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Feeding patterns of age-0 bluefin tuna in the western Mediterranean inferred from stomach-content and isotope analyses

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ABSTRACT: Knowing how migratory pelagic predators use food web resources during early life stages is essential for understanding population dynamics and recruitment. Feeding habits of ~3 to 4 mo old Atlantic bluefin tuna (ABFT) *Thunnus thynnus* from 2 Mediterranean nearshore areas (Cartagena and Cambrils, Spain) showing distinct oceanographic features were investigated using stomach-content (SCA) and stable-isotope (SIA) analyses. SCA showed diet variability between areas and years; further correspondence analysis revealed that epipelagic fishes (primarily anchovy and sardine) constitute the staple diet of age-0 ABFT in Cambrils, whereas mesopelagic fishes (especially myctophids) and squid were consumed in significant amounts by ABFT in Cartagena. δ^{13}C values differed significantly between tissues (white muscle and liver), areas and years (2009 and 2010), while δ^{15}N values were not significantly different. In all cases, mean δ^{13}C values were significantly higher in muscle than in liver, while differences in δ^{13}C between locations and years were also observed in some instances. Estimations of trophic niche width using the antilogarithm of Shannon's entropy, exp(H), and from stable isotope Bayesian ellipses, SEA_c, indicated that niche width was generally larger in samples from Cambrils than in those from Cartagena. Moreover, SEA_c analysis suggested that trophic niches were broader in 2010 than in 2009. Our results indicate a low degree of feeding specialisation and suggest that, like older classes, age-0 ABFT rely on a wide prey range, easily adapting their foraging behaviour to prey availability and distribution in the habitat. This research assesses the potential of using stable isotope techniques combined with SCA for the characterization of spatio-temporal variability in the diet of juvenile top predators.

KEY WORDS: Atlantic bluefin tuna · *Thunnus thynnus* · Diet · Trophic ecology · Stomach-content analysis · Stable-isotope analysis

INTRODUCTION

The Atlantic bluefin tuna (ABFT) *Thunnus thynnus* (Linnaeus, 1758) is a highly migratory pelagic predator that ranges throughout the North Atlantic Ocean and the Mediterranean Sea. Increased fishing mortality due to overexploitation led to a significant decline in ABFT populations in the early 2000s (MacKenzie et al. 2009). Although the latest scientific assessments show signs of recent recovery of the stock (ICCAT 2014), the historical evolution of ABFT biomass and the future response of the population to

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alternative management actions are highly uncertain (Fromentin et al. 2014). For instance, population productivity and recruitment dynamics are still largely unknown, which hinders the development of strategic plans for ABFT conservation. A good knowledge of foraging patterns at early life stages is essential for the understanding of population dynamics and recruitment variations of the stocks. Nevertheless, studies focusing on the feeding habits of larval and young ABFT in the western Mediterranean are scarce, despite this area being one of the main ABFT spawning grounds and an important distribution area for juveniles of this species. The eastern population of ABFT breeds in specific areas of the Mediterranean Sea, the westernmost of which is located around the Balearic Islands (Alemany et al. 2010). ABFT larvae up to a size of ~10 mm (18–20 d old) can be sampled in the Balearic Sea during June and July (Catalán et al. 2011, Reglero et al. 2011), but beyond this size/age, they become inaccessible by ichthyoplankton surveys over the spawning area. A few months afterwards (September through November), age-0 juveniles (3–5 mo old) can be found in nearshore areas along the Iberian Mediterranean coast (Sanz-Brau 1990), and thereafter are no longer encountered in these waters.

In the Balearic breeding ground, ABFT larvae feed primarily on copepod larvae and cladocerans (Catalán et al. 2011), although piscivory (including cannibalism) is also likely to occur (Reglero et al. 2011). Age-0 ABFT captured off the coast of Valencia (eastern Spain) were shown to prey mainly on pelagic fish (Sanz-Brau 1990). Nothing is yet known about the trophic ecology of juvenile tuna during their migration from the breeding ground around the Balearic archipelago to coastal peninsular waters.

The feeding habits of age-0 ABFT have been investigated in the western (Sanz-Brau 1990) and central (Sinopoli et al. 2004, Battaglia et al. 2013a,b) Mediterranean Sea through identification and quantification of prey in gut contents. Sarà & Sarà (2007) also investigated the diets of young ABFT (ages 1 and 2) from the Tyrrhenian Sea using stable-isotope analysis (SIA). Stomach-content analyses (SCA) reveal the composition of recently ingested food, and thus provide small-scale (spatially and temporally) information on foraging patterns of animals. The analysis of stable isotope ratios (especially those of carbon and nitrogen) in diverse animal tissues and non-living structures complements the SCA method. SIA allows for long-term integrated measures of diets assimilated over time (Bearhop et al. 2004), and has thereby become a valuable tool in the study of animal feeding preferences, habitat utilisation and movements. This method relies on the principle that consumers acquire the isotopic composition of their prey, which ultimately reflects the local environmental isotope composition (i.e. the isotopic composition of local primary producers). Therefore, the use of SIA provides valuable information on trophic behaviour and movement patterns of marine migratory organisms that are difficult to investigate in the wild (Ménard et al. 2007, Graham et al. 2010, Olson et al. 2010, Logan et al. 2011, Varela et al. 2013, Young et al. 2015).

The present study combines SCA and SIA to assess inter-annual and regional variations in the feeding patterns of age-0 ABFT in 2 nearshore habitats of the Iberian Mediterranean coast showing different oceanographic features. The tissues used for SIA, white skeletal muscle and liver, have different turnover rates (Graham 2008) and are hence assumed to integrate isotopic compositions over distinct time scales.

