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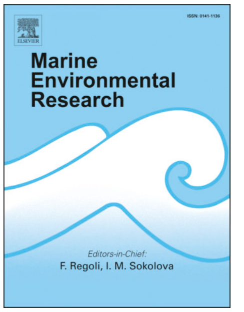

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José L. Varela, Elisa Rojo-Nieto, Joan M. Sorell, Antonio Medina

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Using stable isotope analysis to assess trophic relationships between Atlantic bluefin tuna (*Thunnus thynnus*) and striped dolphin (*Stenella coeruleoalba*) in the Strait of Gibraltar

José L. Varela[a,*], Elisa Rojo-Nieto[b,c], Joan M. Sorell[a], Antonio Medina[a]

[a]Departamento de Biología, Universidad de Cádiz, Campus de Excelencia Internacional del Mar (CEI·MAR), Av. República Saharaui s/n, 11510 Puerto Real, Cádiz, Spain

[b] Departamento de Tecnologías del Medio Ambiente, Universidad de Cádiz, Centro Andaluz de Ciencias y Tecnologías Marinas (CACYTMAR/INMAR), Campus de Excelencia Internacional del Mar (CEI·MAR), Av. República Saharaui s/n, 11510 Puerto Real, Cádiz, Spain

[c]Current address: Department of Cell Toxicology, Helmholtz Centre for Environmental Research – UFZ, Permoserstr. 15, DE-04310, Leipzig, Germany.

*Corresponding author.

E-mail address: joseluis.varela@uca.es

ABSTRACT

Stable isotope analysis (δ^{13}C and δ^{15}N from liver and muscle) was used to assess trophic relationships between Atlantic bluefin tuna (ABFT) (*Thunnus thynnus*) and striped dolphin (SC) (*Stenella coeruleoalbla*) in the Strait of Gibraltar (SoG). δ^{15}N values from ABFT muscle and liver tissues were significantly different from those of dolphin samples, but no for δ^{13}C values. Diet estimation by MixSIAR models from muscle and liver revealed that ABFT fed mainly on squids (*Todaropsis eblanae* and *Illex coindetii*). The shrimp *Pasiphae* sp. Was estimated to be the most important prey-species in the die of SC. Trophic positions estimated from muscle and liver isotopic data suggested that ABFT occupy a higher trophic level than SC. Estimations of isotopic niche, as measured by the standard ellipse area, indicated that ABFT show a broader trophic niche than SC; furthermore, SEAc did not show trophic overlap between both predators. The results of this study suggest that resource partitioning occurs between ABFT and SC in the SoG ecosystem.

Keywords: Trophic ecology, Isotope mixing models, Scombridae, Delphinidae

1. Introduction

The Strait of Gibraltar (SoG) is a pasageway for many migrating species moving from the Atlantic Ocean to the Mediterranean Sea and backwards (Sabatés and Recasens, 2002; Aranda et al., 2013; Abid et al., 2015; Abascal et al. 2016). Moreover, the SoG is a region frequented by large pelagic fishes, such as tunas and billfishes, and cetaceans (Hernández-García, 1995; de Stephanis et al. 2008; Rojo-Nieto et al., 2011; Abid et al., 2017; Sorell et al. 2017; Giménez et al., 2018). This region is characterized by water-mixing processes that cause upwelling events and enhanced primary production (Echevarría et al., 2002), which supports a wide variety of species.

Earlier studies, based on stomach content analysis (SCA) or field observations, have suggested that tunas and dolphins might establish competitive, mutualism or commensalism relationships (Scott and Cattanach, 1998; Das et al., 2000; Clua and Grosvalet, 2001). For example, Scott and Cattanach (1998) reported that tuna-dolphin associations reduce the risk of predation from large sharks, whereas Clua and Grosvlaet (2001) observed that near Azores Islands large Atlantic bluefin tuna (ABFT, *Thunnus thynnus*) gain advantages when feeding with common dolphins (*Dephinus delphis*). In addition, several authors have reported that trophic resource partitioning becomes a strategy commonly used by tuna and dolphin inhabiting the same area (Perrin et al 1973; Hassani et al., 1997). SCA and field observations give detailed data on diet composition, feeding overlap, and consumption rate (Das et al., 2000; Chipps and Garvey, 2006); however, they record trophic information at a relatively brief timescale (Estrada et al., 2005; Logan et al., 2011). For this reason, stable isotope analysis (SIA) has become a useful complement to traditional methods (*i.e.* field observations or stomach content analysis) as it allows for long-term integrated measures of diets assimilated over time (Bearhop et al., 2004).

The carbon stable isotope ratios (δ^{13}C) provide information about dietary sources (Fry, 2006), whereas nitrogen stable isotope ratios (δ^{15}N) are used as indicators of the consumer's trophic position (Post, 2002). δ^{13}C and δ^{15}N can provide trophic information over weeks or months, depending on the tissue turnover rate (Gannes et al., 1998). Relatively slow turnover tissues such as muscle (Hesslein et al., 1993; MacAvoy et al., 2001) integrate information on trophic behavior at time scales of months, whereas liver, which shows a faster turnover rate (Guelinckx et al., 2007; Suzuki et al., 2005), may give information at a shorter time scale (weeks). Moreover, the isotopic composition of predator tissues and their most common prey allow estimation of dietary compositions by applying mixing models (Parnell et al., 2010). Stable isotopes are also used to estimate isotopic niche widths (Bearhop et al., 2004; Newsome et al., 2007; Syväranta et al., 2013), which are measures of dietary diversity.

