

Trophic ecology of elasmobranchs caught off Gujarat, India, as inferred from stable isotopes

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Habitat use and trophic levels were investigated in 13 species of elasmobranch caught off Gujarat, India, through their isotopic composition. Most of the animals were fished commercially and were immature, suggesting that fisheries operate in nursery habitats. All of the sharks analysed except *Rhincodon typus* had a higher estimated trophic level (>3.8) than rays and guitarfish (<3.8), suggesting a diet of bony fish and cephalopods. The trophic level of *Sphyrna lewini* and *R. typus* increased with total length, indicating ontogenetic dietary shifts, but the other species did not follow this trend. According to their $\delta^{13}\text{C}$ values, *R. typus*, *Mobula diabolus*, and, surprisingly, *Rhina ancylostoma* appeared to be the most pelagic species. In comparison, *Stegostoma fasciatum*, *Pristis pectinata*, *Rhinobatos granulatus*, and *Aetomylaeus maculatus* appeared to be the most demersal, inshore species, and their $\delta^{13}\text{C}$ signatures were significantly different from those of the three aforementioned species.

Keywords: $\delta^{13}\text{C}$, $\delta^{15}\text{N}$, India, rays, sharks.

Introduction

In recent decades, elasmobranchs have become a significant part of the fish catch in northwestern India. Catch composition data are not readily available, but the multispecies nature of the fisheries is evident from the literature, which reports that more than 20 shark species (mainly carcharhinids and sphyrnids) are caught regularly (Appukuttan and Nair, 1988). This extensive fishery, given the biological sensitivity of many elasmobranchs (owing to their relatively slow growth rate, low fecundity, and late age of maturity), has markedly reduced the local abundance of several species. Some species, such as the whale shark (*Rhincodon typus*), are now considered endangered (Pravin, 2000).

Despite the extensive catches, baseline information on the biology of the whale shark and other heavily exploited species is fragmentary. Information on the diet and trophic position can contribute to improved understanding of species ecology and the management plans for commercial stocks. Yet, no extensive information on food habits is available for elasmobranchs around India. Published data are limited to qualitative determinations of stomach contents, with no assessment of the relative contribution of each species to biomass intake (Raje *et al.*, 2002). Given the opportunistic feeding of many elasmobranchs, this information is usually insufficient to assess adequately the trophic levels of the various species.

Sharks and their relatives are commonly acknowledged to be top predators in many marine communities (Wetherbee and Cortés, 2004), but quantitative estimates of the trophic level are rare (Cortés, 1999). Traditionally, the trophic position of a species was determined by analysing the stomach contents of available specimens and determining the composition of the diet.

Dietary studies are useful, but can be biased by opportunistic feeding and differential digestion rates of prey, so only reflect the identifiable food ingested at a specific time. Many stomachs from different periods, regions, and size classes are necessary to obtain a general overview of the diet. The measurement of stable nitrogen isotopes in tissues of marine organisms is an alternative approach to estimating the trophic level and is based on the relative abundance of ^{15}N to ^{14}N ($\delta^{15}\text{N}$) in animal tissues, which show an enrichment of ^{15}N in consumers over their prey. Therefore, the $\delta^{15}\text{N}$ of each animal becomes an index of trophic position relative to the $\delta^{15}\text{N}$ of the known trophic position of a primary consumer (Cabana and Rasmussen, 1996). The index provides a general and integrated view of the trophic level at which the species feeds, although it does not provide specific dietary information. To date, relatively few studies have used stable-isotope analysis to estimate the trophic level of elasmobranchs (e.g. Ostrom *et al.*, 1993; Fisk *et al.*, 2002; Estrada *et al.*, 2003, 2006; Domi *et al.*, 2005; MacNeil *et al.*, 2005; Kerr *et al.*, 2006).

In contrast to $\delta^{15}\text{N}$, $\delta^{13}\text{C}$ values vary considerably, with little or no consistent increase with increasing trophic level (Vander Zanden and Rasmussen, 2001). The $\delta^{13}\text{C}$ of aquatic consumers can provide information on the sources of energy (Boutton, 1991) because $\delta^{13}\text{C}$ values are conserved “up the food chain” and vary at the base. That index, therefore, has proven useful in identifying where particular organisms feed; $\delta^{13}\text{C}$ values are typically less negative (more enriched in ^{13}C) in coastal or benthic foodwebs than in more-oceanic foodwebs. In addition, they are lower in fluvial and estuarine ecosystems than in marine ecosystems (Hobson *et al.*, 1997).

Here, we present the results of a survey of the isotopic signatures of 13 elasmobranch species fished off Gujarat, India.

The results were used to estimate the trophic position and to infer the habitat preferences of each species. A clupeiform fish, the Indian ilisha (*Ilisha melastoma*) of known trophic level, was included for comparative purposes. We also compared the trophic positions obtained from stable-isotope compositions with those calculated from published dietary studies to gauge whether the results were consistent.