MATERIALS AND METHODS

Sample collection

Juvenile ABFT were caught by trawling during daylight hours in September to October, at ~1.6–6.4 km off Cartagena (Murcia province, SE Spain; 2008 to 2010, n = 210) and Cambrils (Tarragona province, NE Spain; 2009 and 2010, n = 115) (Fig. 1). The round weight of fish captured off Cartagena

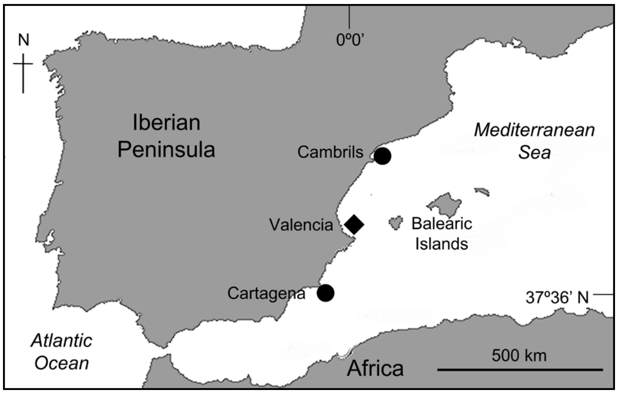

Fig. 1. Age-0 Atlantic bluefin tuna (ABFT) *Thunnus thynnus* were captured in September to October off Cambrils (NE Spain) in 2009 and 2010, and off Cartagena (SE Spain) in 2008 to 2010 (●). In a previous study, Sanz-Brau (1990) sampled ABFT of the same age for stomach-content analysis (SCA) in the nearby Gulf of Valencia (◆), located halfway between Cambrils and Cartagena

ranged between 158 and 1330 g (21 to 43 cm straight fork length), whereas individuals caught off Cambrils ranged between 299.4 and 1388.3 g (25.4 to 37.5 cm straight fork length). Whole stomachs and tissue samples (liver and white dorsal muscle) were removed from each fish and stored at −20°C until analysis.

Stomach-content analysis (SCA)

Once thawed, the stomachs were weighed and cut open to remove their contents. The stomach lining was also weighed after being rinsed and blotted dry. The difference between both masses was considered the total content mass, including gastric liquid, which often contained remains of crustaceans and fish in an advanced digestion stage. The stomach contents were washed through a sieve of 1000 µm mesh size. Each prey item was identified to the lowest possible taxon using specific identification keys based on morphological features and the online database FishBase (Clarke 1986, Todd et al. 1996, Froese & Pauly 2010). For each type of prey, the mass (to the nearest 0.01 g) and the number of individuals were recorded. Remains of hard parts (fish otoliths, cephalopod beaks, and crab claws and carapace) were closely examined to identify partially digested prey. Species for which only hard parts were found were not considered in the correspondence analysis (see 'Statistical analysis' section below), as they are likely to be remains of prey that were ingested several days earlier; however, their mass was taken into account in calculating stomach fullness. Stomach fullness was defined for each predator as the ratio between the mass of the stomach content (g) and the mass of the individual (g).

Stable-isotope analysis (SIA)

Samples of muscle and liver for SIA were obtained from the tuna collected in 2009 and 2010. Following thawing and rinsing with distilled water, the tissue pieces were freeze-dried and powdered. Half of the resulting sample was used directly for δ^{15}N analysis, while the remainder was processed for lipid extraction following Varela et al. (2013) to avoid the effects of lipid content on δ^{13}C measurements. Aliquots of ~1 mg were packed in tin capsules for the determination of carbon and nitrogen isotopic ratios (δ^{13}C and δ^{15}N) by continuous-flow isotope ratio mass spectrometry. Values of δ^{13}C and δ^{15}N are given in parts per thousand (‰) relative to standards (Peterson & Fry 1987).

Statistical analysis

The dietary importance of the different prey was assessed using the index of relative importance (IRI), expressed as a percentage, according to the formula:

$$\%\mathrm{IRI}_i = (\mathrm{IRI}_i \,/\, \sum_{i=1}^{n} \mathrm{IRI}_i) \times 100 \qquad \text{(Pinkas 1971)}$$

where $\mathrm{IRI}_i = (\%\mathrm{Num}_i + \%\mathrm{W}_i) \times \%\mathrm{O}_i$; $\%\mathrm{Num}_i$ = number of individuals of prey item $i \times 100$/total number of all prey items; $\%\mathrm{W}_i$ = weight of prey item $i \times 100$/total weight of all prey items; and $\%\mathrm{O}_i$ = number of stomachs containing prey item $i \times 100$/total number of stomachs.

The trophic niche width was measured for each area and year using the antilogarithm of Shannon's entropy: $N_1 = \exp(H)$, where H is $-\Sigma p_i \ln(p_i)$ (Hill 1973) and p_i is the proportion of the IRI (Acuña & Villarroel 2010); the index N_1 approximates the number of abundant species in the diet (Hill 1973, Chandy et al. 2006). Alternatively, niche width was estimated based on the bidimensional space of δ^{13}C and δ^{15}N values by analyzing stable isotope Bayesian ellipses (SIBER) (Jackson et al. 2011) using the software package SIAR (Parnell et al. 2008) for R (R Core Team 2013).

Geographic and interannual variations in the prey composition of the ABFT diet were assessed through correspondence analysis (CA), which is a multivariate technique based on decomposing the Chi-square statistic associated with a pivot table into orthogonal factors (Benzécri 1973). Prey with %W >5% in at least one of our data subsets were selected for this analysis. CA was performed for all non-empty stomachs, using the mass percentages of the different prey ingested by each tuna. Stomachs with only liquid remains or unidentified prey in an advanced digestion stage were not used in these analyses. Variations in stomach fullness were assessed through general additive models (GAMs), assuming a gamma distribution and a log link function. The packages FactoMineR 1.25 (Husson et al. 2007) and mgcv 1.7–26 (Wood 2010) were used for the CAs and GAMs, respectively.

Variations in isotopic values attributable to the 3 considered factors (tissue, geographic location and year) were assessed using PERMANOVA (permutational MANOVA) with the function 'adonis' in the package vegan for R (Oksanen et al. 2013). Three-factor PERMANOVA based on 999 permutations was performed to conduct univariate permutation-based ANOVA on each dependent variable (δ^{13}C and δ^{15}N). To further examine specific differences in δ^{13}C values between locations and years, means were compared using Student's *t*-test following Levene's test for equality of variances.

RESULTS

Stomach-content analysis (SCA)

Identification of prey in the stomach contents to the species level was not always possible due to the advanced stage of digestion observed in some individuals. In some instances, a large proportion of the soft material consisted of unidentifiable and/or unquantifiable remains which were thus unusable for the calculation of feeding indices.