Trophic relationships between tunas and dolphins have been studied in the Pacific Ocean (Perrin et al. 1973; Scott and Cattanach, 1998; Scott et al. 2012), Atlantic Ocean (Hassani et al., 1997; Das et al., 2000; Clua and Grosvalet, 2001; Pusineri et al., 2008) and Indian Ocean (Ballance and Pitman, 1998; Anderson and Shaan, 1999), but similar investigations have not been undertaken in the Mediterranean Sea. The present study was conducted to investigate the feeding habits, trophic positions and trophic relationships of ABFT and SC residing in the SoG using SIA coupled to Bayesian isotope mixing models.

2. Material and methods

2.1 Sampling

ABFT (n=30), ranging between 127 and 212 cm in straight fork length (SFL), were caught by baitboat from the SoG (Fig. 1) in 2012 and 2013 (Supplementary Material, Table S1).

The curved fork length (CFL) was recorded on board fishing vessels to the nearest cm (Supplementary Material, Table S1), the straight fork length (SFL) being estimated from the equation $SFL = 2.9457 + 0.9442 \times CFL$ (Rodríguez-Marín et al., 2015). SC (n=7) stranded in the Spanish coast of the SoG were necropsied soon after they were found dead (time range from two to 48 h) in 2012 and 2013 (Supplementary Material, Table S2). Tissue samples were only collected from dolphins in "very fresh" or "fresh" conditions (1-2 on a 0-5 scale, 0 when alive). The total length of the dolphins ranged between 121 and 220 cm (Supplementary Material, Table S2).

2.2 Stable isotope analysis

Small pieces of muscle and liver (~ 5 g) were collected from all the sampled animals and stored at -20°C until analysis. Because stomach content data of both predators was not available, the list of prey chosen for SIA was based on the identification of prey in ABFT and dolphin stomachs collected in the area (Varela et al., 2013; Sorell et al., 2017; Giménez et al., 2017, 2018). It consisted of 5 fish species (*Trachurus trachurus*, *Micromesistius poutassou*, *Sardina pilchardus* and *Myctophum punctatum*), 2 squids (*Illex coindetii* and *Todaropsis eblanae*) and 2 crustaceans (*Sergia robusta* and *Pasiphae* sp.). Prey species were collected by trawling in the SoG area during a research cruise carried out in March, 2013, and stored at −20 °C until use.

Muscle and liver samples of the focal species, as well as whole prey were thawed and rinsed with distilled water to remove blood and other impurities. Following freeze-drying, the samples were ground, and aliquots of ∼1 mg were placed into tin capsules for ^{15}N analysis. Before ^{13}C analysis, lipids were extracted from the samples with chloroform:methanol (2:1 v/v) as described by Varela et al. (2012, 2013). The relative abundances of ^{13}C and ^{15}N were measured by a continuous gas flow system using a Thermo Finnigan Elementary Analyzer Flash EA1112 coupled to a Finnigan MAT Delta Plus mass spectrometer. All carbon and nitrogen isotope data are reported in δ notation according to the following equation: $\delta X = [(R_{sample}/R_{standard}) - 1] \times 1000$, where X is ^{13}C or ^{15}N and R is the ratio $^{13}C/^{12}C$ or $^{15}N/^{14}N$ (Peterson and Fry, 1987). Standard materials are Vienna Pee Dee belemnite for carbon and atmospheric N_2 for nitrogen and expressed as parts per thousand (‰) relative to standards (Peterson and Fry, 1987). Precision of either C or N isotopic determinations was ±0.15‰.

2.3 Data analysis

Differences in $\delta^{13}C$ and $\delta^{15}N$ values between species and tissues were analyzed by Student's *t*-test. When normality or homoscedasticity assumptions were violated, Mann-Whitney *U*-test nonparametric test was used. A significance level of $\alpha = 0.05$ was considered for all statistical tests. Statistical analyses were performed using Statgraphics Centurion v16.2.04.

