Fish Aquat Sci 71:984–991

Michael Panneton W (2013) The Mammalian Diving Response: An Enigmatic Reflex to Preserve Life? Physiology 28:284–297

Michael Reynolds R (1993) Physical oceanography of the Gulf, Strait of Hormuz, and the Gulf of Oman-Results from the Mt Mitchell expedition. Mar Pollut Bull 27:35–59

Minagawa M, Wada E (1986) Nitrogen isotope ratios of red tide organisms in the East China Sea: A characterization of biological nitrogen fixation. Mar Chem 19:245–259

Minamikawa S, Iwasaki T, Kishiro T (2007) Diving behaviour of a Baird's beaked whale, Berardius bairdii, in the slope water region of the western North Pacific: First dive records using a data logger. Fish Oceanogr 16:573–577

Minamikawa S, Watanabe H, Iwasaki T (2013) Diving behavior of a false killer whale, Pseudorca crassidens, in the Kuroshio-Oyashio transition region and the Kuroshio front region of the western North Pacific. Mar Mammal Sci 29:177–185

Monroig O, Tocher DR, Navarro JC (2013) Biosynthesis of polyunsaturated fatty acids in marine invertebrates: Recent advances in molecular mechanisms. Mar Drugs 11:3998–4018

Mori Y (2002) Optimal diving behaviour for foraging in relation to body size Body Size Review. J Evol Biol 15:269–276

Motta PJ (1977) Anatomy and Functional Morphology of Dermal Collagen Fibers in Sharks. Copeia 1977:454

Motta PJ, Maslanka M, Hueter RE, Davis RL, la Parra R de, Mulvany SL, Habegger ML, Strother J a, Mara KR, Gardiner JM, Tyminski JP, Zeigler LD (2010) Feeding anatomy, filter-feeding rate, and diet of whale sharks Rhincodon typus during surface ram filter feeding off the Yucatan Peninsula, Mexico. Zoology (Jena) 113:199–212

Motta PJ, Wilga CD (2001) Advances in the Study of Feeding Behaviors, Mechanisms, and

Mechanics of Sharks. Environ Biol Fishes 60:131–156

Moura JF de, Merico A, Montone RC, Silva J, Seixas TG, Godoy JM de O, Saint'Pierre TD, Hauser-Davis RA, Beneditto APM Di, Reis EC, Tavares DC, Lemos LS, Siciliano S (2015) Assessment of trace elements, POPs, 210Po and stable isotopes (δ^{15}N and δ^{13}C) in a rare filter-feeding shark: The megamouth. Mar Pollut Bull 95:402–406

Musyl MK, Brill RW, Boggs CH, Curran DS, Kazama TK, Seki MP (2003) Vertical movements of bigeye tuna (*Thunnus obesus*) associated with islands, buoys, and seamounts near the main Hawaiian Islands from archival tagging data. Fish Oceanogr 12:152–169

Naito Y, Costa DP, Adachi T, Robinson PW, Fowler M, Takahashi A (2013) Unravelling the mysteries of a mesopelagic diet: a large apex predator specializes on small prey (C Franklin, Ed.). Funct Ecol 27:710–717

Naya DE, Arim M, Vargas R (2002) Diet of South american fur seals (*Arctocephalus australis*) in Isla de Lobos, Uruguay. Mar Mammal Sci 18:734–745

Nelson JD, Eckert S a. (2007) Foraging ecology of whale sharks (*Rhincodon typus*) within Bahía de Los Angeles, Baja California Norte, México. Fish Res 84:47–64

Nelson D, McKibben J (1997) An acoustic tracking of a megamouth shark, *Megachasma pelagios*: a crepuscular vertical migrator. Biol Fishes:389–399

Newsome SD, Wolf N, Peters J, Fogel ML (2014) Amino acid δ13C analysis shows flexibility in the routing of dietary protein and lipids to the tissue of an omnivore. Integr Comp Biol 54:890–902

Nicol S, Bowie A, Jarman S, Lannuzel D, Meiners KM, Merwe P van der (2010) Southern Ocean iron fertilization by baleen whales and Antarctic krill. Fish Fish 11:203–209

Nordøy ES, Blix AS (2009) Movements and dive behaviour of two leopard seals (*Hydrurga leptonyx*) off Queen Maud Land, Antarctica. Polar Biol 32:263–270

Noren SR, Williams TM (2000) Body size and skeletal muscle myoglobin of cetaceans: Adaptations for maximizing dive duration. Comp Biochem Physiol - A Mol Integr Physiol 126:181–191

Norman BM, Holmberg JA, Arzoumanian Z, Reynolds SD, Wilson RP, Rob D, Pierce SJ, Gleiss AC, La Parra R De, Galvan B, Ramirez-Macias D, Robinson D, Fox S, Graham R, Rowat D, Potenski M, Levine M, McKinney JA, Hoffmayer E, Dove ADM, Hueter R, Ponzo A, Araujo G, Aca E, David D, Rees R, Duncan A, Rohner CA, Prebble CEM, Hearn A, Acuna D, Berumen ML, Vázquez A, Green J, Bach SS, Schmidt J V. Beatty SJ, Morgan DL (2017) Undersea constellations: The global biology of an endangered marine megavertebrate further informed through citizen science. Bioscience 67:1029–1043

Norman BM, Morgan DL (2016) The return of “Stumpy” the whale shark: two decades and counting. Front Ecol Environ 14:449–450

