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Diet of whale sharks *Rhincodon typus* inferred from stomach content and signature fatty acid analyses

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ABSTRACT: Whale sharks *Rhincodon typus* are large filter-feeders that are frequently observed feeding in surface zooplankton patches at their tropical and subtropical coastal aggregation sites. Using signature fatty acid (FA) analyses from their subdermal connective tissue and stomach content analysis, we tested whether whale sharks in Mozambique and South Africa predominantly feed on these prey and/or what other prey they target. Arachidonic acid (20:4ω6; mean ± SD = 17.8 ± 2.0% of total FA), 18:0 and 18:1ω9c were major FA of whale sharks, while in contrast, coastal epipelagic zooplankton collected near feeding whale sharks had 22:6ω3 (docosahexaenoic acid), 16:0 and 20:5ω3 (eicosapentaenoic acid) as major FA. Stomach contents of 3 stranded sharks were dominated by mysids (61 to 92% of prey items), another one by sergestids (56%), and a fifth stomach was empty. The dominant mysids (82% index of relative importance) were demersal zooplankton that migrate into the water column at night, suggesting night-time feeding by whale sharks. High levels of bacterial FA in whale sharks (5.3 ± 1.4% TFA), indicating a detrital link, potentially via demersal zooplankton, also support night-time foraging activity. High levels of oleic acid (16.0 ± 2.5%) in whale sharks and their similarity with FA profiles of shrimp, mysids, copepods and myctophid fishes from the meso- and bathypelagic zone suggest that whale sharks also forage in deep-water. Our findings suggest that, in the patchy food environment of tropical systems, whale sharks forage in coastal waters during the day and night, and in oceanic waters on deep-water zooplankton and fishes during their long-distance movements.

KEY WORDS: Feeding ecology · Omega 6 fatty acids · Signature lipids · Mysida · Chondrichthyans · Fatty acid biomarkers

INTRODUCTION

Early observations on whale sharks *Rhincodon typus* suggested that they may be omnivores, with phytoplankton and marine algae forming a component of their diets along with zooplankton and small nekton (Wright 1870, Kaikini et al. 1959, Silas & Rajagopalan 1963), although the more recent consensus is that they feed mainly on zooplankton (Stevens 2007, Rowat & Brooks 2012). However,

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almost all the available diet information originates from either observations of whale sharks feeding at the surface, during the day, generally close to the coast (e.g. Nelson & Eckert 2007), or from the stomach contents obtained from a limited number of incidentally caught or stranded specimens (e.g. Silas & Rajagopalan 1963, Rao 1986). Both of these data sources have significant limitations. Whale sharks spend a substantial proportion of their time in the open ocean, and may only briefly visit coastal areas (Heyman et al. 2001, Wilson et al. 2001, Rowat et al. 2011). They undertake frequent deep dives into bathypelagic depths, possibly to feed (Brunnschweiler & Sims 2011), and also forage at night (Taylor 2007) when zooplankton communities change dramatically due to emergence and vertical migration (Alldredge & King 1980, Hays 2003). Coastal observations of surface feeding during the day may therefore not be representative of their predominant feeding behaviours and prey preferences.

There are few direct assessments of the diet of whale sharks from stomach contents, and they often lack detail because of partial digestion of contents. No data have yet been published from current or recent targeted fisheries in Taiwan, China and India, where a substantial sample size could be achieved. Instead, most accounts originate from incidental strandings or catches. The most recent published record of the stomach contents of a whale shark is from a specimen landed in 1983 (Karbhari & Josekutty 1986). Reports range from descriptions of 'finely divided red matter' (Haly 1883) or 'green viscid fluid' (Pai et al. 1983) to 2 more detailed analyses of zooplankton taxa (Silas & Rajagopalan 1963) and prey fish species (Rao 1986). It appears that the stomach contents of whale sharks vary greatly, although the scarcity of available data precludes a conclusive assessment of their diet at this stage. Stomach content analyses require a large sample size to provide quantitative data (Pethybridge et al. 2011), can overestimate certain prey groups (Richardson et al. 2000), and only describe the most recent meal (Iverson et al. 2004) which, for whale sharks, is likely to be of coastal origin since that is where they are stranded or caught. Coastal waters may not represent their main foraging habitat, however, and stomach content analysis alone could result in misleading conclusions about their diet.

Biochemical approaches, such as stable isotope and fatty acid (FA) analyses, provide a longer-term record of an animal's diet. The use of FA signatures as an indirect method of assessing dietary preferences and the trophic ecology of marine animals has increased over the past 2 decades (Budge et al. 2006). Recently, FA analysis has been used to study the diet of elasmobranchs (Schaufler et al. 2005, Pethybridge et al. 2010, Pethybridge et al. 2011, McMeans et al. 2012, Couturier et al. 2013a). The rationale behind this approach is that the FA composition of the prey directly influences the FA signature of the predator (Iverson 2009). This direct influence is because most high trophic level marine animals lack the ability to synthesise particular FA, especially the essential long-chain (≥C20) polyunsaturated FA (LC-PUFA), *de novo* (Sargent et al. 1995, Dalsgaard et al. 2003, Iverson 2009). Although this is a promising technique, dietary FA analyses in elasmobranchs also have limitations. The degree to which elasmobranch predators modify dietary FA prior to storage is not yet known. Predators may also store different amounts of some FA in different tissues. For example, elasmobranch muscle tissue is high in PUFA, while the liver contains more monounsaturated FA (MUFA) (Pethybridge et al. 2010, McMeans et al. 2012). There is currently no information available on differences between subdermal connective tissue and muscle or liver tissue from elasmobranchs.

In a pilot study, Couturier et al. (2013b) presented FA profiles of whale shark subdermal tissue and reef manta ray *Manta alfredi* muscle tissue and showed that both large, planktivorous, pelagic species had high levels of arachidonic acid (ARA; 20:4ω6) and an unusually low ω3/ω6 ratio of <1. The authors indicated that the origin of this signature remained unresolved. Here, we investigate the diet of whale sharks using detailed stomach content analysis of 5 stranded individuals, as well as FA analyses of whale shark subdermal tissue, zooplankton from feeding events, and published FA signatures of other potential prey items including demersal (emergent) zooplankton, fishes, macroalgae, crab larvae, fish eggs, deep-sea plankton, euphausiids, gelatinous zooplankton and suspended organic material. We test whether whale sharks predominantly feed on crustacean zooplankton commonly observed during their feeding events, or what other food sources they may target.

MATERIALS AND METHODS

Stomach contents sample collection

Samples of stomach contents were taken from 5 dead, stranded whale sharks. Three sharks stranded at Pomene, southern Mozambique (22.92° S, 35.58° E)

late on 15 Aug 2009 and were dissected the following night and early morning of 17 Aug. One whale shark was washed up in northern South Africa at Scottburg (30.30° S, 30.76° E) and was dissected on 10 Feb 2010 and another at Sodwana Bay (27.55° S, 32.68° E) was dissected on 5 Aug 2010. Stomach contents were well-mixed *in situ* and large subsamples ('samples' henceforth) were taken and stored in either 95% ethanol or 10% formalin. All samples from South Africa were kept in ethanol, but some of the samples from Mozambique stored in formalin may have degraded somewhat prior to analysis.

Stomach content analysis

Stomach content samples were washed, stained overnight with Rose Bengal, mixed, and 2 ml subsamples taken and analysed in a gridded Petri dish under a stereo-microscope. All identifiable parts were categorised out of 2 subsamples or until at least 100 separate items were counted. Some counts were inferred from certain parts when whole specimens were not available. Numbers of the sergestid *Lucifer* were based on eye pair counts, and mysids were counted from whole specimens plus intact telsons. Crab megalopae were based on intact specimens plus eye pairs because eyes were often separated from the body. Chaetognath hooks in 2 stomach contents (22 and 5) were both defined as one individual worm. The numerical occurrence for each category ($\%N_o$) was calculated as a percentage of total counts. The remainder of the stomach contents was scanned for unusual or whole specimens. The frequency of occurrence ($\%F_o$) was calculated as the percentage of all stomachs containing each category. To generate a prey size spectrum, up to 27 whole specimens per taxon were measured using the microscope eyepiece scale bar. An approximate mean length of the sergestid *Lucifer*, which could not be measured here, was taken from Teodoro et al. (2012). Specimens were in various states of digestion, so weight could not be inferred. We used length^3 and assumed a density of 1 g cm^{-3}, similar to that of water, as a proxy for mass and calculated the mass (%M) as a percentage of total mass. Count was multiplied by length^3 to assess the relative importance of each taxon, and also to calculate the index of relative importance (IRI; Pinkas et al. 1971) per prey category as IRI = $(\%N_o + \%M)\%F_o$, which was then expressed as %IRI (Cortés 1997).

