



# Trophic strategies of three predatory pelagic fish coexisting in the north-western Mediterranean Sea over different time spans

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## ABSTRACT

Understanding the trophic ecology of marine predators is pivotal to assess their ecological role in the ecosystems and develop management actions. Despite the ecological importance of predatory pelagic fish species, the trophic role that individual species play within marine communities in many marine basins, such as the Mediterranean Sea, remains unclear. In the present study, we aimed to provide new integrative information about the trophic ecology at different temporal scales of Atlantic bonito (*Sarda sarda*), little tunny (*Euthynnus alletteratus*) and swordfish (*Xiphias gladius*) in the north-western Mediterranean Sea. To quantify the trophic habits of these three species, we used stable isotopic analysis and isotopic mixing models from different tissues that integrate trophic information at different temporal scales (~1 month for liver, ~ several months for muscle, >1 year for fin). We found clear and consistent differences in the trophic habits among the three species and time spans within the same species. Although preying mainly on clupeiforms, Atlantic bonito and little tunny present a temporal segregation in the species preference, with Atlantic bonito preying mainly on European sardine and round sardinella at long-term, while little tunny preyed mainly on European anchovy. In the other hand, swordfish shows a more generalist trophic strategy with high preference for demersal prey. This study emphasises the utility of this integrative approach for trophic studies due to its capacity for monitoring trophic habits over different time spans.

## 1. Introduction

Understanding the feeding ecology of marine organisms is important for determining their ecological role in the ecosystems and to design optimal management actions. Studies of species-specific trophic characteristics provide critical information about how to manage fisheries sustainably and promote conservation strategies for particular species, taking into account that global marine catches are increasing, especially for pelagic predators (FAO, 2018; Pauly and Zeller, 2016). Large predatory pelagic fish species are usually classified as top predators, although notable differences in their trophic ecology exist (Stergiou and Karpouzi, 2002). Pelagic predators have top-down effects on marine communities, and thus a reduction in their populations could have important impacts on ecosystems (Baum and Worm, 2009; Ward and Myers, 2005).

The trophic role that individual pelagic predator species play within marine communities in many marine basins, such as the Mediterranean Sea, remains unclear, owing to limited research studies (e.g. Stergiou and Karpouzi, 2002; Falautano et al., 2007; Romeo et al., 2009; Navarro

et al., 2017). This is true of swordfish (*Xiphias gladius*), little tunny (*Euthynnus alletteratus*) and Atlantic bonito (*Sarda sarda*). Based on published information, swordfish is considered a generalist predator exploiting prey at different trophic levels as a result of its vertical movements (Abascal et al., 2010; Rosas-Luis et al., 2017). Its diet is composed of fin-fish such as Aulopiformes and Gadiformes, and some cephalopod species (Bello, 1991; Navarro et al., 2017). This species is considered near threatened by the International Union for Conservation of Nature (IUCN) in the Mediterranean Sea (Abdul Malak et al., 2011) and a decline in its populations could trigger serious problems in ecosystem functioning. In contrast, Atlantic bonito and little tunny show a diet basically composed of Clupeoid species [European anchovy (*Engraulis encrasicolus*), round sardinella (*Sardinella aurita*) and European sardine (*Sardina pilchardus*)], which are highly dependent on environmental variability and fishing activities (Campo et al., 2006; Cardona et al., 2015; Coll et al., 2019). Although Atlantic bonito and little tunny exploit similar trophic niches, some trophic segregation in specific prey species consumed between them exists (Navarro et al., 2017). In addition to limited trophic information, most existing

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knowledge is based on information collected during a particular period of time, and thus, it is not known whether the trophic habits of these species change with time (Davis and Pineda-Munoz, 2016). Considering the ecological and economic importance of these three species, further investigation into their feeding habits at different time scales is needed to obtain a complete overview of their trophic habits.