Material and methods

In April and May 2001 and in November 2003, we sampled elasmobranchs at Veraval, the most important fish-landing location in Gujarat (Figure 1), an Indian state that contributes a major proportion of elasmobranch captures (Pillai and Parakkal, 2000; Raje et al., 2002). The harbour at Veraval, which was designed originally for the operation of 800 fishing vessels, is now the base for >3500 fishing vessels, which are engaged in trawling and trammelnetting (Central Pollution Control Board, 2003). The harbour was the main landing site for whale shark before the protection of the species in August 2001.

In all, 13 species were sampled, representing elasmobranchs commonly caught in the area and various trophic levels and habitats. The sample consisted of six species of shark, *Carcharhinus sorrah* ($n = 9$), *Rhizoprionodon acutus* ($n = 10$), *Mustelus manazo* ($n = 10$), *Sphyrna lewini* ($n = 8$), *R. typus* ($n = 19$), and *Stegostoma fasciatum* ($n = 1$), the sawfish *Pristis pectinata* ($n = 2$), and six batoids, *Himantura bleekeri* ($n = 3$), *Aetomylaeus maculatus* ($n = 2$), *Mobula diabolus* ($n = 5$), *Rhina ancylostoma* ($n = 2$), *Rhinobatos granulatus* ($n = 2$), and *Rhynchobatus djiddensis* ($n = 10$). The clupeiform *I. melastoma* ($n = 10$) was used as a baseline for estimating the relative trophic levels of the elasmobranchs.

Whale sharks were sampled ashore after capture by hook and line, before being processed. The other species were obtained from the Veraval fish market the same day that they were caught. All were labelled and preserved in salt until they reached the laboratory, where they were soaked in distilled water (three

times, 10 h each) to remove salt, then deep-frozen until analysis. All specimens were identified to species, their total body length measured, and a sample of muscle collected from each (Table 1).

Stable isotope analysis

For this analysis, a small section of muscle (1 g) was excised in the laboratory. The muscle sections were dried for 3 d at 70°C then ground with a mortar and pestle. The lipids were removed by rinsing the ground tissue several times with 2:1 chloroform:methanol mixture, following the method of Folch et al. (1957). Approximately 1 mg of the powdered sample was weighed in a tin capsule, automatically loaded and combusted at 1000°C, then analysed in a continuous-flow isotope-ratio mass spectrometer (Flash 1112 IRMS Delta C Series EA, Thermo Finnigan).

Results are expressed in delta (δ) notation, in which the relative variations of stable isotope ratios are expressed as parts-per-thousand variations from predefined standards, calculated as $\delta = [(RS/RR) - 1] \times 1000$, where RS is the ratio of the heavy isotope to the light isotope in the sample, and RR is the ratio of the heavy isotope to the light isotope in the standard.

The standards used were Pee Dee Belemnite (V-PDB) calcium carbonate and atmospheric nitrogen (air) for carbon and nitrogen, respectively. International secondary isotopic standards with known $^{13}\text{C}/^{12}\text{C}$ ratios (International Atomic Energy Agency, IAEA, Vienna), namely polyethylene (IAEA CH₇, $\delta^{13}\text{C} = -31.8$ vs. V-PDB), graphite (USGS₂₄, $\delta^{13}\text{C} = -16.1$ vs. V-PDB), and sucrose (IAEA CH₆, $\delta^{13}\text{C} = -10.4 \pm 0.2\text{‰}$ vs. V-PDB), were used for calibration at a precision of 0.2‰. For nitrogen, international secondary isotopic standards of (NH₄)₂SO₄ (IAEA N₁, $\delta^{15}\text{N} = +0.4$ vs. air, and IAEA N₂, $\delta^{15}\text{N} = 20.3$ vs. air) and KNO₃ (IAEA NO₃, $\delta^{15}\text{N} = 4.7$ vs. air) were used to a precision of 0.3‰.

Trophic level (TL_{15N}) calculation and validation

Trophic levels (TL_{15N}) were estimated from the raw $\delta^{15}\text{N}$ values for each elasmobranch species using the equation (Post, 2002) $\text{TL}_{15\text{N}} =$

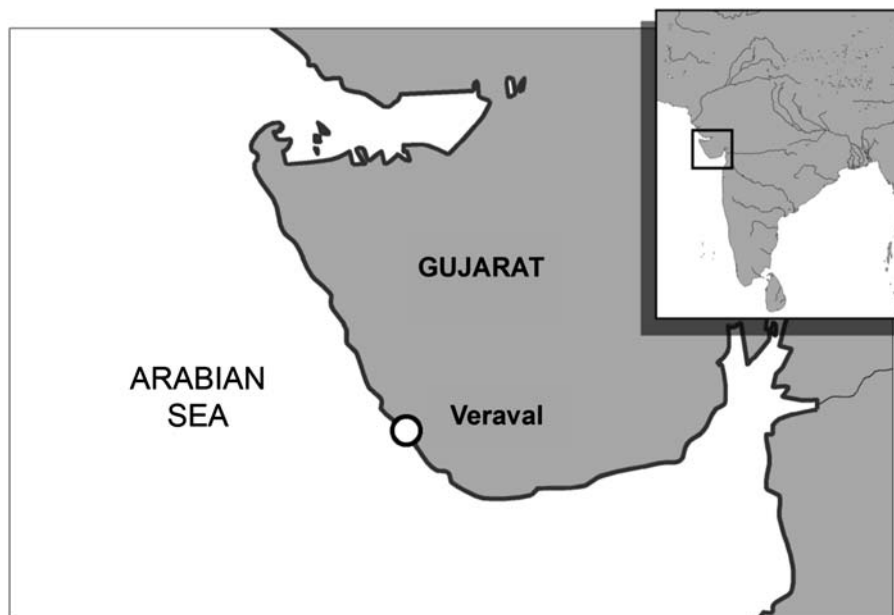


Figure 1. Locator map of the sampling location.

