

# Unravelling the ecological role and trophic relationships of uncommon and threatened elasmobranchs in the western Mediterranean Sea

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**ABSTRACT:** Deficiencies in basic ecological information on uncommon and endangered elasmobranch fishes impair the assessment of their ecological role in marine ecosystems. In this study, we examined the feeding ecology (diet composition and trophic level) and trophic relationships of 22 elasmobranchs (2 Carcharhiniformes, 1 Hexanchiformes, 1 Lamniformes, 3 Myliobatiformes, 6 Rajiformes, 6 Squaliformes and 3 Torpediniformes) present in the western Mediterranean Sea. To obtain a comprehensive view of the feeding ecology of these species, we combined different approaches: stable isotope analyses ( $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values), stomach content analyses and published sources. Our results revealed differences in feeding strategies among elasmobranch groups: skates (Rajiformes) mainly consume crustaceans; sharks (Carcharhiniformes, Hexanchiformes, Squaliformes and Lamniformes) prefer mixed diets composed of cephalopods, crustaceans and fishes; electric rays (Torpediniformes) feed mostly on fishes; and the diet of stingrays (Myliobatiformes) varies between species. Sharks and electric rays show higher trophic positions than skates, and the former occupy similar positions to other apex predators. Skates are more similar to other mesopredator fishes. These new findings offer essential information on the ecological role of several elasmobranchs in the western Mediterranean Sea and provide useful data for managers for future conservation strategies.

**KEY WORDS:** Mediterranean Sea · Rays · Sharks · Skates · Species at risk · Stable isotopes · Trophic ecology · Trophic level

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## INTRODUCTION

Elasmobranch fishes are usually considered keystone species in marine environments, due to their role in maintaining the structure and functioning of food webs (Libralato et al. 2006, Baum & Worm 2009). As predators, elasmobranchs are highly sensitive to ecosystem changes and human impacts such as fishing activity, pollution and habitat degradation (Stevens et al. 2000, Myers & Worm 2003, Dulvy et al. 2014). Therefore, elasmobranchs can be

considered good indicators of ecosystem health (Stevens et al. 2000, Baum & Worm 2009). The decrease in population size of some elasmobranchs may initiate trophic cascades through top-down effects and modify marine communities and ecosystems dramatically (Baum & Worm 2009, Ferretti et al. 2010). For example, the reduced abundance of the pelagic shark *Prionace glauca* was associated with an increased abundance of the pelagic stingray *Pteroplatytrygon violacea* in the tropical Pacific Ocean (Ward & Myers 2005) and a collapse of the

bay scallop fishery in the northwest Atlantic (Myers et al. 2007).

The Mediterranean Sea hosts around 80 species of elasmobranchs and is considered a global hotspot of biodiversity (Abdul Malak et al. 2011, Dulvy et al. 2014). Sharks and rays in the Mediterranean Basin represent approximately 7% of all currently existing elasmobranchs worldwide (Cavanagh & Gibson 2007). However, many species have declined in abundance across the Mediterranean Basin mainly due to degradation, loss of habitats and direct impacts from fishing (Ferretti et al. 2008, Coll et al. 2010, 2013). Currently, 41% of the elasmobranchs in the basin are considered threatened (classified as either Critically Endangered, Endangered or Vulnerable) by the regional assessment of the International Union for the Conservation of Nature (IUCN) (Abdul Malak et al. 2011). In addition, there is an important lack of basic biological and ecological information that precludes the evaluation of their conservation status. Close to 33% of elasmobranchs in the basin are considered Data Deficient (Abdul Malak et al. 2011).

Trophic ecology of marine organisms has been traditionally studied through stomach content analysis (SCA) (Hyslop 1980, Cortés 1999). Although this methodology allows high levels of taxonomic resolution, some marine predators such as elasmobranchs often show a high frequency of empty stomachs, and the prey items that are recovered are often skewed towards those that are more difficult to digest (Pethybridge et al. 2011, Navarro et al. 2014). Moreover, SCA usually requires a large number of stomachs to accurately quantify dietary habits, which can be difficult to obtain, especially for rare, threatened, endangered and protected elasmobranchs (Hyslop 1980, Cortés 1999, Stergiou & Karpouzi 2001). Stable isotope analysis (SIA) of nitrogen ( $\delta^{15}\text{N}$ ) and carbon ( $\delta^{13}\text{C}$ ) has been used as a complementary tool to SCA to study the trophic ecology of marine organisms (Estrada et al. 2006, Hussey et al. 2010, Shiffman et al. 2012, Navarro 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 (Shiffman et al. 2012). Moreover, by combining stable isotope values from consumers with those from their potential prey, isotopic mixing models can be applied to obtain estimates of the relative contribution of each prey item to the diet of the consumer (e.g. Parnell et al. 2013). To use mixing models, previous knowledge of the potential prey of the predator is needed (Phillips et al. 2014). Although outcomes of SCA and isotopic mixing models using SIA should

be interpreted with caution, their combination has already been highlighted as a valuable contribution to a better understanding of the feeding ecology of elasmobranchs (Caut et al. 2013, Navarro et al. 2014, Albo-Puigserver et al. 2015).

In the present study, we aimed to complement and update the available trophic information (dietary habits and trophic position) of 22 uncommon and threatened elasmobranch species present in the northwestern Mediterranean Sea. Specifically, the feeding ecology of these species was examined by combining SCA and SIA of collected individuals and from published diet information. Our study provides new insights into how the different elasmobranch species exploit trophic resources and contributes to a better understanding of the ecological role of these uncommon and threatened elasmobranchs in the Mediterranean Sea.

## MATERIALS AND METHODS

### Study area and sampling procedures

The present study was conducted in the western Mediterranean Sea (Catalan Sea and Gulf of Lions, Fig. 1) between 2011 and 2014, additionally samples collected in the Catalan Sea in 2003 were included. Both the Catalan Sea and the Gulf of Lions are highly productive marine areas due to the combination of the Ebro River and the Rhône River discharges, respectively, and the effect of the Liguro-Provencal-Catalan current along the continental slope (Estrada 1996, Salat 1996). These 2 areas are notably impacted by human activities (Coll et al. 2006, 2012). However, the sampling locations within the Gulf of Lions were in a less exploited area due to its greater distance from the coast and the fact that this area includes a Fishery Restricted Area declared by the General Fisheries Commission for the Mediterranean (Lleonart et al. 2008).

In total, 22 elasmobranch species were collected, including 10 sharks belonging to the following orders: Carcharhiniformes (*Galeorhinus galeus* and *Prionace glauca*), Hexanchiformes (*Hexanchus griseus*), Lamniformes (*Alopias vulpinus*) and Squaliformes (*Centrophorus granulosus*, *Centroscymnus coelolepis*, *Dalatias licha*, *Oxynotus centrina*, *Squalus acanthias* and *Somniosus rostratus*), 6 skates included in the orders Rajiformes (*Dipturus oxyrinchus*, *Leucoraja naevus*, *Raja asterias*, *R. clavata*, *R. montagui* and *R. polystigma*) and 6 rays of the orders Myliobatiformes (*Gymnura altavela*, *Mylio-*

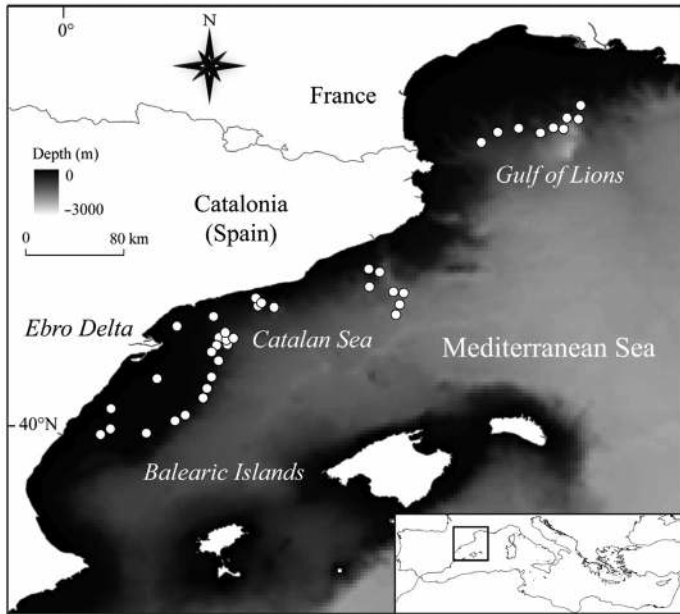


Fig. 1. Study area indicating the sampling locations (white circles)

*batis aquila* and *Mobula mobular*) and Torpediniformes (*Torpedo marmorata*, *T. nobiliana* and *T. torpedo*) (Table 1). Elasmobranchs were obtained opportunistically as bycatch of the commercial trawling fleet in the study area and during 3 experimental oceanographic surveys (conducted under the Dos-Mares and Ecotrans projects of the Spanish Government; Fig. 1).

