

Feeding habits and trophic levels of bluefin tuna *Thunnus thynnus* of different size classes in the Mediterranean Sea

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Summary

Possible changes in diet and trophic levels in relation to size of Mediterranean bluefin tuna, *Thunnus thynnus*, were investigated using labelled carbon ($\delta^{13}\text{C}$) and nitrogen ($\delta^{15}\text{N}$) stable isotopes. Samples were obtained from two locations in the southern Tyrrhenian Sea (Western Mediterranean Sea) in May and October 2004. The $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ analyses revealed at least three significant isotopic groups [small juveniles (0.7–2.2 kg), sub-adults (15–50 kg) and adults (70 to 225 kg)]. $\delta^{13}\text{C}$ was negatively dependent on weight, while $\delta^{15}\text{N}$ was positively dependent on weight [TW = 8.2 (± 0.16) + 0.03 (± 0.0) * $\delta^{15}\text{N}$ (n = 49; r = 0.91; P < 0.001)]. Different prey contribution to the diet was highlighted for each class. The diet of juveniles comprised zooplankton, small pelagic fish and some coastal fish; sub-adults relied on medium pelagic fish, shrimps and cephalopods, and adults relied mainly on cephalopods and larger fish. The trophic level (TL) of tunas belonging to each size class was closely correlated to weight, starting from ca 3.0 TL for Group I and reaching 4.4–4.8 TL for the giants. Bluefin tuna, from small juveniles to giants, showed a shift in feeding preferences due to different use of habitats and food items as a function of the life stage.

Introduction

Bluefin tuna *Thunnus thynnus* are the best example of a fast-growing, long-living vagrant fish, capable of migrating from the Mediterranean to the Atlantic and back (Safina, 1993; Sarà, 1998). For some time now, bluefin tuna have been the subject of extensive debate, as they have been over fished by the industrial fisheries of North America, Europe and Japan (Mather et al., 1995; Block et al., 2001; Chase, 2002). The International Commission for the Conservation of Atlantic Tuna (ICCAT) has regulated tuna fishing since 1970, and has thus encouraged the assimilation of as much data as possible on bluefin tuna biology and ecology to enable better regulation of stocks management. Nevertheless, although much research has been done, there are several aspects of bluefin tuna ecology that remain unclear. A massive research effort has been invested into tracking their trans-ocean movements, using highly sophisticated technological tools (Lutcavage et al., 1999, 2000; Block et al., 2001) and has confirmed that *Thunnus thynnus* uses spawning grounds that differ from its trophic areas (Block et al., 2001). Adult tuna come through the Gibraltar Strait from the eastern Atlantic population (Medina et al., 2002), reaching their Mediterranean spawning grounds in May, where they stay only until late July (Corriero et al., 2003; Karakulak et al., 2004) while small juveniles are massively present in the Mediterranean from late summer to autumn

(Sarà, 1998). It is also known that tuna are opportunistic feeders (*sensu* Rosenzweig, 1981) able to exploit a great variety of resources (Chase, 2002); due to their longevity, their diet would seem to change as they grow. Theory confirms that trophic levels of an organism, because of diet, can vary according to ontogeny, resulting in a shift of position within the food web (Werner and Gilliam, 1984). It is thus possible to infer that the bluefin tuna diet is subject to considerable change, depending on the different trophic positions assumed with each new life stage. However, information on the trophic ecology of each life stage is not complete for this species, and no data are available on the trophic position of bluefin tuna in the different stages of its life history. The trophic position in tuna has been calculated using either ecological models (Pauly et al., 2001) or gut content data (Stergiou and Karpouzi, 2002). In both cases, adult tuna appeared to have trophic level values of over 4.0, thereby assuming a top position in the pelagic food web, but the data available do not describe the effects of ontogeny and size. The stable isotope approach (Das et al., 2000; Jennings et al., 2001; Olson et al., 2004; Estrada et al., 2005) can elucidate some interactions within ecological communities by enabling the assignment of a trophic position to the different components (Post, 2002). As a complementary method to gut content analysis (Pauly et al., 1998; Pinnegar et al., 2003), stable isotopes have already been used to detect ontogenetic diet shifts in fish (e.g. brown trout (Grey, 2001); dusky grouper (Renones et al., 2002); migrating salmon smolts (Kline and Willette, 2002); sardine (Bode et al., 2004). The technique is based on the fact that the carbon isotope ratio ($^{13}\text{C} : ^{12}\text{C}$) of consumers reflects that of their food items (Fry and Sherr, 1984), while the nitrogen isotope ratio ($^{15}\text{N} : ^{14}\text{N}$) exhibits a stepwise enrichment ($\sim 3\text{--}5\%$) with each trophic level (Minagawa and Wada, 1984).