Table 1. The number of individuals measured, total length (TL) and range, and literature-derived biological lengths for the 13 elasmobranch and one reference teleost species in Indian populations for which data are available.

Family	Species	n	Present study		From the literature			Source
			Mean TL (cm)	Range (cm)	Maximum length (cm)	Length at birth (cm)	Length at maturity (cm)	
Carcharhinidae	<i>Carcharhinus sorrah</i>	4	77	70–86	160	50–60	90–95	Raje <i>et al.</i> (2002)
Carcharhinidae	<i>Rhizoprionodon acutus</i>	10	63	54–77	100	30–35	40–70	Raje <i>et al.</i> (2002)
Triakidae	<i>Mustelus manazo</i>	10	51	47–56	220	30	50–68	Yamaguchi and Taniuchi (2000)
Sphyrnidae	<i>Sphyrna lewini</i>	8	68	52–76	420	38–50	140–200	Raje <i>et al.</i> (2002)
Rhincodontidae	<i>Rhincodon typus</i>	18	797	296–1 880	1 800	60	600–900	Pillai and Parakkal (2000)
Stegostomatidae	<i>Stegostoma fasciatum</i>	1	190	190	354	20–36	70	Pillai and Parakkal (2000)
Pristidae	<i>Pristis pectinata</i>	0	–	–	760	–	–	Last and Stevens (1994)
Dasyatidae	<i>Himantura bleekeri</i>	3	73	59–92	105	–	–	Talwar and Jhingran (1991)
Myliobatidae	<i>Aetomylaeus maculatus</i>	2	136	134–139	200	–	54–72	Sommer <i>et al.</i> (1996)
Myliobatidae	<i>Mobula diabolus</i>	2	77	75–79	520	–	–	Raje <i>et al.</i> (2002)
Rhinidae	<i>Rhina ancylostoma</i>	2	208	200–215	300	45	157–178	Vidhayanon (2005)
Rhinobatidae	<i>Rhinobatos granulatus</i>	0	–	–	280	–	–	Sommer <i>et al.</i> (1996)
Rhinobatidae	<i>Rhynchobatus djiddensis</i>	7	124	67–193	310	43–60	110–177	Compagno (1984)
Pristigasteridae	<i>Ilisha melastoma</i>	9	11.74	10.6–12.5	17	–	–	Whitehead (1985)

$TL_{\text{baseline}} + (\delta^{15}\text{N}_{\text{elasmobranch}} - \delta^{15}\text{N}_{\text{baseline}})/3.4$. An appropriate baseline to estimate shifts in the relative trophic level within a single ecosystem is the use of an ecologically related species with a well-understood trophic level (Post, 2002). We used *I. melastoma*, a selective zooplankton-feeder, as the baseline for $TL_{15\text{N}}$ estimations. Its trophic level (TL_{baseline}) is 3.45, calculated from a number of food items as described in FishBase (2010), and its $\delta^{15}\text{N}$ ($\delta^{15}\text{N}_{\text{baseline}}$) is 14.61‰ (mean of ten individuals; Table 2). The assumed value for mean enrichment of $\delta^{15}\text{N}$ per trophic level was taken as 3.4 (Post, 2002).

To validate TL estimates from $\delta^{15}\text{N}$ ($TL_{15\text{N}}$) in Indian elasmobranchs, we compared these values with the diet-based estimates of the trophic level for the same species, as estimated from published prey items (Pauly and Christensen, 1995; FishBase, 2010).

Statistical analysis

The assumption of normality of the samples was checked using a Kolmogorov–Smirnov test, and the homogeneity of variances among sample groups was analysed with a Levene's test. Simple linear regression analyses were carried out between $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ values and length, and between trophic values obtained from different methodologies. A univariate analysis of variance (ANOVA) was conducted separately for both isotopes, followed by multiple comparisons based on Tukey's HSD *post hoc* test. All statistical calculations were carried out using the statistical package SPSS15.

Results

The lengths of 67 of the 83 elasmobranchs for which tissue samples were collected were measured (Table 1). The isotopic values for all species are summarized in Table 2 and illustrated in Figure 2.