After collection, each individual was immediately frozen on board and stored at  $-20^{\circ}\text{C}$ . From each individual, a sample of muscle or fin was collected for the SIA. For the individuals collected during the oceanographic survey in the Catalan Sea (ECOTRANS), we only collected finclips as individuals were released alive after the sampling. Total body length (cm) and body mass (g) of each individual were recorded using a fish measuring board ( $\pm 0.1$  cm) and a digital weighing scale ( $\pm 0.001$  g).

To develop the isotopic mixing models (see the following section), a total of 129 potential prey species

Table 1. Sample size (n) and mean  $\pm$  SD of  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values for 22 elasmobranch species from the western Mediterranean Sea. IUCN conservation status in the Mediterranean Sea is also indicated for each species (DD: Data Deficient; LC: Least Concern; NT: Near Threatened; VU: Vulnerable; EN: Endangered; CR: Critically Endangered; Abdul Malak et al. 2011). The zone (CS: Catalan Sea; GL: Gulf of Lions) and the year when the individuals were collected, sex (M: male; F: female) and tissue analysed are also indicated (Mu: muscle; Fi: fin)

Species	n	Year(s)	IUCN status	$\delta^{13}\text{C}$ (‰)	$\delta^{15}\text{N}$ (‰)	Sex	Zone	Tissue
<b>Carcharhiniformes</b>								
<i>Galeorhinus galeus</i>	3	2012–2013	VU	$-17.38 \pm 0.22$	$14.31 \pm 1.73$	1 (M); 2 (F)	3 (CS)	Mu
<i>Prionace glauca</i>	2	2013	VU	$-16.68 \pm 0.95$	$10.60 \pm 0.06$	2 (M)	2 (CS)	Mu
<b>Hexanchiformes</b>								
<i>Hexanchus griseus</i>	7	2012–2013	VU	$-19.16 \pm 0.65$	$10.03 \pm 0.28$	3 (M); 4 (F)	6 (CS); 1 (GL)	Mu
<b>Lamniformes</b>								
<i>Alopias vulpinus</i>	1	2014	VU	$-17.73$	12	1 (F)	1 (GL)	Mu
<b>Myliobatiformes</b>								
<i>Gymnura altavela</i>	1	2014	CR	$-15.59$	13.90	1 (M)	1 (CS)	Mu
<i>Myliobatis aquila</i>	1	2013	NT	$-16.09$	9.35	1 (M)	1 (CS)	Fi
<i>Mobula mobular</i>	1	2014	EN	$-18.59$	8.59	1 (M)	1 (GL)	Mu
<b>Rajiformes</b>								
<i>Dipturus oxyrinchus</i>	2	2011–2013	NT	$-17.34 \pm 0.95$	$9.43 \pm 0.67$	1 (M); 1 (F)	1 (CS); 1 (GL)	Mu
<i>Leucoraja naevus</i>	3	2013	NT	$-17.44 \pm 0.44$	$9.56 \pm 0.83$	3 (F)	3 (CS)	Mu
<i>Raja asterias</i>	67	2003–2013	LC	$-17.34 \pm 0.67$	$9.11 \pm 0.78$	35 (M); 32 (F)	51 (CS) 16 (LG)	Mu, Fi
<i>Raja clavata</i>	15	2011–2013	NT	$-17.42 \pm 0.44$	$8.86 \pm 0.49$	5 (M); 10 (F)	11 (CS) 4 (GL)	Mu, Fi
<i>Raja montagui</i>	8	2011–2013	LC	$-17.78 \pm 0.58$	$8.20 \pm 0.74$	4 (M); 4 (F)	6 (CS); 2 (GL)	Mu, Fi
<i>Raja polystigma</i>	2	2011–2012	NT	$-17.50 \pm 0.11$	$8.64 \pm 0.61$	1 (M); 1 (F)	1 (CS); 1 (LG)	Mu
<b>Squaliformes</b>								
<i>Centrophorus granulosus</i>	16	2003–2013	VU	$-17.75 \pm 0.65$	$11.34 \pm 0.36$	13 (M); 3 (F)	3 (CS); 13 (GL)	Mu
<i>Centroscymnus coelolepis</i>	24	2012	LC	$-17.83 \pm 0.74$	$10.51 \pm 0.70$	14 (M); 10 (F)	24 (CS)	Mu
<i>Dalatias licha</i>	35	2011–2013	DD	$-18.48 \pm 1.13$	$10.21 \pm 0.57$	17 (M); 18 (F)	17 (CS); 18 (GL)	Mu, Fi
<i>Oxynotus centrina</i>	3	2012–2013	CR	$-17.31 \pm 0.84$	$10.92 \pm 0.49$	3 (F)	1 (CS); 2 (GL)	Mu
<i>Squalus acanthias</i>	4	2011–2012	EN	$-18.22 \pm 1.12$	$10.66 \pm 0.47$	2 (M); 2 (F)	4(LG)	Mu, Fi
<i>Somniosus rostratus</i>	6	2012–2013	LC	$-20.46 \pm 0.75$	$9.95 \pm 0.68$	2 (M); 4 (F)	4 (CS); 2 (GL)	Mu
<b>Torpediniformes</b>								
<i>Torpedo marmorata</i>	18	2003–2013	LC	$-16.48 \pm 0.72$	$11.43 \pm 0.70$	3 (M); 15 (F)	18 (CS)	Mu
<i>Torpedo nobiliana</i>	3	2011–2014	DD	$-16.76 \pm 1.00$	$12.43 \pm 1.23$	3 (F)	1 (CS); 2 (GL)	Mu
<i>Torpedo torpedo</i>	28	2003	LC	$-16.52 \pm 0.65$	$10.98 \pm 0.44$	9 (M); 19 (F)	28 (CS)	Mu

(see Table S1 in the Supplement at [www.int-res.com/articles/suppl/m539p225\\_supp.pdf](http://www.int-res.com/articles/suppl/m539p225_supp.pdf)) were also collected from the northwestern Mediterranean during the experimental oceanographic survey (Ecotrans project) in 2013. For each prey species, a muscle biopsy was collected and stored at  $-20^{\circ}\text{C}$  on board, using the same methods as described above.

### Stomach content analysis

After dissection, each stomach was extracted and weighed with a digital scale ( $\pm 0.01$  g). Each prey found in the stomach was weighed and identified to the lowest taxonomic level possible. Whenever fragments of prey were found, the number of counted individuals was the lowest possible to avoid overestimation of the occurrence of a particular prey. To avoid potential biases associated with opportunistic feeding while the organisms were being captured, we only considered prey items with some evidence of digestion and we removed all prey found in the teeth and mouth of the studied specimens.

To assess the importance of different prey in the diet, the combined index of relative importance (IRI) of species  $i$  (Pinkas 1971) was calculated as follows:

$$\text{IRI}_i = (N_i + W_i) \times \text{FO}_i \quad (1)$$

where  $\text{FO}_i$  is the frequency of occurrence of prey species  $i$  in relation to the total number of stomachs,  $N_i$  is the contribution by number of prey species  $i$  in relation to the whole content of the stomach and  $W_i$  is the wet weight of prey species  $i$  in relation to the whole content of the stomach. The %IRI for each species was divided by the total IRI for all items to obtain the IRI on a percent basis (Cortés 1997). Unidentified prey within each functional group were also included in the estimation of these trophic metrics. Functional group is defined as 'a collection of organisms with similar suites of co-occurring functional attributes they have similar responses to external factors and/or effects on ecosystem processes' (De Bello et al. 2010). The vacuity index, %V, i.e. the percentage of empty stomachs, was also calculated.

### Revision of available diet information

We recorded all published diet information for the 22 elasmobranchs included in this study. To avoid potential geographic differences, we only used diet information of the species from the Mediterranean

Sea. Two electronic databases were used: Web of Science and Google Scholar. From each published study, we recorded different dietary metrics from SCA (%IRI, %FO, %N and occurrence) and from isotopic mixing models (relative contribution of each prey). To standardise and homogenise the data obtained with the literature review, we only considered those prey groups with more than 10% of %IRI.

Although %IRI is the most common and widespread dietary metric used in elasmobranch studies (Cortés 1999), in our case more than 50% of the revised information of our study species did not provide this index. For this reason, when %IRI was not provided, we used %FO, %N, occurrence and isotopic mixing model outputs.