SCA showed differences in the dietary composition between areas and also between years (Tables 1 & 2, Fig. 2). The diet of fish caught off Cartagena (SE Spain, Fig. 1) comprised at least 27 species, including 13 fishes, 5 cephalopods and 9 crustaceans (Table 1). In 2008, cephalopods constituted the most abundant prey group (%IRI = 81.79), *Illex coindetii* being the predominant species (%IRI = 90.51). Fish represented the most abundant prey group in 2009 and 2010 (%IRI = 91.80 and 48.45, respectively). However, while *Sardinella* spp. was predominant in 2009 (%IRI = 83.77), *I. coindetii* (%IRI = 64.57) was the most abundant prey in 2010. Fish, cephalopod, stomatopod and anomuran individuals found in the stomachs were all at an early life stage (either juvenile or larval), whereas the other crustaceans retrieved from the stomach contents were adult forms.

The diet of young tuna collected off Cambrils (NE Spain, Fig. 1) consisted of at least 3 fish species, 1 cephalopod and 7 crustaceans (Table 2). Fish (na-

Table 1. Stomach contents of age-0 Atlantic blue fin tuna (ABFT) *Thunnus thynnus* captured off Cartagena (Murcia, SE Spain). W: weight; Num: number; O: occurrence; IRI: index of relative importance. The trophic niche width is expressed as exp(H), H being the Shannon-Wiener index calculated from IRI

Prey	%W	%Num	%O	IRI	%IRI
2008					
Fishes	5.77	18.56	38.10	926.70	7.37
Capros aper	3.08	9.28	23.81	294.32	3.13
Unidentified fishes	0.70	6.19	19.05	131.14	1.39
Myctophum punctatum	1.97	2.06	9.52	38.39	0.41
Callionymus spp.	0.02	1.03	4.76	4.99	0.05
Cephalopods	91.31	52.58	71.43	10277.80	81.79
Illex coindetii	89.24	48.45	61.90	8523.84	90.51
Unidentified cephalopods	1.10	2.06	4.76	15.04	0.16
Sepietta spp.	0.98	2.06	4.76	14.47	0.15
Crustaceans	2.92	28.87	42.86	1362.22	10.84
Hyperiid amphipods	2.37	15.46	14.29	254.84	2.71
Caridea	0.10	7.22	9.52	69.72	0.74
Gammarid amphipods	0.03	4.12	14.29	59.40	0.63
Pasiphaea spp.	0.28	1.03	4.76	6.23	0.07
Unidentified crustaceans	0.13	1.03	4.76	5.53	0.06
$H = 0.48$, exp(H) = 1.62					
2009					
Fishes	76.23	44.23	46.99	9183.51	91.80
Sardinella spp.	49.01	16.35	16.87	3202.77	83.77
Arctozenus risso	16.09	6.25	0.10	359.63	9.41
Myctophidae	2.80	5.77	9.64	23.97	0.63
Unidentified fishes	2.61	3.85	8.43	16.83	0.44
Ceratoscopelus maderensis	1.58	1.44	2.41	4.77	0.12
Trachurus spp.	1.27	1.44	3.61	3.45	0.09
M. puncatum	1.13	0.48	1.20	1.81	0.05
Notoscopelus elongatus	0.81	1.44	1.20	1.83	0.05
C. aper	0.27	0.96	2.41	0.33	0.01
Sparidae	0.22	1.44	3.61	0.37	0.01
Callionymus spp. larvae	0.00	0.48	1.20	0.00	0.00
Gadiculus argenteus	0.24	0.48	1.20	0.18	0.00
Stomiidae	0.12	0.48	1.20	0.07	0.00
Scorpaenidae	0.05	0.48	1.20	0.03	0.00
Sparid larvae	0.01	0.48	1.20	0.01	0.00
Unidentified larvae	0.03	2.40	7.23	0.07	0.00
Cephalopods	17.36	11.06	20.48	493.44	4.93
I. coindetii	8.84	3.85	8.43	112.15	2.93
Unidentified cephalopods	4.10	1.44	3.45	22.74	0.59
Todarodes sagittatus	2.38	0.96	2.41	7.97	0.21
Bathypolypus spp.	1.31	0.48	1.20	2.36	0.06
Sepiolidae	0.37	2.88	4.82	1.20	0.03
Todaropsis eblanae	0.35	1.44	3.61	0.64	0.02
Crustaceans	6.40	44.71	38.55	327.32	3.27
Pasiphaea spp.	4.47	2.88	4.82	32.91	0.86
Hyperiid amphipod	1.11	18.75	21.69	22.12	0.58
Sergestidae	0.52	5.77	3.61	3.27	0.09
Caridea	0.20	8.17	13.25	1.63	0.04
Gammarid amphipod	0.04	1.92	2.41	0.07	0.00
Anomura	0.03	1.92	2.41	0.06	0.00
Euphausiacea	0.01	0.96	2.41	0.01	0.00
$H = 0.24$, exp(H) = 1.27					

(Table continued on next page)

Table 1 (continued)

Prey	%W	%Num	%O	IRI	%IRI
2010					
Fishes	32.81	63.47	31.13	2997.44	48.45
Pagrus auriga	6.31	31.05	15.09	563.92	17.06
C. aper	3.54	15.07	16.98	316.05	9.56
C. maderensis	5.40	9.13	4.72	68.54	2.07
Trachurus spp.	3.52	1.83	3.77	20.18	0.61
Unidentified fishes	9.17	0.91	1.89	19.03	0.58
A. risso	3.06	1.37	1.89	8.36	0.25
M. punctatum	1.64	2.28	1.89	7.39	0.22
Callionymus spp.	0.09	1.37	1.89	2.76	0.08
Unidentified larvae	0.08	0.46	0.94	0.50	0.02
Cephalopods	65.27	23.74	33.02	2939.24	47.51
I. coindetii	61.17	19.63	26.42	2134.54	64.57
Ommastrephidae	3.62	1.83	2.83	15.40	0.47
Sepiolidae	0.48	2.28	2.83	7.83	0.24
Crustaceans	1.92	12.79	16.98	249.65	4.04
Hyperiid amphipod	1.22	10.96	11.32	137.89	4.17
Stomatopod larvae	0.09	0.91	1.89	1.89	0.06
Pasiphaea spp.	0.59	0.46	0.94	0.99	0.03
Palaemonidae	0.01	0.46	0.94	0.44	0.01
$H = 0.53$, $\exp(H) = 1.70$					