A Bayesian mixing model in R (v3.4.3) (MixSIAR, Stock and Semmens, 2016) was applied to estimate the contribution of different prey to diet. This model estimates the contribution of n sources (prey) to a mixture (predator), and also incorporates the uncertainty in the isotopic signatures of consumers, sources and isotopic discrimination factors (Parnell et al. 2010). Isotopic discrimination factors previously estimated for ABFT and dolphins were used to perform this analysis ($\Delta^{13}C_{sc\,muscle} = 2.0$, $\Delta^{15}N_{sc\,muscle} = 3.0$, $\Delta^{13}C_{ABFT\,muscle} = -0.16 \pm 0.64$, $\Delta^{15}N_{ABFT\,muscle} = 1.64 \pm 0.20$, $\Delta^{13}C_{ABFT\,liver} = 0.42 \pm 0.34$, $\Delta^{15}N_{ABFT\,liver} = 0.68 \pm 0.42$) (Fernández et al., 2011; Varela et al., 2011, 2013). As there is no available data on the $\Delta^{15}N$ and $\Delta^{13}C$ for SC liver tissue, they were estimated using the package SIDER for R (v3.4.3). SIDER uses a phylogenetic regression model based on a compiled dataset to impute (estimate) an isotopic discrimination factors of a consumer (Healy et al., 2017). Prior to running the mixing models, the goodness-of-fit of the data to the model was evaluated using simulated mixing polygons (Smith et al., 2013)

The trophic position (TP) of both predators, ABFT and SC, was calculated from muscle and liver isotopic data according to the equation proposed by Post (2002): $\mathrm{TP} = \lambda + (\delta^{15}\mathrm{N}_{\mathrm{predator}} - \delta^{15}\mathrm{N}_{\mathrm{sec}})/\Delta^{15}\mathrm{N}$, where λ is the trophic position of the organism used as the secondary consumer, $\delta^{15}\mathrm{N}_{\mathrm{predator}}$ and $\delta^{15}\mathrm{N}_{\mathrm{sec}}$ are the δ^{15}N of the predator and secondary consumer, and $\Delta^{15}\mathrm{N}$ is the isotopic discrimination factor for each predator and tissue, taken from earlier studies (Fernández et al., 2011; Varela et al., 2011, 2013). As for MixSIAR, the $\Delta^{15}\mathrm{N}$ for SC liver tissue was estimated with SIDER (Healy et al., 2017). The spotted laternfish *Myctophum puctatum*, an important dietary component of ABFT and dolphins in the SoG area, was used as the secondary consumer, applying values of λ = 3.07 (Corrales et al., 2015) and δ^{15}N = 8.28 (Sorell et al., 2017).

The isotopic niche width and trophic overlap of both species were estimated by Bayesian standard ellipse corrected areas (SEAc) adjusted for small sample size (SEAc) using the SIBER package using the SIBER package (Jackson et al., 2011) of SIAR (Parnell et al., 2010). Unlike other methods for estimating these trophic parameters (e.g. convex hull; Layman et al., 2007), SEAc estimations are less susceptible to outliers (Jackson et al., 2011; Syväranta et al., 2013).

3. Results

Mean isotopic values (±SD) of ABFT and SC tissues are shown in Table 1 and Fig. 2. δ^{15}N values from ABFT muscle and liver tissues were significantly different from those of dolphin samples (*t*-test, $p < 0.01$), whereas no differences were found in δ^{13}C values (*t*-test or *U*-

test, p > 0.05). Significant isotopic differences were also detected between tissues in the two predators (t-test or U-test, p > 0.05), except for SC δ^{13}C values (U-test, p = 0.247).

Isotopic values of prey and predators are plotted in Fig. 2. While ABFT muscle and SC liver δ^{15}N values are highest, the ABFT liver and SC muscle isotope values are intermediate among prey sources. It is worth to note that the two ommastrephid cephalopods (*i.e. I. coindetii* and *T. eblanae*) were grouped into a single prey category.

SIDER estimated values of $\Delta^{15}N = 3.48 \pm 1.57$ and $\Delta^{13}C = 1.39 \pm 1.97$ for SC liver tissue. Otherwise, MixSIAR results from ABFT muscle and liver data estimated that the squids *T. eblan*ae and *I. coindetii* were the main contributors to the diet of ABFT, (Table 3). For SC, the shrimp *Pasiphaea* was estimated to be the most important diet component from muscle and liver isotopic data (Table 3). The mixing polygon simulation indicates that all consumers are located within the 95% mixing region (Fig. 3), therefore the proposed mixing model results are acceptable.

Estimations of ABFT and SC trophic positions (TP) derived from δ^{15}N values of muscle and liver are shown in Table 1. For ABFT, the TP estimated from muscle data ranged from 3.49 to 5.14 (4.44 ± 0.38), whereas the TP calculated from liver ranged from 2.84 to 5.90 (4.39 ± 0.66). For SC, the TP estimations were less variable; thus, while the TP calculated from muscle data ranged from 3.37 to 3.71 (3.50 ± 0.14), the TP estimated from liver ranged from 3.55 to 3.84 (3.76 ± 0.11). The statistical analysis suggested that the ABFT occupies a higher trophic position than the SC (Mann-Whitney U-test, p > 0.05).

Table 4 and Fig. 4 show isotopic niche width and overlap, as measured by the standard ellipse corrected for sample size (SEAc). The isotopic niche width was broader in ABFT than in SC. In both predators, the niche width estimated from liver isotopic data was slightly larger than that estimated from muscle. No significant isotopic overlap between species or tissues was found.