To integrate information from different sources, an index of standardised diet importance was developed ( $P(x)$ ). This index was based on a weighted average of prey importance by study and allowed the incorporation of data from multiple quantitative and qualitative studies. For each species and study ( $i$ ), the prey item ( $x$ ) contributing the most to the diet was assigned the highest score (score = total number of prey items with more than 10% contribution to the diet) and the least important species was assigned a score of 1. A value of total importance of a prey item in the diet of the species was computed according to the weighted sum of the categorisations of all prey groups following:

$$P(x) = \frac{\sum_1^n C_i(x_n)}{C} \quad \text{where: } C = \sum_1^i c_i \quad (2)$$

where  $P$  is the total relative importance of prey  $x$  in each elasmobranch species,  $x$  is the prey item contribution to the diet of more than 10%,  $n$  is the number of prey items considered in the calculation,  $c$  is the score for each prey item and  $i$  is the number of studies considered.

### Stable isotope analysis

Samples (muscle, fin and potential prey) were subsequently freeze-dried and powdered and 0.28 to 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 (LIE-EBD; Spain). Capsules were combusted at  $1020^{\circ}\text{C}$  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 is reported in the conventional delta ( $\delta$ ) per mil notation (‰), relative to Vienna Pee Dee Belemnite ( $\delta^{13}\text{C}$ ) and atmospheric  $\text{N}_2$  ( $\delta^{15}\text{N}$ ). Replicate assays of standards routinely inserted within the sampling sequence indicated analytical measurement errors of  $\pm 0.1\text{‰}$  and  $\pm 0.2\text{‰}$  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 (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. For the samples with a C:N ratio higher than 3.5‰, we corrected the  $\delta^{13}\text{C}$  values to account for the presence of lipids in muscle samples (Logan et al. 2008).

### Isotopic mixing models and isotopic niche analyses

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) based on the isotopic values of each elasmobranch species, and those of their potential prey grouped taxonomically (Table 2). SIAR models allow 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 build the SIAR mixing models, we used the isotopic values of prey collected during the present study in the northwestern Mediterranean. We used the isotopic discrimination factors of  $1.95 \pm 0.26\text{‰}$  for  $\delta^{13}\text{C}$  and  $0.49 \pm 0.32\text{‰}$  for  $\delta^{15}\text{N}$  (Hussey et al. 2010).

As a measure of trophic width, we calculated the Bayesian isotopic standard ellipse areas (SEAs) for Carcharhiniformes, Hexanchiformes, Rajiformes,

Table 2. Sample size (n) and mean  $\pm$  SD of  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values for potential prey of the different target elasmobranchs sampled in the western Mediterranean Sea. Full species names are given in Table 1

Group	n	$\delta^{13}\text{C}$ (‰)	$\delta^{15}\text{N}$ (‰)	Target elasmobranch
Amphipoda	1	-21.57	8.62	<i>R. montagui</i> , <i>R. polystigma</i>
Anguilliformes	1	-19.03	9.78	<i>L. naevus</i> , <i>T. torpedo</i>
Anomura	16	-18.11 $\pm$ 0.40	8.27 $\pm$ 1.52	<i>D. oxyrinchus</i> , <i>G. altavela</i> , <i>M. aquila</i>
Bivalvia	2	-18.84 $\pm$ 0.39	5.90 $\pm$ 0.21	<i>M. aquila</i>
Brachyura	20	-17.37 $\pm$ 3.17	7.59 $\pm$ 0.94	<i>O. centrina</i> , <i>R. asterias</i> , <i>R. clavata</i> , <i>R. polystigma</i>
Cephalopoda	57	-18.85 $\pm$ 0.44	8.64 $\pm$ 0.42	<i>D. licha</i> , <i>D. oxyrinchus</i> , <i>R. clavata</i> , <i>T. marmorata</i>
Clupeiformes	33	-19.21 $\pm$ 0.36	8.26 $\pm$ 0.24	<i>C. granulatus</i> , <i>H. griseus</i> , <i>P. glauca</i> , <i>G. altavela</i> , <i>R. clavata</i> , <i>T. marmorata</i> , <i>T. torpedo</i>
Euphausiacea	1	-20.32	4.65	<i>M. mobular</i>
Gadiformes	24	-19.47 $\pm$ 0.26	8.73 $\pm$ 0.40	<i>C. granulatus</i> , <i>D. licha</i> , <i>G. galeus</i> , <i>H. griseus</i> , <i>P. glauca</i> , <i>R. clavata</i> , <i>T. marmorata</i> , <i>T. nobiliana</i>
Gasteropoda	12	-17.52 $\pm$ 1.60	8.82 $\pm$ 0.98	<i>M. aquila</i>
Isopoda	3	-20.40 $\pm$ 3.50	10.01 $\pm$ 1.14	<i>R. polystigma</i>
Mollusca	75	-18.29 $\pm$ 0.79	8.40 $\pm$ 0.95	<i>R. asterias</i> , <i>T. torpedo</i>
Myctophiformes	2	-20.62 $\pm$ 0.83	8.41 $\pm$ 0.20	<i>D. licha</i> , <i>T. marmorata</i>
Natantia	47	-18.50 $\pm$ 0.84	7.71 $\pm$ 0.59	<i>C. granulatus</i> , <i>C. coelolepis</i> , <i>D. licha</i> , <i>D. oxyrinchus</i> , <i>L. naevus</i> , <i>R. asterias</i> , <i>R. clavata</i> , <i>R. montagui</i> , <i>R. polystigma</i> , <i>T. torpedo</i>
Octopoda	7	-18.31 $\pm$ 1.23	8.55 $\pm$ 0.64	<i>G. galeus</i> , <i>S. acanthias</i>
Osmeriformes	3	-19.19 $\pm$ 0.44	9.30 $\pm$ 1.05	<i>C. granulatus</i>
Perciformes	105	-18.87 $\pm$ 0.88	9.59 $\pm$ 1.00	<i>C. granulatus</i> , <i>H. griseus</i> , <i>P. glauca</i> , <i>G. altavela</i> , <i>R. clavata</i> , <i>T. marmorata</i> , <i>T. torpedo</i>
Pleuronectiformes	19	-19.13 $\pm$ 0.38	9.38 $\pm$ 1.13	<i>L. naevus</i> , <i>T. torpedo</i>
Polychaeta	3	-17.03 $\pm$ 0.55	8.38 $\pm$ 1.74	<i>O. centrina</i> , <i>R. polystigma</i> , <i>T. nobiliana</i> , <i>T. torpedo</i>
Selachii (small sharks)	20	-18.57 $\pm$ 0.50	8.59 $\pm$ 0.59	<i>D. licha</i>
Sepiidae	9	-18.87 $\pm$ 0.88	9.59 $\pm$ 1.00	<i>P. glauca</i>
Teleostei	223	-19.26 $\pm$ 0.63	9.09 $\pm$ 0.80	<i>A. vulpinus</i> , <i>C. coelolepis</i> , <i>S. acanthias</i> , <i>D. oxyrinchus</i> , <i>M. aquila</i> , <i>R. asterias</i>
Teuthida	31	-19.04 $\pm$ 0.40	9.24 $\pm$ 1.08	<i>A. vulpinus</i> , <i>C. granulatus</i> , <i>C. coelolepis</i> , <i>G. galeus</i> , <i>H. griseus</i> , <i>S. acanthias</i> , <i>S. rostratus</i> , <i>P. glauca</i> , <i>M. aquila</i>
Tunicata	10	-19.47 $\pm$ 0.32	8.12 $\pm$ 1.15	<i>S. rostratus</i>

Squaliformes and Torpediniformes (Jackson et al. 2011). Lamniformes and Myliobatiformes were not considered in the analysis due to their limited number of samples. 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 width exploited by the species considered (high values of isotopic standard ellipse areas indicate high trophic width). SEA was calculated using the routine Stable Isotope Bayesian Ellipses (Jackson et al. 2011) incorporated in the SIAR library.

### Trophic level

We estimated the trophic level (TL) of each species and taxonomic order by using SCA (TL<sub>sca</sub>) and SIA (TL<sub>sia</sub>). We estimated the TL<sub>sia</sub> according to the algorithm proposed by Zanden & Rasmussen (2001):

$$TL_{\text{consumer}} = TL_{\text{basal}} + (\delta^{15}\text{N}_{\text{consumer}} - \delta^{15}\text{N}_{\text{basal}}) / \Delta\delta^{15}\text{N} \quad (3)$$

where  $\delta^{15}\text{N}_{\text{consumer}}$  is the value for each elasmobranch and  $\delta^{15}\text{N}_{\text{basal}}$  is that of the bivalve *Pecten jacobaeus* sampled from the northwestern Mediterranean. For the  $\Delta^{15}\text{N}$  values, we used 1.95 (Hussey et al. 2010).