The range of $\delta^{13}\text{C}$ values was from -16.05‰ (for *R. ancylostoma* and *R. typus*) to -12.04‰ (for *S. fasciatum*). Intraspecific variability in $\delta^{13}\text{C}$ was sometimes high (Table 2), as for *A. maculatus* ($n = 2$; s.d. = 0.99) and *R. djiddensis* ($n = 10$; s.d. = 0.94). Comparison of the data among all elasmobranch species revealed significant differences in $\delta^{13}\text{C}$ (ANOVA: $F = 13.81$, $p < 0.0001$). The $\delta^{13}\text{C}$ values of *R. typus*, *R. ancylostoma*, and *M. diabolus* were significantly lower than those of *P. pectinata*, *R. granulatus*, and *A. maculatus* (Tukey's HSD test, $p < 0.05$). The other species did not show significant differences (Table 2, Figure 2).

The range of $\delta^{15}\text{N}$ values in the 13 species was from 13.7‰ (for *R. ancylostoma*) to 17.65‰ (for *P. pectinata*). Relatively high levels of intraspecific variability were found: the maximum $\delta^{15}\text{N}$ s.d. was 1.51 for *R. typus*, followed by 1.13 for *R. ancylostoma* (of which there were just two specimens). Comparison of the data among all elasmobranch species revealed significant differences in $\delta^{15}\text{N}$ (ANOVA; $F = 48.12$, $p < 0.0001$). Pairwise comparisons indicated no differences in $\delta^{15}\text{N}$ among sharks except for *R. typus*, which produced significantly lower values than the other sharks (Tukey's HSD test, $p < 0.05$; Table 2). Except *R. typus*, sharks had higher $\delta^{15}\text{N}$ values than guitarfish and rays, which were indistinguishable from each other. Estimated trophic levels, based on $\delta^{15}\text{N}$ ($TL_{15\text{N}}$), ranged from 3.18 for *R. ancylostoma* to 4.34 for *P. pectinata* (Table 2).

Sample sizes were insufficient to examine ontogenetic changes in the isotopic ratios for most species. *Rhincodon typus* and *S. lewini* were the only species in which total length was related to $\delta^{15}\text{N}$ or $TL_{15\text{N}}$ ($p = 0.022$ and $p = 0.025$, respectively), and only in *R. typus* was length related to $\delta^{13}\text{C}$ ($p = 0.019$; Figure 3), although it is recognized that the samples of *S. lewini* did not cover their overall length range.

Table 2. The number of fish analysed, isotope values, and trophic level (TL_{15N}) for each species sampled, and the results from the ANOVA for significant differences between species means, with species having at least one letter in common indicating no significant difference (Tukey's test, *p* < 0.05).

Species	N	δ ¹³ C					δ ¹⁵ N					TL	s.d. of TL
		Mean	Significant difference	s.d.	Min.	Max.	Mean	Significant difference	s.d.	Min.	Max.		
Sharks													
<i>Carcharhinus sorrah</i>	9	-14.63	abc	0.39	-15.2	-14.0	16.54	bc	0.40	16.1	17.4	4.02	0.12
<i>Rhizoprionodon acutus</i>	10	-14.30	abc	0.46	-15.2	-14.8	16.74	bc	0.46	16.0	17.5	4.07	0.13
<i>Mustelus manazo</i>	10	-14.14	bc	0.51	-15.2	-13.3	15.68	abc	0.43	14.8	16.1	3.76	0.13
<i>Sphyrna lewini</i>	8	-15.50	ab	0.27	-15.9	-15.1	16.35	bc	0.57	15.4	17.2	3.96	0.19
<i>Rhincodon typus</i>	19	-16.05	a	0.81	-17.9	-14.7	14.02	a	1.51	11.0	15.5	3.32	0.58
<i>Stegostoma fasciatum</i>	1	-12.04					16.10					3.89	0.00
Sawfish													
<i>Pristis pectinata</i>	2	-13.30	c	0.14	-13.4	-13.2	17.65	c	0.49	17.3	18.0	4.34	0.15
Rays													
<i>Himantura bleekeri</i>	3	-14.70	abc	0.62	-15.2	-14.0	15.70	abc	0.87	15.1	16.7	3.77	0.26
<i>Aetomylaeus maculatus</i>	2	-13.50	c	0.99	-14.2	-12.8	15.40	ab	0.71	14.9	15.9	3.68	0.21
<i>Mobula diabolus</i>	5	-16.02	a	0.71	-16.7	-14.9	14.86	ab	0.93	13.4	15.8	3.61	0.27
Guitarfish													
<i>Rhina ancylostoma</i>	2	-16.05	a	0.49	-16.4	-15.7	13.70	a	1.13	12.9	14.5	3.18	0.33
<i>Rhinobatos granulatus</i>	2	-13.35	c	0.07	-13.4	-13.3	15.20	ab	0.00	15.2	15.2	3.62	0.00
<i>Rhynchobatus djiddensis</i>	10	-14.81	abc	0.94	-16.3	-13.8	15.48	abc	0.98	14.4	17.6	3.71	0.30
Teleost													
<i>Ilisha melastoma</i>	10	-15.18	ab	0.31	-15.7	-14.8	14.61	ab	0.88	13.0	16.0	3.45	0.26