Table 2. Stomach contents of age-0 ABFT captured off Cambrils (Tarragona, NE Spain). W: weight; Num: number; O: occurrence; IRI: index of relative importance. The trophic niche width is expressed as $\exp(H)$, H being the Shannon-Wiener index calculated from IRI

Prey	%W	%Num	%O	IRI	%IRI
2009					
Fishes	97.86	87.36	40.91	7577.00	98.26
Engraulis encrasicolus	36.72	56.32	22.73	2114.55	54.75
Sardina pilchardus	53.61	26.44	20.45	1637.27	42.39
Sardinella spp.	7.25	2.30	4.55	43.39	1.12
Unidentified larvae	0.29	2.30	4.55	11.75	0.30
Cephalopods	1.40	2.30	2.27	8.41	0.11
Bathypolypus spp.	1.40	2.30	2.27	8.41	0.22
Crustaceans	0.74	10.34	11.36	125.96	1.63
Stomatopoda	0.04	6.90	4.55	31.51	0.82
Phronima sedentaria	0.30	2.30	4.55	11.81	0.31
Brachyura	0.40	1.15	2.27	3.53	0.09
$H = 0.84$, $\exp(H) = 2.31$					
2010					
Fishes	81.49	27.59	30.99	3379.73	54.54
Engraulis encrasicolus	29.97	16.67	16.90	788.26	33.41
Sardina pilchardus	50.67	9.20	12.68	758.91	32.16
Unidentified larvae	0.84	1.72	4.23	10.84	0.46
Cephalopods	12.21	2.87	30.99	467.38	7.54
Unidentified cephalopods	11.99	2.30	5.63	80.50	3.41
Bathypolypus spp.	0.22	0.57	1.41	1.12	0.05
Crustaceans	6.30	69.54	30.99	2350.07	37.92
Stomatopoda	2.85	32.18	15.49	542.85	23.01
Decapod zoeae	0.91	13.22	7.04	99.51	4.22
Caridea	2.30	9.77	2.82	34.00	1.44
Palaemon spp.	0.08	6.32	2.82	18.03	0.76
Decapod phyllosomae	0.10	5.17	2.82	14.84	0.63
Nematoscelis megalops	0.04	2.30	4.23	9.89	0.42
Phrosina semilunata	0.02	0.57	1.41	0.84	0.04
$H = 0.75$, $\exp(H) = 2.11$					

mely *Engraulis encrasicolus* and *Sardina pilchardus*) predominated over the cephalopod and crustacean prey groups in 2009 and 2010 (%IRI = 98.26 and 54.54, respectively), although crustaceans (mainly stomatopods) contributed significantly to the diet in 2010 (%IRI = 37.92). In all years studied, the trophic niche width, measured as $\exp(H)$, was somewhat lower in Cartagena (from 1.27 to 1.70 in 2008 to 2010; Table 1) than in Cambrils (2.31 and 2.11 in 2009 and 2010, respectively; Table 2).

CA was performed on 11 prey groups: *Engraulis encrasicolus*, *Sardina pilchardus*, *Trachurus* spp., *Capros aper*, *Pagrus auriga*, myctophids, *Arctozenus risso*, ommastrephids, stomatopods, gammarid amphipods, carideans, and *Phrosina semilunata*. *E. encrasicolus* and *S. pilchardus* were merged into a single group, as their factorial coordinates were very close in a previous analysis. The first and second factorial axes of this CA synthetize 30.2% of the variability in the data. The first factorial axis opposes *E. encrasicolus*, *S. pilchardus* and *Trachurus* spp. on the negative semi-axis versus all other prey groups on the positive semi-axis (Fig. 2). The highest values on this axis correspond to 8 prey groups with close factorial coordinates on the axis. On the second factorial axis, these 8 prey groups are separated, with *A. risso* having the lowest factorial coordinate, whereas myctophids to ommastrephids and gammarid amphipods form a continuum situated on the positive semi-axis (except for myctophids) (Fig. 2).

The barycenters of the factorial coordinates of the tuna from each year and location (Cartagena 2008 to 2010, Cambrils 2009 and 2010) appear to be also distributed according to these 2 patterns related to the first 2 axes (Fig. 2). The barycenters corresponding to Cambrils have negative values on the first axis, whereas the ones cor-