Tissue sample collection

Biopsies of 24 live, unrestrained whale sharks were taken at Praia do Tofo in southern Mozambique (23.85° S, 35.54° E) between June and August 2011. Whale shark samples were from 16 immature males, 2 mature males and 6 females, ranging from 500 to 850 cm estimated total length (TL). We used a hand spear with a modified tip that penetrated up to ~2 cm into the connective tissue to extract biopsies (0.13 ± 0.01 g; mean ± SE) laterally between the 1st and 2nd dorsal fin. With a lack of captive feeding studies examining how closely FA signatures of various predator tissues relate to their prey, we worked under the assumption that these subdermal tissue samples are representative of muscle lipid FA profiles, which in turn are indicative of, and provide information on, the diet of whale sharks. We acknowledge that subdermal tissue has not previously been used to infer diet in elasmobranchs. We deem this a valid approach, considering the results from a concurrent study showing that muscle tissue of reef manta rays and subdermal tissue of whale sharks have similar FA profiles (Couturier et al. 2013b). In addition, obtaining information on a threatened and protected species, such as the whale shark, from biopsies of live animals with little impact on their welfare is an important benefit of this approach.

For a local comparison of zooplankton and whale shark signature FA profiles from Praia do Tofo, qualitative zooplankton samples were collected in November and December 2011 using either a 10 cm diameter, 100 µm mesh hand-held net towed by a swimmer, or a 50 cm diameter 200 µm mesh net towed horizontally from a boat. Gelatinous zooplankton and some macrozooplankton groups were separated from the samples prior to storage. Three categories of plankton were distinguished: feeding, non-feeding and shelf-break samples. Feeding zooplankton samples were collected from just below the surface within 5 m of a feeding whale shark, and included mixed samples and separate zooplankton specimens: a shrimp, chaetognath, gelatinous and gastropod zooplankton. Non-feeding samples were collected from the same location when whale sharks were not present or not feeding, and included mixed samples and separate specimens of decapod larvae and copepods. Shelf-break samples were collected in 300 m deep water off the continental shelf ~15 km east of Praia do Tofo. Vertically integrated samples to 50, 100 and 200 m depth were collected with a 200 µm mesh net. Whale shark and zooplankton samples were immediately put on ice and stored for 38 to 108 d and 54 to 99 d, respectively, at –20°C prior to analysis.

Lipid extraction and lipid class determination

Lipid extraction was performed using the modified Bligh & Dyer (1959) method with a 1-phase methanol:chloroform:water (2:1:0.8 by volume) overnight extraction. Phases were separated by adding water and chloroform to achieve a final solvent ratio of 1:1:0.9 methanol-chloroform-water. After the phases partitioned, total lipids were recovered from the lower chloroform phase by rotary evaporation of chloroform *in vacuo* at ~40°C. The resulting total lipid extracts (TLE) were concentrated to dryness by application of a stream of inert nitrogen gas. Samples were weighed to determine total lipid content as % lipid and as mg g^{-1} of sample wet weight (ww). Lipids were re-diluted in chloroform and stored at −20°C prior to further analyses. Lipid class compositions were determined using an Iatroscan Mark V TH10 thin layer chromatograph coupled with a flame ionisation detector (FID). For each sample, the TLE was spotted and the chromarods were developed in a polar solvent system (60:10:0.1 by volume, hexane:diethyl-ether:acetic acid) for 25 min. A standard solution containing known quantities of wax esters, triacylglycerols, free fatty acids, sterols and phospholipids (Nu-Chek Prep) was run with the samples. The chromarods were oven-dried for 10 min at 100°C and analysed immediately. Peaks were identified by comparison of their retention factor with the standards. Peak areas were quantified using SIC-480II Iatroscan™ Integrating Software v.7.0-E (System Instruments Co., Mitsubishi Chemical Medicine). Peak areas were transformed to mass per µl spotted based on pre-determined linear regressions and further converted to mg of lipid per g of tissue ww.

Fatty acid determination

An aliquot of the TLE was transmethylated with 3 ml methanol:hydrochloric acid:chloroform (10:1:1 by volume) and heated at ~80°C for 2 h to produce fatty acid methyl esters (FAME). After cooling and adding 1 ml Milli-Q water, FAME were extracted 3 times with 1.8 ml hexane:dichloromethane (4:1 by volume). Samples were reduced to dryness under a nitrogen stream and a C_{19} FAME internal injection standard solution (Alltech Associates) was added prior to instrumental analyses. Gas chromatography (GC) was performed on an Agilent Technologies 7890B GC equipped with a non-polar Equity™-1 fused silica capillary column (15 m × 0.1 mm i.d., 0.1 µm film thickness), an FID, a split/splitless injector and an Agilent Technologies 7683 B Series autosampler. Helium was the carrier gas. Samples were injected in splitless mode at an oven temperature of 120°C. After injection, oven temperature was raised to 270°C at 10°C min^{-1} and finally to 300°C at 5°C min^{-1}. Peaks were quantified with ChemStation software (Agilent Technologies). GC results are typically subject to an error of up to ±5% of individual component areas. Component identities were confirmed with GC mass-spectrometry (GC-MS) using a Finnigan ThermoQuest GCQ GC-MS system (Finnigan) fitted with an on-column injector and using Thermoquest Xcalibur software. Other operating conditions were as previously described (Lee Chang et al. 2012).

Signature fatty acid analyses

Fatty acids were expressed as percentage of total FA (%TFA) and presented as mean ± SD. Of the full profile, 15 FA with a concentration of >1% TFA in the mean whale shark profile were used in the following analyses. Principle component analyses (PCA) were applied to FA profiles to explore similarities among whale sharks, other similar predators, and their observed and hypothesised prey. PCA also ranked the contribution of each FA to the separation, based on eigenvector coefficients in the linear combinations of variables making up the PCs. The most important FA for a principle component are shown on PCA plots and were arbitrarily defined as having eigenvector coefficents >|0.175|. No pre-treatment was applied to the signature FA data prior to computing a resemblance matrix based on Bray-Curtis similarity. Hierarchical cluster analysis, based on the group average, was performed and the results applied to the PCA plots by showing the similarity clusters. Analysis of similarity among groups (ANOSIM; 1-way; 999 max. permutations) was performed on similarity matrices, with interpretation focusing on the ANOSIM-R value rather than significance level because of the small numbers of replicates. ANOSIM-R values >0.75 indicate strong separation between groups, and <0.25 are barely separated groups. Similarity percentage analyses (SIMPER; 1-way Bray-Curtis similarity, 90% cut-off) were calculated for zooplankton and whale sharks from Tofo to examine which FA contributed most to the separation. *t*-tests were used to assess whether the means of a particular FA of 2 groups were significantly different. Analyses and plots were produced using PRIMER v6 (Primer-E).

