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Trophic habits of an abundant shark in the northwestern Mediterranean Sea using an isotopic non-lethal approach

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A B S T R A C T

Studying the feeding ecology of an organism is essential to understanding its ecological role in the ecosystem. Although the small-spotted catshark (*Scyliorhinus canicula*) is widely studied, most feeding studies have been conducted using invasive techniques, such as the analysis of stomach contents. Moreover, information from the Mediterranean Sea is surprisingly scarce and not up to date. Here, we studied the feeding ecology of the small-spotted catshark in the northwestern Mediterranean Sea using stable isotopes (nitrogen and carbon isotopic values) from blood samples, with individuals released alive in the area of capture after sampling. In overall for the population of small-spotted catshark, the isotopic values were −19.01 ± 1.12‰ and 8.03 ± 0.61‰ for $\delta^{13}C$ and for $\delta^{15}N$, respectively. Results reveal a diet mainly composed of euphausiids, with sex and size variations. Results confirm the ecological role of the small-spotted catshark as a mesopredator, which holds a trophic position similar to skates and rays in the study area, but lower than the other demersal and pelagic sharks analysed. The trophic behaviour of the small-spotted catshark indicates its high trophic plasticity, which could allow this species to thrive in highly exploited environments. Our methodological approach, which did not damage the target species, presents new possibilities for conducting ecological studies with other elasmobranchs in the Mediterranean Sea, a highly exploited area that hosts many threatened and rare species.

1. Introduction

Investigating the trophic ecology of an organism is essential to understanding its ecological role in the ecosystem. Among marine predators, sharks are an important component of marine ecosystems (Ferretti et al., 2010; Heithaus et al., 2008). They frequently play a predatory role and their removal affects the structure and functioning of marine ecosystems (Baum et al., 2003; Stevens et al., 2000). Sharks present a large variety of ecological strategies and feeding behaviours and their ecological roles vary between species and regions (Cortés, 1999; Simpfendorfer et al., 2001). The Mediterranean Sea is an area of great diversity for these marine predators (Coll et al., 2010). However, many Mediterranean shark species have declined as a consequence of the degradation and loss of habitats or due to the direct impacts of fishing (Aldebert, 1997; Coll et al., 2010; Ferretti et al., 2008; Navarro et al., 2016). Currently, around 49% of the Mediterranean sharks are considered threatened by the regional assessment of the International Union for the Conservation of Nature (Abdul Malak, 2011).

Despite these declines, there are shark species in the Mediterranean Sea that are more resilient to the impact of human activities and that persist in highly impacted areas (Aldebert, 1997; Navarro et al., 2016). This is the case of the small-spotted catshark (*Scyliorhinus canicula*), the most abundant shark in the Mediterranean Sea and Eastern North Atlantic (Compagno, 1984). However, recent stock assessments from the Ligurian and Tyrrhenian Seas have indicated that the fishing mortality of this species is currently greater than the mortality ensuring a maximum sustainable yield (GFCM, 2015; Serena et al., 2014). Therefore, a shift in its conservation status may be underway.

Despite the relatively high abundance of the small-spotted catshark, accurate information about the feeding ecology of this species, and in some areas in particular such as the western Mediterranean Sea, is relatively scarce (Macpherson, 1981; Valls et al., 2011). Previous information from diet studies conducted in the Mediterranean Sea and the Atlantic Ocean indicates that this species is an opportunistic predator that exploits a wide range of benthic crustaceans and demersal fish (Lyle, 1983; Mnasri et al., 2012; Olaso et al., 1998; Valls et al., 2011). Although the small-

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spotted catshark also preys on fin-fish, the importance of crustaceans in its diet appears to vary spatially and ontogenetically (Lyle, 1983; Valls et al., 2011). The small-spotted catshark is a mid-level predator and can be preyed upon by other sharks, such as the kitefin shark *Dalatias licha* (Barría et al., 2015; Navarro et al., 2014). It generally has a low commercial value and is marginally marketed for human consumption in some areas of the Mediterranean Sea, including Turkey and Spain (Basusta et al., 2005). Because the small-spotted catshark is the most abundant demersal shark in the Mediterranean Sea, it is fundamental to advance our understanding of its ecological role.

The trophic ecology of elasmobranchs has traditionally relied on stomach content analysis (Cortés, 1999; Stergiou and Karpouzi, 2002). Although this type of analysis provides high levels of taxonomic resolution, it requires dead individuals. Currently, a large proportion of sharks and rays are rare or threatened (Dulvy et al., 2014) and the use of lethal techniques are not recommended. As an alternative, non-lethal methodologies based on stable isotopes of nitrogen ($\delta^{15}N$) and carbon ($\delta^{13}C$) performed on particular animal tissues, such as blood or fins, have been powerful tools to study different aspects of the feeding ecology of marine predators (Layman et al., 2012; Shiffman et al., 2012; Tilley et al., 2013). $\delta^{15}N$ and $\delta^{13}C$ values are indicators of the consumers' trophic positions and dietary sources, respectively (Layman et al., 2012). This is based on the fact that $\delta^{15}N$ and $\delta^{13}C$ values are transformed from dietary sources to consumers in a predictable manner and integrate the diet of the consumer over a longer time period, depending the turnover rate of the tissue analysed (Caut et al., 2013; Shiffman et al., 2012). In addition, by combining stable isotope values for consumers with those of their potential prey (after further qualitative analysis), isotopic mixing models can be applied to interpreting isotopic values by estimating the relative contribution of each prey item to the diet of the consumer (Parnell et al., 2010).