The main objective of the present study was to investigate the feeding habits and trophic levels of juvenile to adult *Thunnus thynnus* in the Mediterranean, thereby contributing to information about the species' life history. The specific aims were: (i) to highlight possible ontogeny-related shifts in diet and trophic levels; (ii) to document possible differences in habitats occupied during different life stages; and (iii) to create the first isotopic background regarding the Mediterranean life stage of this species.

Materials and methods

Data collection and samplings

Samples of bluefin tuna were obtained from two locations in the southern Tyrrhenian (Western Mediterranean Sea) in May and October 2004. Tuna weighing from 15 to 225 kg (n = 35) were collected on 3 May 2004 by means of traditional tuna

traps typical of the Mediterranean (Tonnara, Sarà, 1998). The trap nets were positioned off the western coast of Sicily (S. Cusumano, Trapani; Lat. 37.9°N; Long. 12.5°E). Small juveniles ($n = 14$) were caught with a sardine purse seine (a 10 m long spoon and two lateral wings, each 40 m long and mesh ranging from 93 mm at the beginning of the wings to 54 mm near the bunt; Sinopoli et al., 2004) in the Gulf of Palermo (about 40 nm eastward of S. Cusumano; Lat. 38.2°N; Long. 13.1°E) between 3 September and 1 October 2004. We were forced to collect tuna at different times of the year, as juvenile and adult tuna are not simultaneously present in the Mediterranean due to their biological cycle (see Introduction). In an attempt to gain a plausible isotopic picture of tuna diet, stomach contents were collected from all tuna for analyses. However, 65% of the tuna collected had partially digested stomach contents or empty stomachs, either due to regurgitation caused by fishing operations (Chase, 2002), or to the length of time spent in the traps prior to capture. Prey identification was carried out to the lowest possible taxonomic level. Fragmented prey identification was based on the number of eyes, mouth parts, tails or other anatomical parts traceable to single specimens (Sinopoli et al., 2004). Thus, wherever possible, items found in full stomachs were analysed for their isotopic contents, while for the other items plausibly belonging to the diet of tuna, isotopic signatures were obtained from Pinnegar et al. (2003), which represents the most complete isotopic review to date for the Mediterranean.

Stable isotopic analyses

Once collected, prey samples and bluefin muscle (20–50 g; excised from the area just before the caudal fin; 90% white

tissue) were immediately frozen (-20°C). For analysis, the samples were rinsed with distilled water, acidified in HCl, re-rinsed in distilled water and dried at 60°C for the time required to reach a constant weight. They were then ground with a mortar and pestle. Isotopic analyses were performed using a Finnigan Delta-S isotope ratio mass spectrometer. Isotopic values were expressed in parts per thousand as deviations from standards (Pee Dee belemnite limestone for $\delta^{13}\text{C}$ and nitrogen in air for $\delta^{15}\text{N}$): $\delta^{13}\text{C}$ or $\delta^{15}\text{N} = [(R_{\text{sample}}/R_{\text{standard}}) - 1] \times 10^3$, where $R = {}^{13}\text{C}/{}^{12}\text{C}$ or ${}^{15}\text{N}/{}^{14}\text{N}$.

Statistical analyses

ANOVA was used to test the null hypothesis of no difference among size class and isotope content in tuna muscle. Size classes (class, 7 levels) were treated as a fixed factor and seven sampling replicates were taken randomly for each class. The heterogeneity of variance was tested using Cochran's C test prior to the ANOVA. The Student-Newman-Keuls (SNK) test enabled the appropriate means comparison. The software Statistica rel. 5.0/99 edition (StatSoft) was used to run the ANOVA and regressions between size classes and trophic levels. In order to identify the most important carbon and nitrogen sources responsible for the isotopic composition of the tuna, we performed isotope multi-source mixing models applied to isotopic data from each class (Phillips and Gregg, 2003). To achieve this, class-averaged isotopic values of tuna as targets in the model, and isotopic signals of potential organic matter sources (Table 1) as main end-members, were used. ISO-SOURCE software (Phillips and Gregg, 2003; Newsome et al., 2004; Sarà et al., 2004) was used to calculate all mixing models. Stable isotope estimates of trophic level (TL) were