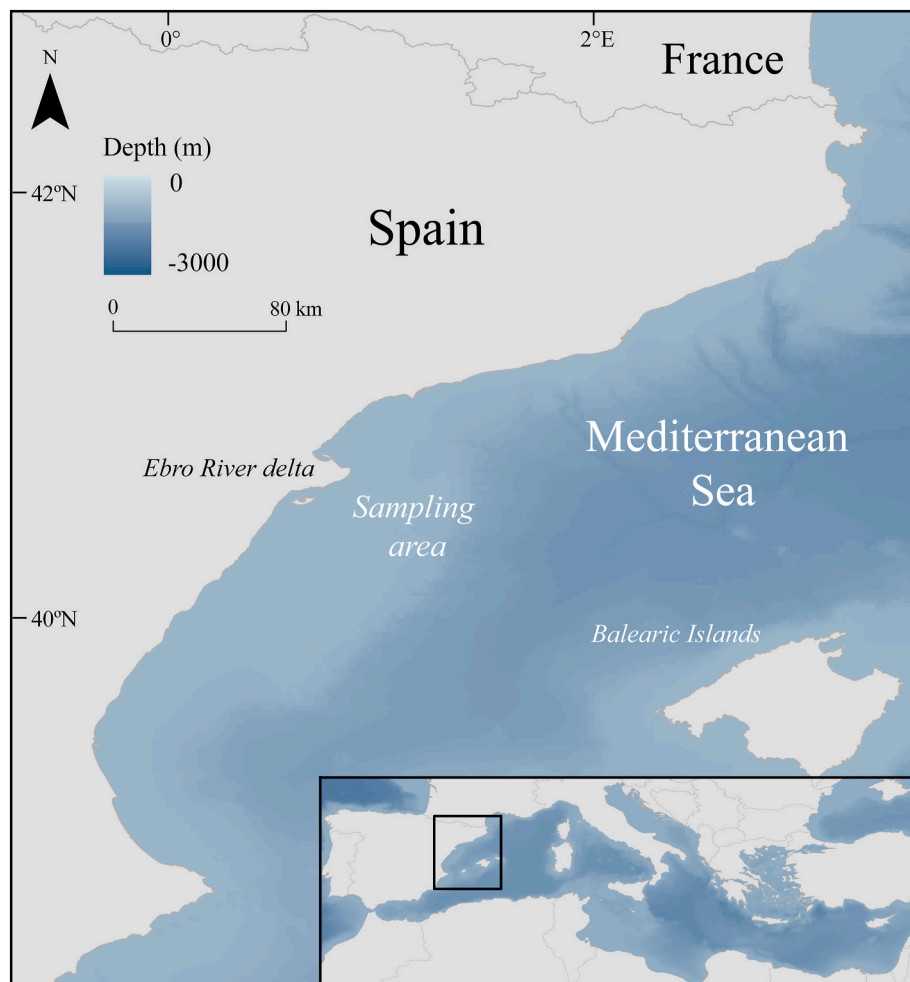
The trophic ecology of marine predators has been traditionally studied using stomach content analysis, which has the advantage of providing high taxonomic resolution (Hyslop, 1980). However, stomach contents represent only part of what an animal has eaten recently and can be skewed due to differences in the digestibility of prey (Hyslop, 1980). In the last decade, studies using stomach content analysis have been complemented with the analysis of stable isotopes of nitrogen ( $\delta^{15}\text{N}$ ) and carbon ( $\delta^{13}\text{C}$ ) values (Phillips et al., 2014). This approach is based on the fact that  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  values are transformed from dietary sources to consumers in a predictable manner (Phillips et al., 2014).  $\delta^{15}\text{N}$  is used as a proxy of trophic levels while  $\delta^{13}\text{C}$  values show little change with trophic levels but are useful indicators of the dietary source of carbon (Layman et al., 2012). Depending on the tissue analysed, it integrates different time periods over weeks (i.e. the liver) or months (i.e. the muscle or the fin) (MacNeil et al., 2005). Isotopic mixing models combining stable isotope values of consumers in different tissues and different periods with those from their potential prey (Phillips et al., 2014) can be applied to obtain estimates of the relative contribution of each prey item to the estimated diet of the consumers at different time-scales (MacNeil et al., 2005; Navarro et al., 2014).

In the present study, we aimed to provide new integrative information about the trophic ecology at different temporal scales of Atlantic bonito, little tunny and the swordfish in the north-western Mediterranean Sea, complementing previous results obtained at short-term scale with these three species based on stomach content analysis (Navarro et al., 2017). Specifically, we aimed to investigate if the trophic strategies of these three pelagic fish predators changed along different temporal-scales. Also, with these trophic information, we examined the degree of segregation/overlap in the trophic niche between these three species along different periods. We hypothesize that the relationship and exploitation of the trophic niche of these three species will change through time (Navarro et al., 2017). To examine the feeding strategies of these three marine predators at different temporal scales, we analysed stable isotopic values, used isotopic mixing models and estimated the trophic niche in different tissues that integrate different temporal information of their diet (~1 month for liver, ~several months for muscle, ~1 year for fin; (Davis and Pineda-Munoz, 2016; Madigan et al., 2012). Our study provides new insights into the ways in which Atlantic bonito, little tunny and the swordfish exploit trophic resources and contributes to an understanding of their ecological role within the community.