Table 1. *Rhincodon typus*. Potential prey items for whale sharks, the rationale for their inclusion, the reference for this and references to corresponding fatty acid signatures taken from the literature

Prey item	Rationale	Reference	FA signature reference
Acartia copepods	Direct feeding observation	Nelson & Eckert (2007)	Cotonnec et al. (2001), Escribano & Perez (2010)
Amphipods	Reported in stomach contents	This study	Jeffs et al. (2004), Richoux et al. (2005)
Bathypelagic shrimps	Deep diving for foraging	Brunnschweiler & Sims (2011)	Lewis (1967)
Brachyuran eggs	Whale shark faecal analysis	Meekan et al. (2009)	Figueiredo & Narciso (2008), Torres et al. (2008)
Chaetognaths	Direct feeding observation	Rowat et al. (2011)	Jeffs et al. (2004)
Copepods	Reported in stomach contents	This study	Jeffs et al. (2004), Cass et al. (2011)
Cumaceans	Emergent zooplankton possibly important	This study	Würzberg et al. (2011)
Cuttlefish	Reported in stomach contents	van Kampen (1908)	Nichols et al. (2002)
Decapod larvae	Reported in stomach contents	Silas & Rajagopalan (1963)	Jeffs et al. (2004)
Deep-sea fishes	Deep diving for foraging	Brunnschweiler & Sims (2011)	Lewis (1967), Jeffs et al. (2004)
Euphausiids	Faecal analysis	Jarman & Wilson (2004)	Nichols et al. (2002), Jeffs et al. (2004)
Fishes	Direct feeding observation	Duffy (2002)	Lewis (1967), Nichols et al. (2002)
	Saurida: reported in stomach contents	van Kampen (1908)	Ozogul et al. (2011)
Fish eggs	Direct feeding observation	Heyman et al. (2001)	Tamaru et al. (1992), Jeffs et al. (2004), Nguyen et al. (2012)
Gelatinous zooplankton	Direct feeding observation	Heyman et al. (2001)	Holland et al. (1990), Nichols et al. (2003), Jeffs et al. (2004)
Macroalgae	Reported in stomach contents	This study	Johns et al. (1979), Allan et al. (2010)
Mysids	Reported in stomach contents	This study	Richoux et al. (2005), Herrera et al. (2011)
Sergestids (e.g. *Lucifer*)	Reported in stomach contents	This study	Petursdottir et al. (2008)
Small plankton	Incidental ingestion	–	Escribano & Perez (2010)
Suspended matter	Incidental ingestion	–	Cotonnec et al. (2001), Allan et al. (2010)
Thraustochytrids	Incidental ingestion	–	Lee Chang et al. (2012)
Others	Ostracod: reported in stomach contents	This study	Jeffs et al. (2004)
	Pteropod: reported in stomach contents	Silas & Rajagopalan (1963)	Jeffs et al. (2004)
	Stomatopod larvae: direct observation	Rowat et al. (2011)	Jeffs et al. (2004)

FA signatures of potential prey items and other comparison marine animals were collated from the literature and converted to %TFA where appropriate. Comparative signatures from the reef manta ray *Manta alfredi* (n = 13; Couturier 2013b), leatherback turtle *Dermochelys coriacea* (n = 1, neutral- and phospholipids; Holland et al. 1990), ocean sunfish *Mola mola* (n = 2; Hooper et al. 1973), fin whale *Balaenoptera physalus*, harp seal *Pagophilus groenlandica* (means; Ackman et al. 1971), humpback whale *Megaptera novaengliae* (means of n = 2 to 17; Waugh et al. 2012), and 15 species of deep-sea chondrichthyans (means of n = 1 to 10; Pethybridge et al. 2010) were obtained as context for the results from whale sharks.

For dietary investigations, all zooplankton samples from Mozambique were included in addition to literature FA signatures of potential and observed prey groups (Table 1). For example, whale sharks have been observed feeding on *Acartia* copepods in Mexico (Nelson & Eckert 2007); we therefore included FA signatures of *Acartia* (Cotonnec et al. 2001, Escribano & Perez 2010) and other copepod species (Jeffs et al. 2004, Cass et al. 2011). Only prey FA profiles containing essential FA ARA, eicosapentaenoic acid (EPA; 20:5ω3) and docosahexaenoic acid (DHA; 22:6ω3) were used in the analysis. This exploratory approach has limitations, including the small number of signature FA profiles available, the use of data that may not be from the exact prey species or location in question, and potential differences in analytical methods used.

Several hypothetical mixed signature FA profiles were calculated to explore potential prey mixes for whale sharks. Mix 1 included all prey items within 40% Bray-Curtis similarity of whale sharks. Mix 2 reflected zooplankton from observed feeding events and was a mean of all zooplankton samples collected at Praia do Tofo while whale sharks were feeding. Mix 3 was a proportional mean including prey

groups found in our stomach content analysis, based on the %IRI. Mix 4 was a proportional mean of the main prey categories of other stomach content reports, based on number of samples in that category. Mix 5 was a hypothetical diet of 30% daytime zooplankton, 20% demersal zooplankton (nighttime), 20% deep-water fishes, 20% bathypelagic crustaceans and 10% gelatinous zooplankton. The total lipid content of the respective prey groups was taken into account in these mixes. For example, in Mix 3, the %IRI of mysids was 82% and their total lipid content was 20.1% of dry weight, resulting in a coefficient of 0.92 for mysids; this coefficient was much smaller for the less numerous amphipods (%IRI = 7; lipid content = 9.3%; coefficient = 0.03) (see Appendix 1 for details).

RESULTS

Stomach contents

Of the 5 whale shark stomach contents, 4 were dominated by zooplankton, while 1 whale shark had a largely empty stomach aside from containing some macroalgal fragments. We put these findings into context with all other available whale shark stomach content reports from the literature (Fig. 1, Table 2). Eighteen prey categories were identified in our 4 stomach contents, of which mysids, the sergestid *Lucifer* spp., and copepods were most numerous (Table 3). Mysids dominated the %IRI (82.1%), followed by gammarid amphipods (6.9%) and isopods (4.3%). Ostracods, fish eggs, isopods and algal fragments were recorded in low numbers in 3 of the 4 stomachs (Table 3). Mysids numerically dominated the stomach contents of both whale sharks from South Africa and 1 individual from Mozambique, constituting 61 to 92% of total counts (Fig. 2). The mysid dominance in these samples is illustrated more clearly when considering the size of identified prey items, with large mysids accounting for 98 to 100% of the integrated mass (count × length^3). Similarly, the sergestid *Lucifer* dominated the 1 other sample numerically, and even more so when considering their estimated integrated mass (Fig. 2). Interestingly, all 3 whale sharks stranded together in Mozambique contained different prey items: one empty stomach, one containing mostly *Lucifer*, and one with mainly mysids.

Lipid class composition

The lipid class profile (expressed as mean ± SD% of TLE) of whale sharks was dominated by phospholipids (68.1 ± 10.9%) and sterols (21.4 ± 3.6%). A mean of all zooplankton collected at Praia do Tofo was high in phospholipids (43.2 ± 18.6%) and free FA (34.1 ± 19.9%; Table 4). Minor lipid classes for whale sharks included free FA (5.3%), triacylglycerols (2.8%) and wax esters (2.3%), while for zooplankton they were sterols (9.8%), triacylglycerols (9.7%) and wax esters (3.3%; Table 4).

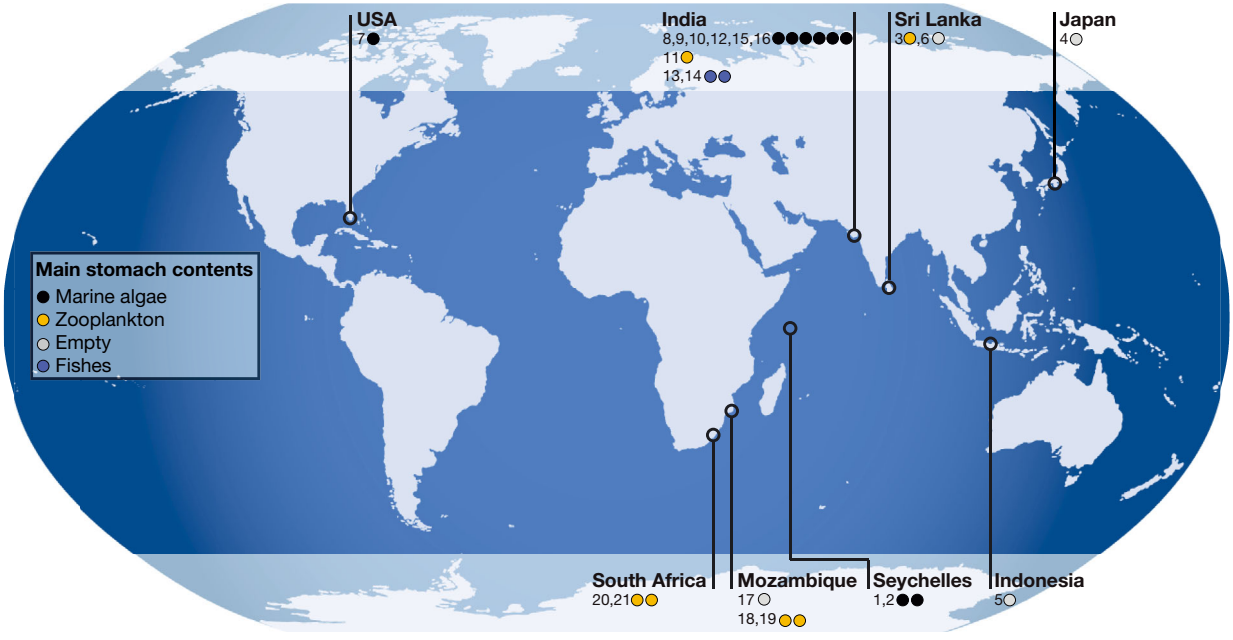

Fig. 1. *Rhincodon typus*. Records of whale shark stomach contents (see Table 2)