Nowacek DP, Johnson MP, Tyack PL, Shorter KA, McLellan WA, D. AP (2001) Buoyant balaenids: the ups and downs of buoyancy in right whales. Proc R Soc B Biol Sci 268:1811–1816

O’Neil JM, Roman MR (1992) Grazers and associated organisms of *Trichodesmium*. Mar Pelagic Cyanobacteria Trichodesmium other Diazotrophs:61–73

Ohizumi H, Kuramochi T, Kubodera T, Yoshioka M, Miyazaki N (2003) Feeding habits of Dall’s porpoises (*Phocoenoides dalli*) in the subarctic North Pacific and the Bering Sea basin and the impact of predation on mesopelagic micronekton. Deep Res Part I Oceanogr Res Pap 50:593–610

Okuyama J, Seminoff JA, Dutton PH, Benson SR (2016) Fine-Scale Monitoring of Routine Deep Dives by Gravid Leatherback Turtles during the Internesting Interval Indicate a Capital Breeding Strategy. Front Mar Sci 3

Olin JA, Hussey NE, Grgicak-Mannion A, Fritts MW, Wintner SP, Fisk AT (2013) Variable δ^{15}N Diet-Tissue Discrimination Factors among Sharks: Implications for Trophic Position, Diet and Food Web Models. PLoS One 8

Orlov AM, Baitalyuk AA (2014) Spatial distribution and features of biology of Pacific sleeper shark Somniosus pacificus in the North Pacific. J Ichthyol 54:526–546

Otani S, Naito Y, Kawamura A, Kawasaki M, Nishiwaki S, Kato A (1998) Diving behavior and performance of Harbor porpoises, Phocoena phocoena, in Funka Bay, Hokkaido, Japan. Mar Mammal Sci 14:209–220

Panigada S, Zanardelli M, Canese S, Jahoda M (1999) How deep can baleen whales dive? Mar Ecol Prog Ser 187:309–311

Papastamatiou YP, Iosilevskii G, Leos-Barajas V, Brooks EJ, Howey LA, Chapman DD, Watanabe YY (2018) Optimal swimming strategies and behavioral plasticity of oceanic whitetip sharks. Sci Rep 8:1–12

Parrish FA, Abernathy K, Marshall GJ, Buhleier BM (2002) Hawaiian Monk Seals (*Monachus*

Schauinslandi) Foraging in Deep-Water Coral Beds. Mar Mammal Sci 18:244–258

Parrish CC, Pethybridge H, Young JW, Nichols PD (2014) Deep-Sea Research II Spatial variation in fatty acid trophic markers in albacore tuna from the southwestern Pacific Ocean - A potential ‘ tropicalization ’ signal.

Parsons TR (1963) Suspended organic matter . Fish Res Board Canada, Biol Station Nanaimo, BC

Perrin WF (2009) Pantropical spotted dolphin: *Stenella attenuata*. Encycl Mar Mamm:819–821

Peterson SH, Ackerman JT, Costa DP (2015) Marine foraging ecology influences mercury bioaccumulation in deep-diving northern elephant seals. Proc R Soc B Biol Sci 282:20150710

Peterson BJB, Fry B (1987) Stable isotopes in ecosystem studies. Annu Rev Ecol Syst 18:293–320

Pethybridge H, Daley RK, Nichols PD (2011) Diet of demersal sharks and chimaeras inferred by fatty acid profiles and stomach content analysis. J Exp Mar Bio Ecol 409:290–299

Pethybridge H, Daley R, Virtue P, Butler EC V. Cossa D, Nichols PD (2010) Lipid and mercury profiles of 61 mid-trophic species collected off south-eastern Australia. Mar Freshw Res 61:1092

Pethybridge HR, Parrish CC, Bruce BD, Young JW, Nichols PD (2014) Lipid, fatty acid and energy density profiles of white sharks: Insights into the feeding ecology and ecophysiology of a complex top predator. PLoS One 9

Pethybridge HR, Parrish CC, Morrongiello J, Young JW, Farley JH, Gunasekera RM, Nichols PD, Bearhop S, Adams CE, Waldron S, Fuller R a. Macleod H, Brillinger DR, Stewart BS, Christiansen H, Fisk A, Hussey N, Hayes JM, Jackson AL, Inger R, Parnell AC, Bearhop S, Jaspers C, Acuña JL, Brodeur RD, Layman CA, Arrington DA, Montaña CG, Post DM, Boucek R, Hammerschlag-peyer CM, Lorrain A, Graham BS, Popp BN, Allain V, Olson RJ, Hunt BP V, Potier M, Fry B, Galván-Magaña F, Menkes CER, Kaehler S, Ménard F, Moore DS, McCabe GP, Craig B, Hesterberg T, Monaghan S, Clipson A, Epstein R, Pethybridge HR, Parrish CC, Morrongiello J, Young JW, Farley JH, Gunasekera RM, Nichols PD, Quiros AL, Reviews RR, Semmens BX, Ward EJ, Moore JW, Darimont CT, Lecea A De, Fennessy S, Smit A, Christiansen H, Fisk A, Hussey N, Jackson AL, Inger R, Parnell AC, Bearhop S, Layman CA, Boucek R, Hammerschlag-peyer CM, Lorrain A, Graham BS, Popp BN, Allain V, Olson RJ, Hunt BP V, Potier M, Fry B, Galván-Magaña F, Menkes CER, Kaehler S, Ménard F, Moore DS, McCabe GP, Craig B, Hesterberg T, Monaghan S, Clipson A, Epstein R, Bearhop S, Adams CE, Waldron S, Fuller R a. Macleod H, Brillinger DR, Stewart BS, Lecea A De, Fennessy S, Smit A, Hayes JM, Semmens BX, Ward EJ, Moore JW, Darimont CT, Reviews RR, Jaspers C, Acuña JL, Brodeur RD, Rautio M (2007) Tourist compliance to a Code of Conduct and the resulting effects on whale shark (*Rhincodon typus*) behavior in Donsol, Philippines. J Anim Ecol 37:102–108