Table 1

All organic matter potentially evident in the diet composition of bluefin tuna in the Mediterranean. (a) Isotopic composition of potential diet items as measured in the present study, collected from stomach contents, or by fishing, or extrapolated from Pinnegar et al. (2003). (b) Main trophic pooled categories resulting from mixed equations

Item	n	Size (cm)	$\delta^{13}\text{C}$	SD	$\delta^{15}\text{N}$	SD	Source
(a)							
<i>Sarda sarda</i> larvae	100	2.0	-19.0	0.0	4.4	0.2	This study
<i>Sarda sarda</i> juveniles	8	8.0	-17.5	0.2	7.6	0.6	This study
<i>Sarda sarda</i> adults	13	14.0	-18.2	0.1	7.5	0.2	This study
<i>Scomber scomber</i>	6	25.0	-20.4	0.1	11.2	0.1	This study
<i>Loligo vulgaris</i>	6	17.0	-19.5	0.4	9.6	0.5	This study
<i>Sepia</i> sp.	5	10.0	-18.7	0.1	8.0	0.0	This study
Shrimp	10	5.0	-18.5	0.1	8.0	0.0	This study
<i>Todares</i> sp.	8	24.0	-18.4	0.1	10.2	0.2	This study
<i>Cromis cromis</i> juveniles	–	–	-19.1	–	6.9	–	Pinnegar et al., 2003
<i>Cromis cromis</i> adults	–	–	-19.0	–	4.6	–	Pinnegar et al., 2003
Boops boops	–	–	-18.7	–	6.7	–	Pinnegar et al., 2003
<i>Atherina presbyter</i>	–	–	-16.5	–	7.7	–	Pinnegar et al., 2003
<i>Spicara maena</i>	–	–	-19.6	–	7.0	–	Pinnegar et al., 2003
<i>Salpa sarpa</i> juveniles	–	–	-17.4	–	5.4	–	Pinnegar et al., 2003
<i>Salpa sarpa</i> adults	–	–	-16.1	–	6.5	–	Pinnegar et al., 2003
<i>Mullus</i> sp.	–	–	-15.2	–	8.6	–	Pinnegar et al., 2003
<i>Scorpena</i> sp.	–	–	-16.6	–	7.5	–	Pinnegar et al., 2003
<i>Coris julis</i>	–	–	-16.2	–	8.6	–	Pinnegar et al., 2003
Pooled categories			$\delta^{13}\text{C}$	±	$\delta^{15}\text{N}$	±	
(b)							
Small pelagic fish (<i>Sarda</i> larvi)	–	–	-19.0	0.0	4.4	0.2	
Medium pelagic fish (<i>Sarda</i> juv + ad)	–	–	-17.8	0.4	7.6	0.4	
Large pelagic fish (<i>Scomber scomber</i>)	–	–	-20.4	0.1	11.2	0.1	
Cephalopods (<i>Loligo</i> + <i>Todares</i>)	–	–	-19.5	0.4	9.9	0.5	
Nekto-benthic fish (from <i>Cromis</i> to <i>Coris</i>)	–	–	-17.4	0.2	6.9	1.3	
Micro-zooplankton < 200 μm	–	–	-22.4	–	3.6	–	Pinnegar et al., 2003
Meso-zooplankton > 200 μm	–	–	-21.7	–	4.3	–	Pinnegar et al., 2003

calculated assuming a constant per-trophic-level fractionation of 3.4‰ (Post, 2002; Pinnegar et al., 2003) because of its reliably consistent pattern, irrespective of whether the animal concerned was a herbivore, carnivore or detritivore (Post, 2002). In order to calculate trophic levels, we chose the averaged value for zooplankton of $3.64 \pm 0.23\text{‰}$ as a trophic baseline, which corresponded to the mean value of zooplankton isotopic values reported in Pinnegar et al. (2003).