Table 2. *Rhincodon typus*. Records of whale shark stomach contents with reference to Fig. 1 and the main prey found

Ref.	Contents	Source
1, 2	Marine algae	Wright (1870)
3	Finely divided red matter	Haly (1883)
4	Mainly empty, sucking fish and a wooden pole	Kishinouye (1901)
5	Mainly empty, cuttlefish bones, small gobies and lizard fishes	van Kampen (1908)
6	Empty	Southwell (1912/13)
7	Marine algae and digested food material	Gudger (1932)
8, 9	Marine algae	McCann (1954)
10	Greenish matter, with some marine algae	Kaikini et al. (1959)
11	Zooplankton, fish, marine algae, sand	Silas & Rajagopalan (1963)
12	Green matter	Seshappa et al. (1972)
13, 14	Anchovies and sardines, zoo- and phytoplankton	Rao (1986)
15	Green fluid	Pai et al. (1983)
16	Marine algae, fishes, crustaceans, molluscs and a sucker fish	Karbhari & Josekutty (1986)
17–19	Mysids, sergestid *Lucifer*, and one mainly empty with some marine algae	This study
20, 21	Mysids	This study

Fatty acids

Overall, whale shark tissue contained saturated FA (SFA; 37.4% TFA) as the major FA group, followed by PUFA (32.4%) and MUFA (30.2%). In contrast, the profile differed markedly in zooplankton, which had more PUFA (43.4%), with SFA at 38.1% and MUFA lower at 18.2% (Table 5). Of the 48 FA identified, 32 and 31 were found in greater than trace amounts (>0.2%) in whale sharks and plankton, respectively. Major FA for whale sharks were ARA, 18:0 and 18:1ω9c, and major FA for zooplankton were DHA, 16:0 and EPA, in decreasing order of abundance. Bacterial fatty acids, which include iso- and anteiso branched FA plus 15:0 and 17:0 FA (Budge & Parrish 1998), were higher in whale sharks (5.3 ± 1.4%) than zooplankton (3.0 ± 0.8%; t = 7.56, p < 0.001). The strongest differences between mean signatures of the observed prey and predator were among the PUFA, with whale sharks having ~6 times higher levels of ARA, and 10 and 9 times lower levels of EPA and DHA, respectively (Table 5). A similarity percentage analysis between plankton and whale sharks supported this, with DHA (21%), ARA (17%), 18:1ω9c

Table 3. *Rhincodon typus*. Stomach contents of 4 whale sharks from southern Africa, with counts and percentages from subsamples, and mean sizes ± SE (mm), with number of samples in brackets. Other taxa found in the samples (n) are indicated with +

	Whale shark #2		**Whale shark #3**		**Whale shark #4**		**Whale shark #5**	
Sex (LT in cm):	Female (630 cm)		Female (820 cm)		Male (830 cm)		Male (770 cm)	
Location:	Pomene, Mozambique		Pomene, Mozambique		Scottburg, South Africa		Sowdana, South Africa	
Stomach contents (%IRI)	Count (%)	Mean size	Count (%)	Mean size	Count (%)	Mean size	Count (%)	Mean size
Sergestids *Lucifer* (1.6)	96 (55.8%)		1 (1%)				5 (3%)	
Mysids (82.1)	+		62 (60.8%)	6.40±0.47 (3)	92 (92%)	9.4±0.17 (20)	149 (90.3%)	9.10±0.17 (20)
Copepods (1.3)	36 (20.9%)	1.27±0.08 (27)	20 (19.6%)	1.37±0.06 (4)	1 (1%)	0.91±0.14 (2)	+	0.84±0.07 (6)
Fish eggs (0.3)	10 (5.8%)	0.62±0.07 (3)	1 (1%)	0.40 (1)			4 (2.4%)	
Ostracods (0.3)	15 (8.7%)	0.74±0.02 (16)	1 (1%)	1.46±0.66 (2)			+	0.98±0.04 (2)
Chaetognaths (0.01)	1 (0.6%)						+	
Spermatophores (0.2)	14 (8.1%)	1.15±0.03 (3)					+	1.62 (1)
Amphipods (6.9)	+	4.13±0.63 (2)			1 (1%)	5.60±1.58 (4)		
Fish scales (0.06)	+		+				6 (3.6%)	
Stomatopod larvae (2.5)	+	4.60±1.15 (3)						
Algal fragments	+		+		+			
Brachyuran eyes	+							
Gastropod shells	+							
Isopods (4.3)			+	4.70±0.2 (2)	3 (3%)	4.17±0.40 (9)	+	2.96±0.18 (6)
Megalopae (0.6)			16 (15.7%)	2.65±0.09 (14)				
Fish bones			+	9.00 (1)				
Foraminiferans (0.02)					3 (3%)		1 (0.6%)	
Bivalves							+	

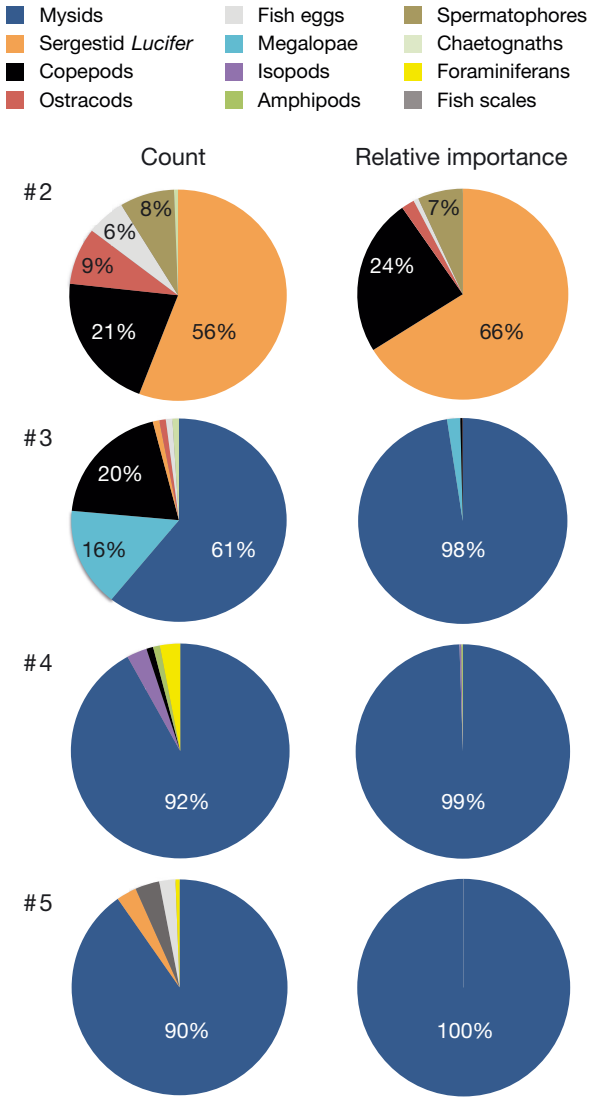

Fig. 2. *Rhincodon typus*. Stomach content analysis of 4 whale sharks from Mozambique and South Africa, with counts and relative importance (count × size^3) for each major taxon and percentages >5% shown as numbers

Table 4. *Rhincodon typus*. Mean ± SE lipid class compositions of whale shark (n = 24) and zooplankton (n = 29) samples from Praia do Tofo, expressed as % and mass of sample wet weight. Note that wax esters may include coeluting hydrocarbons and steryl esters