In addition, we calculated the TL of elasmobranch species using the SCA results (TL<sub>sca</sub>) using the following equation:

$$TL_j = 1 + \sum_{i=1}^n DC_{ji} - TL_i \quad (4)$$

where  $j$  is the predator of prey  $i$ ,  $DC_{ji}$  is the fraction of prey  $i$  in the diet of predator  $j$ , and  $TL_i$  is the trophic level of prey  $i$ . The  $TL_i$  values used for prey species were obtained from previous modelling studies conducted in the northwestern Mediterranean (Coll et al. 2006, Navarro et al. 2011, Bănarău et al. 2013).

### Statistical analyses

Differences between groups (taxonomic orders) based on %IRI and between groups and species within groups based on  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  values were tested using semi-parametric permutation multivariate analyses of variance tests (PERMANOVA test) on the Euclidean distance matrix (Anderson et al. 2008). When significant differences between groups or species were found, pairwise tests were performed. If the number of unique permutations was less than 100, Monte Carlo tests were used. PERMANOVA allows for the analysis of complex designs (multiple factors and their interaction) without the constraints of normality, homoscedasticity or the need for a large

number of variables as in sampling units of traditional ANOVA tests. The method calculates a pseudo- $F$  statistic directly analogous to the traditional  $F$ -statistic for multifactorial univariate ANOVA models, using permutation procedures to obtain  $p$ -values for each term in the model (Anderson et al. 2008). PERMANOVA tests were carried out with PRIMER-E 6 software.

In addition, the potential relationships between IUCN categories (Critically Endangered, Vulnerable, Endangered, Near Threatened, Least Concern, Data Deficient) and TL<sub>sia</sub> of each elasmobranch species and between TL<sub>sia</sub> and TL<sub>sca</sub> were assessed using the Pearson product-moment correlation coefficient. This correlation coefficient is a measure of statistical dependence between 2 variables, ranging between  $-1$  and  $1$  (negative and positive correlation, respectively). Previous to this analysis, the number of samples and the body length were normalised using the natural logarithm to smooth the differences between the data. The Pearson product-moment correlation coefficient was also used to determine potential relationships between  $\delta^{15}\text{N}$  values and body length of the specimens of each taxonomic order. Results were considered significant at  $p < 0.05$ .

## RESULTS

### Stomach content analysis

In total, 224 stomachs were collected from 251 individuals (see Table 1). The vacuity index differed between species and ranged from 100% for *Prionace glauca* and 78.6% for *Torpedo torpedo* to 0% for *Galeorhinus galeus* and the skates *Dipturus oxyrinchus*, *Leucoraja naevus*, *Raja clavata*, *R. montagui* and *Gymnura altavela* (Tables S2–S4 in the Supplement at [www.int-res.com/articles/suppl/m539p225\\_supp.pdf](http://www.int-res.com/articles/suppl/m539p225_supp.pdf)).

We found significant differences in the stomach content composition based on %IRI between taxonomic orders (pseudo- $F_{5,12} = 2.36$ ,  $p = 0.003$ ). In particular, the stomach contents were significantly different between Squaliformes and Rajiformes (pseudo- $t = 1.57$ ,  $p = 0.004$ ), between Squaliformes and Torpediniformes (pseudo- $t = 2.61$ ,  $p = 0.007$ ) and between Rajiformes and Torpediniformes (pseudo- $t = 2.21$ ,  $p = 0.01$ ).

At the species level, cephalopods were the most important prey group for *Centrophorus granulosus* (%IRI = 55.0%,  $N = 14$ ), *Centroscymnus coelolepis* (%IRI = 57.3%,  $N = 53$ ), *Squalus acanthias* (%IRI =

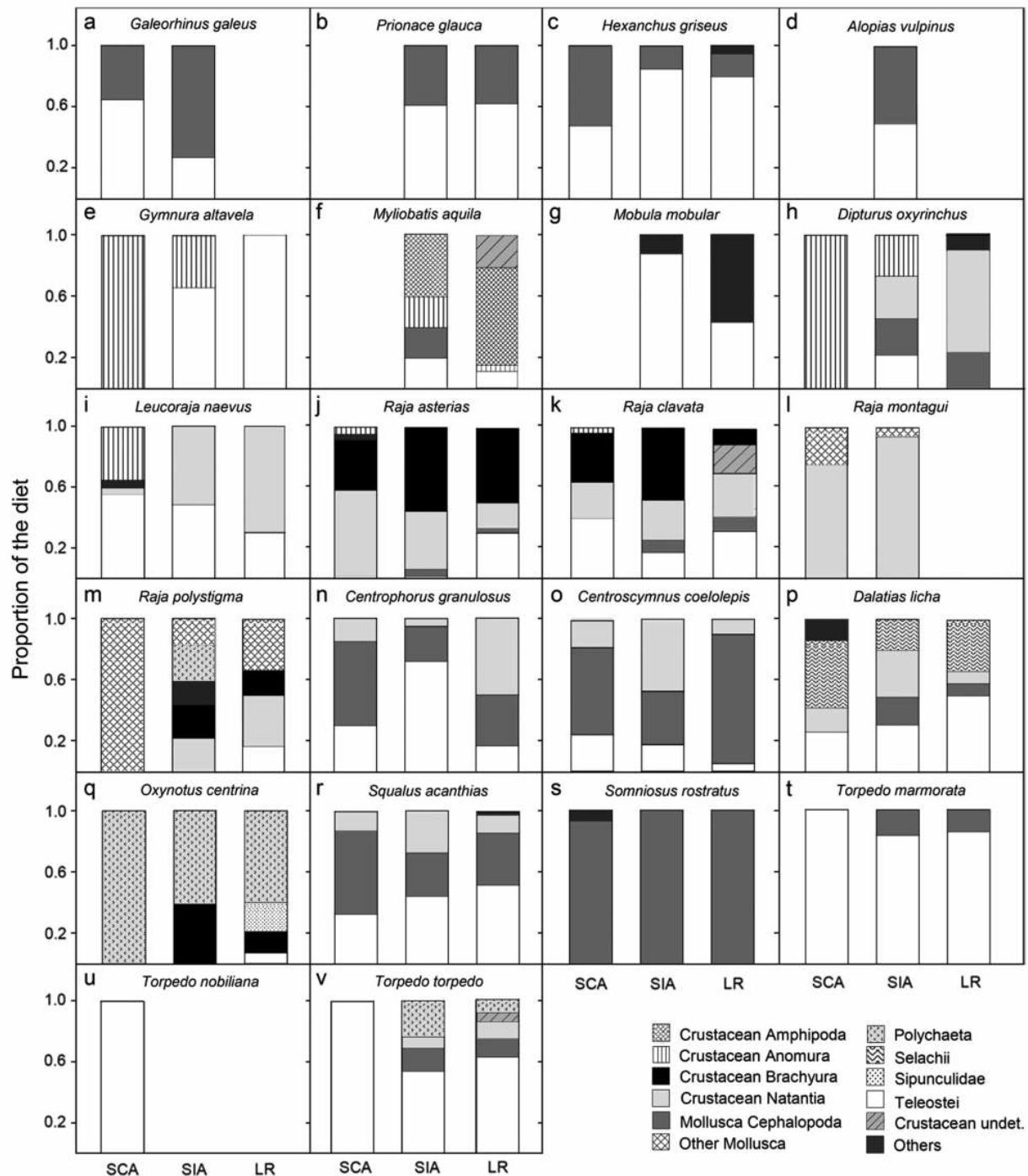


Fig. 2. Proportion of principal prey groups of each elasmobranch species based on stomach content analysis (SCA) conducted in the present study (index of relative importance), the proportion of importance of each prey estimated with stable isotope analysis (SIA) mixing models and stomach content results expressed as a proportion of each prey from the literature review (LR). (a,b) Carcharhiniformes, (c) Hexanchiformes, (d) Lamniformes, (e–g) Myliobatiformes, (h–m) Rajiformes, (n–s) Squaliformes and (t–v) Torpediniformes. Undet: undetermined

54.6%, N = 4), *Somniosus rostratus* (%IRI = 93.0%, N = 5) (Squaliformes) and *Hexanchus griseus* (%IRI = 52.1%, N = 6) (Hexanchiformes) (Table S2, Fig. 2). Teleostei (fin-fish) was the most important prey

group in the stomach of *G. galeus* (%IRI = 64.6%, N = 1, Table S2) (Carcharhiniformes), *Torpedo marmorata*, *T. nobiliana* and *T. torpedo* (%IRI = 100 for the 3 species; N = 16, N = 3, N = 28, respectively,