Results

Tuna collected in the present study belong to seven size classes ranging from small juveniles (not older than 1 month, class I) to at least 11 years (class VI; Table 2). Each size class (I–VII) was significantly different from the others (ANOVA; Table 3). Further ANOVA carried out on isotopic data separated specimens belonging to each size class from an isotopic (i.e. trophic) point of view. Specimens of classes I and II showed no differences with regard to both carbon (Fig. 1) and nitrogen (Fig. 2), whereas the carbon specimens of classes III–V were similar. The nitrogen specimens of classes III, IV and V were similar among themselves, but also to specimens of class VI. Lastly, specimens of classes VI and VII showed no differences in regard to carbon, and specimens of class VII showed significant differences only with nitrogen. The relationship between weight and isotopes was tested with a linear regression model including rough data on total tuna collected ($n = 49$). Carbon was negatively dependent on weight as follows: $TW = -17.7 (\pm 0.11) - 0.006 (\pm 0.0) * \delta^{13}C$ [$n = 49$;

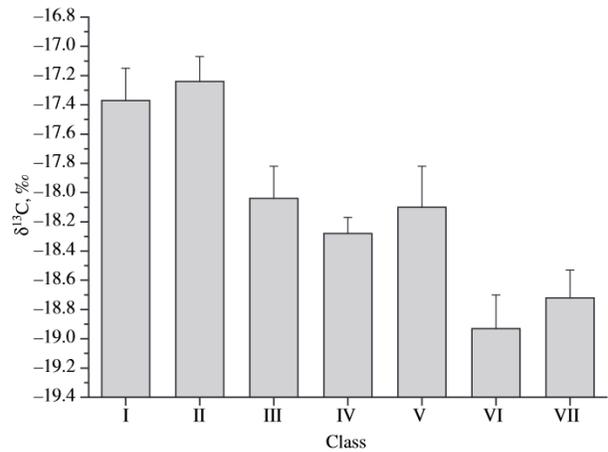


Fig. 1. Mean carbon isotopic composition ($\delta^{13}C$, ‰; \pm SE) of each size class of bluefin tuna analysed (see Table 2 for size class limits)

$r = 0.58$; $P < 0.001$], while nitrogen was positively dependent on weight according to the following equation: $TW = 8.2 (\pm 0.16) + 0.03 (\pm 0.0) * \delta^{15}N$ [$n = 49$; $r = 0.91$; $P < 0.001$].

Tuna diet composition and tuna trophic levels

Mixing model outcomes are qualitatively synthesised in Table 4, while in Fig. 3, a $\delta^{13}C$ – $\delta^{15}N$ scatter plot, including all potential organic sources in the tuna diets, is shown. The

Table 2

Statistics of bluefin tuna size classes analysed in the present study and corresponding isotopic values [n , sample size; TW, mean total weight of each class; TL, estimated trophic level by means of Post (2002) formula; SD, standard deviation; *TL calculated using Mediterranean baseline of 3.64]

Size class	Age	Weight range	TW	SD	$\delta^{13}C$	SD	$\delta^{15}N$	SD	TL	SD
I	0+	0.7–0.9	0.8	0.0	-17.4	0.2	7.2	0.3	3.0	0.2
II	0+	1.0–2.2	1.7	0.2	-17.2	0.2	7.7	0.3	3.2	0.2
III	2	15.0–30.0	21.4	1.7	-18.0	0.2	9.5	0.2	3.7	0.2
IV	3	31.0–40.0	34.8	0.3	-18.3	0.1	9.7	0.2	3.8	0.1
V	4	41.0–50.0	42.9	2.2	-18.1	0.3	10.0	0.2	3.9	0.2
VI	6–9	70.0–130.0	90.2	8.1	-18.9	0.2	10.4	0.2	4.0	0.2
VII	10–13	178.0–225.0	196.6	6.7	-18.7	0.2	13.1	0.2	4.8*	0.1
All tuna		0.7–225.0	55.4	65.5	-18.1	0.8	9.7	1.9	3.8	0.6

Table 3

ANOVA testing whether (a) weight (TW) of bluefin tuna considered in the present study and their isotopic composition ($\delta^{13}C$ and $\delta^{15}N$) were different (* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; ns, not significantly different [$P > 0.05$]; ($\sqrt{\quad}$), data log transformed [$\log x + 1$]) and (b) outcome of Student-Newman-Keuls test

(a) Source	d.f.	TW			$\delta^{13}C$			$\delta^{15}N$		
		MS	F	P-value	MS	F	P-value	MS	F	P-value
Size class (CL)	6	21.29	955.04	***	2.78	10.30	***	25.83	71.19	***
Residuals	42	0.02			0.27			0.36		
Cochran's C-test				*($\sqrt{\quad}$)			ns			ns