4. Discussion

Feeding ecology studies based on stomach contents of stranded animals have been questioned because they report inaccurate information of unhealthy and poorly fed specimens (Ross, 1984; Selzer et al., 1986). However, fasting conditions apparently do not affect marine mammals (Gómez-Campos et al. 2011, Payo-Payo et al., 2013). Nitrogen and carbon isotopic ranges observed in muscle and liver of both predators were broader than those reported in earlier studies carried out in the western Mediterranean Sea (Payo-Payo et al., 2013; Medina et al., 2015), and narrower than those reported in the Alboran Sea. This suggests that these species have a more varied diet in the SoG. Otherwise, the lack of $\delta^{13}C$ differences between predators may indicate that they fed on either similar prey (Matley et al., 2015), or different prey with similar $\delta^{13}C$ values.

MixSIAR models from muscle and liver data estimated that the diet of ABFT was mainly composed of ommastrephid squids (*T. eblanae* and *I. coindetii*). These results are in agreement with those a previous study carried out on ABFT caught by trap in the SoG (Varela et al., 2013). The different results obtained from the two distinct tissues may be related to their different turnover rates, so that they would provide information at two distinct time scales (Varela et al., 2014). Regarding SC, the decapod crustacean *Pasiphaea* sp. was estimated to be the most important prey source. Conversely, Gómez-Campos et al. (2011) estimated that hake (*Merluccius merluccius*) contributed to 60.3% of the diet of mature SC sampled in the western Mediterranean Sea. Nevertheless, in this analysis the authors did not included decapod shrimps, which have been reported as common prey of SC in the Mediterranean Sea (Würtz and Marrale, 1993; Dede et al., 2015; Aznar et al., 2017). Although decapod crustaceans show lower caloric and nutrient content than fish and squid (Cartes et al., 2008), they may occur at high densities in shallow waters at night (Sardou et al., 1996; Vestheim and Kaartvedt, 2009), becoming an important food resource of upper-level predators. It is noteworthy that decapod crustaceans, however, were not found in gut contents of common and bottlenose dolphins (*Tursiops truncatus*) stranded in the SoG region (Giménez et al., 2017, 2018).

A significantly higher TP was estimated for ABFT (4.44-4.39) compared to SC (3.50-3.76), suggesting that ABFT feed on preys located at higher trophic positions. These values can be compared to TP estimates in killer whales (*Orcinus orca*) ocurring in the SoG. Considering skin $\delta^{15}N$ values of 12.66‰ for *O. orca* sampled in the area (Esteban et al., 2016), and a prey-skin discrimination factor of 3.05‰ (Caut et al., 2011), we can conclude that this delphinid occupies a higher trophic postion than ABFT and SC (TP = 4.53; calculated using the equation of Post (2002)). Other studies, in fact, have shown that killer whales are capable of feeding on tuna and dolphin (Esteban et al., 2014; Bolaños-Jiménez et al., 2014; De Stephanis et al., 2015).

The trophic diversity estimated by the Bayesian standard ellipse corrected areas (SEAc) (Jackson et al., 2011) suggests that ABFT shows a more euryphagous diet in the SoG. The estimated trophic overlap between both predators was low, indicating that these species play different trophic roles in the SoG ecosystem. This finding is not unexpected, since tunas and dolphins inhabiting the same habitat tend to reduce their trophic competition by feeding on different preys or locations (Hassani et al., 1997; Das et al., 2000). In fact, a study based on sonic- and radio-tracking data reported that tunas and dolphins feed at different depths and times in the eastern Pacific Ocean (Scott et al, 2012).

Although the results reported in the present study suggest that stable isotope are suitable for assessing feeding habits, trophic positions and trophic relationships of ABFT and SC in the SoG, there are several caveats that should be accounted for. Firstly, the number of individuals of each potential prey species is fairly low, and thus the high intra-specific or temporal isotopic variability of these preys has not been considered. Secondly, the low number of muscle and liver samples analysed, especially for SC, did not allow us to study seasonal variations of the trophic relationships. Further studies, therefore, should be conducted to investigate seasonal shifts in the feeding behavior of these two predators.

5. Conclusions

To our knowledge, the present study provides the first information about the trophic relationship between ABFT and SC in the SoG. Our findings indicate that resource partitioning occurs between both predators, ABFT feeding on preys at higher trophic positions. Furthermore, estimates of the trophic level suggest that ABFT and SC in the SoG can be better classified as mesopredators rather than top predators.

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Figure captions

Fig. 1. Map of the study region showing the approximate location of samplings of stranded SC (●) and fishing zone of ABFT (dashed area).

Fig. 2. Mean δ^{13}C and δ^{15}N values of prey, ABFT and SC. MP, *Micromesistius poutassou*; MyP, *Myctophum punctatum*; Psp, Pasiphae sp.; SP, *Sardina pilchardus*; SQ; Squids (*Illex coindetii* and *Todaropsos eblanae*); SR, *Sergia robusta*; TT; *Trachurus trachurus*.

Fig. 3. Mixing model polygon results. Stable isotope mixing model polygons for a) ABFT muscle, b) ABFT liver, c) SC muscle and D) SC liver. ABFT and SC are represented with black dots and potential prey species with white dots. Colored region represents the 95% confidence interval.