Table S4) (Torpediniformes), *Leucoraja naevus* (%IRI = 54.7%, N = 3, Table S3) and *Raja clavata* (%IRI = 40.6%, N = 6, Table S3) (Rajiformes). Prey of the groups Selachii (representing a group of small demersal sharks) and Polychaeta were the most important prey of *Dalatias licha* (%IRI = 45.3%, N = 30, Table S2) and *Oxynotus centrina* (%IRI = 100%, N = 3, Table S2), respectively (Fig. 2) (Squaliformes). Decapod crustaceans were the most important prey of *R. asterias* (%IRI = 56.3%, N = 39, Table S3) and *R. montagui* (%IRI = 75.6%, N = 8, Table S3) (Rajiformes). Crustaceans of the group Anomura were a unique prey group found in *Dipturus oxyrinchus* (Rajiformes) and *Gymnura altavela* (Myliobatiformes) (for both species, %IRI = 100%, N = 1, Tables S3 & S4, respectively), and crustaceans of the group Amphipoda were a unique prey found in the stomach of *R. polystigma* (%IRI = 100%, N = 2; Table S3, Fig. 2) (Rajiformes).

#### Dietary insights from the literature

We found a total of 49 studies published between 1971 and 2014 documenting the diet of 18 of the 22 elasmobranchs considered in the present study. Of those, 3 works corresponded to studies of stomach contents in Carcharhiniformes, 2 in Hexanchiformes, 8 in Myliobatiformes, 17 in Rajiformes and 12 in Squaliformes, and 7 studies were based on the diet of Torpediniformes (Table S5).

Based on the available published diet data, the diet of Carcharhiniformes and Hexanchiformes were composed mainly of fishes and cephalopods (Fig. 2). Myliobatiformes included crustaceans, molluscs and small fishes in their diet. The diet of Rajiformes was basically composed of crustaceans; Squaliformes included cephalopods, fishes and crustaceans. The diet of Torpediniformes was composed mainly of fishes (Table S6, Fig. 2).

#### Stable isotope analysis

$\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values differed among orders ( $\delta^{13}\text{C}$ : pseudo- $F_{6,244} = 27.04$ ,  $p < 0.001$ ;  $\delta^{15}\text{N}$ : pseudo- $F_{6,244} = 57.60$ ,  $p < 0.001$ ; Fig. 3, Table 3). In particular, Torpediniformes and Squaliformes showed the highest and lowest  $\delta^{13}\text{C}$ , respectively (Fig. 3), whereas Carcharhiniformes showed the highest  $\delta^{15}\text{N}$  values and Rajiformes the lowest values (Fig. 3). At an intragroup level, we found significant differences in  $\delta^{15}\text{N}$  values between Rajiformes species ( $\delta^{15}\text{N}$ : pseudo- $F_{5,91} =$

2.83,  $p = 0.02$ , Fig. 3, Table 3). Significant differences in  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values were found in Squaliformes ( $\delta^{13}\text{C}$ : pseudo- $F_{5,82} = 9.87$ ,  $p < 0.001$ ;  $\delta^{15}\text{N}$ : pseudo- $F_{5,82} = 9.66$ ,  $p < 0.001$ ; Fig. 3, Table 3). In the case of Torpediniformes, we only found statistical differences in  $\delta^{15}\text{N}$  values (pseudo- $F_{2,46} = 7.58$ ,  $p < 0.001$ ; Fig. 3) between species (Table 3).  $\delta^{15}\text{N}$  values showed a positive relationship to body length in Squaliformes and Torpediniformes ( $p = 0.002$ ,  $p < 0.001$ , respectively), but not in Hexanchiformes and Rajiformes (Fig. 4).

#### Isotopic mixing models

Bayesian isotopic mixing model outputs suggested that the proportion of the Teleostei prey group (fish) was high in *C. granulosus* and *H. griseus* (Fig. 2). In *G. altavela*, *C. granulosus* and *T. marmorata*, fishes of the Perciformes represented the most important prey group. Clupeiformes showed a high proportion in the diet of *H. griseus*, *P. glauca* and *Mobula mobular* (Table 4). Cephalopods showed the highest proportion in the diet of *G. galeus* and were the second-most common prey group estimated for *H. griseus*, *S. acanthias* and *A. vulpinus* (Fig. 2). Polychaeta was estimated as an important prey of *O. centrina*, *R. polystigma* and *T. torpedo* (Fig. 2), while decapod crustaceans were an important prey group for *D. oxyrinchus*, *L. naevus*, *R. montagui*, *R. asterias* and *R. clavata* (Table 4).

#### Trophic position and trophic width

$\text{TL}_{\text{sca}}$  and  $\text{TL}_{\text{sia}}$  ranged between  $\text{TL}_{\text{sca}} = 3.1$  and  $\text{TL}_{\text{sia}} = 3.2$  for *O. centrina* and *R. montagui*, respectively, to  $\text{TL}_{\text{sca}} = 4.9$  and  $\text{TL}_{\text{sia}} = 6.3$  for *G. galeus* (Tables S2 & S3, Fig. 5). Between orders, we found that both  $\text{TL}_{\text{sca}}$  and  $\text{TL}_{\text{sia}}$  showed a positive correlation ( $R^2 = 0.47$ ,  $p < 0.05$ ; Fig. 6), with higher TL values in Carcharhiniformes ( $\text{TL}_{\text{sca}} = 4.94$ ;  $\text{TL}_{\text{sia}} = 5.6 \pm 1.22$  [mean  $\pm$  SD]), followed by Lamniformes ( $\text{TL}_{\text{sia}} = 5.12$ ), Hexanchiformes ( $\text{TL}_{\text{sca}} = 4.68$ ;  $\text{TL}_{\text{sia}} = 4.2 \pm 0.3$ ), Squaliformes ( $\text{TL}_{\text{sca}} = 4.31 \pm 0.61$ ;  $\text{TL}_{\text{sia}} = 5.6 \pm 1.22$ ), Torpediniformes ( $\text{TL}_{\text{sca}} = 4.48 \pm 0.02$ ;  $\text{TL}_{\text{sia}} = 4.93 \pm 0.38$ ), Myliobatiformes ( $\text{TL}_{\text{sca}} = 4.14$ ;  $\text{TL}_{\text{sia}} = 4.41$ ) and Rajiformes ( $\text{TL}_{\text{sca}} = 3.68 \pm 0.38$ ;  $\text{TL}_{\text{sia}} = 3.6 \pm 0.4$ ).

SEAs differed among Carcharhiniformes ( $\text{SEA} = 4.36\%$ ), Hexanchiformes ( $0.98\%$ ) and Rajiformes ( $1.14\%$ ; Fig. 3), whereas Squaliformes ( $2.33\%$ ) and Torpediniformes ( $1.36\%$ ) overlapped in their SEAs (Fig. 7).