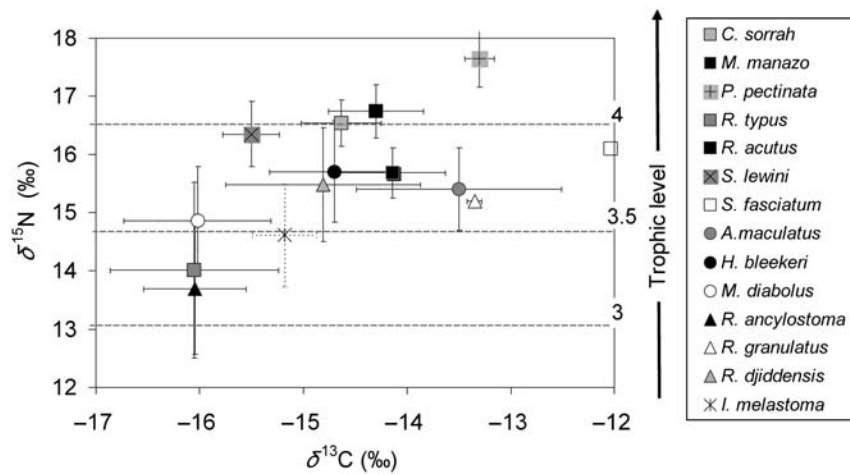


Figure 2. Biplot (δ¹³C–δ¹⁵N) of isotopic compositions (mean ± s.d.) of all the fish sampled.

Stable-isotope analysis was validated by the fact that there was a significant positive correlation between isotope-based estimates of the trophic level (TL_{15N}, current study) and the diet-based trophic levels (*r* = 0.79, *p* < 0.002; Figure 4). The slope of the best-fit regression line was not significantly different from 1, and the intercept was also not significantly different from 0 at a 95% confidence interval (TL = 0.74 + 0.78 TL_{15N}, *p* < 0.003, adjusted *r* = 0.77).

Discussion

Many of the individuals analysed in the current study were immature (Table 1), which is evident when the lengths of our samples are compared with size-at-birth and *L*_{max} values (using data for Indian populations, where available). All individuals of at least three of the species studied here (*S. lewini*, *C. sorrah*, and *M. manazo*) had lengths less than the species' typical lengths at

maturity, suggesting that fishing activities take place at least partially in nursery habitats. For example, the mean length of the captured *S. lewini* (67.75 cm) was only slightly greater than the size-at-birth (38–50 cm). *Sphyrna lewini* is probably the most common hammerhead shark inhabiting Indian seas, and the smallest young are found close inshore and move into deeper water as they grow. Raje *et al.* (2002) reported that in Kerala, 98% of *S. lewini* caught by gillnets then landed from 1990 to 1993 ranged in length from 40 to 60 cm, similar to the size range found here (52–76 cm). These data reinforce the conclusion that newborns and juveniles of this species are being fished in India, although the restricted sampling periods and the gears used may have influenced our interpretation of which species were caught. Similar circumstances have been described in the Gulf of Mexico (Ruíz, 1983; Bonfil, 1997; Madrid *et al.*, 1997), and off Hawaii (Duncan and Holland, 2006) and Mauritania (Ducrocq, 1998).