## 2. Material and methods

### 2.1. Sampling procedure

Samples of muscle, liver and fin of swordfish ( $n = 25$ ), little tunny ( $n$



**Fig. 1.** Sampling area in the north-western Mediterranean Sea. The geographic position of the studied area in the Mediterranean Basin is also indicated in the bottom right corner.

= 16), and Atlantic bonito ( $n = 22$ ) were collected and stored at  $-20^{\circ}\text{C}$  during spring and summer 2012 in the western Mediterranean Sea (Fig. 1). All individuals were sampled on-board on longline, purse seine and gillnet fishing vessels working in the study area (see further explanation in Navarro et al., 2017). This region is one of the most important fishing areas in the Mediterranean Sea (Leonart and Maynou, 2003) influenced by two marine currents, the Liguro-Provençal-Catalan and the Balearic Current, both close to the coast (Salat, 1996).

## 2.2. Stable isotope analyses

All tissues were freeze-dried and powdered, and 0.3–0.4 mg of each sample was packed into tin capsules. Isotopic analyses were performed at the Laboratory of Stable Isotopes of the Estación Biológica de Doñana - CSIC (Sevilla, Spain). Samples were combusted at  $1020^{\circ}\text{C}$  using a continuous flow isotope-ratio mass spectrometry system (Thermo Electron) by means of a Flash HT Plus elemental analyser interfaced with a Delta V Advantage mass spectrometer. Stable isotope ratios were expressed in the standard  $\delta$ -notation (‰) relative to Vienna Pee Dee Belemnite ( $\delta^{13}\text{C}$ ) and atmospheric  $\text{N}_2$  ( $\delta^{15}\text{N}$ ). Based on laboratory standards, the measurement error was  $\pm 0.1$  and  $\pm 0.2$  for  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$ , respectively. The standards used were EBD-23 (cow horn, internal standard), LIE-BB (whale baleen, internal standard) and LIE-PA (razor-bill feathers, internal standard). These laboratory standards were previously calibrated with international standards supplied by the International Atomic Energy Agency (IAEA, Vienna). The %C and %N of all samples were also determined. Because lipid content could affect the  $\delta^{13}\text{C}$  values by depleting the real values, we extracted the lipid content from the liver samples using chloroform: methanol (2:1 v/v) before the isotopic analyses (Bodin et al., 2009). After, we checked that the C:N ratios (a proxy of lipid content) of the liver without lipids, muscle and fin tissues were lower than 3.5%, a threshold that indicated that the lipid content of the tissue do not affect the  $\delta^{13}\text{C}$  values (Logan et al., 2008).

## 2.3. Isotopic mixing model and isotopic niche

The proportional contribution of each prey item to the estimated diet of each of the species and each tissue was estimated using MixSIAR Bayesian isotopic mixing model (Stock et al., 2018; Stock and Semmens, 2016). Models were run with a generalist type prior, 3 Markov chain Monte Carlo (MCMC) chains of 300,000 draws and a burn-in of 200,000 draws. The convergence of models was checked using both Gelman-Rubin and Geweke diagnostics (Stock et al., 2018; Stock and Semmens, 2016). Diet-tissue-discrimination factor values (DTDF) for each of the tissues (muscle and fin:  $\Delta\delta^{15}\text{N} = 1.9 \pm 0.4\text{‰}$ ,  $\Delta\delta^{13}\text{C} = 1.8 \pm 0.3\text{‰}$  for, liver:  $\Delta\delta^{15}\text{N} = 1.1 \pm 0.6\text{‰}$ ,  $\Delta\delta^{13}\text{C} = 1.2 \pm 0.3\text{‰}$ ) were obtained from the Pacific Bluefin tuna (*Thunnus orientalis*) under controlled conditions (Madigan et al., 2012). Because we did not used specific-DTDF values for each of the three studied species, the estimated diet based on the isotopic mixing models could have certain bias. To run the MixSIAR model, values of the main potential prey were taken from the results of stomach content analysis reported in Navarro et al. (2017). For little tunny and Atlantic bonito, European sardine, European anchovy and round sardinella were selected. For swordfish, Aulopiformes, Clupeiformes, cephalopods and Gadiformes were used (see Table 1 for stable isotope values). Isotopic values of the potential prey were taken from a reference isotopic that contains up to 128 species collected in the area of study (Barría et al., 2015).  $\delta^{13}\text{C}$  and/or  $\delta^{15}\text{N}$  values differed among the different prey groups (ANOVA tests,  $p < 0.05$ , see Table 1).