Petursdottir H, Gislason A. Falk-Petersen S, Hop H, Svavarsson J (2008) Trophic interactions of the pelagic ecosystem over the Reykjanes Ridge as evaluated by fatty acid and stable isotope analyses. Deep Sea Res Part II Top Stud Oceanogr 55:83–93

Pierce SJ, Méndez-Jiménez A, Collins K, Rosero-Caicedo M, Monadjem A (2010a) Developing a Code of Conduct for whale shark interactions in Mozambique. Aquat Conserv Mar Freshw Ecosyst 20:782–788

Pierce SJ, Méndez-Jiménez A, Collins K, Rosero-Caicedo M, Monadjem A (2010b) Developing a Code of Conduct for whale shark interactions in Mozambique. Aquat Conserv Mar Freshw Ecosyst 20:782–788

Podlesak DW, McWilliams SR (2006) Metabolic Routing of Dietary Nutrients in Birds: Effects of Diet Quality and Macronutrient Composition Revealed Using Stable Isotopes. Physiol Biochem Zool 79:534–549

Ponganis PJ (2015) Diving Behavior. Cambridge University Press

Post DM (2002) Using stable isotopes to estimate trophic position: models, methos, and assumptions. Ecology 83:703–718

Post DM, Layman CA, Arrington DA, Takimoto G, Quattrochi J, Montaña CG (2007a) Getting to the fat of the matter: Models, methods and assumptions for dealing with lipids in stable isotope analyses. Oecologia 152:179–189

Post DM, Layman C a, Arrington DA, Takimoto G, Quattrochi J, Montaña CG (2007b) Getting to the fat of the matter: models, methods and assumptions for dealing with lipids in stable isotope analyses. Oecologia 152:179–89

Potier M, Bach P, Ménard F, Marsac F (2013) Deep-Sea Research II In fl uence of mesoscale features on micronekton and large pelagic fi sh communities in the Mozambique Channel.

Pravin P (2000) Whale shark in the Indian coast-Need for conservation. Curr Sci 79:310–315

Prince ED, Goodyear CP (2006) Hypoxia-based habitat compression of tropical pelagic fishes. Fish Oceanogr 15:451–464

Pütz K, Cherel Y (2005) The diving behaviour of brooding king penguins (*Aptenodytes patagonicus*) from the Falkland Islands: Variation in dive profiles and synchronous underwater swimming provide new insights into their foraging strategies. Mar Biol 147:281–

290

Queiroz N, Humphries NE, Noble LR, Santos AM, Sims DW (2010) Short-term movements and diving behaviour of satellite-tracked blue sharks *Prionace glauca* in the northeastern Atlantic Ocean. Mar Ecol Prog Ser 406:265–279

Raclot T, Groscolas R (1998) Fatty acid evidence for the importance of myctophid fishes in the diet of king penguins, *Aptenodytes patagonicus*. 33:523–533

Raj RP, Peter BN, Pushpadas D (2010) Oceanic and atmospheric influences on the variability of phytoplankton bloom in the Southwestern Indian Ocean. J Mar Syst 82:217–229

Ramírez-Macías D, la Parra-Venegas R de, Remolina-Suarez F, Trigo-Mendoza M, Vazquez-Juarez R (2012) Patterns in composition , abundance and scarring of whale sharks *Rhincodon typus* near Holbox Island ,. 80:1401–1416

Ramírez-Macías D, Queiroz N, Pierce SJ, Humphries NE, Sims DW, Brunnschweiler JM (2017) Oceanic adults, coastal juveniles: tracking the habitat use of whale sharks off the Pacific coast of Mexico. PeerJ 5:e3271

Ramírez-macías D, Vázquez-haikin A, Vázquez-juárez R (2012) Whale shark Rhincodon typus populations along the west coast of the Gulf of California and implications for management. 18:115–128

Randall DJ (1970) Gas exchange in fish. Fish Physiol 4:253–292

Rau GH, Sweeney RE, Kaplan IR (1982) Plankton 13C:12C ratio changes with latituda: differences between northern and southern oceans. Deep Res 29:1035–1039

Reich KJ, Bjorndal K a, Bolten AB (2007) The “lost years” of green turtles: using stable isotopes to study cryptic lifestages. Biol Lett 3:712–4

Reisinger RR, Keith M, Andrews RD, Bruyn PJN de (2015) Movement and diving of killer whales (*Orcinus orca*) at a Southern Ocean archipelago. J Exp Mar Bio Ecol 473:90–102

Riley MJ, Hale MS, Harman A, Rees RG (2010) Analysis of whale shark Rhincodon typus aggregations near South Ari Atoll, Maldives Archipelago. Aquat Biol 8:145–150