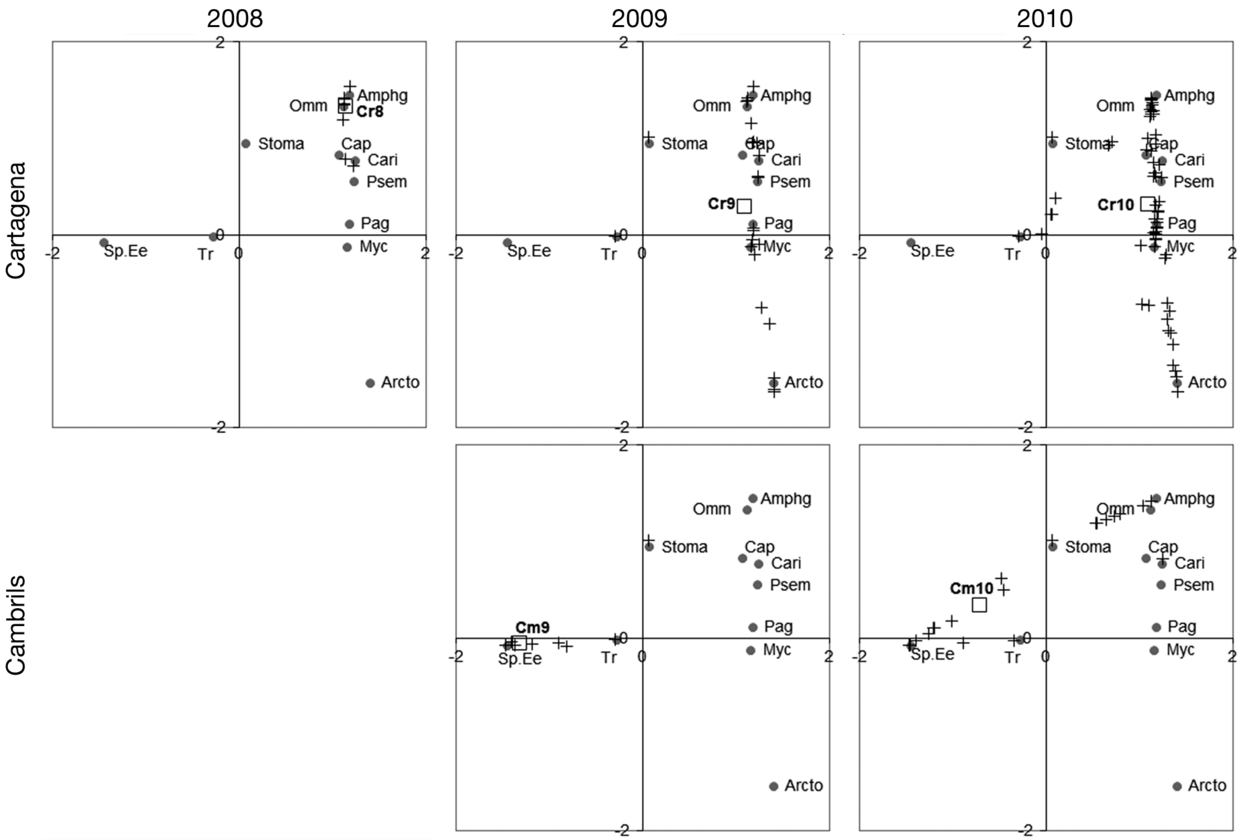

Fig. 2. Correspondence analysis of the weight percentages of the main ABFT prey (●) by predator in Cambrils (Cm) and Cartagena (Cr) in 2008 to 2010. (+) Predators (same cross represents predators with identical factorial coordinates), (□) barycenters for each year and region. Sp: *Sardina pilchardus*, Ee: *Engraulis encrasicolus*, Tr: *Trachurus* spp., Stoma: stomatopods, Omm: ommastrephids, Amphg: gammarid amphipods, Cap: *Capros aper*, Cari: carideans, Psem: *Phrosina semilunata*, Pag: *Pagrus auriga*, Myc: myctophids, Arcto: *Arctozenus risso*

responding to Cartagena have positive values on this axis for all 3 years. The distribution of the individual factorial coordinates partially confirms these patterns. Individuals sampled near Cartagena are distributed mainly along the gradient related to the second axis (Fig. 2). On the other hand, individuals sampled near Cambrils are distributed along a diagonal gradient between anchovy and sardine on one side and ommastrephid cephalopods and gammarid amphipods on the other, particularly in the case of the individuals sampled in 2010.

Stomach fullness appeared to be influenced by tuna size (fullness increasing for larger individuals up to 35 cm; Table 3) and by sampling location (higher fullness ratios in fish from Cartagena compared to those from Cambrils; Fig. 3).

Stable-isotope analysis (SIA)

C and N stable isotope ratios were measured in white muscle and liver tissues of the tuna collected off Cartagena and Cambrils in 2009 and 2010. δ^{13}C values ranged from −18.03 to −17.51‰ for muscle and from −18.45 to −17.89‰ for liver. δ^{15}N values ranged from 8.29 to 9.01‰ for muscle and from 8.39

Table 3. Generalized additive model of stomach fullness of the sampled juvenile ABFT as a function of sampling location and straight fork length (FL)

Parametric coefficients	Estimate	SE	*t*-value	Pr(>|*t*|)
Cambrils (intercept)	0.007402	0.00123	6.018	5.66×10^{-9}
Cartagena (difference)	0.006129	0.001695	3.616	0.000356
Smooth term	edf	Ref.df	*F*	p-value
s(FL)	1.652	2.066	5.736	0.00336

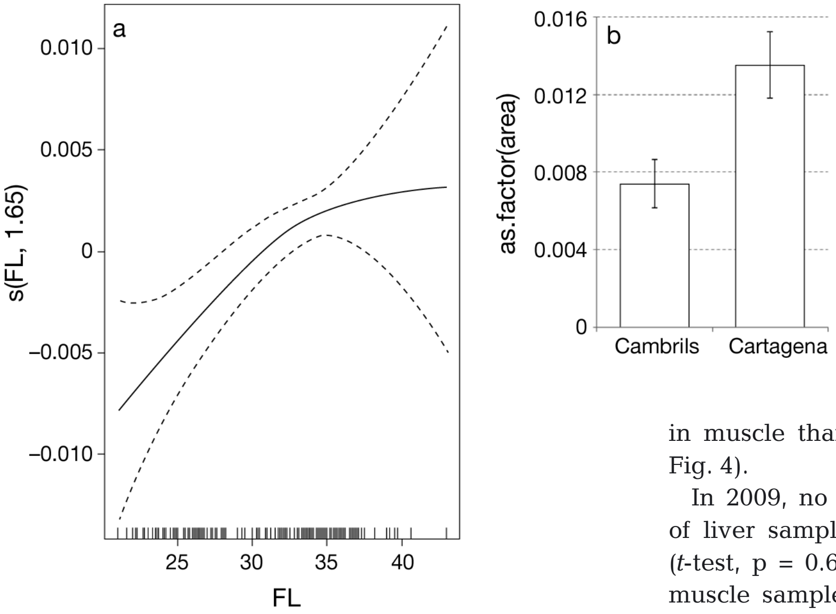

Fig. 3. Generalized additive model of stomach fullness of the sampled juvenile ABFT as a function of (a) straight fork length (FL), and (b) sampling location; s is the partial effect corresponding to the smooth term (FL) on stomach fullness, and as.factor is the partial effect corresponding to the factor term (sampling area) on stomach fullness. Dashed lines in (a) and vertical bars in (b) indicate 1 SE

to 8.68‰ for liver (Tables 4 & 5). Three-factor permutational ANOVA indicated that there were significant differences in $\delta^{13}C$ between tissues, locations and years (Table 6), but no differences were detected for $\delta^{15}N$ (Table 6). Comparisons of means showed that in all cases, $\delta^{13}C$ values were significantly higher in muscle than in liver (*t*-test, $p \leq 0.002$; Table 4, Fig. 4).