As a measure of trophic width, we calculated the standard ellipse areas corrected for the sample size ( $\text{SEA}_C$ ; area containing 40% of the data) as well as the Bayesian isotopic ellipse area ( $\text{SEA}_B$ ) for each tissue for each species (Jackson et al., 2011). This metric represents a measure of the total amount of isotopic niche exploited by a particular consumer and is thus a proxy for the extent of trophic diversity (or trophic width) exploited by the species considered (high values of  $\text{SEA}_C$  and  $\text{SEA}_B$

**Table 1**

Mean and standard deviation of  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  values of the main prey group used in the MixSIAR model for Atlantic bonito (*Sarda sarda*), little tunny (*Euthynnus alletteratus*) and swordfish (*Xiphias gladius*). The results of ANOVA and post-hoc Tukey HSD tests are shown. Means differing significantly ( $P < 0.05$ ) are indicated by letters (a, b, c) in the case of differences among prey species.

Predator	Prey	n	$\delta^{15}\text{N}$ (‰)	$\delta^{13}\text{C}$ (‰)
Atlantic bonito, Little tunny	European anchovy	20	$7.62 \pm 0.63\text{a}$	$-18.87 \pm 0.25\text{a}$
	Round sardinella	10	$8.43 \pm 0.43\text{b}$	$-18.77 \pm 0.18\text{a}$
	European sardine	19	$8.78 \pm 0.55\text{b}$	$-19.66 \pm 0.51\text{b}$
	ANOVA test	F	14.93	29.13
		p-value	<0.001	<0.001
Swordfish	Aulopiformes	10	$7.89 \pm 0.39\text{a}$	$-19.15 \pm 0.69\text{a}$
	Cephalopoda	20	$9.01 \pm 1.01\text{c}$	$-18.98 \pm 0.49\text{b}$
	Clupeiformes	49	$8.17 \pm 0.76\text{a,b}$	$-19.43 \pm 0.12\text{a}$
	Gadiformes	13	$8.83 \pm 0.25\text{b}$	$-19.45 \pm 0.12\text{a}$
	ANOVA test	F	12.53	7.08
		p-value	<0.001	<0.001

indicate high trophic width). Overlap between  $\text{SEA}_B$  of different pair of tissues of the same species and between different species for each tissue were calculated. The overlap between two  $\text{SEA}_B$  was calculated as percentage of the area overlapped respect to the total area of A and B ( $\text{SEA}_B$  was computed using 1000 posterior draws; Jackson et al., 2011). The  $\text{SEA}_B$  uses multivariate ellipse-based Bayesian metrics. Bayesian inference techniques allow for robust statistical comparisons between data sets with different sample sizes. Isotopic standard ellipse areas were calculated using the SIBER package (SIBER, Jackson et al., 2011). R software v.3.6.1 was used for the isotopic mixing models and isotopic niche analyses.

## 2.4. Statistical analyses

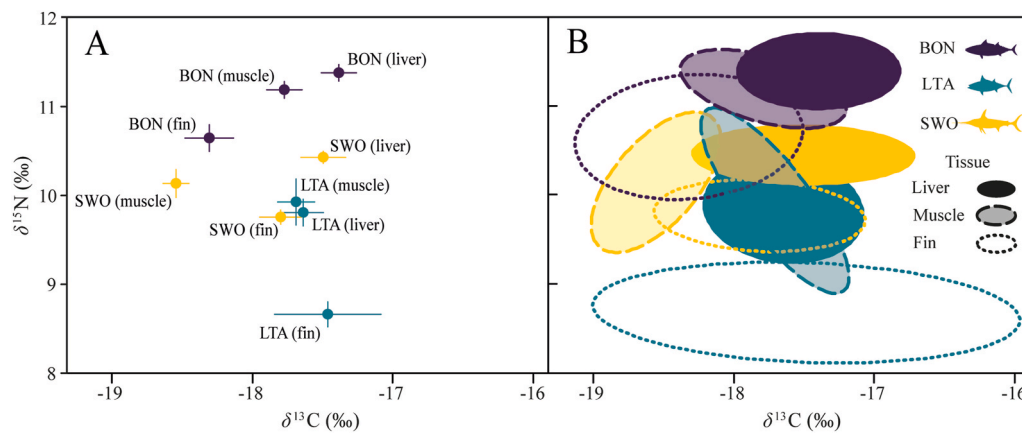
Differences in  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values between species and tissues (after the correction for fractionation values between tissues) within the same species were tested using ANOVA and Tukey post-hoc tests. To correctly interpret and compare isotopic values between liver and muscle-fin, we adjusted the  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values of muscle by the difference between the DTDF of liver and muscle obtained from the Pacific Bluefin tuna (*Thunnus orientalis*) under controlled conditions. Moreover, data were checked for normality and heteroscedasticity. A significance level of  $p = 0.05$  was considered for all statistical tests. SPSS v.23 was used to conduct the statistical analyses.