(b)

SNK test

TW

Class I < II < III < IV < V < VI < VII

$\delta^{13}C$

Class I = II > III = IV = V > VI = VII

$\delta^{15}N$

Class I = II < III = IV = V = VI < VII

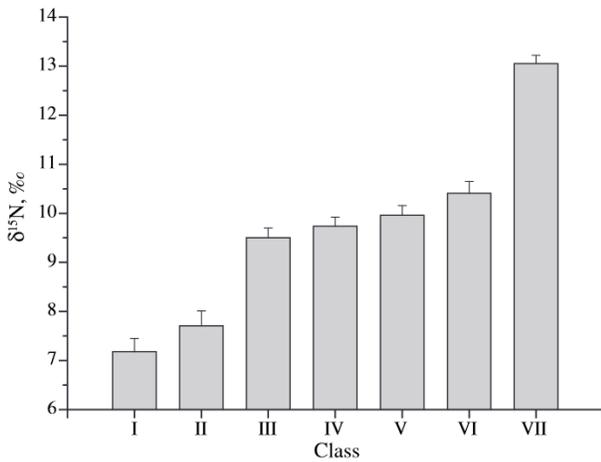


Fig. 2. Mean nitrogen isotopic composition ($\delta^{15}\text{N}$, ‰; \pm SE) of each size class of bluefin tuna analysed (see Table 2 for size class limits)

diet of size classes I and II appeared to be similar, with a high contribution from zooplankton, small pelagic fishes and coastal fish, while the other sources contributed little. A broad range, consisting of medium pelagic fish, shrimps, cephalopods and coastal fish, affected the isotopic compositions of size classes III–V. In contrast, class VI tuna showed a major contribution of cephalopods and large fish, while the other sources were negligible. Class VII tuna relied almost exclusively on pelagic fishes (large, rather than medium size) and cephalopods. The trophic level of tuna belonging to each size class was calculated. Trophic levels were correlated ($P < 0.05$; Fig. 4) to weight as tuna size classes assumed different trophic levels, starting from about three (class I–II), representing a likely baseline level for this species (disregarding the larvae stage), and reaching 4.8 for the giant tuna (class VII), which represents the top level.

Discussion

Mediterranean bluefin tuna exhibited size-specific changes in isotopic composition as displayed by the significant relationships between isotopes and size. The relationship between nitrogen and size was notable, as younger tuna displayed nitrogen-depleted values (~7.0–8.0‰), while values in older tuna were about two times more enriched (~13.0‰). This pattern indicates a considerable accumulation of ^{15}N , which can be explained either by (a) physiological changes or (b)

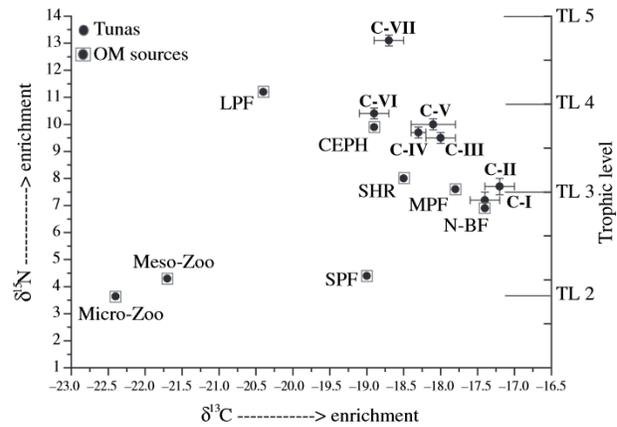


Fig. 3. $\delta^{13}\text{C}$ – $\delta^{15}\text{N}$ scatter-plot with isotopic composition of tuna (circle \pm SE) and organic matter sources (squares with circles inside) potentially evident in tuna isotopic composition. Trophic Level (TL) calculated by using the Mediterranean baseline is also drawn. [SPF, small-size pelagic fishes; MPF, medium-size pelagic fishes; LPF, large-size pelagic fishes; N-BF, nekto-benthic fishes; CEPH, cephalopods; SHR, shrimps; Micro-Zoo, the trophic baseline represented by microzooplankton (organisms smaller than 20 μm); Meso-Zoo, mesozooplankton (organisms larger than 200 μm)]