Robin Baird, Webster DL, Schorr GS, McSweeney DJ (2008) Diel Variation In Beaked Whale Diving Behavior

Robinson DP (2015) The ecology of whale sharks Rhincodon typus within the Arabian Gulf and the Gulf of Oman. PhD dissertation, Heriot Watt University, Edinburgh, UK

Robinson PW, Costa DP, Crocker DE, Gallo-reynoso JP, Champagne CD, Fowler MA, Goetsch C, Goetz KT, Hassrick JL, Kuhn CE, Maresh JL, Maxwell SM, Mcdonald BI, Hu LA, Peterson SH, Simmons SE, Teutschel NM, Villegas-amtmann S, Yoda K (2012) Foraging Behavior and Success of a Mesopelagic Predator in the Northeast Pacific Ocean : Insights from a Data-Rich Species , the Northern Elephant Seal. 7

Robinson SA, Hindell MA (1996) Foraging ecology of gentoo penguins *Pygoscelis papua* at Macquarie Island during the period of chick care. Ibis (Lond 1859) 138:722–731

Robinson DP, Jaidah MY, Bach S, Lee K, Jabado W, Rohner CA, March A, Caprodossi S (2016) Population Structure , Abundance and Movement of Whale Sharks in the Arabian Gulf and the Gulf of Oman. PLoS One 11:e0158593

Robinson DP, Jaidah MY, Bach SS, Rohner CA, Jabado RW, Ormond R, Pierce SJ (2017) Some like it hot: Repeat migration and residency of whale sharks within an extreme natural environment. PLoS One 12:1–23

Robinson DP, Jaidah MY, Jabado RW, Lee-brooks K, El- NMN, Malki AA Al, Elmeer K, Mccormick PA, Henderson AC, Pierce SJ, Ormond RFG (2013) Whale Sharks, *Rhincodon typus*, Aggregate around Offshore Platforms in Qatari Waters of the Arabian Gulf to Feed on Fish Spawn. PLoS One 8:e58255

Robinson C, Steinberg DK, Anderson TR, Arístegui J, Carlson CA. Frost JR, Ghiglione J-F, Hernández-León S, Jackson GA. Koppelmann R, Quéguiner B, Ragueneau O, Rassoulzadegan F, Robison BH, Tamburini C, Tanaka T, Wishner KF, Zhang J (2010) Mesopelagic zone ecology and biogeochemistry – a synthesis. Deep Sea Res Part II Top Stud Oceanogr 57:1504–1518

Robison BH (2009) Conservation of deep pelagic biodiversity. Conserv Biol 23:847–858

Rodhouse P (1990) Cephalopod fauna of the Scotia Sea at South Georgia: potential for commercial exploitation and possible consequences. Antarct Ecosyst Ecol Chang Conserv:289–298

Rohner CA (2012) A global whale shark hotspot in southern Mozambique : population structure , feeding ecology , movements and environmental drivers.

Rohner C a. Armstrong a. J, Pierce SJ, Prebble CEM, Cagua EF, Cochran JEM, Berumen ML, Richardson a. J (2015a) Whale sharks target dense prey patches of sergestid shrimp off Tanzania. J Plankton Res 37:352–362

Rohner CA, Armstrong AJ, Pierce SJ, Prebble CEM, Cagua EF, Cochran JEM, Berumen ML, Richardson AJ (2015b) Whale sharks target dense prey patches of sergestid shrimp off

Tanzania. J Plankton Res 37:352–362

Rohner C, Couturier LIE, Richardson AJ, Pierce SJ, Prebble CEM, Gibbons MJ, Nichols PD (2013) Diet of whale sharks Rhincodon typus inferred from stomach content and signature fatty acid analyses. Mar Ecol Prog Ser 493:219–235

Rohner CA, Flam AL, Pierce SJ, Marshall AD (2017) Steep declines in sightings of manta rays and devilrays (*Mobulidae*) in southern Mozambique. PeerJ Prepr:1–32

Rohner C, Pierce S, Marshall A, Weeks S, Bennett M, Richardson AJ (2013) Trends in sightings and environmental influences on a coastal aggregation of manta rays and whale sharks. Mar Ecol Prog Ser 482:153–168

Rohner CA, Richardson AJ, Jaine FRA, Bennett MB, Cliff G, Robinson DP, Reeve-Arnold KE, Pierce SJ (2017) Satellite tagging highlights the importance of productive Mozambican coastal waters to the ecology and conservation of whale sharks. PeerJ Prepr:1–36

Rohner CA, Richardson AJ, Jaine FRA, Bennett MB, Weeks SJ, Cliff G, Robinson D, Pierce SJ (2017) Satellite tagging highlights the importance of productive Mozambican coastal waters to the ecology and conservation of whale sharks.