## 3. Results

### 3.1. Isotopic differences between species and tissues

We found that Atlantic bonito showed the highest values of  $\delta^{15}\text{N}$  followed by swordfish and little tunny in the three analysed tissues (Fig. 2, Tables 2 and 3). In the case of  $\delta^{13}\text{C}$  values, the pattern was similar with the exception of the  $\delta^{13}\text{C}$  values of liver, which did not show differences among the three species (Fig. 2, Tables 2 and 3). When comparing between each tissue within each species we found significant differences in the  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  values for all species (Fig. 2, Tables 2 and 3). Overall, muscle showed higher values than fin and liver for  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  values in the three species (Fig. 2, Tables 2 and 3).

The niche breadth, measured by the  $\text{SEA}_C$ , was higher in fin of little



**Fig. 2.** (A) Mean and standard error of  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values and (B) standard ellipse. Areas for each tissue (muscle, liver, fin) of Atlantic bonito (BON), little tunny (LTA) and swordfish (SWO) sampled in the northwestern Mediterranean Sea. In the case of liver, both  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  values are adjusted for the trophic enrichment factor between muscle-fin and liver.

**Table 2**

Mean and standard deviation of  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  in muscle, liver and fin of Atlantic bonito, little tunny and swordfish sampled in the NW Mediterranean Sea in 2012. In the case of liver, both  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  values are adjusted for the trophic enrichment factor between muscle-fin and liver. Standard ellipse areas corrected by sample size (SEAc) are also indicated. The results of post-hoc Tukey HSD tests are shown by the subscripts. Means differing significantly ( $P < 0.05$ ) are indicated by letters (a, b, c) in the case of differences of the same tissue between species and by numbers (1, 2, 3) in the case of different tissue within the same species –the means of species or tissue with the same letter (for species) or number (for tissues) were not significantly different (the ANOVA tests are shown in Table 3).

Predator	N	Tissue	$\delta^{15}\text{N}$ (‰)	$\delta^{13}\text{C}$ (‰)	SEAc (‰ <sup>2</sup> )
Atlantic bonito	22	Liver	11.38 ± 0.43 <sub>a,1</sub>	−17.41 ± 0.58 <sub>a,1,2</sub>	0.84
	22	Muscle	11.19 ± 0.44 <sub>a,2</sub>	−17.79 ± 0.58 <sub>a,2</sub>	0.73
	22	Fin	10.64 ± 0.70 <sub>a,1</sub>	−18.32 ± 0.81 <sub>a,1</sub>	1.83
Little tunny	16	Liver	9.81 ± 0.60 <sub>b,1</sub>	−17.65 ± 0.56 <sub>a,1</sub>	1.14
	16	Muscle	9.93 ± 1.03 <sub>b,2</sub>	−17.71 ± 0.52 <sub>a,1</sub>	1.04
	16	Fin	8.67 ± 0.56 <sub>b,1</sub>	−17.48 ± 1.51 <sub>b,1</sub>	2.81
Swordfish	25	Liver	10.43 ± 0.34 <sub>c,1</sub>	−17.51 ± 0.79 <sub>a,1</sub>	0.88
	25	Muscle	10.14 ± 0.78 <sub>b,2</sub>	−18.55 ± 0.45 <sub>b,2</sub>	0.92
	25	Fin	9.76 ± 0.39 <sub>c,1</sub>	−17.81 ± 0.74 <sub>a,1</sub>	0.97

tunny (SEAc = 2.81), followed by fin of Atlantic bonito (1.83) (Fig. 2, Table 2). Among species, we found low niche overlap based on SEAc values between Atlantic bonito, little tunny for the three analysed tissues

**Table 3**

Results of the ANOVA tests comparing  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  values between each species (Atlantic bonito, little tunny and swordfish) for each tissue (liver, muscle and fin) and between each tissue (muscle, liver and fin) for each species (Atlantic bonito, little tunny and swordfish). In the case of liver, both  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  values are adjusted for the trophic enrichment factor between muscle-fin and liver.

Parameter	Liver			Muscle			Fin		
	F	gl	P	F	gl	P	F	gl	P
$\delta^{15}\text{N}$ (‰)	59.32	2	<0.001	16.19	2	<0.001	58.06	2	<0.001
$\delta^{13}\text{C}$ (‰)	0.63	2	0.52	17.87	2	<0.001	3.37	2	0.04
Parameter	Atlantic bonito			Little tunny			Swordfish		
	F	gl	P	F	gl	P	F	gl	P
$\delta^{15}\text{N}$ (‰)	8.51	2	0.001	11.52	2	<0.001	5.87	2	0.001
$\delta^{13}\text{C}$ (‰)	3.51	2	0.03	1.16	2	0.31	7.47	2	0.004

(Fig. 2, Table 4). The highest overlap was found between little tunny and swordfish in the SEAc calculated with liver isotopic values (Fig. 2, Table 4). Within species, the highest niche overlap was found between liver and muscle in Atlantic bonito and little tunny (19–31% overlap) and between muscle and fin in Atlantic bonito (10.8%) (Fig. 2, Table 4).