Lipid class	Whale sharks % TLE ± SE	Zooplankton % TLE ± SE
Free fatty acids	5.4 ± 0.7	34.1 ± 4.1
Phospholipids	68.1 ± 2.2	43.2 ± 3.8
Sterols	21.4 ± 0.7	9.8 ± 1.2
Triacylglycerols	2.8 ± 0.9	9.7 ± 3.6
Wax esters	2.3 ± 0.8	3.3 ± 1.1
Lipid content (mg g^{-1})	1.8	7.4

Table 5. *Rhincodon typus*. The mean fatty acid (FA) profile (% of TFA) of whale sharks (n = 24) and zooplankton (n = 31), grouping all FA < 0.2% as others

Fatty acid	Whale shark Mean (±SE)	Zooplankton Mean (±SE)
SFA		
14:0	0.6 (0.1)	4.5 (0.6)
i15:0	0.3 (0.0)	
15:0	0.4 (0.0)	0.8 (0.1)
i16:0	0.2 (0.0)	
16:0[b]	12.2 (0.4)	21.1 (0.7)
17:0[b]	1.5 (0.1)	1.5 (0.1)
i18:0[b]	1.2 (0.1)	
18:0[b]	17.7 (0.3)	7.7 (0.5)
i19:0	0.3 (0.0)	
20:0	0.4 (0.0)	0.7 (0.2)
22:0	0.9 (0.1)	0.6 (0.1)
23:0	0.6 (0.0)	0.2 (0.0)
24:0	1.0 (0.0)	0.6 (0.1)
Others[c]	0.2 (0.0)	0.4 (0.0)
Total SFA	**37.4 (0.1)**	**38.1 (0.2)**
MUFA		
16:1ω9c	0.6 (0.0)	0.3 (0.2)
16:1ω7c[b]	1.9 (0.2)	4.2 (0.4)
16:1	0.2 (0.1)	–
17:1ω8c[ab]	1.6 (0.2)	0.3 (0.0)
17:1	0.5 (0.0)	
18:1ω9c[b]	16.0 (0.5)	5.4 (0.6)
18:1ω7c[b]	4.2 (0.3)	2.8 (0.3)
19:1	0.6 (0.0)	
20:1ω11c		1.6 (0.6)
20:1ω9c	0.7 (0.0)	0.5 (0.1)
20:1ω7c		0.4 (0.1)
22:1ω11c		0.3 (0.1)
22:1ω9c	0.2 (0.0)	0.3 (0.1)
22:1ω7c	0.3 (0.0)	0.2 (0.0)
24:1ω9c[b]	2.3 (0.1)	1.1 (0.2)
24:1ω7c	0.4 (0.0)	
Others[c]	0.5 (0.0)	0.8 (0.0)
Total MUFA	**30.2 (0.1)**	**18.2 (0.2)**
PUFA		
ω3		
18:4ω3		1.1 (0.2)
18:3ω3		1.0 (0.1)
20:5ω3 (EPA)[b]	1.2 (0.1)	11.5 (0.8)
20:4ω3		0.6 (0.1)
21:5ω3		0.3 (0.0)
22:6ω3 (DHA)[b]	2.8 (0.2)	22.0 (1.8)
22:5ω3[b]	2.5 (0.1)	2.0 (0.5)
ω6		
18:3ω6		0.3 (0.0)
20:4ω6 (ARA)[b]	17.8 (0.4)	2.6 (0.4)
20:3ω6	0.3 (0.0)	0.3 (0.0)
20:2ω6		0.4 (0.1)
22:5ω6[b]	1.0 (0.1)	1.0 (0.1)
22:4ω6[b]	6.2 (0.2)	0.4 (0.1)
Others[c]	0.6 (0.0)	
Total PUFA	**32.4 (0.1)**	**43.4 (0.3)**

[a]Includes a17:0
[b]Included in the PCA analyses
[c]Other FA for whale sharks include: 14:1ω5c, a15:0, 16:1ω5, 18:3ω6, 18:4ω3, 18:3ω3, 18:1ω7t, 18:1ω5c, 20:4ω3, 20:2ω6, 20:1ω11c, 20:1ω7c, 21:5ω3, 22:1ω11c, 24:1ω11c
[c]Other FA for zooplankton include: 14:1ω5c, i15:0, a15:0, i16:0, 16:1, 16:1ω5c, 17:1, i18:0, 18:1ω7t, 18:1ω5c, i19:0, 19:1, 24:1ω11c, 24:1ω7c

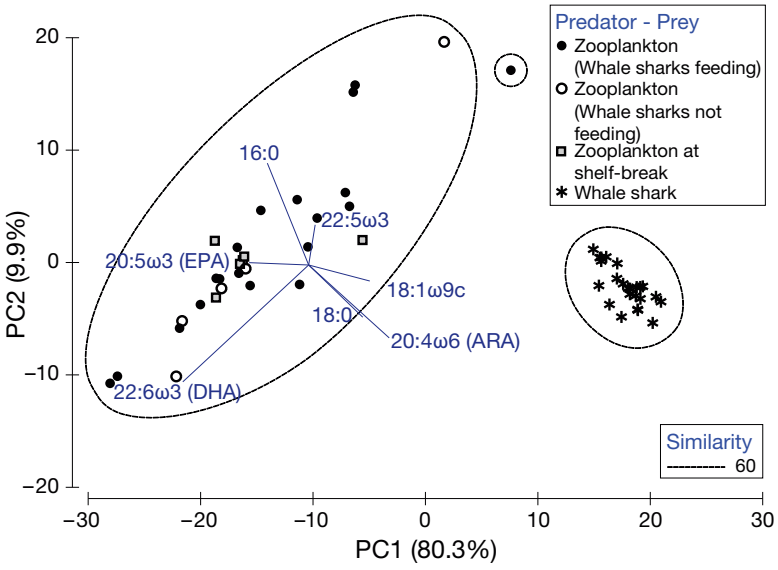

Fig. 3. First and second principle components of whale shark *Rhincodon typus* and zooplankton signature fatty acid (FA) profiles from Praia do Tofo (including all FA > 1% TFA), with 60% similarity clusters indicated. Fatty acids contributing most to the separation (eigenvector coefficient >|0.175|) are shown on the plot

(12%) and EPA (11%) contributing most to the separation.

Whale sharks grouped separately from all plankton samples collected locally at Praia do Tofo (ANOSIM-R > 0.96; Fig. 3). There was no separation among zooplankton samples when whale sharks were feeding and when they were not (ANOSIM-R = −0.12) or with samples from the shelf break (ANOSIM-R < 0.21). PCA and SIMPER analyses demonstrated that higher levels of 18:0, ARA and 18:1ω9c in whale sharks, and high levels of 16:0, DHA and EPA in zooplankton, resulted in the separation between predator and observed prey.

Signature FA profiles of whale sharks were also different from profiles of a suite of other potential prey groups (ANOSIM-R > 0.83; Fig. 4). High levels of 18:1ω9c and ARA separated the whale sharks from most of the potential prey categories. Bathypelagic shrimps had even higher levels of those FA, resulting in whale sharks grouping towards the centre of the plot (Fig. 4). Although all prey groups were significantly different from whale sharks, several potential prey species grouped within 40% similarity to the predator. These included all bathypelagic shrimps and mysids (Lophogastridae, Oplophoridae and Pasiphaeidae) and sergestids *Sergestes arcticus*, as well as some copepods *Rhincalanus nasutus*, fish eggs *Mugil cephalus*, deep-sea fishes *Myctophym nitidulum*, cumaceans *Diastylidae* sp. and *Nannastacidae* sp., gelatinous zooplankton *Chelophyes appendiculata*, decapod larva *Jasus edwardsii* phyllosoma, subsurface suspended matter and macroalgae (Phaeophyta and Chlorophyta).