In this study, the main objective was to examine the trophic ecology of the small-spotted catshark in the northwestern Mediterranean Sea using stable isotope analyses on blood samples obtained from free-living individuals. Specifically, we investigated whether small-spotted catsharks showed age- (juveniles vs. adults) and sex- (males vs. females) related diet differences. To evaluate the ecological role of this species in relation to other elasmobranchs living in the area, we compared the isotopic values of the small-spotted catshark to published isotopic information in other species.

2. Materials and methods

2.1. Study area and sampling procedure

The present study was conducted in the northwestern Mediterranean Sea (Catalan Sea; Fig. 1). This is a relatively high productive area in the Mediterranean due to the combination of the organic matter contributions from the Ebro River and the effect of the Liguro-Proveçal-Catalan current along the continental slope (Salat, 1996). This area is also highly impacted by habitat degradation and human activities, such as fishing (Coll et al., 2012; Navarro et al., 2015).

During July of 2013, a total of 62 small-spotted catshark (*Scyliorhinus canicula*) individuals were caught in the study area during an experimental demersal fishing cruise (held under the ECOTRANS project; Institute of Marine Sciences ICM-CSIC, Spain). Once caught, the individuals were kept in tanks with a continuous flow of seawater and were sampled within 1 h after capture (Valls et al., 2016). The body length (±0.1 cm), body mass (±0.1 g) and sex (visually examining external reproductive organs) were recorded for each individual. The age of each sampled small-spotted catshark was classified based on their total body length (TL) as juveniles (TL < 37 cm) and adults (TL ≥ 37 cm) (Leloup and Olivereau, 1951). For each individual, we extracted 0.3 ml of blood from the caudal region using 0.5 ml syringes and immediately we separated the red cells from the plasma fraction by centrifugation. We did not use any anesthetic during the sampling. The red cells were frozen at −70 °C until isotopic determination. After blood sampling and body size measurements, each individual was returned to the water tank for 30 min and released alive in the area of capture. The total length of the small-spotted catsharks sampled ranged from 17 cm to 50.5 cm; 32 of them were males and 30 were females (Table 1).

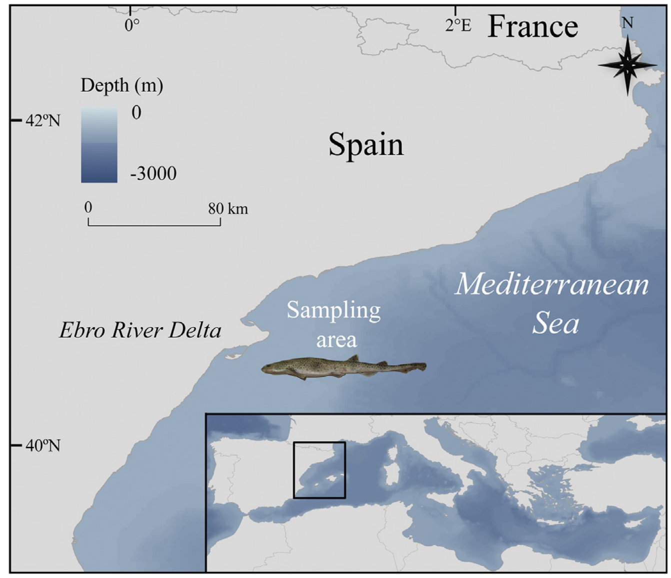

Fig. 1. Map of the study area (northwestern Mediterranean Sea) indicating the sampling area.

2.2. Stable isotope analyses and isotopic mixing model

Blood samples were subsequently freeze-dried and powdered and 0.28–0.33 mg of each sample was packed into tin capsules. Isotopic analyses were performed at the *Laboratorio de Isótopos Estables* of the *Estación Biológica de Doñana* at CSIC (Spain). Samples were combusted at 1020 °C using a continuous flow isotope ratio mass spectrometry system by means of a Flash HT Plus elemental analyser coupled to a Delta-V Advantage isotope ratio mass spectrometer via a CONFLO IV interface (Thermo Fisher Scientific). The isotopic composition was reported in the conventional delta (δ) per mil notation (‰), relative to Vienna Pee Dee Belemnite ($\delta^{13}C$) and atmospheric N_2 ($\delta^{15}N$). Replicate assays of standards routinely inserted within the sampling sequence indicated analytical measurement errors of ±0.1‰ and ±0.2‰ for $\delta^{13}C$ and $\delta^{15}N$, respectively. The standards used were EBD-23 (cow horn, internal

Table 1
Number of individuals sampled, mean and standard deviation of total body length and isotopic values of small-spotted catshark from the northwestern Mediterranean Sea.