### 3.2. Isotopic mixing model outputs

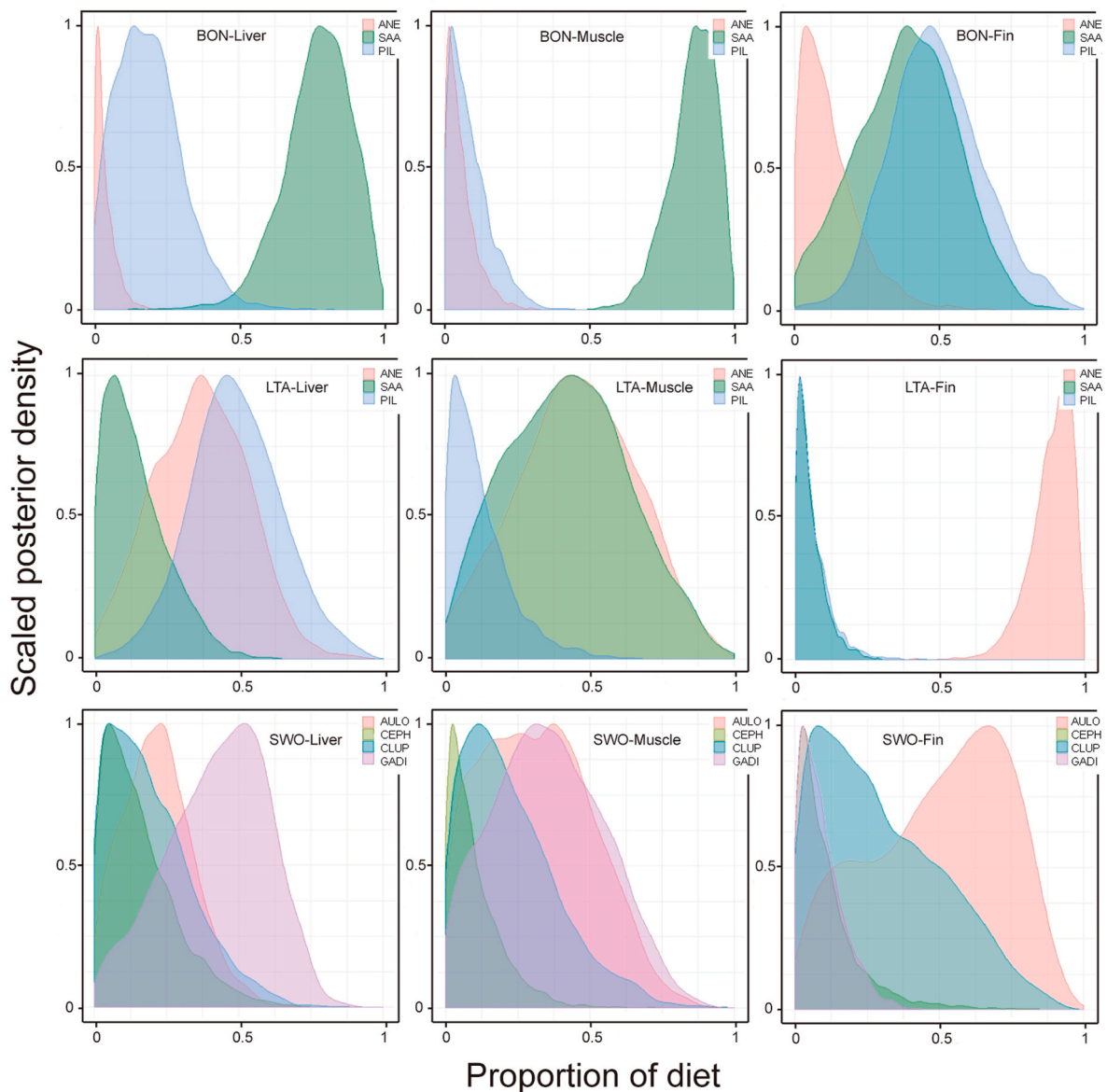
The potential contribution of each prey estimated by MixSIAR models differed between species and also between the tissues of each species (Fig. 3). Specifically, for Atlantic bonito we found that round

**Table 4**

Median percentage values of SEAB overlap between each pair of species and same tissue and between tissues for each species. 25% and 75% percentiles are reported in parenthesis. In the case of liver, both  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  values are adjusted for the trophic enrichment factor between muscle-fin and liver.