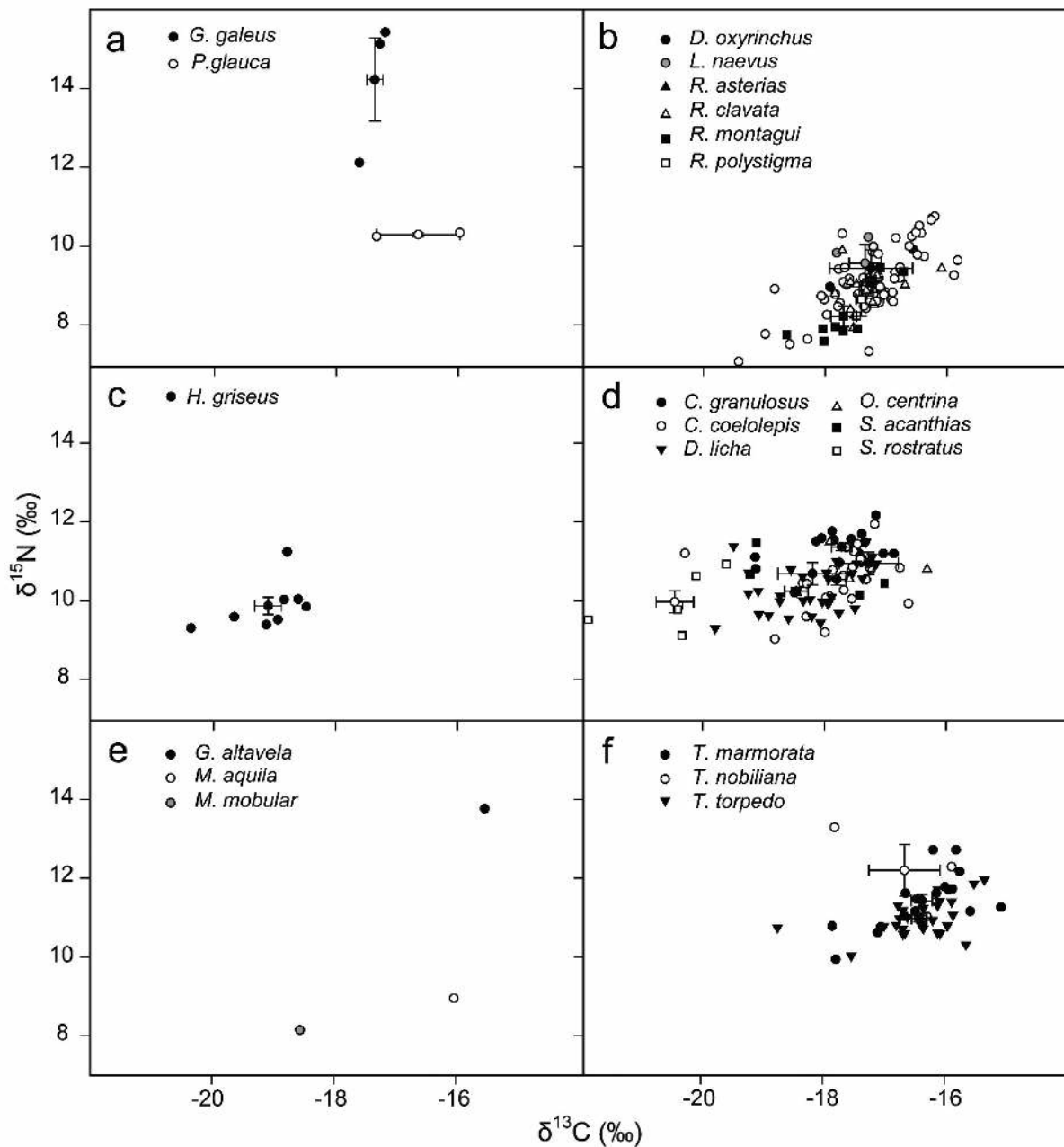


Fig. 3. Mean  $\pm$  SD of  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  values for (a) Carcharhiniformes, (b) Rajiformes, (c) Hexanchiformes, (d) Squaliformes, (e) Myliobatiformes and (f) Torpediniformes from the western Mediterranean Sea. Full species names are given in Table 1

### Trophic ecology and conservation status

According to the IUCN categories for the Mediterranean Sea (Froese & Pauly 2014), the 22 species collected in the present study were classified as Data Deficient (3 species), Near Threatened (5 species), Vulnerable (4 species), Endangered (2 species) and Critically Endangered (2 species) (Table 1). In addition, we included 6 species considered to be of Least

Concern but with deficiencies in ecological data availability in the region.

We found a positive but non-significant relationship between  $\text{TL}_{\text{sia}}$  and the degree of threat as categorised by the IUCN classification ( $R = 0.42$ ,  $p = 0.06$ ; Fig. 7). *Dalatias licha* ( $\text{TL}_{\text{sia}} = 4.21$ ), *G. galeus* ( $\text{TL}_{\text{sia}} = 6.31$ ) and *T. nobiliana* ( $\text{TL}_{\text{sia}} = 5.35$ ) were not included because available information was insufficient to accurately assess their extinction risk (Data Deficient).

Table 3. PERMANOVA results showing the significant isotopic differences based on pairwise tests between taxonomic orders, and between species within the orders. Full species names are given in Table 1

$\delta^{13}\text{C}$ between orders	Squaliformes	Hexanchiformes	Torpediniformes	
Carcharhiniformes	0.026	0.001		
Hexanchiformes	0.035			
Rajiformes	<0.001	<0.001	<0.001	
Myliobatiformes	0.031	0.011		
Torpediniformes	<0.001	<0.001		
$\delta^{15}\text{N}$ between orders	Squaliformes	Hexanchiformes	Rajiformes	Torpediniformes
Carcharhiniformes	<0.001	0.007	<0.001	0.004
Lamniformes	0.043	0.025	<0.001	
Myliobatiformes			0.015	
Rajiformes	<0.001	<0.001		<0.001
Torpediniformes	<0.001	<0.001		
$\delta^{15}\text{N}$ between Rajiformes	<i>L. naevus</i>	<i>R. asterias</i>	<i>R. clavata</i>	
<i>R. montagui</i>	0.024	0.002	0.022	
$\delta^{13}\text{C}$ between Squaliformes	<i>D. licha</i>	<i>S. rostratus</i>		
<i>C. granulatus</i>	0.016	<0.001		
<i>C. coelolepis</i>	0.013	<0.001		
<i>D. licha</i>		<0.001		
<i>O. centrina</i>		<0.001		
<i>S. acanthias</i>		0.005		
$\delta^{15}\text{N}$ between Squaliformes	<i>C. coelolepis</i>	<i>D. licha</i>	<i>S. acanthias</i>	<i>S. rostratus</i>
<i>C. granulatus</i>	<0.001	<0.001	0.007	<0.001
<i>O. centrina</i>		0.046		
$\delta^{15}\text{N}$ between Torpediniformes	<i>T. torpedo</i>			
<i>T. marmorata</i>	0.009			
<i>T. nobiliana</i>	0.005			

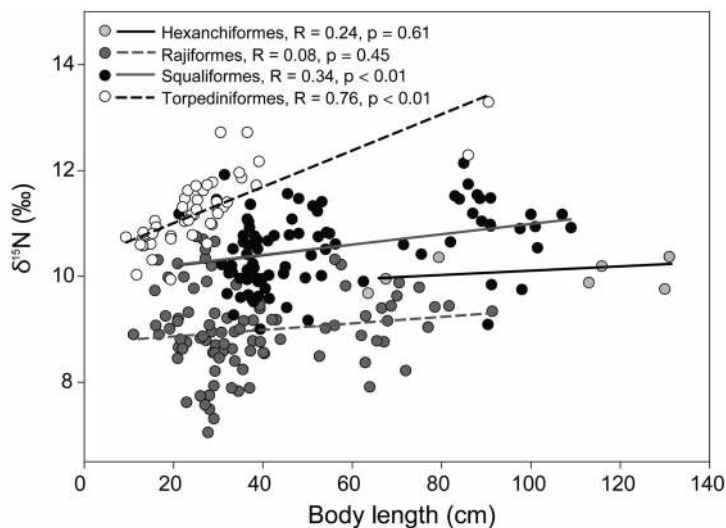


Fig. 4. Relationships between  $\delta^{15}\text{N}$  values and body length in Hexanchiformes, Rajiformes, Squaliformes and Torpediniformes from the western Mediterranean Sea

## DISCUSSION

In this study, we present new information on the feeding ecology of 22 species of uncommon and threatened elasmobranchs in the western Mediterranean Sea. SCA data provided information with a higher taxonomic resolution. SIA results allowed us to characterise the feeding habits from a functional point of view. The information from the literature enabled us to summarise the main prey of the species in different habitats of the Mediterranean Sea and complemented the information for those species with low sample sizes. Although our study may have limitations due to the small sample size of some species, obtaining a large sample size of rare elasmobranchs is difficult, and using com-

Table 4. Relative contribution of potential prey to the diet of elasmobranchs from the western Mediterranean estimated with SIAR isotopic mixing models. Contributions are designated as the estimated low 95% highest density region (hdr), mean contribution and high 95% hdr. Full species names are given in Table 1