Rohner CA, Richardson AJ, Jaine FRA, Bennett MB, Weeks SJ, Cliff G, Robinson DP, Reeve-Arnold KE, Pierce SJ (2018) Satellite tagging highlights the importance of productive Mozambican coastal waters to the ecology and conservation of whale sharks. PeerJ 6:e4161

Rohner CA, Richardson AJ, Marshall AD, Weeks SJ, Pierce SJ (2011) How large is the world's largest fish? Measuring whale sharks Rhincodon typus with laser photogrammetry. J Fish Biol 78:378–385

Rohner CA, Richardson AJ, Prebble CEM, Marshall AD, Bennett MB, Weeks SJ, Cliff G, Wintner SP, Pierce SJ (2015) Laser photogrammetry improves size and demographic estimates for whale sharks. PeerJ 3:e886

Rohner C. Weeks S. Richardson AJ, Pierce S. Magno-Canto M. Feldman G, Cliff G, Roberts MJ (2018) Oceanographic influences on a global whale shark hotspot in southern Mozambique. PeerJ PrePints

Roman J, McCarthy JJ (2010) The whale pump: Marine mammals enhance primary productivity in a coastal basin. PLoS One 5

Rosen DA. Winship AJ, Hoopes LA (2007) Thermal and digestive constraints to foraging behaviour in marine mammals. Philos Trans R Soc B Biol Sci 362:2151–2168

Rowat D, Brooks KS (2012) A review of the biology, fisheries and conservation of the whale shark Rhincodon typus. J Fish Biol 80:1019–56

Rowat D, Brooks K, March A, McCarten C, Jouannet D, Riley L, Jeffreys G, Perri M, Vely M, Pardigon B (2011) Long-term membership of whale sharks (*Rhincodon typus*) in coastal aggregations in Seychelles and Djibouti. Mar Freshw Res 62:621

Rowat D, Meekan MG, Engelhardt U, Pardigon B, Vely M (2006) Aggregations of juvenile whale sharks (*Rhincodon typus*) in the Gulf of Tadjoura, Djibouti. Environ Biol Fishes 80:465–472

Rowat D, Speed CW, Meekan MG, Gore MA. Bradshaw CJ a. (2009) Population abundance and apparent survival of the Vulnerable whale shark Rhincodon typus in the Seychelles aggregation. Oryx 43:591

Russell NJ (2013) Polyunsaturated Fatty acids marine bacteria - a dogma rewritten. Microbiology 145:767–779

Saba GK, Steinberg DK (2012) Abundance, Composition, and Sinking Rates of Fish Fecal Pellets in the Santa Barbara Channel. Sci Rep 2:1–6

Saito H, Aono H (2014) Characteristics of lipid and fatty acid of marine gastropod *Turbo cornutus*: High levels of arachidonic and n-3 docosapentaenoic acid. Food Chem 145:135–144

Sardenne F, Bodin N, Chassot E, Amiel A, Degroote M, Hollanda S, Pethybridge H (2016) Trophic niches of sympatric tropical tuna in the Western Indian Ocean inferred by stable isotopes and neutral fatty acids. Prog Oceanogr 146:75–88

Sargent J, Bell G, McEvoy L, Tocher D, Estevez A (1999) Recent developments in the essential fatty acid nutrition of fish. Aquaculture 177:191–199

Satterfield FR, Finney BP (2002) Stable isotope analysis of Pacific salmon: Insight into trophic status and oceanographic conditions over the last 30 years. Prog Oceanogr 53:231–246

Schaefer KM, Fuller DW (2007) Vertical movement patterns of skipjack tuna (*Katsuwonus pelomis*) in the eastern equatorial Pacific Ocean, as revealed with archival tags. Fish Bull 105:379–389

Schaefer KM, Fuller DW, Block BA (2011) Movements, behavior, and habitat utilization of yellowfin tuna (*Thunnus albacares*) in the Pacific Ocean off Baja California, Mexico, determined from archival tag data analyses, including unscented Kalman filtering. Fish Res 112:22–37

Schleimer A, Araujo G, Penketh L, Heath A, McCoy E, Labaja J, Lucey A, Ponzo A (2015)

Learning from a provisioning site: code of conduct compliance and behaviour of whale sharks in Oslob, Cebu, Philippines. PeerJ 3:e1452

Schmidt K, Atkinson A, Steigenberger S, Fielding S, Lindsay MCM, Pond DW, Tarling GA, Klevjer TA, Allen CS, Nicol S, Achterberg EP (2011) Seabed foraging by Antarctic krill : Implications for stock assessment , bentho-pelagic coupling , and the vertical transfer of iron. 56:1411–1428

Schmidt J V, Chen C, Sheikh SI, Meekan MG, Norman BM, Joung S (2010) Paternity analysis in a litter of whale shark embryos. 12:117–124

Schmidt J V. Schmidt CL, Ozer F, Ernst RE, Feldheim KA, Ashley M V. Levine M (2009) Low genetic differentiation across three major ocean populations of the whale shark, *Rhincodon typus*. PLoS One 4

Schorr GS, Falcone E a, Moretti DJ, Andrews RD (2014) First long-term behavioral records from Cuvier's beaked whales (*Ziphius cavirostris*) reveal record-breaking dives. PLoS One 9:e92633

Schreer JF, Kovacs KM (1997) Allometry diving capacity. Can J Zool 75:339–358

Scott MD, Hohn a a, Westgate a J, Nicolas JR, Whitaker BR, Campbell WB (2001) A note on the release and tracking of a rehabilitated pygmy sperm whale *Kogia breviceps*. J Cetacean Res Manag 3:87–94

Sepulveda CA, Aalbers SA, Ortega-Garcia S, Wegner NC, Bernal D (2011) Depth distribution and temperature preferences of wahoo (*Acanthocybium solandri*) off Baja California Sur, Mexico. Mar Biol 158:917–926