SPECIES	Liver	Muscle	Fin
Atlantic bonito-little tunny	0	0 (0.00–3.17)	0
Atlantic bonito-swordfish	0	0 (0.00–1.01)	2.65 (0.00–10.25)
Little tunny-swordfish	10.99 (1.09–24.15)	1.42 (0.00–10.58)	0 (0.00–1.34)
TISSUE	Atlantic bonito	Little tunny	Swordfish
Liver-muscle	19.71 (8.32–34.10)	31.05 (21.01–38.59)	2.41 (0.00–7.74)
Liver-fin	1.48 (0.00–6.89)	0.05 (0.00–3.72)	1.48 (0.00–10.33)
Muscle-fin	10.82 (2.91–20.39)	2.96 (0.27–7.14)	4.50 (0.28–12.80)





**Fig. 3.** MixSIAR results showing the posterior density distribution of proportional contributions of the potential prey group to the total diet for each tissue (liver, muscle and fin) and species (Atlantic bonito-BON, little tunny-LTA and swordfish-SWO). In Atlantic bonito and little tunny, European anchovy (ANE) is represented in pink, round sardinella (SAA) is in green and European sardine (PIL) is in blue. In the case of swordfish, “AULO” refers to Aulopiformes, “CEPH” to Cephalopods, “CLUP” to Clupeiformes and “GADI” to Gadiformes. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

sardinella was the main important prey group for muscle (85%) and liver (77%), whereas for fin, European sardine (49%) showed similar importance to round sardinella (49%) (Fig. 3). In the case of the little tunny, the isotopic mixing models indicated that European anchovy was the main prey estimated for liver (74%) and fin (80%), whereas for muscle, the importance of round sardinella (42%) was similar to European anchovy (45%) (Fig. 3, Table 3). For swordfish, the results showed a more diverse diet with some preference for Gadiformes in the case of muscle (42%) and liver (36%), or for Aulopiformes in fin samples (49%) (Fig. 3).

#### 4. Discussion

We present new information on the trophic ecology of little tunny, Atlantic bonito and swordfish in the western Mediterranean Sea at different temporal scales (~1 month for liver, ~several months for muscle and >1 year for fin). Information that complement a previous

diet study conducted with the same species in the same study area based on stomach content analysis (Navarro et al. 2017). We found clear and consistent differences in the trophic habits among the three species based on their stable isotopic values, isotopic niches and isotopic mixing model outputs. Atlantic bonito and little tunny exploit particular pelagic fish prey species and swordfish shows a more generalist trophic strategy. Moreover, dietary habits and trophic niches occupied by each fish predator were different depending on the temporal scale.

The temporal variation of estimated diet composition in Atlantic bonito is reflected in its  $\delta^{15}\text{N}$ ,  $\delta^{13}\text{C}$  and trophic niche (standard ellipse area, SEA) values of fin, which differed from the other tissues. It is known that Atlantic bonito mainly consumes Clupeiformes, such as round sardinella (Campo et al., 2006; Navarro et al., 2017). However, previous studies did not find evidence of temporal variation in the diet of Atlantic bonito based on stomach content results (e.g. Campo et al., 2006). We found that although the main prey for this species in the short- (liver) and medium-term (muscle) is round sardinella, in the

long-term (fin) the isotopic mixing models revealed that European sardine is also an important prey for Atlantic bonito. These results are probably related to differences in prey availability within the foraging area exploited by Atlantic bonito. Round sardinella prefers warm waters and reaches its highest abundance and thus, availability for pelagic predator species, in the study area in summer (Sabatés et al., 2006). On the other hand, sardine prefers colder waters and spawns in winter in the study area (Palomera et al., 2007). Since all individuals of Atlantic bonito were obtained between mid-spring and summer periods (May to September; see Materials and Methods), the higher contribution of round sardinella in muscle and in liver probably reflects a higher availability of this prey in the foraging area during the warm period, while sardine could be more present in its diet during winter. For this reason, the contribution of sardine is higher in the mixing models for the fin tissue and also the niche width in this tissue is wider than in the other two tissues, since the stable isotopes measured in fin are giving us an average of a longer period that include more diversity of preys if diet change through time.