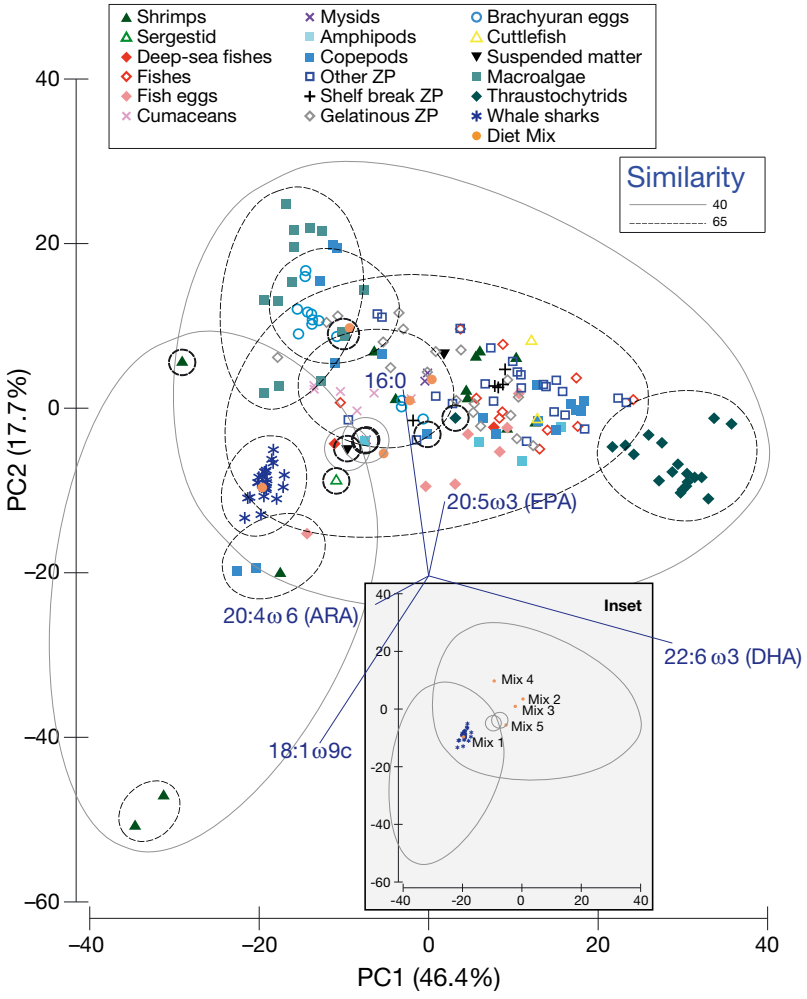

Fig. 4. First and second principal components of whale shark *Rhincodon typus* and potential prey signature fatty acid (FA) profiles (including all fatty acids > 1% TFA), with 40% and 65% similarity clusters indicated. Fatty acids contributing most to the separation are indicated on the plot (eigenvector coefficient >|0.175|). The inset shows the same plot with the position of the hypothetical mix FA profiles. Zp = zooplankton

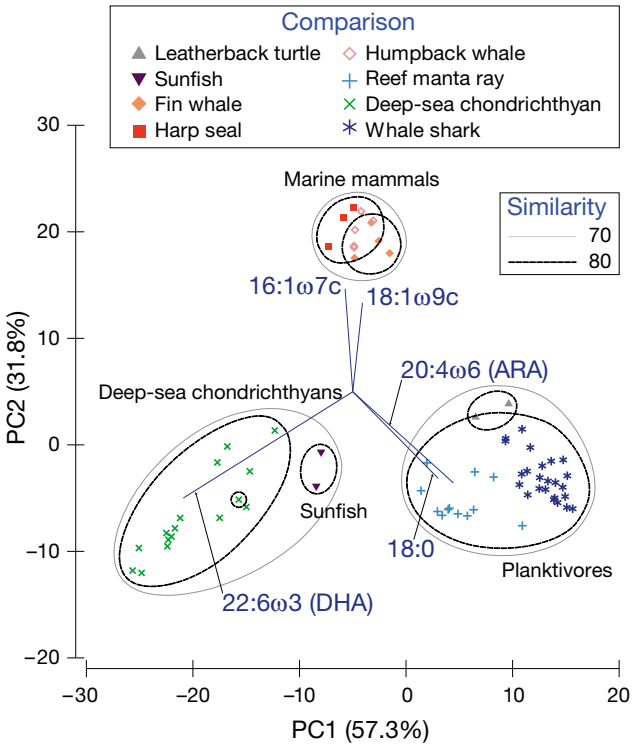

Fig. 5. First and second principle component of signature fatty acid (FA) profiles (>1% TFA) from whale sharks *Rhincodon typus* in comparison with chondrichthyans, planktivores, marine mammals and other marine species (data from Ackman et al. 1971, Hooper et al. 1973, Holland et al. 1990, Pethybridge et al. 2010, Waugh et al. 2012, Couturier 2013b). The 2 data points for the leatherback turtle represent the neutral- and phospholipid fractions for the pectoral muscle of a single animal. Fatty acids contributing most to the separation (eigenvector co-efficient >|0.175|) are shown on the plot

A hypothetical signature FA profile of all samples within 40% similarity to whale sharks (Mix 1) grouped among whale shark profiles (Fig. 4). Other mixed profiles (Mixes 2 to 5) grouped separate from whale sharks, with Mix 5 closest to the predator.

When comparing with other species, signature FA profiles of whale sharks grouped close to reef manta rays, and separately from other categories (Bray-Curtis similarity = 80%; Fig. 5). Whale shark profiles were, however, significantly different from reef manta rays (ANOSIM-R = 0.89), mainly due to lower levels of DHA (SIMPER 24% dissimilarity) and higher levels of ARA (14% dissimilarity). Leatherback turtles grouped closest to the 2 planktivorous elasmobranch species, but were significantly separate (ANOSIM-R > 0.97). In general, deep-sea chondrichthyans had higher levels of DHA than whale sharks, while marine mammals had higher levels of 16:1ω7c, 18:1ω9c and EPA (Fig. 5).

DISCUSSION

Although most research activity on whale shark diet has focused on their daytime surface feeding on zooplankton in coastal areas, evidence from signature FA and stomach content analyses presented here indicate that other prey are likely to be important contributors to their diet. Specifically, demersal macrozooplankton, deep-water fishes and deep-water macrozooplankton may play additional important roles. We caution against generalisation at this stage, however, as whale shark tissue samples were limited to one area (southern Mozambique) and to a relatively small size range (500 to 850 cm estimated total length). Further comparisons among other sites and with smaller or larger whale sharks will likely show geographic and ontogenetic patterns in their diet. Nevertheless, our results provide a new perspective on the diet of the world's largest fish.

Lipid classes

Whale shark samples had low levels of triacylglycerols, which are typically the main energy storage lipids in fishes (Sheridan 1988). Our findings for the subdermal tissue of whale sharks are similar to that observed for muscle of other tropical and temperate shark species, where only low levels of triacylglycerols generally occurred, and phospholipids dominated (Nichols et al. 1998, Mooney et al. 2002). As other elasmobranchs store mostly triacylglycerols in their liver (Pethybridge et al. 2010), our findings for subdermal tissue do not necessarily mean that whale sharks have low lipid storage. Further biochemical investigations using different tissues would clarify where and how much storage lipid is present in whale sharks. Zooplankton had unusually high amounts of free fatty acids. Considering the challenging field conditions in Mozambique, this high level is likely to be caused by lipid degradation during storage. The samples generally contained high levels of PUFA, indicating degradation was restricted to lipid class composition alone, consistent with other field-based studies (Phleger et al. 2007, Young et al. 2010).

Comparing whale sharks with other large marine predators

In addition to the unusually high levels of ω6 PUFA in whale sharks first reported in Couturier et al. (2013b), we show here that the full FA profile of

whale sharks also differs from other marine animals. The FA profile of reef manta rays was closest to that of whale sharks. Reef manta rays are ecologically similar to whale sharks in that they are both large, filter-feeding elasmobranchs that live mainly in tropical and sub-tropical waters (Stevens 2007, Marshall et al. 2009). This combination of characteristics is unique, since other large filter-feeders forage mostly in temperate to polar waters where, in contrast to tropical areas, their planktonic prey accumulate large lipid stores (Lee & Hirota 1973, Kattner & Hagen 1995). The FA profile of the leatherback turtle (Holland et al. 1990), another large zooplanktivore that regularly moves large distances in tropical to temperate waters (e.g. Bailey et al. 2012), grouped closest to the 2 filter-feeding elasmobranchs. Similar to whale sharks, leatherback turtles had high levels of ARA but in contrast, they also had high levels of EPA. This is likely to be due to their reliance on gelatinous zooplankton, especially jellyfish (Houghton et al. 2006), which also contain relatively high levels of ARA, EPA and DHA (Holland et al. 1990, Nichols et al. 2003, van der Bank et al. 2011). A suite of deep-sea chondrichthyans (Pethybridge et al. 2010) and the sunfish (Hooper et al. 1973) had higher levels of DHA than whale sharks, and marine mammals (Ackman et al. 1971, Waugh et al. 2012) contained more 16:1ω7, 18:1ω9 and EPA than whale sharks.