Size	n	Body length (cm)	$\delta^{15}N$ (‰)	$\delta^{13}C$ (‰)
Juvenile male	18	31.79 ± 6.29	7.73 ± 0.47	−19.44 ± 1.67
Adult male	14	46.21 ± 1.99	8.73 ± 0.58	−18.38 ± 0.78
Juvenile female	16	30.13 ± 3.28	7.63 ± 0.33	−19.07 ± 0.67
Adult female	14	41.05 ± 3.24	8.19 ± 0.34	−19.01 ± 0.67

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standard), LIE-BB (whale baleen, internal standard) and LIE-PA (razorbill feathers, internal standard). These laboratory standards were previously calibrated with international standards supplied by the International Atomic Energy Agency (IAEA, Vienna). To avoid potential interference from the chemical treatment to remove urea content, we did not remove the urea from the tissues. Because all samples showed a C:N ratio lower than 3.5‰ we did not correct the $\delta^{13}C$ values to account for the presence of lipids in muscle samples (Post et al., 2007).

To interpret the isotopic values of each species from a trophic point of view, we applied the SIAR Bayesian isotopic mixing model (Stable Isotope Analysis in R, 4.1.3; Parnell et al., 2010). The SIAR model estimates the potential contribution of each prey in the diet of the consumer, in our case based on the blood isotopic values of small-spotted catshark and its potential prey grouped taxonomically (Table 2). This model runs in the free software R (R Development Core Team, 2009) and allows the inclusion of sources of uncertainty in the data, in particular the variability in the stable isotope ratios of the predator and the potential prey (Parnell et al., 2010). To develop the SIAR model, values of the potential prey were taken from a reference isotopic library (corrected mathematically for lipid content) that contains up to 128 species collected in the same study area during the same experimental demersal fishing cruise (ECOTRANS project; Barría et al., 2015). The main potential prey (Table 2) were selected according to the information gathered from studies conducted previously by Valls et al. (2011) and Macpherson (1981) in the northwestern Mediterranean. We used different tissue discrimination factors from each prey type experimentally estimated for the nursery catshark (*Scyliorhinus stellaris*), a closely related species (Caut et al., 2013). In this study, Caut et al. (2013) calculated the tissue discrimination factors between the blood of nursery catshark and the muscle of their potential preys. Specifically, the tissue discrimination factors (mean ± standard deviation) used were: Annelida 1, $\delta^{13}C = 2.74 \pm 0.02$‰ and $\delta^{15}N = 1.38 \pm 0.21$‰ for Polychaeta; Mollusca, $\delta^{13}C = 2.62 \pm 0.04$‰ and $\delta^{15}N = 2.12 \pm 0.13$‰ for Cephalopoda; Caridea, $\delta^{13}C = 2.75 \pm 0.02$‰ and $\delta^{15}N = 1.01 \pm 0.08$‰ for Euphausiacea and Natantia; Teleostei, $\delta^{13}C = 2.89 \pm 0.01$‰ and $\delta^{15}N = 1.79 \pm 0.07$‰ for Fishes; and for Reptantia an average between Anomura and Brachyura ($\delta^{13}C = 2.79 \pm 0.05$‰ and $\delta^{15}N = 2.14 \pm 0.18$‰) was used.

As a measure of trophic width, we calculated the Bayesian isotopic ellipse area based on the individual isotopic values (Jackson et al., 2011). This metric represents a measure of the total amount of isotopic niche exploited by a particular predator and is thus a proxy for the extent of trophic diversity (or trophic width) exploited by the species considered (high values of isotopic standard ellipse areas indicate high trophic width). Isotopic standard ellipse areas and their overlap were calculated using the routine Stable Isotope Bayesian Ellipses (SIBER; Jackson et al., 2011) incorporated in the SIAR library (Stable Isotope Analysis in R, Parnell et al., 2010).

Table 2
Sample size (n), mean and standard deviation of $\delta^{13}C$ and $\delta^{15}N$ values of the potential prey of small-spotted catshark sampled in the northwestern Mediterranean Sea (based in Barría et al., 2015).