Species	Potential prey	Low 95% hdr	Mean% contribution	High 95% hdr	Species	Potential prey	Low 95% hdr	Mean% contribution	High 95% hdr
<b>Carcharhiniformes</b>					<i>R. montagui</i>	Amphipoda	0	0.06	0.15
<i>G. galeus</i>	Gadiformes	0	0.27	0.56		Natantia	0.84	0.94	1.01
	Octopoda	0.02	0.40	0.74	<i>R. polystigma</i>	Amphipoda	0	0.17	0.35
	Teuthida	0	0.33	0.65		Brachyura	0	0.22	0.41
<i>P. glauca</i>	Clupeiformes	0	0.22	0.43		Isopoda	0	0.16	0.34
	Gadiformes	0	0.21	0.40		Natantia	0	0.22	0.41
	Perciformes	0	0.18	0.37	Polychaeta	0	0.24	0.45	
	Sepida	0	0.20	0.38	<b>Squaliformes</b>				
	Teuthida	0	0.19	0.38	<i>C. granulatus</i>	Clupeiformes	0	0.07	0.19
<b>Hexanchiformes</b>						Gadiformes	0	0.10	0.25
<i>H. griseus</i>	Clupeiformes	0.12	0.46	0.88		Osmeriformes	0.01	0.20	0.38
	Gadiformes	0	0.29	0.55		Perciformes	0.12	0.34	0.55
	Perciformes	0	0.10	0.30		Natantia	0	0.07	0.17
	Teuthida	0	0.15	0.38	Teuthida	0.01	0.21	0.40	
<b>Lamniformes</b>					<i>C. coelolepis</i>	Natantia	0	0.47	0.43
<i>A. vulpinus</i>	Teleostei	0.02	0.50	0.96		Teleostei	0.31	0.17	0.65
	Teuthida	0.04	0.50	0.97		Teuthida	0.06	0.35	0.59
<b>Myliobatiformes</b>					<i>D. licha</i>	Cephalopoda	0	0.16	0.36
<i>G. altavela</i>	Clupeiformes	0	0.33	0.65		Gadiformes	0	0.14	0.29
	Perciformes	0	0.34	0.65		Myctophiformes	0	0.19	0.38
	Anomura	0	0.33	0.65		Natantia	0.14	0.31	0.48
<i>M. aquila</i>	Teleostei	0	0.19	0.39		Selachii	0	0.20	0.40
	Anomura	0	0.20	0.38	(small sharks)				
	Bivalvia	0	0.20	0.40	<i>O. centrina</i>	Brachyura	0.03	0.39	0.75
	Gasteropoda	0	0.20	0.39		Polychaeta	0.25	0.60	0.97
	Teuthida	0	0.20	0.39	<i>S. acanthias</i>	Natantia	0	0.27	0.55
<i>M. mobular</i>	Clupeiformes	0.02	0.34	0.59		Octopoda	0	0.28	0.57
	Euphausiacea	0	0.10	0.19		Teleostei	0.10	0.45	0.77
	Myctophiformes	0.30	0.56	0.88	<i>S. rostratus</i>	Teuthida	0	0.31	0.63
<b>Rajiformes</b>						Tunicata	0.37	0.69	1.01
<i>D. oxyrinchus</i>	Anomura	0	0.26	0.50	<b>Torpediniformes</b>				
	Cephalopoda	0	0.24	0.47	<i>T. marmorata</i>	Cephalopoda	0	0.17	0.38
	Natantia	0	0.28	0.54		Clupeiformes	0	0.09	0.24
	Teleostei	0	0.21	0.44		Gadiformes	0	0.11	0.31
<i>L. naevus</i>	Anguiliformes	0	0.23	0.51		Myctophiformes	0	0.05	0.15
	Natantia	0.14	0.52	0.92		Perciformes	0.31	0.58	0.82
	Pleurenctiformes	0	0.25	0.53	<i>T. nobiliana</i>	Gadiformes	0	0.34	0.73
<i>R. asterias</i>	Brachyura	0.43	0.55	0.67		Polychaeta	0.27	0.66	1.03
	Mollusca	0	0.05	0.13	<i>T. torpedo</i>	Anguiliformes	0.02	0.20	0.36
	Natantia	0.23	0.38	0.53		Clupeiformes	0	0.06	0.17
	Teleostei	0	0.02	0.06		Perciformes	0	0.16	0.31
<i>R. clavata</i>	Brachyura	0.30	0.47	0.64		Pleurenctiformes	0	0.11	0.24
	Cephalopoda	0	0.08	0.22		Mollusca	0	0.15	0.29
	Clupeiformes	0	0.08	0.21	Natantia	0	0.07	0.17	
	Gadiformes	0	0.06	0.15	Polychaeta	0.09	0.24	0.38	
	Natantia	0.02	0.26	0.49					
Perciformes	0	0.05	0.13						

plementary approaches is probably the only way to advance our knowledge about their ecological role.

Overall, the shark species seem to have similar diets, mainly composed of fishes and cephalopods. However, they may also include other sharks in their

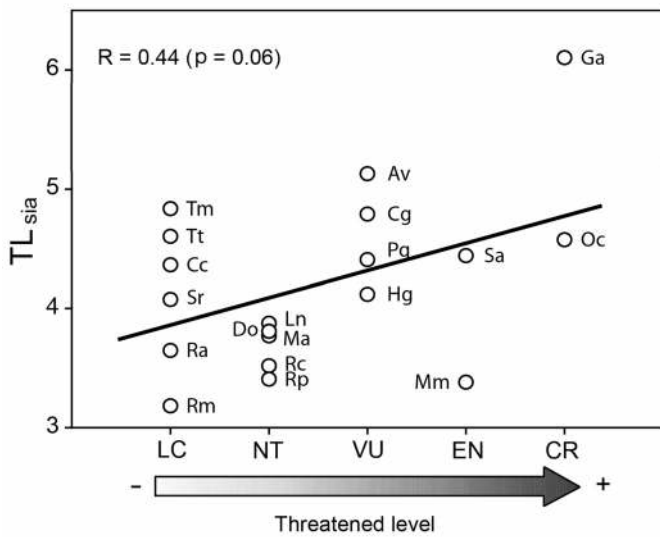


Fig. 5. Relationships between the threatened category according to the IUCN conservation status (Abdul Malak et al. 2011) and the trophic level of 19 elasmobranch species estimated with stable isotope analysis (TL<sub>sia</sub>): *Prionace glauca* (Pg), *Hexanchus griseus* (Hg), *Alopias vulpinus* (Av), *Centrophorus granulosus* (Cg), *Centroscyrnus coelolepis* (Cc), *Oxynotus centrina* (Oc), *Squalus acanthias* (Sa), *Somniosus rostratus* (Sr), *Dipturus oxyrinchus* (Do), *Leucoraja naevus* (Ln), *Raja asterias* (Ra), *R. clavata* (Rc), *R. montagui* (Rm), *R. polystigma* (Rp), *Gymnura altavela* (Ga), *Myliobatis aquila* (Ma), *Mobula mobular* (Mm), *Torpedo marmorata* (Tm) and *T. torpedo* (Tt). LC: Least Concern; NT: Not Threatened; VU: Vulnerable; EN: Endangered; CR: Critically Endangered

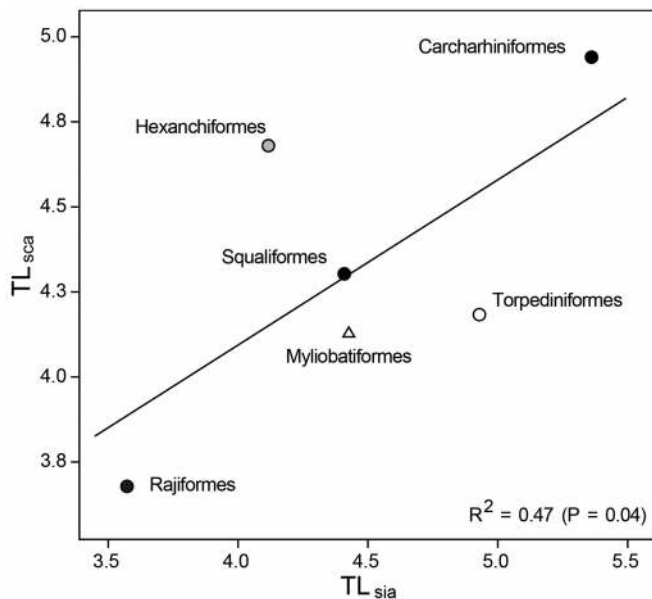


Fig. 6. Relationships between the trophic level estimated with stomach content analysis (TL<sub>sca</sub>) and with stable isotope analysis (TL<sub>sia</sub>) for Carcharhiniformes, Hexanchiformes, Squaliformes, Myliobatiformes, Torpediniformes and Rajiformes from the western Mediterranean Sea