Comparing FA profiles of whale sharks with those of their observed or hypothesised prey further highlighted the unusual nature of the FA profile of whale sharks. They generally contained more ARA and 18:1ω9, but less EPA and DHA than their prey. Marine zooplankton usually have high levels of PUFA from the ω3 family, with an ω3/ω6 ratio in dominant groups, such as mysids or calanoid copepods, of 7 to 18 (Dalsgaard et al. 2003, Brett et al. 2009). Some prey groups are notable exceptions to this general rule, which we explore below in the context of whale shark ecology.

Herbivorous whale sharks?

Marine macroalgae have often been reported from whale shark stomachs, and we also found algal fragments in 3 stomach contents. Some marine macroalgae contain high levels of ARA, and were the only potential diet items investigated here that had high concentrations of ARA similar to whale sharks (t = 1.04, p = 0.32). However, considering the overwhelming observational evidence (e.g. Nelson & Eckert 2007, Motta et al. 2010) and a mouth morphology adapted to filter feeding (Gudger 1941, Paig-Tran et al. 2011), whale sharks clearly are not herbivores. The high occurrence of macroalgae in stomach contents is likely due to incidental ingestion of broken-off floating pieces that do not get digested as quickly as invertebrate or fish prey. Comparisons of the concentrations of ARA alone are misleading, because the full FA profiles of most macroalgae grouped separate to those of whale sharks, although 3 specimens were within 40% similarity to whale sharks. Based on these additional considerations for macroalgae, we propose that the link from macroalgae to whale sharks is likely to be via microheterotrophs in the sediment and the detrital food web to demersal zooplankton (see below).

Feeding at depth

Whale sharks are commonly observed at the ocean surface; however, they have recently been tracked to dive to bathypelagic (>1000 m) depths (Graham et al. 2006, Brunnschweiler & Sims 2011). While whale sharks spend a lot of their time in the epipelagic zone, these deep dives are intriguing and have been hypothesised to be foraging related (Brunnschweiler & Sims 2011). Signature FA results further support the deep-water foraging hypothesis. Of the potential prey groups we compared with whale sharks, FA signatures of deep-water species were among those grouping closest to the sharks. These included bathypelagic shrimps and mysids (Lophogastridae, Oplophoridae and Pasiphaeidae) caught between 1000 and 4000 m depth (Lewis 1967), cumaceans from 600 m depth (Würzberg et al. 2011), copepods from between 200 and 300 m depth (Cass et al. 2011) and the deep-water fish *Myctophum nitidulum* from 50 to 1000 m depth (Lewis 1967). This trend was not unanimous, with some bathypelagic fishes and copepods from similar depths grouping further away from whale sharks. We highlight the limitation that these comparative FA profiles for potential prey items were from different areas and seasons, which likely influenced their signatures (Dalsgaard et al. 2003). Our study is presently limited by the scarcity of FA profiles of potential prey from southeastern Africa or other tropical and subtropical areas.

The level of oleic acid (18:1ω9) generally increases with depth (Lewis 1967). Bathypelagic crustaceans had as much as 77% (of TFA) oleic acid (Lewis 1967). Other specimens with a high (>20%) oleic acid content included the copepod species from 200 to 300 m depth (Cass et al. 2011), deep-water fishes *Myctophum*

nitidulum and *Leuroglossus stilbus* (Lewis 1967), as well as plankton from an upwelling zone in Chile (Escribano & Perez 2010), fish eggs (Tamaru et al. 1992, Nguyen et al. 2012) and a brown algae, *Dictyota dichotoma* (Johns et al. 1979). Whale sharks also contained high levels of oleic acid (16.0 ± 2.5% TFA)—more than the surface plankton collected at Praia do Tofo (5.4 ± 3.5% TFA; $t = 13.01$, $p > 0.001$). This comparison further supports the idea that whale sharks gain some of their nutrition from prey that spend at least part of their day in waters deeper than ~200 m. Myctophid fishes could be such a potential prey group. Myctophids are among the most abundant mesopelagic fishes, are widely distributed, and many migrate vertically from hundreds of metres depth during the day to 100 to 200 m depth at night (Watanabe et al. 1999, Catul et al. 2011). Myctophids are also important prey for many large predators including penguins and seals (Reid & Arnould 1996, Raclot et al. 1998). While the overall FA signatures of deep-living prey and whale sharks are reasonably similar and could be linked by the diel vertical migration of the prey and the deep-diving behaviour of sharks, these particular prey do not explain the high ARA content found in whale sharks. Deep-sea fishes and bathypelagic crustaceans were low in PUFA and contained only 0.8 ± 0.8 and 1.8 ± 2.0% of ARA, respectively (Lewis 1967, Jeffs et al. 2004).

Feeding at night

Mysids were the dominant prey in stomach contents of both whale sharks from northern South Africa and one shark from southern Mozambique. Mysids are part of the demersal zooplankton that avoid visual predators during the day by sheltering in or on the benthos and migrating into the water column at night (Alldredge & King 1977, Porter & Porter 1977, Ohlhorst 1982). This functional group of zooplankton often plays a major role in coastal ecosystems, including coral reefs, kelp forests and sub-tropical bays (Alldredge & King 1977, Hammer 1981, Jacoby & Greenwood 1989). The vertical migration of demersal zooplankton is not uniform across different groups. For example, in a subtropical sand flat environment, mysids vertically migrate throughout the night, while amphipods emerge at specific times to avoid moonlight (Alldredge & King 1980). While most of the demersal zooplankton biomass is found close to the bottom at night, larger species move higher into the water column (Alldredge & King 1985). The dominance of large mysids in the whale shark stomach contents therefore indicates that they may feed extensively at night on demersal macrozooplankton. Some tracking evidence supports this hypothesis, with a shark tracked in southern Mozambique staying deeper at night than during the day while it was in shallow coastal waters (Brunnschweiler et al. 2009). Direct observational evidence is not available, and will be difficult to attain since this feeding behaviour would occur sub-surface, and introduced light would deter some demersal zooplankton and attract other plankton.

The high concentrations of bacterial FA in the whale shark tissue (5.3 ± 1.4% TFA) supported the notion that demersal zooplankton is part of the diet of whale sharks. Iso- and ante-iso branched and odd-chain FA are relatively common in bacteria and a subgroup of heterotrophic eukaryotes (the thraustochytrids; Lee Chang et al. 2011), but are generally rare in eukaryotes (Perry et al. 1979). The presence of bacterial FA in higher trophic level species indicates a link to the detrital and heterotrophic food chain (Lee Chang et al. 2011, Lee Chang et al. 2012), since bacteria colonise sinking particulate matter after plankton blooms (Morris 1984, Skerratt et al. 1995) and are found in high concentrations in sediments (Santangelo et al. 2000, Raghukumar 2002). Thraustochytrids can also occur at abundance in these environments. Of the prey groups in Fig. 4, tropical thraustochytrids (mean 25.5% TFA; Lee Chang et al. 2011, Lee Chang et al. 2012), suspended particulate matter (7.6% TFA; Cotonnec et al. 2001, Allan et al. 2010) and brachyuran eggs (6.9% TFA; Figueiredo & Narciso 2008, Torres et al. 2008) were the only groups with higher concentrations of bacterial FA than whale sharks. While brachyuran larvae are part of the diet of at least some whale sharks (Meekan et al. 2009), suspended particulate matter could be ingested by whale sharks in large quantities when filter-feeding. Bacterial and heterotrophic-derived FA could also be transferred when whale sharks ingest demersal zooplankton that feed within the sediment during the day.