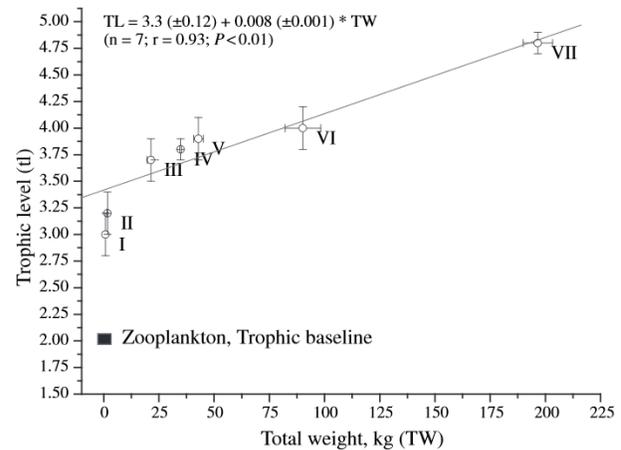


Fig. 4. Relationship between total weight (TW, kg) and trophic level using the Mediterranean baseline (Trophic Level, TL) including the seven size classes of tuna sampled. Regression line and its model are also reported

different habitat exploitation and resource use (Livingstone, 1982; Werner and Gilliam, 1984). The relationship between stable isotope signatures and body size theoretically occurs as

Table 4

Estimated contributions of all sources potentially included in bluefin tuna diet (–, source potentially compatible with isotopic signatures of individuals but with small contribution; +, primary source; NO, source not admissible). Results are from mixing equations (two isotopes and three to four end-members according to Phillips and Gregg, 2003 and all related literature) and from classical extrapolation (see $\delta^{13}\text{C}$ – $\delta^{15}\text{N}$ scatter plot presented in Fig. 3)

Class	Plankton	Small pelagic fish	Coastal benthopelagic	Medium pelagic fish	Shrimps	Cephalopods	Larger pelagic fish
I	+	+	+	–	+	NO	NO
II	+	+	+	–	+	NO	NO
III	NO	–	+	+	+	–	NO
IV	NO	–	+	+	+	–	NO
V	NO	–	+	+	+	–	NO
VI	NO	NO	NO	+	–	+	+
VII	NO	NO	NO	–	–	+	+

a result of the changing allocation of isotopes or from changes in tissue turnover rates during ontogeny (Hesslein et al., 1993; Overman and Parrish, 2001). The marked retention of the heavier isotope (^{15}N) in bluefin tuna, scaled to size, was probably due to shifts in diet and habitat rather than to physiological changes (Cocheret de la Morinière et al., 2003). Indeed, bluefin tuna are fast-growing organisms and show a far-ranging migratory pattern from the Atlantic to the Mediterranean. The size effect in resource use has already been well documented in fish (Pinnegar and Polunin, 2000; Renones et al., 2002; Bode et al., 2004), and it is thought to be associated with/or caused by changes in feeding habitat (Werner and Gilliam, 1984). Size differences were not evident for tuna caught in the North-western Atlantic (Estrada et al., 2005), whose isotopic composition was quite constant ($\delta^{15}\text{N}$ ca. 14.0‰ , ranging from 35 to 196 kg). The higher isotopic values ($\delta^{15}\text{N} \sim 3\text{--}5\text{‰}$ more) in the Atlantic in comparison to the Mediterranean specimens and the absence of size effect could be explained by a local and latitudinal effect of ^{15}N enrichment of the Atlantic waters (Olson et al., 2004) and by assuming that Atlantic tuna reach the trophic levels of giants more quickly (after only 2 or 3 years) than Mediterranean tuna. In contrast, Mediterranean tuna showed a shift in their diet, mainly as a consequence of habitat change. Starting from an age of 1 month to several years, bluefin tuna showed wide variation in nitrogen signatures, explainable only by the fact that they were able to exploit a plethora of resources available from the bottom to the surface of the water column and from coastal to offshore environments (Sinopoli et al., 2004). Although scant data exists on the use of stable isotopes for tuna, some studies have reported on their trophic choices using gut content analysis (Chase, 2002; Sinopoli et al., 2004). For example, young-of-the-year specimens collected in the south Tyrrhenian (Sinopoli et al., 2004) had an essentially piscivorous diet, with secondary contributions from invertebrates and benthic resources (Stergiou and Karpouzi, 2002). Accordingly, small tuna (classes I and II) diet was influenced by both pelagic and benthic-pelagic resources, which is typical of fish living close to the coastline and exploiting the entire water column from bottom to surface (small pelagic fishes and macroplankton; see Table 4). The diet of Group II tunas (15–50 kg; size classes I–III) relied mainly on pelagic resources, with a large contribution from pelagic invertebrates such as cephalopods and shrimps. Bluefin tuna studied by Estrada et al. (2005) were shown to feed on medium-size pelagic prey, but they also relied on krill and zooplankton although Atlantic tuna did not appear to include any coastal related isotopic signatures in their diet. Thus, a difference is evident in the diet of tuna of the same size from the western Atlantic and from the Mediterranean, and may be proof of the opportunistic behaviour typical of this species (Rosenzweig, 1981). Classes VI and VII tuna were significantly different in their ^{15}N content (10.4‰ and 13.1‰ , respectively). In light of recent isotopic data by Estrada et al. (2005), reporting higher nitrogen content than in the Mediterranean, it is possible to infer the origin of >178 kg tuna analysed in the present study. Giant tuna spend most of their time in the Atlantic, entering the Mediterranean through the Gibraltar Strait to reach their spawning grounds. Isotopes provide time-integrated information on the composition developed in the months of an animal's life prior to analysis (Gannes et al., 1997). Thus higher nitrogen values of the class VII giant tuna might reflect the isotopic composition of their previous life environment, i.e. the Atlantic, which is slightly richer in ^{15}N than the Mediterranean. Such a hypothesis could