Species	n	$\delta^{13}C$ (‰)	$\delta^{15}N$ (‰)
REPTANTIA			
Dardanus arrosor	3	−17.89 ± 0.33	9.66 ± 0.25
Munida intermedia	3	−18.31 ± 0.54	6.38 ± 0.83
Munida rutllanti	3	−18.48 ± 0.31	6.59 ± 0.24
Pagurus prideaux	3	−17.38 ± 0.33	8.56 ± 0.23
Goneplax rhomboides	3	−18.34 ± 1.32	8.29 ± 0.48
Liocarcinus depurator	3	−18.32 ± 0.16	8.30 ± 0.48
Macropipus tuberculatus	3	−18.49 ± 0.69	7.44 ± 0.16
Macropodia longipes	3	−17.81 ± 0.57	6.47 ± 0.26
Medorippe lanata	3	−17.77 ± 0.27	9.12 ± 0.20
Monodaeus couchii	1	−21.4	6.44
EUPHAUSIACEA			
Meganyctiphanes norvegica	3	−20.55 ± 0.24	5.01 ± 0.31
CEPHALOPODA			
Sepietta oweniana	3	−19.41 ± 0.25	8.32 ± 0.43
Rossia macrosoma	3	−18.95 ± 0.89	7.98 ± 1.78
Sepiola affinis	3	−19.43 ± 0.26	8.82 ± 1.00
FISHES			
Capros aper	3	−20.15 ± 0.26	8.46 ± 0.39
Cepola macrophthalma	3	−20.42 ± 0.05	8.14 ± 0.04
Engraulis encrasicolus	10	−18.97 ± 0.17	8.09 ± 0.33
Gadiculus argenteus	3	−19.43 ± 0.13	8.85 ± 0.69
Lepidorhombus boscii	3	−19.11 ± 0.39	8.10 ± 0.71
Lepidorhombus whiffiagonis	1	−20.13	8.86
Spicara smaris	3	−19.15 ± 0.57	9.58 ± 1.04
Symphurus nigrescens	2	−18.74 ± 0.30	10.00 ± 0.59
Trachurus trachurus	10	−19.12 ± 0.11	9.13 ± 0.19
NATANTIA			
Alpheus glaber	3	−17.97 ± 0.84	7.82 ± 0.24
Chlorotocus crassicornis	3	−19.71 ± 0.63	6.99 ± 0.47
Pasiphaea sivado	3	−19.47 ± 0.27	6.63 ± 0.63
Plesionika antigai	3	−19.34 ± 0.37	7.20 ± 0.27
Processa canaliculata	3	−19.14 ± 0.14	7.75 ± 0.36
Solenocera membranacea	4	−18.35 ± 0.64	8.46 ± 0.74
POLYCHAETA			
Aphrodita aculeata	3	−17.03 ± 0.55	8.38 ± 1.74

2.3. Statistical analyses

We tested the effect of sex (female vs. male) and age (juveniles vs. adults) on stable isotope values of the small-spotted catshark using ANOVA tests. The interaction between sex and age was also tested in the model. The assumptions of ANOVA were checked with a Kolmogorov-Smirnov test for normality and a Levene test for homogeneity of variances. All analyses were performed with IBM-SPSS Statistics version 23 statistical software. A significance level of $p < 0.05$ was used for all tests.

2.4. Comparison of the trophic niche of small-spotted catshark with other sympatric elasmobranchs

To understand the ecological role of the small-spotted catshark in relation to other elasmobranchs, we compared our results with information of sharks and rays coexisting in the same area. Specifically, we compared their trophic position (based on the isotopic values) with published muscle isotopic information of the Selachiformes: Common thresher shark (*Alopias vulpinus*), gulper shark (*Centrophorus granulosus*), Portuguese dogfish (*Centroscymnus coelolepis*), kitefin shark (*Dalatias licha*), tope shark (*Galeorhinus galeus*), bluntnose sixgill shark (*Hexanchus griseus*), angular roughshark (*Oxynotus centrina*), blue shark (*Prionace glauca*), little sleeper shark (*Somniosus rostratus*) and spiny dogfish (*Squalus acanthias*); and the Batoids: Lognosed skate (*Dipturus oxyrinchus*), spiny butterfly ray (*Gymnura altavela*), cuckoo skate (*Leucoraja naevus*), giant devil ray (*Mobula mobular*), common eagle ray (*Myliobatis aquila*), starry ray (*Raja asterias*), thornback ray (*Raja clavata*), spotted ray (*Raja montagui*), speckled ray (*Raja polystigma*), marbled electric ray (*Torpedo marmorata*), electric ray (*Tetronarce nobiliana*) and common torpedo (*Torpedo torpedo*) (Barría et al., 2015). To avoid potential biases associated to differences in the isotopic assimilation between blood and muscle tissues, the blood isotopic values of small-spotted catshark were corrected for the difference between the trophic discrimination factors of muscle ($\Delta\delta^{15}N = 3.49$‰, $\Delta\delta^{13}C = -1.81$‰) and blood ($\Delta\delta^{15}N = 3.19$‰, $\Delta\delta^{13}C = 0.70$‰) experimentally estimated by Caut et al. (2013).

Table 3
Summary of the ANOVA tests examining the variation in blood stable isotopes between sexes (males and females) and ages (juveniles and adults) for small-spotted catsharks sampled in the northwestern Mediterranean Sea.