Need for a diverse diet for a large, warm-water filter feeder

Results of whale shark stomach content and FA analyses presented here have shown that whale sharks feed on a variety of zooplankton prey, which is also supported by observational evidence (see Rowat & Brooks 2012). The reliance of this large predator on different prey groups means that no single matching prey FA profile exists. Of the hypothetical prey mix

FA profiles, the *post hoc*-determined Mix 1 (>40% similarity to the whale shark profile) was the only one that grouped with the whale shark profiles, with Mix 5 (30% surface zooplankton, 20% demersal zooplankton, 20% deep-sea fishes, 20% deep-sea crustaceans and 10% gelatinous zooplankton) being the next closest. Other mixes calculated without reference to our FA results grouped further away and showed that inferences from stomach contents or surface feeding events alone are not representative of their diet. FA analysis provides an informative time-averaged view of a predator's diet (Dalsgaard et al. 2003), which can be especially important for wide-ranging species that are difficult to observe for much of their lives. The fact that FA signatures of surface, daytime zooplankton do not match that of whale sharks substantiates this point.

Whale sharks, together with manta rays, have the unique challenge of being large, pelagic filter feeders searching for prey in the tropics and sub-tropics—comparatively nutrient-poor environments (Sarmiento & Gruber 2006) where plankton abundance strongly varies through time and space (Lalli & Parsons 1997). Targeting blooms of plankton at the surface in coastal areas is one strategy whale sharks use (e.g. Nelson & Eckert 2007). These blooms are ephemeral, so that whale sharks may have to move large distances between blooms. The present study indicates that other food sources, such as vertically migrating or mesopelagic fishes and zooplankton (both from offshore waters), or demersal zooplankton at the coast, are likely to be major prey items for whale sharks. As high concentrations of zooplankton are patchy and ephemeral in tropical waters, the search for food is likely the main driver for a complex diet comprising different foraging habitats and prey groups. This feeding strategy also helps explain why whale sharks move long distances and dive to deep waters.

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Appendix 1. Hypothetical prey mixes for Fig. 4, with the prey species, the relevant FA profile reference, the relative importance (RI) to this mix, the lipid content (LC; % of dry weight) and the reference for LC, the proportion of lipid content for this mix (PLC) and the proportion coefficient (PC; this coefficient is multiplied with the %TFA of a FA of each prey item, and the sum of these products is the value used for that FA)

Prey species	FA reference	RI	LC	LC reference	PLC	PC
Mix 1						
Bathypelagic shrimp - *Pasiphaea* sp.	Lewis (1967)	1	8.33	[a]	4.91	0.05
Bathypelagic shrimp - *Gnathophausia gracilis*	Lewis (1967)	1	8.33	[a]	4.91	0.05
Bathypelagic shrimp - *Acanthephyra curtirostris*	Lewis (1967)	1	8.33	[a]	4.91	0.05
Bathypelagic shrimp - *Acanthephyra curtirostris*	Lewis (1967)	1	8.33	[a]	4.91	0.05
Copepod - *Rhincalanus nasutus* (Gulf of California)	Cass et al. (2011)	1	9.4	Cass et al. (2011)	5.54	0.06
Copepod - *Rhincalanus nasutus* (tropical NE Pacific)	Cass et al. (2011)	1	8.8	Cass et al. (2011)	5.19	0.05
Fish eggs - *Mugil cephalus* (seawater outdoor) eggs	Tamaru (1992)	1	21.92	[b]	12.92	0.13
Other zooplankton - *Jasus edwardsii* phyllosoma stage 7	Jeffs et al. (2004)	1	27.2	Jeffs et al. (2004)	16.03	0.16
Cumacean - *Nannastacidae* sp.	Wuerzberg et al. (2011)	1	2.3	Wuerzberg et al. (2011)	1.36	0.01
Cumacean - *Diastylidae* sp.	Wuerzberg et al. (2011)	1	1.1	Wuerzberg et al. (2011)	0.65	0.01
Deep-sea fish - *Myctophum nitidulum*	Lewis (1967)	1	8.2	[c]	4.83	0.05
Sergestid - *Sergestes arcticus*	Petursdottir et al. (2008)	1	20	Petursdottir et al. (2008)	11.79	0.12
Green algae	Couturier et al. (unpubl. data)	1	20.63	[d]	12.16	0.12
Brown algae - *Hormorsira banksii*	Johns et al. 1979	1	20.63	[d]	12.16	0.12
Brown algae	Couturier et al. (unpubl. data)	1	20.63	[d]	12.16	0.12
Subsurface suspended matter	Cotonnec et al. 2001	1	1	[e]	0.59	0.01
Gelatinous zooplankton - *Chelophyes appendiculata*	Jeffs et al. 2004	1	1.4	Jeffs et al. (2004)	0.83	0.01
Sum			169.63		1.16	

[a] = No lipid content available in Lewis (1967), substituted with Oplophoridae (n = 6) from Lee & Hirota (1973)
[b] = No lipid content available in Tamaru (1992), substituted with other fish eggs of 6 spp. from Nguyen (2012), Jeffs et al. (2004), Ortega & Mourente (2010)
[c] = No lipid content available in Lewis (1967), substituted with another myctophid from Jeffs et al. (2004)
[d] = Lipid content derived from a mean of 3 brown algae from Tabarsa et al. (2011)
[e] = Lipid content not available; estimate

Appendix 1 (continued)

Prey species	FA reference	RI	LC	LC reference	PLC	PC
Mix 2						
Mixed sample 1	This study	1	1.99	This study	43.02	0.43
Mixed sample 2	This study	1	0.10	This study	2.08	0.02
Mixed sample 3	This study	1	0.13	This study	2.72	0.03
Mixed sample 4	This study	1	0.14	This study	3.09	0.03
Mixed sample 5	This study	1	0.06	This study	1.29	0.01
Mixed sample 6	This study	1	0.04	This study	0.80	0.01
Mixed sample 7	This study	1	0.07	This study	1.52	0.02
Jellyfish	This study	1	0.01	This study	0.16	0.00
Aurelia sp.	This study	1	0.00	This study	0.06	0.00
Ctenophores	This study	1	0.00	This study	0.05	0.00
Ctenophores	This study	1	0.00	This study	0.00	0.00
Salpes	This study	1	0.04	This study	0.77	0.01
Salpes	This study	1	0.11	This study	2.38	0.02
Diacavolinia sp. 1	This study	1	0.15	This study	3.22	0.03
Diacavolinia sp. 2	This study	1	0.15	This study	3.31	0.03
Other gastropod 1	This study	1	0.87	This study	18.89	0.19
Other gastropod 2	This study	1	0.66	This study	14.30	0.14
Shrimp	This study	1	0.06	This study	1.35	0.01
Chaetognath	This study	1	0.05	This study	1.00	0.01
Sum			4.63			1.00
Mix 3						
Mysids	This study	0.82	23.06	Richoux et al. (2005), Herrera et al. (2011)	18.91	0.92
Amphipods	This study	0.07	9.28	Jeffs et al. (2004), Richoux et al. (2005)	0.65	0.03
Stomatopods	This study	0.03	13.4	Jeffs et al. (2004)	0.40	0.02
Sergestid *Lucifer*	This study	0.02	20	Petursdottir et al. (2008)	0.40	0.02
Copepods	This study	0.01	12.98	Jeffs et al. (2004), Cass et al. (2011)	0.13	0.01
Sum			78.72		20.4906	1
Mix 4						
Marine algae (n = 9)[f]	This study	0.53	20.06	Tabarsa et al. (2012)	10.63	0.71
Zooplankton (n = 6)[g]	This study	0.35	11.86	Jeffs et al. (2004), Cass et al. (2011), Richoux et al. (2005)	4.15	0.28
Fishes (n = 2)	This study	0.12	0.89	Nichols et al. (2002), Ozogul et al. (2011)	0.11	0.01
Sum		1	32.81		14.89	1.00
Mix 5						
Daytime zooplankton	This study	0.3	11.86	Jeffs et al. (2004), Cass et al. (2011), Richoux et al. (2005)	3.56	0.40
Demersal zooplankton	This study	0.2	8.58	Wurzberg et al. (2011), Richoux et al. (2005), Herrera et al. (2011)	1.72	0.19
Deep-sea fishes	This study	0.2	8.2	Jeffs et al. (2004)	1.64	0.18
Bathypelagic crustaceans	This study	0.2	8.33	Lee & Hirota (1973)	1.67	0.19
Gelatinous zooplankton	This study	0.1	2.9	Hooper et al. (1990), Jeffs et al. (2004)	0.29	0.03
Sum		1	39.87		8.87	1.00

[f] = number of stomach contents containing this group (Fig. 1)
[g] = contains amphipods, copepods, ostracods, stomatopod, phyllosoma, chaetognath, pteropod

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