In 2009, no significant differences in $\delta^{13}C$ values of liver samples were observed between locations (*t*-test, p = 0.654; Table 4), whereas $\delta^{13}C$ values of muscle samples from Cartagena were significantly higher than those from Cambrils (*t*-test, p = 0.003). In 2010, mean $\delta^{13}C$ values of both muscle and liver samples collected from Cartagena were significantly lower than those from Cambrils (*t*-test, p < 0.001; Table 4). It is noteworthy that the $\delta^{13}C$ and $\delta^{15}N$ values measured in muscle of the specimens captured off Cartagena in 2009 were the highest among all isotopic values recorded (Tables 4 & 5). As a result, both liver and muscle mean isotopic values of the specimens from Cambrils in that year lay between the lower (liver) and upper (muscle) values of fish from Cartagena (Fig. 4a).

Table 4. Mean $\delta^{13}C$ and $\delta^{15}N$ (±SD) of age-0 ABFT captured off Cartagena (Murcia, SE Spain) and Cambrils (Tarragona, NE Spain) in September to October 2009 and 2010. The right column shows p-values from Student's *t*-tests comparing $\delta^{13}C$ means between Cartagena and Cambrils, assuming equal (*t*) or unequal (*t**) variances. p-values from comparisons between muscle and liver $\delta^{13}C$ means are shown beneath each sampling location

	Cartagena (n = 10)	Cambrils (n = 25)	p-value
2009			
$\delta^{13}C$			
Muscle	−17.51 ± 0.23	−17.74 ± 0.17	*t*, p = 0.003
Liver	−17.92 ± 0.12	−17.89 ± 0.14	*t*, p = 0.654
p-value	*t*, p = 0.002	*t*, p = 0.002	
$\delta^{15}N$			
Muscle	9.01 ± 0.50	8.58 ± 0.43	
Liver	8.65 ± 0.32	8.46 ± 0.37	
	Cartagena (n = 13)	Cambrils (n = 25)	p-value
2010			
$\delta^{13}C$			
Muscle	−18.03 ± 0.12	−17.70 ± 0.23	*t**, p < 0.001
Liver	−18.45 ± 0.23	−17.96 ± 0.31	*t*, p < 0.001
p-value	*t**, p < 0.001	*t*, p = 0.001	
$\delta^{15}N$			
Muscle	8.29 ± 0.69	8.47 ± 0.93	
Liver	8.39 ± 0.49	8.68 ± 1.05	

Table 5. Comparison of mean $\delta^{13}C$ and $\delta^{15}N$ (±SD) between 2009 and 2010. The right column shows p-values from Student's *t*-tests, assuming equal (*t*) or unequal (*t**) variances

	2009 (n = 10)	2010 (n = 13)	p-value
Cartagena			
$\delta^{13}C$			
Muscle	−17.51 ± 0.23	−18.03 ± 0.12	*t*, p < 0.001
Liver	−17.92 ± 0.12	−18.45 ± 0.23	*t*, p < 0.001
$\delta^{15}N$			
Muscle	9.01 ± 0.50	8.29 ± 0.69	
Liver	8.65 ± 0.32	8.39 ± 0.49	
	2009 (n = 25)	2010 (n = 25)	p-value
Cambrils			
$\delta^{13}C$			
Muscle	−17.74 ± 0.17	−17.70 ± 0.23	*t*, p = 0.502
Liver	−17.89 ± 0.14	−17.96 ± 0.31	*t**, p = 0.260
$\delta^{15}N$			
Muscle	8.58 ± 0.43	8.47 ± 0.93	
Liver	8.46 ± 0.37	8.68 ± 1.05	

Table 6. Three-factor permutational ANOVA for $\delta^{13}C$ and $\delta^{15}N$ data

	df	SS	MS	Pseudo-*F*	R^2	p-value
$\delta^{13}C$						
Tissue	1	0.0017466	0.00174665	37.381	0.18426	0.001
Location	1	0.0007458	0.00074582	15.962	0.07868	0.001
Year	1	0.0006786	0.00067856	14.522	0.07158	0.002
Residuals	135	0.0063080	0.00004673		0.66547	
Total	138	0.0094791			1	
$\delta^{15}N$						
Tissue	1	0.000001	0.00000074	0.00037	0.00000	0.9988
Location	1	0.000092	0.00009185	0.04625	0.00034	0.8689
Year	1	0.002838	0.00283834	1.42908	0.01047	0.2279
Residuals	135	0.268127	0.00198613		0.98919	
Total	138	0.271058			1	

Comparing between years (Table 5, Fig. 4), the $\delta^{13}C$ values of the samples from Cartagena were significantly higher in 2009 than in 2010 (*t*-test, $p < 0.001$), whereas no significant differences in $\delta^{13}C$ values of both muscle (*t*-test, $p = 0.502$) and liver (*t*-test, $p = 0.260$) were observed between years for fish from Cambrils.

Table 7 shows data on isotopic niche width, as measured by the standard ellipse area (SEA) and the area of the standard ellipse corrected for sample size (SEA_c). The trophic niche width estimated by SEA_c was generally larger in fish from Cambrils than in those from Cartagena, with the exception of the result obtained from muscle samples in 2009. SEA_c also indicated that the trophic niches in 2010 were wider than in 2009, although the SEA_c values obtained from muscle samples of fish from Cartagena showed the opposite pattern.

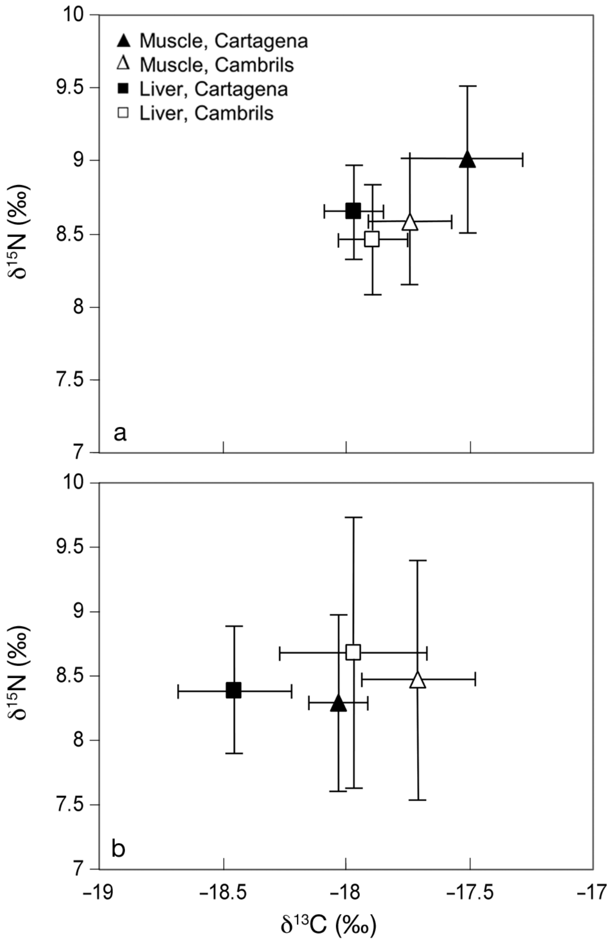

Fig. 4. Mean $\delta^{13}C$ and $\delta^{15}N$ values (±SD) of muscle and liver from age-0 ABFT captured off Cambrils (NE Spain) and Cartagena (SE Spain) in (a) 2009 and (b) 2010