Fig. 4. δ^{13}C and δ^{15}N bi-plots for ABFT and SC tissues (circles, ABFT muscle; triangles, ABFT liver; pluses, SC muscle; crosses, SC liver). Ellipses represent the standard ellipse corrected area (SEAc) estimated for ABFT muscle (solid line), ABFT liver (dashed line), SC muscle (dotted line), and SC liver (twodashed line).

Table 1

Isotopic values (δ^{15}N and δ^{13}C presented in ‰) and trophic position (mean ± SD) of ABFT and SC by tissue type. The right column shows p-values obtained from comparisons of means between species; *p*-values resulting from comparisons between tissues are shown in rows beneath the compared data. t, Student's *t*-test; U, Man-Whitney *U*-test.

		ABFT	STD	*p* value
δ^{15}N (‰)	Muscle	11.43 ± 0.63	10.59 ± 0.46	< 0.01 (t)
	Liver	10.08 ± 0.45	11.58 ± 0.40	< 0.01 (t)
***p* value**		<0.001 (t)	< 0.001 (t)	
δ^{13}C (‰)	Muscle	-17.62 ± 0.27	-17.53 ± 0.47	0.548 (t)
	Liver	-17.29 ± 0.48	-17.35 ± 0.64	0.848 (U)
***p* value**		< 0.001 (U)	0.247 (U)	
TP	Muscle	4.44 ± 0.38	3.50 ± 0.14	< 0.001 (U)
	Liver	4.39 ± 0 .68	3.76 ± 0.11	< 0.05 (U)
***p* value**		0.756 (U)	< 0.01 (U)	

Table 2

Mean ± SD of δ^{15}N and δ^{13}C values (presented in ‰) of the prey species considered in the SIAR mixing-models. n, number of individuals.

Preys	δ^{15}N (‰)	δ^{13}C (‰)	Weight (g)
Todaropsis eblanae (n = 3)	10.39 ± 0.57	-17.31 ± 0.22	188.03±15.31
Illex coindetii (n=2)	10.11 ± 0.70	-17.49 ± 0.29	190.30±10.03
Trachurus trachurus (n = 3)	10.58 ± 0.54	-16.99 ± 1.92	135.59±12.26
Micromesistius poutassou (n = 3)	10.38 ± 0.26	-17.99 ± 0.51	167.79±13.23
Sardina pilchardus (n=3)	10.09 ± 0.77	-18.05 ± 0.87	42.61±8.78
Myctophum punctatum (n = 3)	9.18 ± 0.66	-18.28 ± 0.48	3.51±0.78
*Sergia robusta (*n = 3)	7.84 ± 0.61	-18.29 ± 1.00	1.05±0.30
Pasiphaea sp. (n = 2)	6.17 ± 0.10	-20.18 ± 0.20	0.69±0.28

Table 3

Dietary contribution of common prey sources based on MixSiar model for ABFT and SC liver and muscle tissues. Values are presented as mean proportion estimates with 5% and 97.5% confidence intervals.