Parameter	Effect	$F_{[df]}$	*p*
δ^{15}N (‰)	Sex	$6.74_{[1,128]}$	0.01
	Age	$39.06_{[1,60]}$	<0.001
	Sex × age	$2.06_{[1,60]}$	0.16
δ^{13}C (‰)	Sex	$0.14_{[1,60]}$	0.71
	Age	$4.63_{[1,60]}$	0.04
	Sex × age	$3.13_{[1,60]}$	0.10

3. Results

3.1. Isotopic differences

δ^{15}N values differed significantly between sexes and ages, whereas δ^{13}C values only differed significantly between ages (Tables 1 and 3; Fig. 2A). Adults showed higher δ^{15}N isotopic values than juveniles (Tables 1 and 3; Fig. 2). Males showed higher δ^{15}N values than females (Tables 1 and 3; Fig. 2A). Moreover, we found that both δ^{15}N and δ^{13}C values showed a positive relationship to body length ($P < 0.01$; Fig. 3).

Regarding the standard ellipse areas (SEA; a proxy of the trophic width) and similar to the isotopic values, we found differences between sexes and ages (Fig. 2B). Juvenile (SEA = 2.46‰^{2}) and adult males (1.41‰^{2}) showed the widest isotopic niche; whereas the isotopic niche was smaller in juvenile (0.72‰^{2}) and adult females (0.70‰^{2}) (Fig. 2).

3.2. Isotopic mixing model results

The feasible contribution of each potential prey estimated by SIAR models clearly revealed that crustaceans are the most important assimilated prey for the small-spotted catshark, independent of sex and age, followed by cephalopods and fish (Table 4, Fig. 4A). Within crustaceans, SIAR outputs indicated that the Euphausiacea group is the most important prey for males and females, and juveniles and adults (Table 4, Fig. 4), followed by the Natantia and Reptantia groups (Table 4, Fig. 4B).

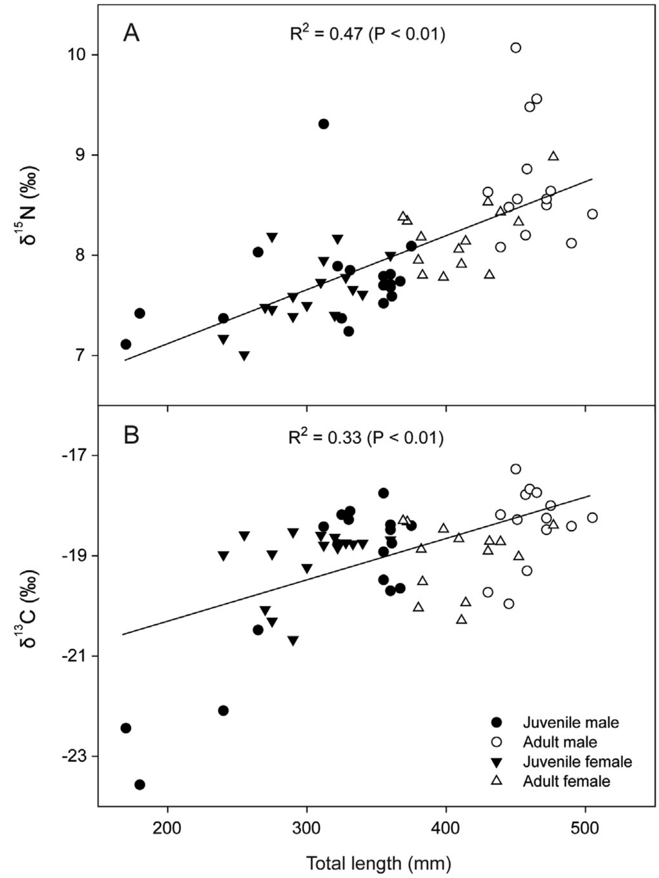

Fig. 3. Relationships between δ^{15}N, δ^{13}C and total length values in small-spotted catshark sampled in the northwestern Mediterranean Sea.