explain the difference in nitrogen signatures among size classes in the Mediterranean and the similarity of signatures between the Mediterranean giants and the Atlantic tuna.

Trophic levels for Mediterranean tuna

Size-related shifts in the diet of bluefin tuna, obtained from isotopic data, also subtly reflected trophic levels (Fig. 4). Tuna displayed a wide range of trophic levels, shifting from about TL 3.0 for juveniles to about TL 4.8 for adults (by using the Mediterranean trophic baseline; 3.64‰) or about TL 4.4 if using the Atlantic baseline ($\sim 5.0\text{‰}$; Bode et al., 2004). To date, there is no information available on tuna trophic position in the Mediterranean, as highlighted by Pinnegar et al. (2003). The only isotope dataset regarding tuna provided by Estrada et al. (2005) cannot be used for comparison, as these authors used another baseline (10.2‰ given by a pelagic fish, *Ammodytes americanus*; trophic level 3). Classes I and II (group I) clustered around the same TL (~ 3.1). Such a value was lower (3.1 as opposed to 4.2) than that calculated by Stergiou and Karpouzi (2002) for specimens of the same age, although they described a diet very similar to that extrapolated isotopically in the present study. This discrepancy might not only be due to the bias in the different approaches (mass-balance modelling and gut contents vs. stable isotopes) in calculating TLs, but also to local differences in the isotopic composition of food web components. The coastal environment where our juveniles were trapped is highly variable, receiving a range of organic matter input (e.g. terrigenous or anthropogenic). Thus local differences in carbon and nitrogen sources might affect the isotopic configuration of the coastal food webs, thereby amplifying the differences due to the different approaches. Our Group II tuna had a TL of about 3.8, a value very similar to that reported by Estrada et al. (2005), but lower than the values reported by Stergiou and Karpouzi (2002). The differences in the latter case are smaller because the environment where tuna of this size live probably included offshore areas, of which the physical homogeneity would limit the bias due to local differences.

Classes VI and VII had a TL of 4.0 and 4.4–4.8, respectively, placing them at the top of marine pelagic food webs. These values were higher than the values for western Atlantic tuna found by Estrada et al. (2005). Whether using the same Mediterranean baseline (3.6‰) or a more enriched one, considering the differences between the Atlantic and the Mediterranean, the TL of our adult tuna would still be higher than *Neothunnus alalunga* (~ 4.0), *Stenella coeruleoalba* (~ 4.0) and *Delphinus delphis* (~ 4.3) caught in the eastern part of the Atlantic (Bay of Biscay, Das et al., 2000). Giant tuna, however, being organisms whose food tends to be a mixture of low- and high-TL organisms ($\text{TL} = 3.5\text{--}4.5$), can reach a trophic position comparable with most of the Atlantic cetaceans and sharks (Estrada et al., 2003).

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