ABFT

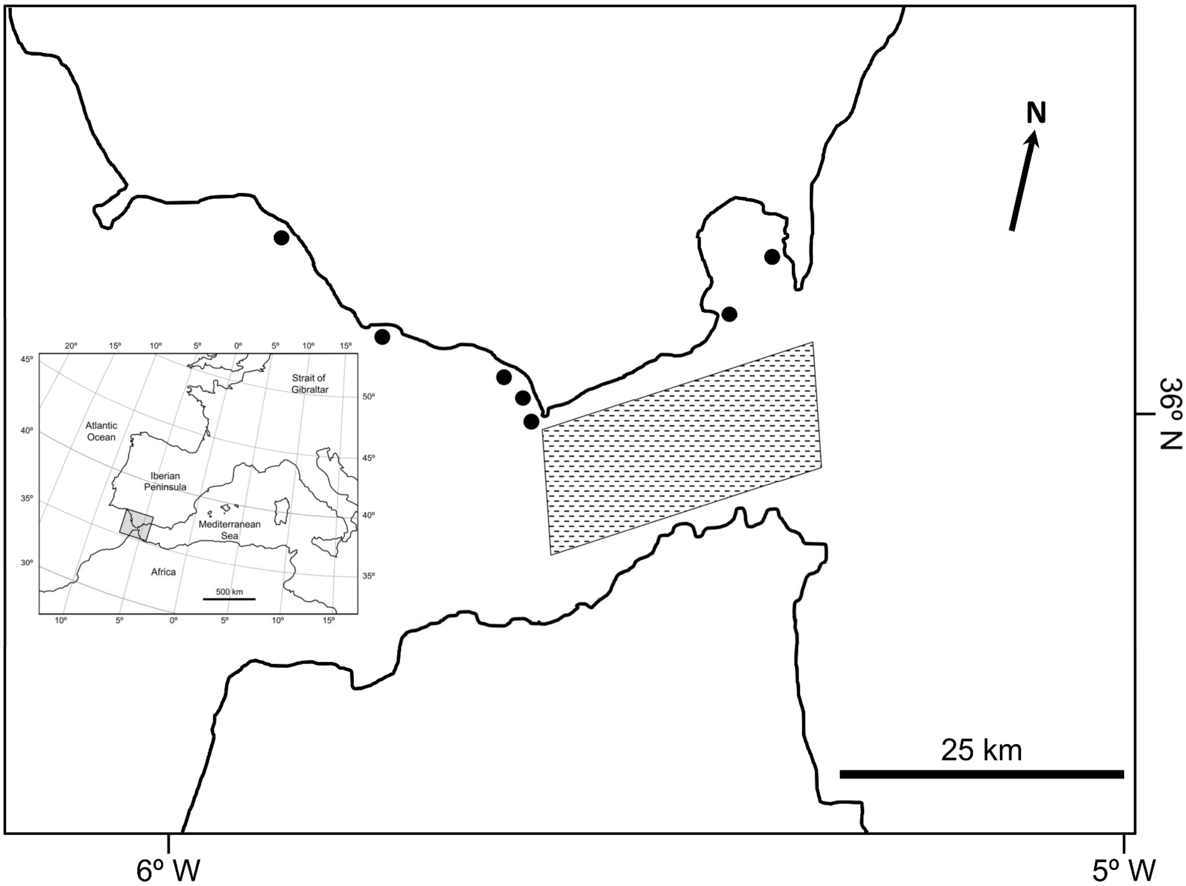
	Muscle			Liver		
Preys	**5%**	**97.5%**	**mean (%)**	**5%**	**97.5%**	**mean (%)**
Squids (*Illex coindetii* and *Todaropsis eblanae*)	3.6	51.0	27.8	11.8	55.8	31.4
Trachurus trachurus	3.3	26.0	12.0	4.5	35.6	16.6
Micromesistius poutassou	4.5	45.5	19.3	0.2	23.8	6.1
Sardina pilchardus	3.2	35.3	14.9	1.8	38.2	11.5
Myctophum punctatum	1.7	39.3	12.5	2.5	49.2	18.2
Sergia robusta	0.8	24.1	9.8	0.6	24.8	8.1
Pasiphaea sp.	0.4	11.2	3.8	1.8	15.6	8.2

SC

	Muscle			Liver		
Preys	**5%**	**97.5%**	**mean (%)**	**5%**	**97.5%**	**mean (%)**
Squids (*Illex coindetii* and *Todaropsis eblanae*)	1.4	16.5	7.2	1.4	32.6	11.0
Trachurus trachurus	3.8	32.9	15.0	1.4	22.5	8.3
Micromesistius poutassou	1.3	27.1	10.0	1.4	27.2	11.5
Sardina pilchardus	0.8	31.9	10.1	1.3	36.5	12.0
Myctophum punctatum	1.8	46.8	15.2	0.3	28.9	9.8
Sergia robusta	1.2	31.33	11.2	3.3	45.3	17.8
Pasiphaea sp.	16.4	45.8	31.4	12.6	46.5	29.6

Table 4. Trophic niche width and overlap of ABFT and SC, as estimated by SIBER (Stable Isotope Bayesian Ellipses in R) analysis of muscle and liver isotopic values. SEAc, corrected standard ellipse area.