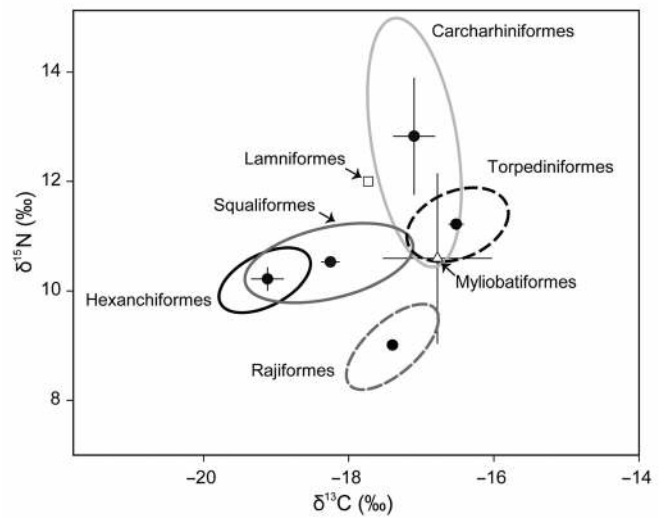


Fig. 7. Mean ± SD of δ<sup>15</sup>N and δ<sup>13</sup>C values and the standard ellipse areas of 7 elasmobranch orders from the western Mediterranean Sea

diet, such as observed in the kitefin shark *Dalatias licha* (Squaliformes) (Navarro et al. 2014), or polychaetes, crustaceans and shark eggs such as in the angular roughshark *Oxynotus centrina* (Squaliformes) (Barrull & Mate 2001). The little sleeper shark *Somniosus rostratus* (Squaliformes) is also a specialist predator that feeds mostly on cephalopods, especially squids (Golani 1986). In contrast to sharks, the skates analysed in the present study (Rajiformes) feed principally on crustaceans, which is consistent with Ebert & Bizzarro (2007), who showed that skates are epibenthic predators specializing on invertebrates. Stingrays (Myliobatiformes) prey on different trophic groups, while *Gymnura altavela* (Myliobatiformes) feeds mainly on fishes such as other butterfly rays (Yokota et al. 2013). *Myliobatis aquila* (Myliobatiformes) feeds on molluscs (Capapé et al. 1992, Jardas et al. 2004), and *Mobula mobular* (Myliobatiformes) filters small pelagic crustaceans through modified gills (Couturier et al. 2012). Electric rays (Torpediniformes) feed mainly on fishes that they capture actively through electric discharges (Lowe et al. 1994). The largest electric rays show high TLs, similar to the TL reported for the Mediterranean by Stergiou & Karpouzi (2001).

Differences observed in the diet composition between taxonomic orders in this study may be explained by the available resources in the habitat exploited by each functional group (Barnett et al. 2012). Usually, when resources are shared in a restricted environment, species will adapt and modify their feeding behaviour to coexist in the same area (Lowe

et al. 1996, Heithaus 2001, Motta & Wilga 2001, Dean et al. 2007). In this way, elasmobranchs can feed on different prey groups and reduce competition for available food resources (Carrassón & Cartes 2002, Heupel et al. 2007, Navarro et al. 2014).

Understating the trophic position of elasmobranchs is important to investigate their ecological role in relation to other organisms in the ecosystem (Cortés 1999, Stergiou & Karpouzi 2001). We found differences in the TLs between taxonomic orders using both the SCA and SIA approaches. Sharks, in particular those of the orders Carcharhiniformes and Lamniformes, showed the highest TLs close to cetaceans, seabirds and other marine fishes such as bluefin tuna *Thunnus thynnus* and the anglerfishes *Lophius budegassa* and *L. piscatorius* in Mediterranean ecosystems (Coll et al. 2006, Navarro et al. 2011, Tecchio et al. 2013). This highlights their potential role as apex predators in the marine Mediterranean ecosystem (Ferretti et al. 2008). The results from the SIA showed that, among sharks, *Galeorhinus galeus* (Carcharhiniformes) and *Centrophorus granulosus* (Squaliformes) had the highest TLs. This was not as clearly observed in the SCA results, likely due to the low number of stomach samples available. Electric rays (Torpediniformes) also showed high TLs, probably because they selectively feed on fishes with high  $\delta^{15}\text{N}$  content. These results suggest that electric rays may also play an important role as top predators in the Mediterranean Sea. In addition, electric rays (*Torpedo marmorata*, *T. torpedo* and *T. nobiliana*) have similar TLs to sharks of the order Squaliformes, and these values were in some cases higher, i.e. *Hexanchus griseus* or *Centroscyrnus coelolepis*. Skates (Rajiformes) had a significantly lower TL (TL<sub>sca</sub>) than the other elasmobranchs we studied, illustrating that they could be considered as mesopredators in the western Mediterranean Sea. Our results differed from those of Ebert & Bizzarro (2007), mainly due to the absence of small demersal sharks in the comparative analyses. In the NW Mediterranean Sea, skates may occupy a similar TL to small sharks such as *Galeus melastomus*, *Etmopterus spinax* and *Scyliorhinus canicula* (Cortés 1999, Polunin et al. 2001, Albo-Puigserver et al. 2015).

For many fish species and sharks, there is a positive relationship between body size and trophic position (Romanuk et al. 2011, Heithaus et al. 2013). In the present study, this positive relationship was significant in Torpediniformes and Squaliformes, whereas in Rajiformes and Hexanchiformes, this relationship was not significant. These results highlight the fact

that size may not always be a good proxy for trophic position in elasmobranchs.

Stingrays (Myliobatiformes) display a wide trophic range. However, due to the small sample size it was not possible to determine their trophic width based on the isotopic area. Despite this, we observed that these species play different ecological roles due to their diverse feeding strategies: *M. mobular* is an epipelagic manta ray that feeds on zooplankton (Couturier et al. 2012), and its TL is expected to be lower than other elasmobranchs; *M. aquila* has a TL similar to skates, probably because it feeds on organisms of low TL such as filter feeding molluscs as was previously documented in other eagle rays such as *Myliobatis freminvillei*, *M. australis* and *M. californica* (Jacobsen & Bennett 2013). In contrast to the other Myliobatiformes of our study, *G. altavela* showed a TL similar to other sharks, such as *G. galeus*, which mainly feeds on bony fishes.

Although the trophic niche width differed between orders, we found a clear overlap in the trophic niche between them with the exception of Rajiformes. The species of this order have a smaller trophic niche, and it was therefore segregated from the other elasmobranch orders in the present study. This occurs because the species of this group have similar feeding behaviour and consequently have a lower trophic width, similar to other specialist species, such as tunnids in the Mediterranean Sea (Medina et al. 2015).

Overfishing and habitat degradation may have profoundly altered populations of sharks, skates and rays (Stevens et al. 2000, Ferretti et al. 2010, Dulvy et al. 2014). These alterations have led to a substantial increase in the number of threatened species, and the study of species at risk has become more challenging due to the difficulty in obtaining a sufficient number of samples (Smart et al. 2013, Barriá et al. 2015). The effect of the disappearance of top predators in the Mediterranean Sea is only partially known and may involve changes in species composition in the prey community or other top predators as has been documented in other marine ecosystems (Stevens et al. 2000). In the western Mediterranean Sea, several elasmobranchs, including demersal species, have been fished as non-target species in demersal trawlers without knowing the real consequences of the removal of these organisms from the food web (Navarro et al. 2015).

Based on our results, the potential effects of the decline of elasmobranchs could be different between species. Although we observed differences in the trophic position between species, sharks (Carcharhiniformes, Hexanchiformes, Lamniformes and

Squaliformes) and rays (Myliobatiformes and Torpediniformes) can be considered as top predators, and their declines may generate trophic cascades and changes in the community structure such as releases of mesopredator prey populations (Myers et al. 2007). In contrast, skates (Rajiformes) can be considered mesopredators, as they link the different food web compartments and TLs in marine ecosystems (Matich et al. 2011). These cascade effects may be more complex than simply top-down or bottom-up alterations in the food web considering the overall behaviour of predators, such as migration timing and duration of residency by sex or age (Navia et al. 2010, Mejía-Falla & Navia 2011, Bornatowski et al. 2014). These may have strong effects on behaviour, health and survival, and ultimately may have ecological impacts within populations of other species with which they interact (Ritchie et al. 2012, Andrews & Harvey 2013).

Studies of feeding ecology can contribute to our understanding of the community structure and ecological interactions of elasmobranchs in marine ecosystems (Winemiller 1989, Krebs 1999). Here we have shown that the use of stomach content and isotopic analyses, in combination with extant reviews, may establish general resource use patterns and describe interspecific differences among poorly studied and difficult to sample uncommon and threatened elasmobranch fishes. Prior to this study, information on the trophic ecology of Mediterranean elasmobranchs was only available for a few species, a limitation that had precluded the inclusion of particular species in previous food-web models in the Mediterranean Basin. Our results include dietary information that will allow an exploration of the ecological role of these elasmobranchs in the Mediterranean Sea. Moreover, by using ecological models, the potential effect of fishing activity can be evaluated (such as in Coll et al. 2013). These results can provide useful data for managers to conduct an appropriate assessment and thus conservation actions of these species.

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