Sepulveda CA, Knight A, Nasby-Lucas N, Domeier ML (2010) Fine-scale movements of the swordfish *Xiphias gladius* in the Southern California Bight. Fish Oceanogr 19:279–289

Sequeira AMM, Mellin C, Meekan MG, Sims DW, Bradshaw CJA (2013) Inferred global connectivity of whale shark *Rhincodon typus* populations. J Fish Biol 82:367–389

Sequeira A, Mellin C, Rowat D, Meekan MG, Bradshaw CJA (2012) Ocean-scale prediction of whale shark distribution. Divers Distrib 18:504–518

Shepard ELC, Ahmed MZ, Southall EJ, Witt MJ, Metcalfe JD, Sims DW (2006) Diel and tidal rhythms in diving behaviour of pelagic sharks identified by signal processing of archival tagging data. Mar Ecol Prog Ser 328:205–213

Shiffman DSA, Gallagher AJA, Boyle MDC (2012) Stable isotope analysis as a tool for elasmobranch conservation research : a primer for non-specialists.

Siegel V (2005) Distribution and population dynamics of *Euphausia superba*: Summary of recent findings. Polar Biol 29:1–22

Sigsgaard EE, Nielsen IB, Bach SS, Lorenzen ED, Robinson DP, Knudsen SW, Pedersen MW, Jaidah M Al, Orlando L, Willerslev E, Møller PR, Thomsen PF (2016) Population characteristics of a large whale shark aggregation inferred from seawater environmental DNA. Nat Publ Gr 1:1–4

Simpfendorfer CA, Heupel MR, White WT, Dulvy NK (2011) The importance of research and public opinion to conservation management of sharks and rays: A synthesis. Mar Freshw Res 62:518–527

Sims DW (2008) Sieving a Living : A Review of the Biology , Ecology and Conservation Status of the Plankton-Feeding Basking Shark *Cetorhinus Maximus*. Adv Mar Biol 54:3–5

Sims DW, Queiroz N, Doyle TK, Houghton JDR, Hays GC (2009a) Satellite tracking of the World's largest bony fish, the ocean sunfish (*Mola mola L.*) in the North East Atlantic. J Exp Mar Bio Ecol 370:127–133

Sims DW, Southall EJ, Richardson AJ, Reid PC, Metcalfe JD (2003) Seasonal movements and behaviour of basking sharks from archival tagging : no evidence of winter hibernation. 248:187–196

Sleeman JC, Meekan MG, Wilson SG, Polovina JJ, Stevens JD, Boggs GS, Bradshaw CJA (2010a) To go or not to go with the flow: Environmental influences on whale shark movement patterns. J Exp Mar Bio Ecol 390:84–98

Smith GJD, Gaskin DE (1974) The diet of harbor porpoises (*Phocoena phocoena (L.)*) in coastal waters of Eastern Canada, with special reference to the Bay of Fundy. Can J Zool 52:777–782

Sokoll S, Holtappels M, Lam P, Collins G, Schlüter M, Lavik G, Kuypers MMM (2012) Benthic nitrogen loss in the Arabian Sea off Pakistan. Front Microbiol 3:1–17

Soldevilla M, Hildebrand JA, Frasier K, Aichinger Dias L, Martinez A, Mullin K, Rosel P, Garrison L (2017) Spatial distribution and dive behavior of Gulf of Mexico Bryde's whales: potential risk of vessel strikes and fisheries interactions. Endanger Species Res 32:533–550

Speed CW, Meekan MG, Field IC, McMahon CR, Abrantes K, Bradshaw CJ a. (2011) Trophic ecology of reef sharks determined using stable isotopes and telemetry. Coral Reefs

Speed CW, Meekan MG, Rowat D, Pierce SJ, Marshall AD, Bradshaw CJA (2008) Scarring patterns and relative mortality rates of Indian Ocean whale sharks. J Fish Biol 72:1488–1503

Spitz J, Cherel Y, Bertin S, Kiszka J, Dewez A, Ridoux V (2011) Prey preferences among the community of deep-diving odontocetes from the Bay of Biscay, Northeast Atlantic. Deep Sea Res Part I Oceanogr Res Pap 58:273–282

Sterling JT, Ream RR (2004) At-sea behavior of juvenile male northern fur seals (*Callorhinus ursinus*). Can J Zool 82:1621–1637

Stevens JD (1986) Whale shark (*Rhincodon typus*) biology and ecology : A review of the primary literature.

Stevens JD, Bradford RW, West GJ (2010) Satellite tagging of blue sharks (*Prionace glauca*) and other pelagic sharks off eastern Australia: Depth behaviour, temperature experience and movements.

Stewart JD, Hoyos-Padilla EM, Kumli KR, Rubin RD (2016) Deep-water feeding and behavioral plasticity in Manta birostris revealed by archival tags and submersible observations. Zoology 119:406–413

Stewart JD, Rohner CA, Araujo G, Avila J, Fernando D, Forsberg K, Ponzo A, Rambahiniarison JM, Kurle CM, Semmens BX (2014) Trophic overlap in mobulid rays: insights from stable isotope analysis. 11:109–117

Sutton TT (2013) Vertical ecology of the pelagic ocean: classical patterns and new perspectives. J Fish Biol 83:1508–27

Tanakaa H, Ohshimoa S, Nobuo T, Ichimarub T (2009) Investigation of the geographical origin and migration of anchovy Engraulis japonicus in Tachibana Bay, Japan : A stable isotope approach. Fish Res 102:217–220