DISCUSSION

The SCA results show that the diet of age-0 ABFT is variable, consisting of a broad range of prey items dominated by fishes, although some invertebrate species (cephalopods and small crustaceans) contribute substantially to their diet. In particular, cephalopods accounted for 65 to 91% (by weight) of the diet of juveniles caught off Cartagena in 2008

Table 7. Trophic niche widths of age-0 ABFT captured off Cambrils (Tarragona, NE Spain) and Cartagena (Murcia, SE Spain) in September to October 2009 and 2010, as estimated by SIBER analysis of muscle and liver isotopic values. SEA: standard ellipse area; SEA_c: corrected standard ellipse area. The right column shows statistical differences in SEA

Group	SEA	SEA_c	Group differences Probability (%)
Muscle			
Cambrils 2009 (1)	0.19	0.20	1 vs. 2 (99.39) 1 vs. 3 (97.21) 1 vs. 4 (97.66)
Cambrils 2010 (2)	0.58	0.61	2 vs. 3 (45.43) 2 vs. 4 (42.87)
Cartagena 2009 (3)	0.36	0.41	3 vs. 4 (48.23)
Cartagena 2010 (4)	0.25	0.27	
Liver			
Cambrils 2009 (1)	0.17	0.17	1 vs. 2 (99.99) 1 vs. 3 (98.66) 1 vs. 4 (98.61)
Cambrils 2010 (2)	0.96	1.00	2 vs. 3 (35.48) 2 vs. 4 (20.82)
Cartagena 2009 (3)	0.11	0.13	3 vs. 4 (38.67)
Cartagena 2010 (4)	0.36	0.41	

and 2010. Sanz-Brau (1990), in contrast, found that age-0 ABFT caught nearby (Fig. 1) preyed mainly on pelagic fish species, which is similar to what we observed in the specimens sampled off Cambrils in 2009 and 2010 and off Cartagena in 2009. The occurrence of sharp fluctuations in the amount of cephalopods consumed by juvenile tuna is not surprising, since cephalopod abundance can be highly affected by environmental changes that cause marked inter-annual demographic variations (Vila et al. 2010, Silva et al. 2011). These observations suggest that age-0 ABFT also exhibit a generalist feeding behaviour, just like adult and subadult ABFT (see Chase 2002, Salman & Karakulak 2009, Logan et al. 2011, Battaglia et al. 2013a, Varela et al. 2014), and rely largely on the abundance and availability of potential prey in their habitat.

Among fishes, epipelagic species (e.g. sardine, anchovy and sardinella) were abundant, especially in the stomach contents of juvenile ABFT from Cambrils, whereas mesopelagic species (mainly myctophids) were a significant part of the consumed fish in individuals caught off Cartagena as reflected by the first axis of the CA. Such differences may be due to the different widths of the continental shelf between the 2 areas. Cambrils is close to the wide shelf of the Ebro River delta, which is rich in epipelagic fishes, while the continental shelf off Cartagena is narrow, so that mesopelagic species in this area are likely to be found closer to the shore. In the CA, prey items related to the positive part of the first axis are all mesopelagic, but the second axis reflects a different typology within them, as it separates a strictly mesopelagic species (*Arctozenus risso*) from a group of vertically migrating prey that are present in mesopelagic waters during the day and are near the surface at nighttime. Tuna can prey upon these organisms either during daytime (implying a diving behaviour) or at night (Battaglia et al. 2013a,b). Sinopoli et al. (2004) suggested that the presence of mesopelagic fishes, such as myctophids or paralepidids, in the stomachs of age-0 ABFT was related to vertical movements to deep waters. In support of this, Brill et al. (2002) and Galuardi & Lutcavage (2012) have shown that juvenile ABFT measuring between 66 and 145 cm in curved fork length are capable of diving as deep as 800 m, although they spend the majority of their time swimming in shallow waters above 20 m. Therefore, we concur with Karakulak et al. (2009) and Battaglia et al. (2013a,b) that schools of mesopelagic fishes, along with other vertically migrating pelagic organisms such as planktonic crustaceans and squids, become vulnerable to tuna and other predators that occur in shallow waters from dusk to dawn. The occasional presence of demersal species in gut contents of fish caught off Cartagena (e.g. *Capros aper*) suggests that age-0 ABFT are capable of performing diurnal deep dives in search of potential prey. The differences observed in stomach fullness and the likelihood of diving behaviour off Cartagena suggest that the juvenile ABFT in this area offset the energetic cost of deep diving with higher caloric intakes reflected by larger amounts of ingested prey.

The data provided by SIA agree to some extent with the results of SCA regarding differences in the diet consumed by age-0 ABFT in different locations and years. Most $\delta^{13}C$ values showed significant differences between fish from Cartagena and Cambrils, although $\delta^{15}N$ values were similar. This may indicate that the fish were exploiting different dietary sources but were feeding at similar trophic levels (provided that the food web structure and the baseline isotopic composition of the respective habitats were alike). As regards potential inter-annual trophic changes, the $\delta^{13}C$ values measured in samples from Cartagena differed between 2009 and 2010, whereas no isotopic ratios were significantly different between 2009 and 2010 in Cambrils samples. Such data are consistent with the results of SCA, which indicated dietary variations across the 3 years of sampling off Cartagena (2008 to 2010), but also showed that the diet of ABFT juveniles foraging off Cambrils hardly changed between 2009 and 2010 and was based mostly on sardine and anchovy (>80% by weight). The high consumption of these species off Cambrils may be due to the fact that the northwest Mediterranean area near the Ebro River is a productive habitat, where these pelagic fishes form large aggregations (Lloret et al. 2004, Morote et al. 2010, Tugores et al. 2010). Thus, the great predominance of sardine and anchovy in gut contents of the juvenile ABFT collected in this zone presumably reflects an opportunistic feeding strategy.