In the case of little tunny, and similar to the Atlantic bonito, differences in the trophic habits at different temporal scales were found. In all tissues, European anchovy has a high importance in the diet of little tunny. The results are in accordance with what was found in the previous study that analysed the composition of the diet of this species and individuals based on stomach content analyses (Navarro et al., 2017). At short medium and long term Atlantic bonito and little tunny segregated its isotopic niche. The differences in the main prey exploited by Atlantic bonito and little tunny could be a potential ecological mechanism to reduce the competence for similar prey between them, allowing that both species coexisting in the same pelagic habitat (Navarro et al., 2017; Romeo et al., 2009). The widest isotopic niche width due to high variability of  $\delta^{13}\text{C}$  was found at long term (fin), when the main clupeiform contributing to the estimated diet is the European anchovy. Since fin tissue integrates several months of trophic information, the high variability of  $\delta^{13}\text{C}$  could be a consequence of changes in the feeding ground of little tunny or between inshore and offshore pelagic domains during the year (Mele et al., 2016).

Similar to previous studies (Navarro et al., 2017; Romeo et al., 2009), we found that swordfish includes different demersal fin-fish species in its diet, such as Aulopiformes or Gadiformes and pelagic fin-fish such as Clupeiformes. This explains the partial overlap found between little tunny and swordfish at short term, both exploiting Clupeiformes. At long-term the most exploited prey group was Aulopiformes. The exploitation of demersal species is in line with the more negative  $\delta^{13}\text{C}$  values found in muscle of swordfish than little tunny and Atlantic bonito, indicating that this species mainly uses demersal waters (Layman et al., 2012). This trophic strategy clearly reflects the vertical movements of swordfish in the water column (Abascal et al., 2010). However, when comparing between tissues, more depleted values of carbon were observed in muscle, followed by liver. On the other hand, fin tissue had more enriched values of  $\delta^{13}\text{C}$ . Thus, in the estimations of diet at on a long-term scale, the prey group Gadiformes, comprised mainly of demersal species, did not make an important contribution to the total estimated diet, while at the mid- and short-term, this prey group was the most important. These results suggest that the exploitation of demersal resources and the contribution to the demersal-pelagic coupling by swordfish could be variable depending on the season. In contrast to previous studies conducted in Atlantic, Pacific and Mediterranean waters (Bello, 1991; Hernández-García, 1995; Rosas-Luis et al., 2017, 2016), the isotopic mixing models indicated a low level of importance of cephalopods in the estimated diet of the swordfish at different time scales. This difference with previous studies might be explained by the hard parts structures, such as the beak of cephalopods, that could have been overestimated in the visual analysis of stomach content due to the accumulation of this hard structures in the stomach wall folds.

Comparing the  $\delta^{15}\text{N}$  values as a proxy of trophic level (Navarro et al., 2011) with other large predatory fish species, such as tunas in the

Mediterranean Sea, we found that the three pelagic fish studied have similar  $\delta^{15}\text{N}$  values for muscle tissue. For example, albacore (*Thunnus alalunga*) a predator that mainly consumes European anchovy, shows  $\delta^{15}\text{N}$  values in muscle ( $9.40 \pm 0.30\text{‰}$ ; Goñi et al., 2011) similar to the little tunny, which also feeds on anchovy. Another example is bluefin tuna (*Thunnus thynnus*), an opportunistic and generalist predator that feeds on a variety of fish and invertebrates, mainly on teleost and squids (*Todaropsis eblanae* and *Illex coindetii*), with  $\delta^{15}\text{N}$  muscle values ( $10.51 \pm 0.97\text{‰}$ ; Varela et al., 2011, 2018) and a trophic position (4.44; Varela et al., 2011, 2018) similar to that reported in the present study for swordfish, which is also a generalist predator.

This study provides new data on the feeding strategies of three predatory pelagic fish species—Atlantic bonito, little tunny and swordfish—in the north-western Mediterranean Sea. Estimated diet at different temporal scales highlighted the importance of small pelagic fish for Atlantic bonito and little tunny throughout the year. Based on our isotopic data and isotopic mixing models, swordfish could be considered a benthopelagic predator, exploiting demersal and pelagic prey resources. Our study emphasises the utility of this integrative approach for trophic studies due to its capacity for monitoring dietary habits over different time spans. These data provide new information about the ecological role that Atlantic bonito, little tunny and swordfish play in the Mediterranean food webs, which is essential to the design of more effective management actions.

### Ethical approval

The authors declare that all experimental procedures were conducted in strict accordance with good animal practice as defined by the current Spanish, Catalanian and European legislation.

### CRediT authorship contribution statement

**Joan Navarro:** Conceptualization, Methodology, Formal analysis, Writing - original draft. **Marta Albo-Puigserver:** Methodology, Formal analysis, Writing - original draft. **Pau E. Serra:** Formal analysis, Writing - original draft. **Raquel Sáez-Liante:** Conceptualization, Methodology. **Marta Coll:** Conceptualization, Methodology, Formal analysis, Writing - original draft.

### Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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