Tanakol R, Yazici Z, Sener E, Sencer E (1999) Fatty acid composition of 19 species of fish from the Black Sea and the Marmara Sea. Lipids 34:291–297

Thompson D, Buck CD, McConnell BJ, Garrett J (1998) Foraging behaviour and diet of lactating female southern sea lions (*Otaria flavescens*) in the Falkland Islands. J Zool London 246:135–146

Thomson JA, Araujo G, Labaja J, McCoy E, Murray R, Ponzo A (2017) Feeding the world’s largest fish: highly variable whale shark residency patterns at a provisioning site in the Philippines. R Soc Open Sci 4:170394

Thorrold SR, Afonso P, Fontes J, Braun CD, Santos RS, Skomal GB, Berumen ML (2014a)

Extreme diving behaviour in devil rays links surface waters and the deep ocean. Nat Commun 5:1–7

Thorrold SR, Afonso P, Fontes J, Braun CD, Santos RS, Skomal GB, Berumen ML (2014b) Extreme diving behaviour in devil rays links surface waters and the deep ocean. Nat Commun 5:4274

Thygesen UH, Sommer L, Evans K, Patterson TA (2016) Dynamic optimal foraging theory explains vertical migrations of bigeye tuna. Ecology 97:1852–1861

Thys TM, Ryan JP, Dewar H, Perle CR, Lyons K, O'Sullivan J, Farwell C, Howard MJ, Weng KC, Lavaniegos BE, Gaxiola-Castro G, Miranda Bojorquez LE, Hazen EL, Bograd SJ (2015) Ecology of the Ocean Sunfish, *Mola mola*, in the southern California Current System. J Exp Mar Bio Ecol 471:64–76

Tibiriçá Y, Birtles A, Valentine P, Miller DK (2011) Diving Tourism in Mozambique: An Opportunity at Risk? Tour Mar Environ 7:141–151

Treasure A, Roquet F, Ansorge I, Bester M, Boehme L, Bornemann H, Charrassin J-B, Chevallier D, Costa D, Fedak M, Guinet C, Hammill M, Harcourt R, Hindell M, Kovacs K, Lea M-A, Lovell P, Lowther A, Lydersen C, McIntyre T, McMahon C, Muelbert M, Nicholls K, Picard B, Reverdin G, Trites A, Williams G, Bruyn PJN de (2017) Marine Mammals Exploring the Oceans Pole to Pole: A Review of the MEOP Consortium. Oceanography 30:132–138

Trueman CN, Johnston G, O'Hea B, MacKenzie KM (2014) Trophic interactions of fish communities at midwater depths enhance long-term carbon storage and benthic production on continental slopes. Proc R Soc B 281:20140669

Trueman CN, Mackenzie KM, Palmer MR (2012) Identifying migrations in marine fishes through stable-isotope analysis. J Fish Biol 81:826–47

Trueman CN, MacKenzie KM, St John Glew K (2017) Stable isotope-based location in a shelf sea setting: accuracy and precision are comparable to light-based location methods. Methods Ecol Evol 8:232–240

Turnbull SD, Randell JE (2006) Rare Occurrence of a *Rhincodon typus* (Whale shark) in the Bay of Fundy, Canada. Northeast Nat 13:57–58

Tyminski JP, La Parra-Venegas R De, Cano JG, Hueter RE (2015) Vertical movements and patterns in diving behavior of whale sharks as revealed by pop-up satellite tags in the Eastern Gulf of Mexico. PLoS One 10

Ulrike Griebel (2002) Color Vision in Marine Mammals: A Review. Vienna Sch Mar Biol A Tribut to Jörg Ott:73:87

Vaudo JJ, Byrne ME, Wetherbee BM, Harvey GM, Jr AM, Shivji MS (2017) Horizontal and vertical movements of white marlin, *Kajikia albida* tagged off the Yucatan Peninsula. ICES J Mar Sci

Vignaud TM, Maynard JA, Leblois R, Meekan MG, Vázquez-Juárez R, Ramírez-Macías D, Pierce SJ, Rowat D, Berumen ML, Beeravolu C, Baksay S, Planes S (2014) Genetic structure of populations of whale sharks among ocean basins and evidence for their historic rise and recent decline. Mol Ecol 23:2590–2601

Villegas-Amtmann S, Costa DP (2010) Oxygen stores plasticity linked to foraging behaviour and pregnancy in a diving predator, the Galapagos sea lion. Funct Ecol 24:785–795

Villegas-Amtmann S, Costa DP, Tremblay Y, Salazar S, Aurioles-Gamboa D (2008) Multiple foraging strategies in a marine apex predator, the Galapagos sea lion *Zalophus wollebaeki*. Mar Ecol Prog Ser 363:299–309

Voigt CC, Rex K, Michener RH, Speakman JR (2008) Nutrient routing in omnivorous animals tracked by stable carbon isotopes in tissue and exhaled breath. Oecologia 157:31–40

Wallace BP, Seminoff J a. Kilham SS, Spotila JR, Dutton PH (2006) Leatherback turtles as oceanographic indicators: stable isotope analyses reveal a trophic dichotomy between ocean basins. Mar Biol 149:953–960

Walli A, Teo SLH, Boustany A, Farwell CJ, Williams T, Dewar H, Prince E, Block B a (2009) Seasonal movements, aggregations and diving behavior of Atlantic bluefin tuna (*Thunnus thynnus*) revealed with archival tags. PLoS One 4:e6151