It is worth noting that in all the fish examined, regardless of sampling location or year, the N isotopic ratios of the 2 tissues were similar, while the C isotopic ratios in muscle were enriched relative to liver. Nevertheless, Varela et al. (2012) showed that the $\delta^{13}C$ values of muscle and liver became eventually similar in individuals of the same age reared for 90 d on a single prey. This appears to indicate that the juvenile tuna studied here were not at equilibrium with the food sources in their habitats, and might have arrived recently at the respective feeding grounds.

The diet diversity measured by the Shannon entropy-based index exp(*H*) showed a broader trophic niche for Cambrils (estimating the existence of 2.31 and 2.11 abundant species in the diet in 2009 and 2010, respectively) than for Cartagena (with 1.62, 1.27 and 1.70 abundant species present in the diet in 2008, 2009 and 2010, respectively). Therefore, while exp(*H*) values were rather similar between years in the specimens caught off Cambrils, the trophic niche width for the groups of samples from Cartagena was appreciably smaller in 2009 than in 2008 and 2010. Bearhop et al. (2004) proposed the variance of isotopic ratios of carbon and nitrogen as a good indicator of trophic niche width, giving rise to further stable isotope-based metrics to describe niche structure, including niche width (Layman et al. 2007, Jackson et al. 2011). In agreement with the estimations based on diet diversity from SCA, the SEA_c data derived from SIBER (Stable Isotope Bayesian Ellipses; Jackson et al. 2011) suggested that the trophic niche of individuals collected off Cambrils was wider than that of fish from Cartagena. Moreover, the analysis of SEA_c suggested generally broader trophic niches in 2010. However, the high value of SEA_c obtained from the muscle samples of Cartagena in 2009 is inconsistent with this pattern. The marked difference in trophic niche width obtained with the 2 different tissues in this sampling group could possibly be explained by recent diet shifts that might not have been integrated into the isotopic composition of muscle due to its slower turnover rate compared to liver. Nevertheless, these results should be interpreted cautiously, since the low number of samples (≤25) is likely to reduce the accuracy of the method (Syväranta et al. 2013).

One of the multiple ecological applications of SIA is in making inferences on the distributions and movements of animals through distinct habitats by comparing their stable-isotope composition to that of their local prey, which in turn reflects the local environmental isotope composition (Fry et al. 2003, Graham et al. 2010). This approach has been used to elucidate foraging behaviour and movements of marine top predators, including tunas (Estrada et al. 2005, Graham et al. 2007, 2010, Ménard et al. 2007, Sarà & Sarà 2007, Graham 2008, Olson et al. 2010, Logan et al. 2011, Cardona et al. 2012, Varela et al. 2014). Gradual variations in white muscle isotopic values of yellowfin tuna (mostly δ^{15}N) across latitudinal gradients have been found in the Indian and Pacific Oceans (Ménard et al. 2007, Olson et al. 2010). Such variations result from the propagation of isotopic signatures from the base of the food web, along the food chain, going up to top predators. Although this study spans a small spatial scale, since Cambrils and Cartagena are <500 km apart in straight-line distance, differences in isotopic values could be present due to potential local environmental variations. As river discharges appear to greatly influence isotopic signatures (Graham et al. 2010, Chouvelon et al. 2012), the proximity of Cambrils to the highly productive area around the Ebro River delta would favour increased isotopic values in this zone. Another likely cause of δ^{13}C differences between both areas is the existence of a high-nutrient tongue of water from the Atlantic Ocean (Rodríguez et al. 1998) that reaches the coast of Cartagena. This oceanographic feature may lead to differences in the phytoplankton community, causing a shift in the isotopic baseline towards the lower δ^{13}C values that characterize Atlantic waters compared to the Mediterranean (Goñi et al. 2011).

In age-0 ABFT collected from the Tyrrhenian Sea, Sarà & Sarà (2007) found muscle δ^{13}C values that were very similar to those obtained in this study (−18.0 to −17.5‰ vs. −18.03 to −17.51‰), whereas their mean muscle δ^{15}N values (7.2 to 7.7‰) were lower than the mean N isotopic ratios measured here (8.29 to 9.01‰). It is unlikely that such differences are due to distinct feeding strategies or trophic structures, but they are probably linked to specific environmental processes occurring in each area. Although the juvenile tuna were sampled in different years, and significant inter-annual isotopic variations cannot be ruled out, the relatively large differences in δ^{15}N values between these 2 Mediterranean areas suggest that the N stable isotope ratio is particularly sensitive to spatial variations and is thus a robust indicator of the feeding area (sensu Chouvelon et al. 2012).

Performing SIA in tissues with distinct turnover rates allows the integration of stable-isotope signatures over different time scales. Turnover rates vary as a function of the metabolic features of the organs and the individual's growth rate. In generalist feeders like tunas, tissues with high turnover rates are more informative because they would reflect recent feeding changes more appropriately (Hobson & Clark 1992, Bearhop et al. 2004). Because muscle has a slower turnover rate than liver, it integrates stable isotope ratios over a longer period of time (Graham 2008). Therefore, it seems unlikely that the muscle tissue isotopic signatures reflect only local sources, but they also incorporate isotopic values from previous feeding on prey captured in distant foraging grounds. Nevertheless, it should be taken into consideration that the fast growth rate of ABFT at early life stages is likely to quickly reduce the isotopic differences between organs, so that the temporal window integrated by the 2 tissues would be narrower in juveniles (particularly in age-0 individuals) than in adults.

Environmental changes may significantly affect the relationships within food webs (Young et al. 2015), hence ecosystem-based approaches are essential in predicting the impacts of potential perturbations and contribute to the conservation of marine communities. The present study reveals that the food sources of juvenile ABFT are site-specific and may change with time. It also underlines the potential of using SIA in combination with SCA for the characterization of diet variability in top predators considered in a spatio-temporal context. These techniques may help us better understand trophic interactions in marine pelagic habitats and build realistic ecosystem models.

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