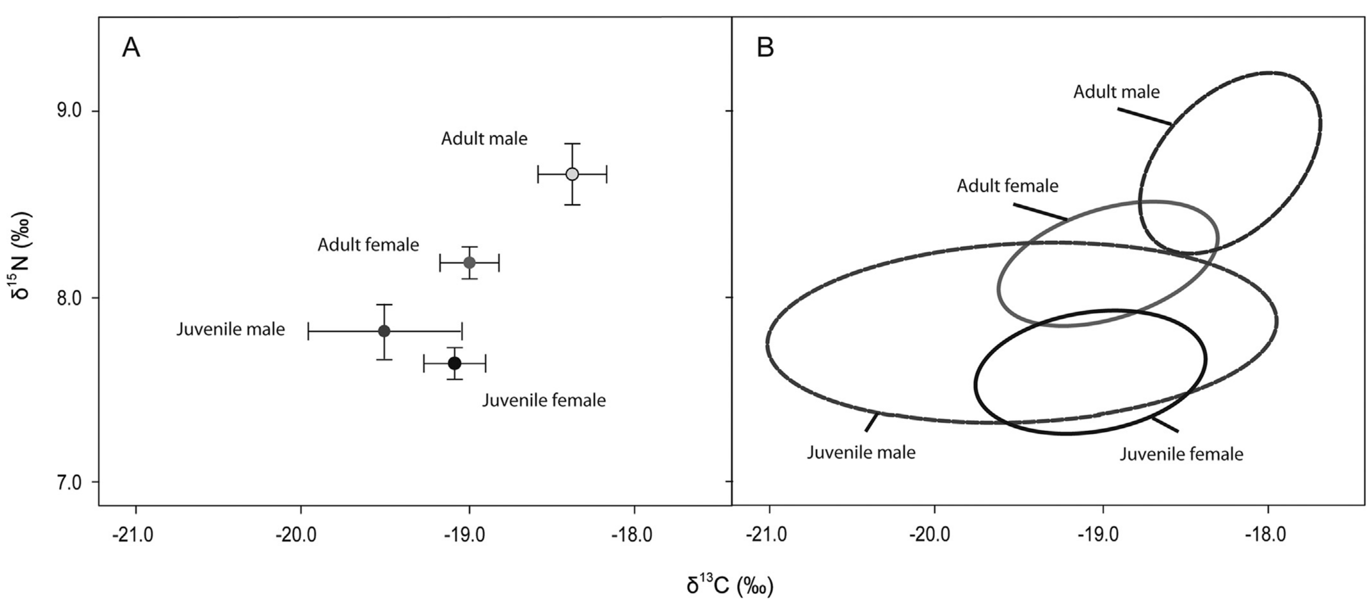

Fig. 2. Mean and standard error of δ^{15}N and δ^{13}C values (A) and the standard ellipse areas (B) of small-spotted catshark sampled in the northwestern Mediterranean Sea.

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Table 4
The relative contribution (%) of the potential prey to the diet of small-spotted catshark from the northwestern Mediterranean Sea estimated with SIAR isotopic mixing models. Contributions are designated as estimated in the low 95% highest density region (hdr), the mean contribution, and the high 95% hdr.

Group	Resources	Low 95% hdr	Mean % contribution	High 95% hdr
Male juvenile				
	Cephalopoda	0	0.10	0.24
	Euphausiacea	0.44	0.54	0.63
	Fishes	0	0.09	0.22
	Natantia	0	0.13	0.33
	Polychaeta	0	0.06	0.18
	Reptantia	0	0.08	0.21
Male adult				
	Cephalopoda	0	0.20	0.40
	Euphausiacea	0.20	0.31	0.42
	Fishes	0	0.16	0.34
	Natantia	0	0.14	0.32
	Polychaeta	0	0.07	0.19
	Reptantia	0	0.11	0.28
Female juvenile				
	Cephalopoda	0	0.11	0.23
	Euphausiacea	0.50	0.58	0.65
	Fishes	0	0.09	0.21
	Natantia	0	0.11	0.27
	Polychaeta	0	0.04	0.12
	Reptantia	0	0.07	0.19
Female adult				
	Cephalopoda	0	0.14	0.29
	Euphausiacea	0.34	0.44	0.52
	Fishes	0	0.13	0.29
	Natantia	0	0.14	0.33
	Polychaeta	0	0.05	0.15
	Reptantia	0	0.10	0.25

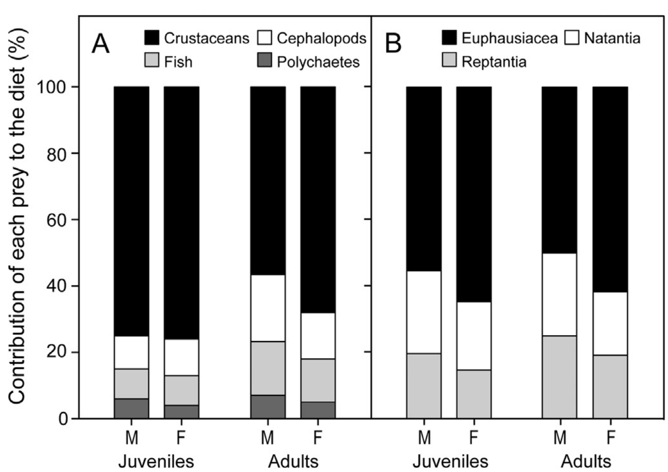

Fig. 4. Mean proportional contribution of different prey groups (A) and different crustaceans groups (B) to the diet of small-spotted catshark in the northwestern Mediterranean Sea based on the results of the SIAR model.

3.3. *Isotopic comparison with other sympatric elasmobranch species*

δ^{15}N values and trophic level ranged between 8.20 ± 0.74‰ in the Batoid *R. montagui* and 14.31 ± 1.73‰ in the Selachiforme *G. galeus* (Fig. 5A and Fig. 5B). δ^{13}C values ranged between −20.46 ± 0.75‰ in the Selachiforme *S. rostratus* and 15.59‰ in the Batoid *G. altavela* (Fig. 5A and B). Regarding small-spotted catshark all of the different sub-groups analysed in this study (males and females, juveniles and adults) were placed in an isotopic niche lower than Selachiformes and similar to the Batoids (Fig. 5 A and Fig. 5B).