Watt CA, Orr J, Ferguson SH (2016) A shift in foraging behaviour of beluga whales Delphinapterus leucas from the threatened Cumberland Sound population may reflect a changing Arctic food web. Endanger Species Res 31:259–270

Watwood SL, Miller PJO, Johnson M, Madsen PT, Tyack PL (2006) Deep-diving foraging behaviour of sperm whales (*Physeter macrocephalus*). J Anim Ecol 75:814–25

Webb TJ, Berghe E vanden, O'Dor R (2010) Biodiversity's big wet secret: The global distribution of marine biological records reveals chronic under-exploration of the deep pelagic ocean. PLoS One 5:1–6

Wells RS, Manire CA, Byrd L, Smith DR, Gannon JG, Fauquier D, Mullin KD (2009) Movements and dive patterns of a rehabilitated Risso's dolphin, *Grampus griseus*, in the Gulf of Mexico and Atlantic Ocean. Mar Mammal Sci 25:420–429

Weng KC, Castilho PC, Morrissette JM, Landeira-Fernandez AM, Holts DB, Schallert RJ, Goldman KJ, Block BA (2005) Satellite Tagging and Cardiac Physiology Reveal Niche Expansion in Salmon Sharks. Science (80-) 310:104–106

Wenzel FW, Polloni PT, Craddock JE, Gannon DP, Nicolas JR, Read AJ, Rosel PE (2013) Food habits of Sowerby's beaked whales (*Mesoplodon bidens*) taken in the pelagic drift gillnet fishery of the western North Atlantic. Fish Bull 111:381–389

Whitehead H (2001) Analysis of animal movement using opportunistic individual identifications : Application to sperm whales. Ecology 82:1417–1432

Whitehead H (2009) SOCPROG programs: analysing animal social structures. Behav Ecol Sociobiol 63:765–778

Williams JL (2017) Multidisciplinary Insights into the Conservation and Biology of Sea Turtles in Mozambique Thesis submitted by. :322

Williams AJ, Allain V, Nicol SJ, Evans KJ, Hoyle SD, Dupoux C, Vourey E, Dubosc J (2015) Vertical behavior and diet of albacore tuna (*Thunnus alalunga*) vary with latitude in the South Pacific Ocean. Deep Res Part II Top Stud Oceanogr 113:154–169

Williams SM, Holmes BJ, Tracey R, Pepperell JG, Domeier ML, Bennett MB (2017) Environmental influences and ontogenetic differences in vertical habitat use of black marlin (*Istiompax indica*) in the southwestern Pacific Subject Category : Subject Areas : Author for correspondence : R Soc Open Sci 4

Wilson RP (1985) The Jackass Penguin (*Spheniscus demersus*) as a pelagic predator. Mar Ecol - Prog Ser 25:219–227

Wilson RP, Culik BM, Adelung D, Spairani HJ, Coria NR (1991) Depth utilisation by breeding Adelie penguins, *Pygoscelis adeliae*, at Esperanza Bay, Antarctica. Mar Biol 109:181–189

Wilson SG, Polovina JJ, Stewart BS, Meekan MG (2005) Movements of whale sharks (Rhincodon typus) tagged at Ningaloo Reef, Western Australia. Mar Biol 148:1157–1166

Wilson SG, Polovina JJ, Stewart BS, Meekan MG (2006) Movements of whale sharks (*Rhincodon typus*) tagged at Ningaloo Reef, Western Australia. Mar Biol 148:1157–1166

Wilson R. Puetz K, Bost C. Culik B. Bannasch R, Reins T, Adelung D (1993) Diel dive depth in penguins in relation to diel vertical migration of prey: whose dinner by candle light? Mar Ecol

Prog Ser 94:101–104

Witt MJ, Broderick AC, Johns DJ, Martin C, Penrose R, Hoogmoed MS, Godley BJ (2007) Prey landscapes help identify potential foraging habitats for leatherback turtles in the NE Atlantic. Mar Ecol Prog Ser 337:231–243

Witteveen BH, Foy RJ, Wynne KM, Tremblay Y (2008) Investigation of foraging habits and prey selection by humpback whales (*Megaptera novaeangliae*) using acoustic tags and concurrent fish surveys. Mar Mammal Sci 24:516–534

Wolf N, Carleton SA, Martínez del Rio C (2009) Ten years of experimental animal isotopes ecology. Funct Ecol 23:17–26

Wolf N, Newsome SD, Peters J, Fogel ML (2015) Variability in the routing of dietary proteins and lipids to consumer tissues influences tissue-specific isotopic discrimination. Rapid Commun Mass Spectrom 29:1448–1456

Womersley FC, Leblond ST, Rowat DRL (2016) Scarring instance and healing capabilities of whale sharks and possible implications. In: Q Science. Proc The 4th International Whale Shark Conference.

Woodworth P a. Schorr GS, Baird RW, Webster DL, McSweeney DJ, Hanson MB, Andrews RD, Polovina JJ (2012) Eddies as offshore foraging grounds for melon-headed whales (*Peponocephala electra*). Mar Mammal Sci 28:638–647

Ziegler JA, Dearden P, Rollins R (2015) Participant crowding and physical contact rates of whale shark tours on Isla Holbox, Mexico. J Sustain Tour 24:616–636