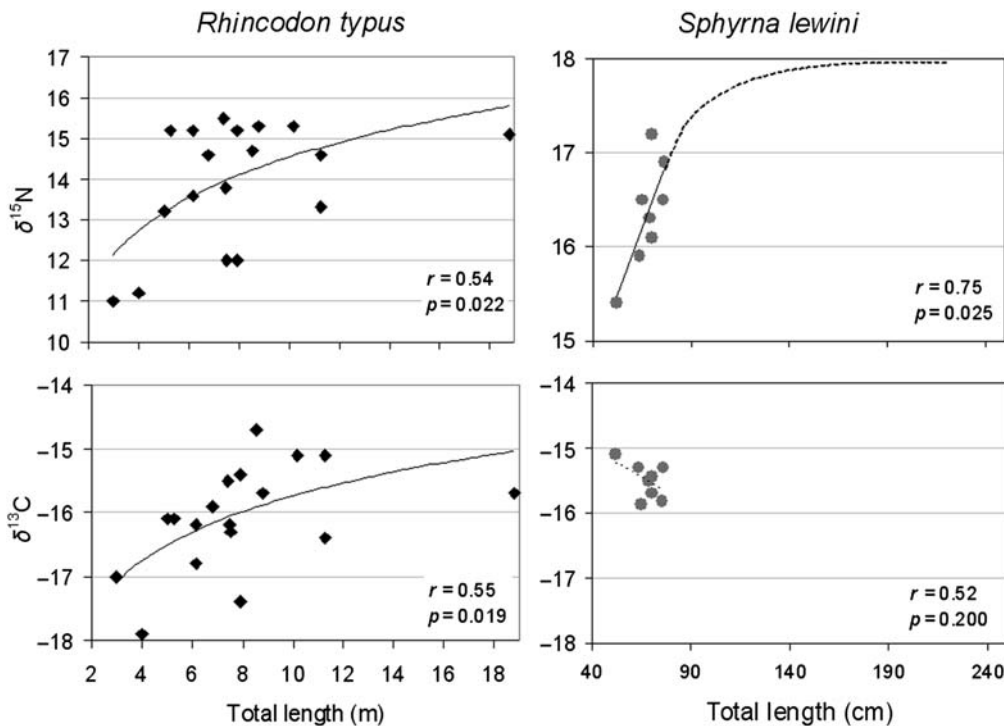


Figure 3. Relationships between $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ and length in (left) *R. typus* [regression lines: $\delta^{15}\text{N} = 10.01 + 1.97 \log(\text{length})$ and $\delta^{13}\text{C} = -18.30 + 1.11 \log(\text{length})$] and (right) *S. lewini* (regression line: $\delta^{15}\text{N} = 12.5 - 0.57 \text{ length}$). The dashed line is the hypothetical $\delta^{15}\text{N}$ trend for larger *S. lewini*.

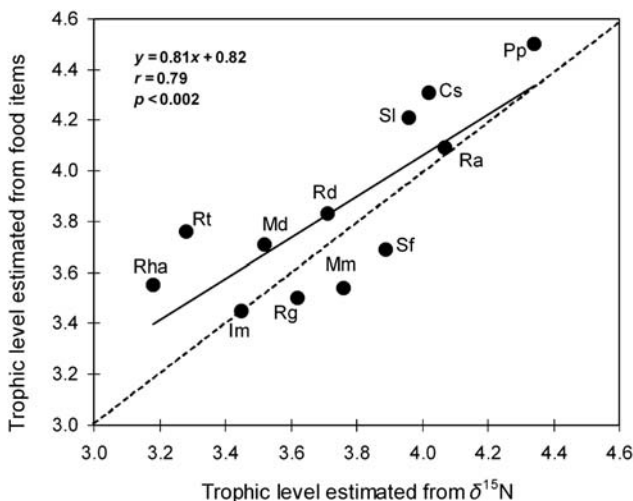


Figure 4. Comparison of isotope-calculated trophic levels ($\text{TL}_{15\text{N}}$) with trophic levels calculated from food items as reported in FishBase (2010). Cs, *Carcharhinus sorrah*; Ra, *Rhizoprionodon acutus*; Mm, *Mustelus manazo*; Sl, *Sphyrna lewini*; Rt, *Rhincodon typus*; Sf, *Stegostoma fasciatum*; Pp, *Pristis pectinata*; Md, *Mobula diabolus*; Rha, *Rhina ancylostoma*; Rg, *Rhinobatos granulatus*; Rd, *Rhynchobatus djiddensis*; and Im, *Ilisha melastoma*. The 1:1 line (dashed) is shown for comparison.

Interspecific variation in stable-isotope composition

The inferred habitats and the prey of each species (adapted from FishBase, 2010) were compared with the results of stable-isotope analysis (Table 3). Based on their $\delta^{13}\text{C}$ values (Table 3), *R. typus*

(−16.05), *M. diabolus* (−16.02), and *R. ancylostoma* (−16.05) are the most pelagic or epipelagic species. Although this conclusion is consistent with the known habitats for the first two species (Table 3), the last species is generally found on sandy and muddy substrata (Michael, 1993). However, it is sometimes found in the water column (Michael, 1993), consistent with our results. Given the small sample size, further study of this species could be informative.

On the other hand, *S. fasciatum* (−12.04), *P. pectinata* (−12.04), *R. granulatus* (−13.35), and *A. maculatus* (−13.5) seem to be the most inshore and benthic species. Their $\delta^{13}\text{C}$ signatures are significantly different from those of the three above-mentioned pelagic species (Table 2). Most of the species sampled are coastal, and differences in their $\delta^{13}\text{C}$ values would reflect differences between pelagic and benthic habitats rather than between oceanic and coastal habitats.

The significant difference in $\delta^{15}\text{N}$ (or TL) between species groups shows that sharks in this area feed at a higher trophic level than rays and guitarfish. All sharks except *R. typus* had a trophic level >3.8 (Figure 2), suggestive of a diet of bony fish and cephalopods. In general, the information matched well with the data listed in Table 3.

The sawfish *P. pectinata* was at the highest trophic level ($\text{TL} = 4.34$), and the reasons for this are unclear. Most guitarfish and rays had a trophic level <3.8, with the ray *H. bleekeri* having the highest value ($\text{TL} = 3.77$). Unfortunately, we could find no dietary data for this species in the literature, so its high TL value leads us to hypothesize that in addition to typical zoobenthos (bottom crustaceans and molluscs), it may feed partly on bony fish and/or cephalopods. This feeding strategy is found in *R. djiddensis* ($\text{TL} = 3.71$), which exhibits similar levels

Table 3. Food items and feeding habitats (in other regions) of the sampled elasmobranchs extracted from FishBase (2010), including the mean values of TL_{15N} and δ¹³C found in the current study.