4. Discussion

In this study, we present information on the feeding ecology of the small-spotted catshark, for the first time gathered through the analysis of stable isotopes in blood from free-living individuals. Although isotopic methodology in the study of the feeding ecology of sharks is increasing (Hussey et al., 2010; Shiffman et al., 2012), the use of non-lethal methods such as the analysis of isotopic values in blood is very scarce (Malpica-Cruz et al., 2013; Matich and Heithaus, 2014). However, this approach may be very useful for the study of the trophic ecology of threatened or rare elasmobranch species. Although our study may have limitations as diet-tissue discrimination factors with some preys, this study can be of great ecological importance because this species has high biomass in the NW Mediterranean Sea and its ecological role as predator can be key in this ecosystem.

In a previous study of the diet of small-spotted catsharks from the western Mediterranean Sea, Macpherson (1981) found that the most important prey were the teleosts, followed by Natantia and euphasusiids. Three decades later, Valls et al. (2011) found that euphausiids were the most important prey followed by Reptantia, polychaetes and teleosts. Our isotopic results confirm that the small-spotted catshark is a carnivorous predator and that crustaceans, in particular euphausiids, are especially important in its diet (in agreement with Valls et al., 2011). In the western Mediterranean, crustaceans are generally an important food resource for elasmobranch species present in the continental shelf and slope waters, where the availability of this resource is high. This is the case for the starry ray (*Raja asterias*) and the thornback ray (*Raja clavata*) (Barría et al., 2015; Navarro et al., 2013). However, the importance of crustaceans reported in our study contrasts with the results of the study conducted in the western Mediterranean 30 years ago using stomach content analyses, which indicated that fish were the main prey for small-spotted catshark (Macpherson, 1981), but are in agreement with those from Valls et al. (2011). This difference in the importance of fish in the diet of the species in the past could be due to a decrease in fish abundance in the western Mediterranean as a result of high fishing pressure in recent decades (Cartes et al., 2013; Coll et al., 2006). Moreover, the abundance of crustaceans may explain the current spatial distribution of small-spotted catshark in the western Mediterranean (Navarro et al., 2016).

The diet of small-spotted catshark seems to differ between areas according to prey availability. For example, in the North Sea the small-spotted shark feeds on hermit crabs, cockles and whelks (Lyle, 1983), and in the North Atlantic their main prey are decapod crustaceans (Martinho et al., 2012). In the Mediterranean Sea, according to the results of the present study and Valls et al. (2011), they mainly feed on euphausiids. Small-spotted catsharks have also been described to have the ability to exploit some species present in discards from bottom trawling fishing operations (Olaso et al., 1998, 2002). Therefore, by showing a wider adaptability of their niche, small-spotted catsharks are probably able to exploit the most abundant resources. This trophic adaptability, in addition to other factors such as their high survivorship when discarded from bottom trawl fisheries (Revill et al., 2005; Rodriguez-Cabello et al., 2005) or their high fecundity (Capapé et al., 2008), may explain the presence of this species in areas that are highly impacted by fisheries (Navarro et al., 2016). On the contrary, more sensitive sharks may have disappeared from these areas (Heithaus et al., 2008; Revill et al., 2005). One clear example is the reduction of the nursehound (*Scyliorhinus stellaris*), a species closely related to the small-spotted catshark which has virtually disappeared from some highly exploited areas of the western Mediterranean Sea (Coll et al., 2014; Maynou et al., 2011), probably because of its lower trophic

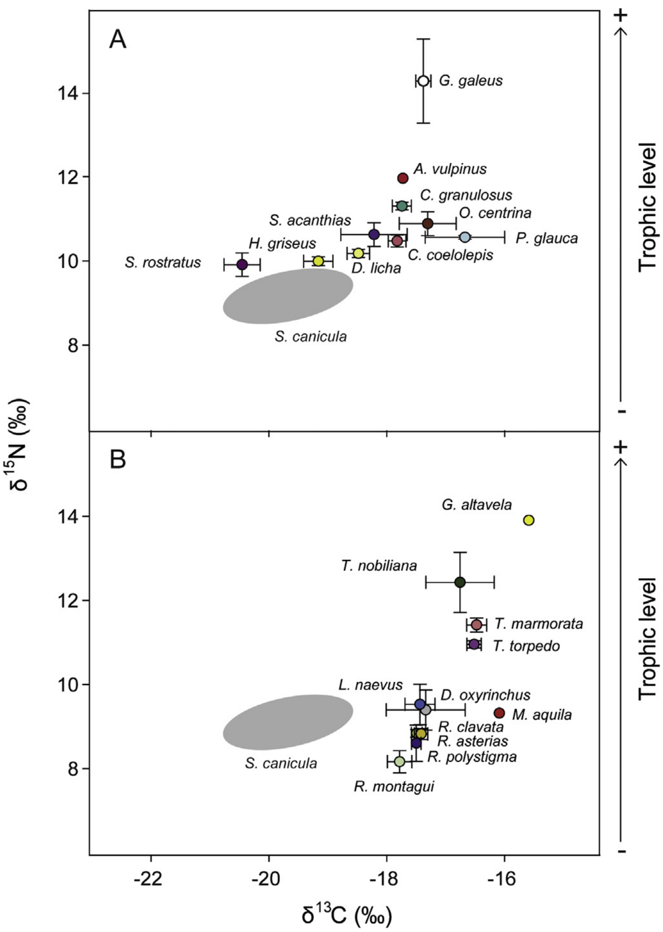

Fig. 5. Mean and standard error of δ^{15}N and δ^{13}C values of small-spotted catshark from the present study (shaded area) and other sharks (A) and skates and rays (B) from a published study conducted in the northwestern Mediterranean Sea (Barría et al., 2015).