Group	SEAc	SEAc Overlap
ABFT		
Muscle (I)	0.55	I vs II (< 0.01)
		I vs III (0.01)
		I vs IV (0.23)
Liver(II)	0.67	II vs III (0.03)
		II vs IV (< 0.01)
SC		
Muscle (III)	0.48	III vs IV (< 0.01)
Liver (IV)	0.53	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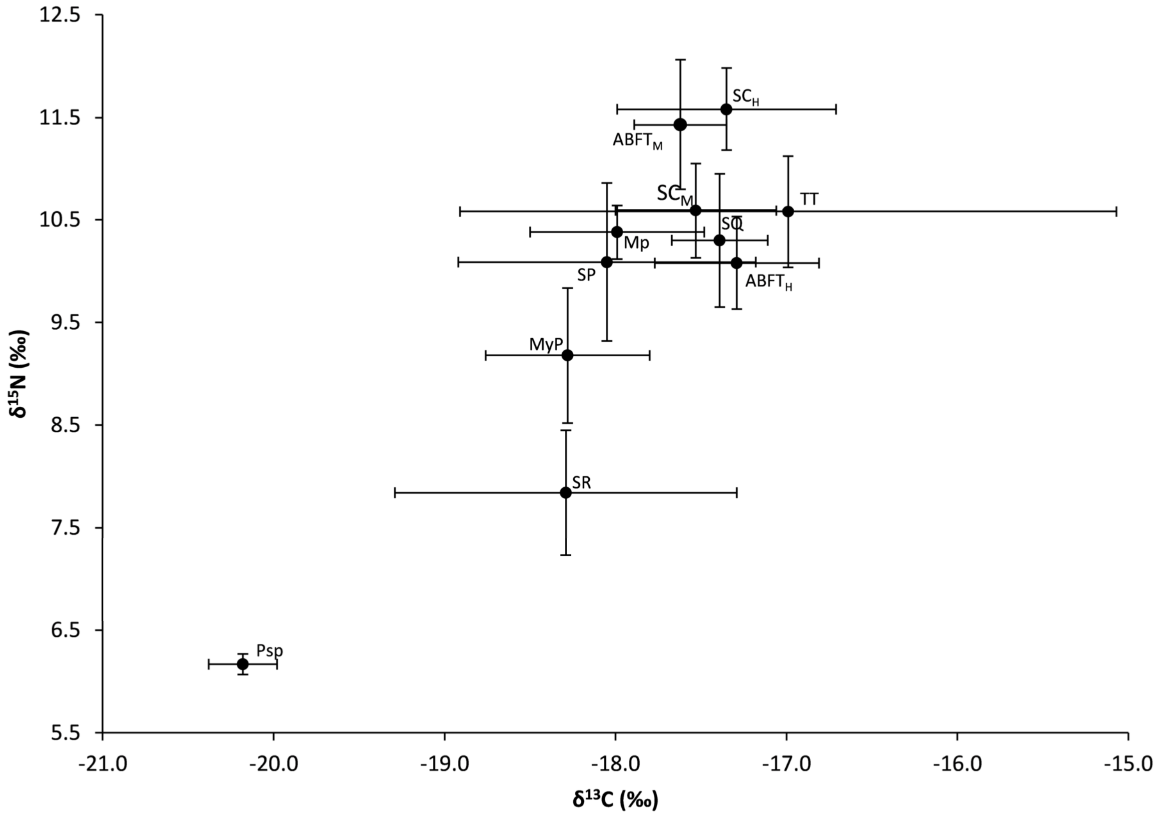
6º W
5º W
Strait of Gibraltar
Atlantic Ocean
Iberian Peninsula
Mediterranean Sea
Africa
500 km