Species	Habitat	δ ¹³ C	Food	TL _{15N}
<i>Carcharhinus sorrah</i> , Spot-tail shark	Continental and insular shelves, primarily near reefs, sometimes offshore	−14.63	Prefers teleosts, but also feeds on cephalopods and crustaceans	4.02
<i>Rhizoprionodon acutus</i> , Milk shark	Continental shelf, often off sandy beaches	−14.30	Small pelagic and benthic teleosts, cephalopods, and other invertebrates	4.07
<i>Mustelus manazo</i> , Starspotted smoothhound	Intertidal zone, on mud and sand substrata, down to a depth of at least 360 m	−14.14	Bottom invertebrates (crustaceans), and teleosts	3.76
<i>Sphyrna lewini</i> , Scalloped hammerhead	Coastal pelagic, semi-oceanic, often entering enclosed bays and estuaries	−15.50	Fish, cephalopods, sharks, and rays	3.96
<i>Rhincodon typus</i> , Whale shark	Offshore and close inshore, sometimes entering lagoons or coral atolls	−16.05	Plankton and nekton	3.32
<i>Stegostoma fasciatum</i> , Zebra shark	Inshore, on sand, rubble, or coral substrata of continental and insular shelves	−12.04	Prefers molluscs, but also feeds on small teleosts, crustaceans, and sea snakes	3.89
<i>Pristis pectinata</i> , Smalltooth sawfish	Commonly seen in bays, lagoons, estuaries, and river mouths	−13.30	Nekton, finfish, and teleosts	4.34
<i>Himantura bleekeri</i> , Bleeker's whipray	Inshore, on soft substrata to at least 30 m deep. Enters estuaries	−14.70	Not known	3.77
<i>Aetomylaeus maculatus</i> , Mottled eagle ray	Inshore, inhabiting mangrove creeks and protected sandy channels	−13.50	Bottom crustaceans and molluscs	3.68
<i>Mobula diabolus</i> , Devil fish	Epipelagic, over continental shelves and near oceanic islands	−16.02	Small pelagic fish and crustaceans, and zooplankton	3.61
<i>Rhina ancylostoma</i> , Bowmouth guitarfish	Coastal areas and on coral reefs, close inshore, on sand and mud substrata	−16.05	Bottom crustaceans and molluscs	3.18
<i>Rhinobatos granulatus</i> , Sharpnose guitarfish	From the intertidal to offshore continental shelves	−13.35	Zoobenthos	3.62
<i>Rhynchobatus djiddensis</i> , Giant guitarfish	Occurs inshore and in shallow estuaries	−14.81	Crabs, lobsters, bivalves, and small fish	3.71
<i>Ilisha melastoma</i> , Indian ilisha	Coastal waters, enters estuaries	−15.18	Zooplankton and crustaceans	3.45

of δ¹⁵N and δ¹³C (Figure 2). Benthic invertebrates are the main prey of *R. granulatus* (TL = 3.62). However, the δ¹⁵N and δ¹³C values (Figure 2) of *R. granulatus* are similar to those of *A. maculatus* (TL = 3.68), and these two species are known to frequent similar habitats (Table 3). Therefore, we infer that they feed on a similar diet of shellfish and crustaceans. *Rhina ancylostoma* (TL = 3.18), *M. diabolus* (TL = 3.52), and *R. typus* (TL = 3.28) were the species feeding at the lowest trophic levels. Their values of δ¹⁵N were close to that of *I. melastoma* (planktonic), suggesting a diet similar to that of the bony fish, i.e. crustaceans and zooplankton, consistent with literature reports. Whale sharks, for example, sieve a vast quantity of plankton to obtain their food, although nektonic prey and occasionally tuna and squid are also components of their diet (Compagno, 1984; Last and Stevens, 1994; Colman, 1997).

Intraspecific variation in stable-isotope composition

Of the 13 elasmobranch species analysed here, only five yielded sufficient data ($n \geq 7$; Table 1) to evaluate intraspecific variation in δ¹⁵N (or trophic level) and δ¹³C with respect to total body length. Values of δ¹³C varied with length only in *R. typus*, and values of δ¹⁵N (or trophic level) only in *R. typus* and *S. lewini*. Our failure to detect any other trends was probably the consequence of the small sample size and restricted length ranges available. Further studies on ontogenetic changes in isotopic signatures are desirable.

Correlations between the δ¹³C value and length in whale shark indicated important δ¹³C changes during ontogeny. Moreover, the lower values of δ¹³C observed in the youngest sharks may indicate geographic segregation; yearling whale sharks retained an offshore pelagic isotopic signal of −17.5‰, but in adults, the

carbon-isotopic composition changed to a more enriched coastal signal (−15.5‰), indicating a transition from pelagic life to a relatively more inshore habitat (AB, unpublished data).

Sphyrna lewini, of which just eight animals were measured (all immature), showed a non-significant tendency for δ¹³C to decrease with length. This seems to suggest that neonatal sharks inhabit coastal habitats and move offshore as they grow, confirming a pattern described previously (Compagno, 1984). To verify this trend, however, larger animals need to be sampled. The trend in δ¹³C observed for this species seems to be opposite to that of whale sharks.