plasticity, as well as its larger size and patchy distribution (Ellis et al., 2009).

Despite the fact that the outputs of the isotopic mixing models revealed similar trophic habits for all the sub-groups analysed, we found some isotopic differences between sexes and ages. Male adults showed higher δ^{15}N values than females and juveniles, probably due to a higher predation of male adults on fishes, as has been observed off the coast of Portugal, Atlantic Ocean (Martinho et al., 2012) or to a higher predation of females and juveniles on preys of lower trophic level such as euphausiids (Rodríguez-Cabello et al., 2007). These sexual and age-related isotopic differences could also be related to the differences in the size of the sampled individuals. In fact, we found a clear correlation between isotopic values and body size. This pattern is common in other sharks, such as in the sandbar shark (*Carcharhinus plumbeus*) in the North Atlantic Ocean (Shiffman et al., 2014) and the blue shark in the Indian Ocean (Rabehagasoa et al., 2012).

We also found isotopic differences between males and females, probably due to differences in the reproductive requirements in females during the sampling period (summer), related to mating and subsequent egg formation (Wearmouth and Sims, 2008; Jardas, 1972). Differences in the diet between sexes of small-spotted catsharks may also be related to a sexual spatial segregation as previously found in other sharks, such as the spiny dogfish, the kitefin shark and the tiger shark (*Galeocerdo cuvier*) (Hanchet, 1991; Matallanas, 1982; Simpfendorfer et al., 2001).

We found a significant relationship between δ^{13}C and size. This could be because larger individuals were feeding in different habitats than small individuals (Heithaus et al., 2013). Juvenile and adult individuals live on different bathymetric strata in the Mediterranean Sea; juveniles inhabit areas below 100–200 m, while in shallower waters the population is composed mainly of adults (D'Onghia et al., 1995; Massutí and Moranta, 2003). Ontogenetic shifts in the diet were also observed in our study, where juveniles had a wider trophic spectrum than adults, indicating a more diverse diet. Juveniles probably have a greater range of movement or they tend to be more generalist, feeding on available resources. On the contrary, adult individuals could be staying in the same areas because they need to remain with other adults to reproduce (Sims et al., 2001; Whitney et al., 2004). Furthermore, larger individuals have a greater ability to select high-energy preys and with higher δ^{15}N values, which could explain this ontogenetic change (Cortes et al., 1996; Webber and Cech, 1998).

When comparing the trophic niche of small-spotted catshark with other sympatric elasmobranchs, we found that the small-spotted catshark was much closer to Rajiformes (skates) than to other Selachiformes (sharks). The similarity between the small-spotted catshark and skates, such as the starry ray or the thornback ray, could be due to the fact that most mesopredators are generalist species that feed primarily on crustaceans (Barría et al., 2015; Navarro et al., 2013). The clear differences between the small-spotted catshark and the other sharks from the western Mediterranean Sea analysed in this study is that these species feed on prey of higher trophic levels (Barría et al., 2015) and they show highly specialised diets. This is evident in the kitefin shark, which consumes small-sized sharks including small-spotted catshark (Barría et al., 2015; Navarro et al., 2014), and the angular rough-shark that feeds on egg cases of elasmobranchs (Barrull and Mate, 2001; Guallart et al., 2015). Therefore, the small-spotted catshark and most of the skate species, such as the cuckoo skate, the starry ray or the thornback ray, can be considered mesopredators in our ecosystem; that is, medium-sized and middle-trophic level predators which both is predated upon and predates. Other demersal sharks (e.g., the gulper shark, the Portuguese dogfish, the kitefin shark and the tope shark) could be considered top predators (Barría et al., 2015).

In conclusion, the present study provides, for the first time, information on the diet of the small-spotted catshark in the western Mediterranean Sea using the analysis of stable isotopes in blood, a non-lethal methodology. Results reveal that the small-spotted catshark diet is mainly composed of euphausiids, with subtle sex and size variations. They showed a large trophic plasticity when compared to the other species, which may allow this species to live in highly exploited environments. The study confirms the ecological role of the species as a mesopredator in the Western Mediterranean Sea, a role similar to that played by skates and rays. The methodological approach used here to sample the studied individuals without damaging them presents new possibilities for future ecologic studies with threatened or rare elasmobranchs in the Mediterranean Sea.

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