12.5
11.5
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7.5
6.5
5.5
δ15N (‰)
SCH
ABFTM
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TT
SQ
Mp
SP
ABFTH
MyP
SR
Psp
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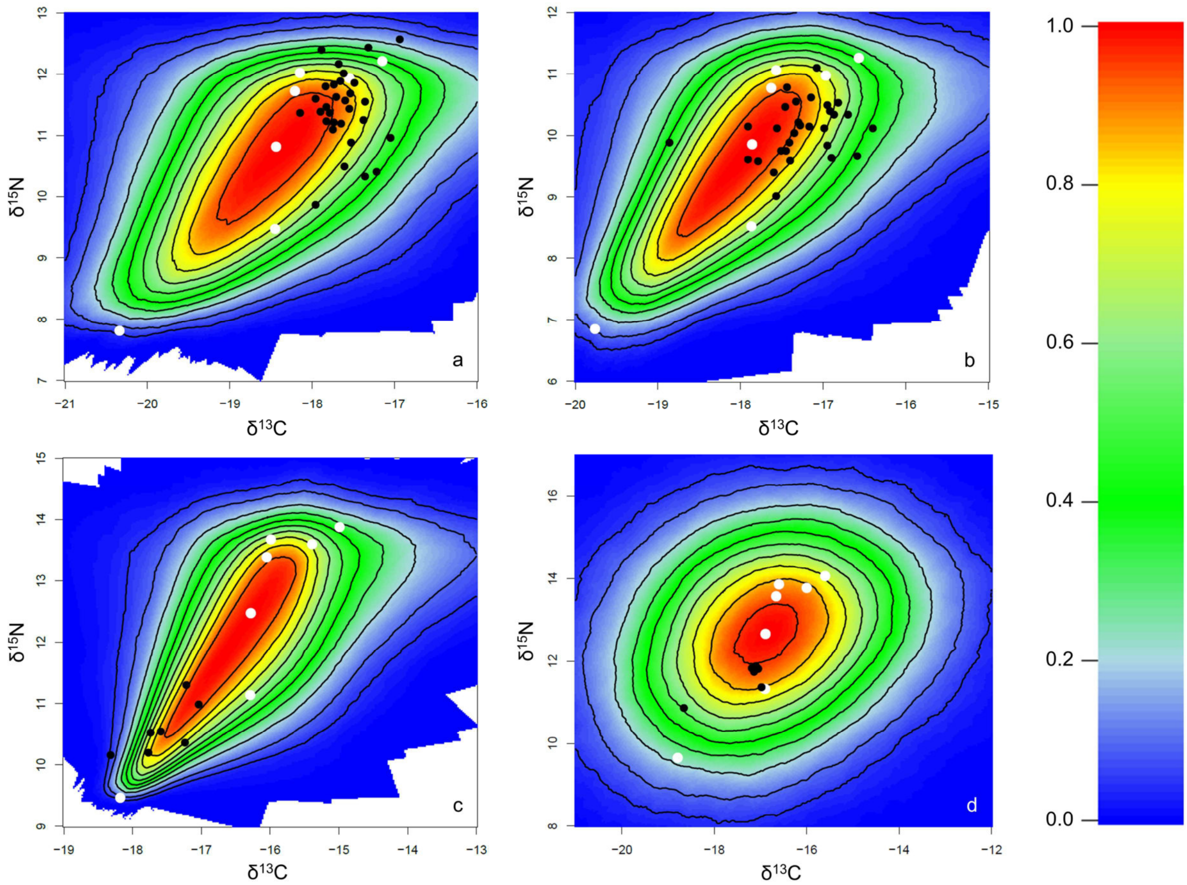
-15.0
δ13C (‰)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δ13C
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b
c
d
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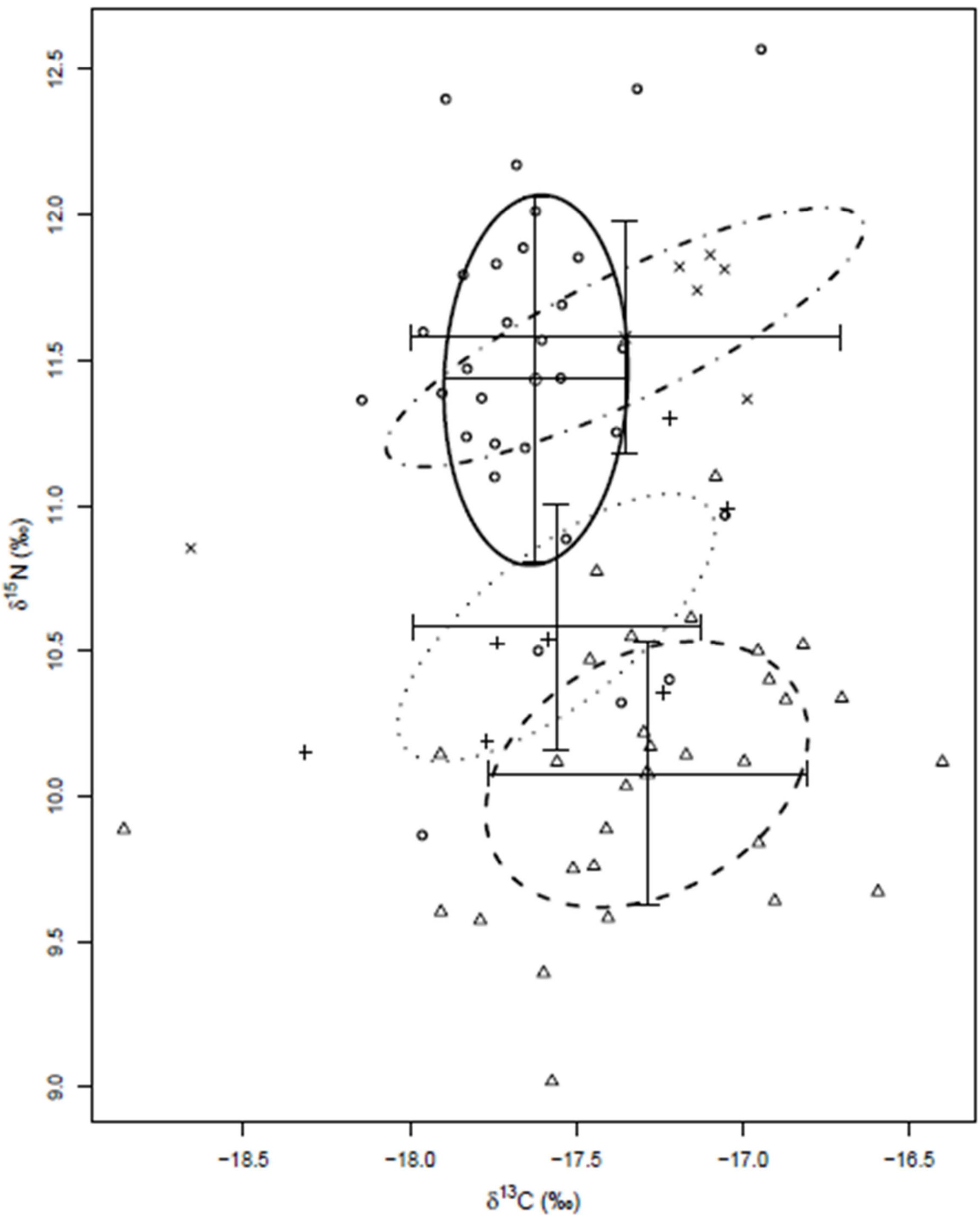
0.8
0.6
0.4
0.2
0.0


12.5
12.0
11.5
11.0
10.5
10.0
9.5
9.0
δ15N (‰)
−18.5
−18.0
−17.5
−17.0
−16.5
δ13C (‰)

Highlights

Trophic relationships between bluefin tuna and striped dolphin were assessed by SIA

SIAR mixing-models estimated that ABFT fed mainly on squid and horse mackerel

Decapod shrimp was estimated to be the main dietary component for STD

TP estimations suggested that ABFT occupy higher trophic levels than STD

Resource partitioning occurs between ABFT and STD