A positive relationship between δ¹⁵N and size has been demonstrated for several fish species. This relationship has generally been attributed almost exclusively to ontogenetic changes in feeding habits, a trend that is seemingly common in fish. Its occurrence in many elasmobranchs is expected because as they increase in size, other modifications take place, such as changes in the size of the jaws, teeth, and stomachs, swimming speed, habitat utilization, movement patterns, energy requirements, hunting skills, and vulnerability to predation. These changes result in larger elasmobranchs having increased exposure to more diverse prey (Wetherbee and Cortés, 2004).

It was expected that the δ¹⁵N (or TL_{15N}) values of whale sharks would increase with fish length. Moreover, animals < 4 m long differed significantly in the δ¹⁵N value from larger animals (the TL_{15N} values for the two length groups were 2.7 and 3.4, respectively; AB, unpublished data). The higher values of muscle δ¹⁵N (or TL_{15N}) found in animals > 4 m may result in part from the decrease in the growth rate and concomitant increase in the δ¹⁵N diet–tissue discrimination factor with maturation, as suggested for other fish species (Trueman et al., 2005).

Similar to whale sharks and despite the small size of the animals analysed here, *S. lewini* exhibited increasing $\delta^{15}\text{N}$ (or TL) values with length (Figure 3; $p < 0.03$). Our hypothesis is that TL continues to increase at an attenuated rate until the shark's hunting skills improve as it grows (to a length of ~ 150 cm). Therefore, the trophic level of adults would be expected to be higher than that of juveniles. For example, if adult *S. lewini* reached a $\delta^{15}\text{N}$ of 18‰ (as the observed trend in Figure 3 suggests), then the corresponding TL would be 4.45. These results are consistent with those of Cabrera Chávez and Castillo Géniz (2000), who reported *S. lewini* to be a generalist feeder with ontogenetic dietary shifts. Those authors pointed out too that the smaller animals fed primarily on shrimps, the mid-size animals mainly on crabs, and the largest ones primarily on teleosts.

Mustelus manazo, *R. acutus*, and *R. djiddensis* were the only other species with a sample size large enough to examine trends in $\delta^{15}\text{N}$ with fish length. However, there was seemingly no trend for these species. Previous studies also found no ontogenetic dietary changes in various species of sharks (Wetherbee and Cortés, 2004). For *M. manazo*, an important reason for this lack of a trend may have been the narrow range of lengths (most of the animals sampled were immature). However, in Japanese *M. manazo*, prey availability is more important than shark size in determining prey composition (Yamaguchi and Taniuchi, 2000). Both results suggest that this species may not exhibit ontogenetic dietary change. Also, species of *Mustelus* elsewhere in the world seem to be specialized crustacean feeders (Ellis *et al.*, 1996), supporting this finding.

Although our sample of *R. acutus* was representative of a wider range of lengths than that of *M. manazo*, no relationship was found between length and trophic level in that species either. These data are consistent with those of Patokina and Litvinov (2005), who found no dependence between size and dietary composition of *R. acutus* in waters off Sierra Leone. It is worth noting that ontogenetic changes in diet have been recorded for some carcharhinid species (Ellis and Musick, 2007), but not for others (Cliff and Dudley, 1991).

We could find no literature documenting ontogenetic dietary change in *R. djiddensis*, and although our data suggest no such change, a larger sample number is needed to confirm this as fact.

Comparison between trophic level estimates

The mean TL estimates based on $\delta^{15}\text{N}$ ($\text{TL}_{15\text{N}}$) were directly compared with the mean TL estimates from dietary studies for each species. A highly significant relationship was found between the two estimates despite the small sample size for all species studied here. There were two exceptions, *R. ancylostoma* and *R. typus*, where the $\text{TL}_{15\text{N}}$ was lower than the TL obtained from dietary analyses. The fact that we had access to just two *R. ancylostoma* may have resulted in a poor estimate of the $\text{TL}_{15\text{N}}$ value for that species. The two varied widely in $\delta^{15}\text{N}$ (s.d. 1.3), corresponding to divergent $\text{TL}_{15\text{N}}$ estimates of 2.83 and 3.41. The latter value is much closer to that obtained from dietary analyses (3.55). *Rhincodon typus* also displayed a difference between the two estimates, but because that shark does show an increase in TL with length and most of our sample were immature, the result is understandable. Further analysis that includes more adults of this species might reveal a higher $\text{TL}_{15\text{N}}$, similar to that obtained from dietary analyses.

Studies of the biology of Indian elasmobranchs are scarce, probably because of the difficulty in obtaining samples. The

absence of a targeted fishery makes the availability of these species accidental (Varma, 1999). Moreover, the collection of stomach contents from fish caught in the wild and fish held in captivity is particularly difficult in elasmobranchs because of the logistical requirements of extended field sampling and the complexity of keeping the fish in captivity (Wetherbee and Cortés, 2004). These difficulties complicate the study of elasmobranch diet and trophic pathways. The strong correlation between the two estimates of the trophic level validates the ability of baseline-adjusted ^{15}N ratios to represent trophic position in elasmobranchs.

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