



# Feeding ecology of a Mediterranean endemic mesopredator living in highly exploited ecosystems

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## ABSTRACT

Knowledge of marine predator trophic ecology is essential for defining their ecological role and trophic position in ecosystems. Based on their trophic habits, sharks and batoids occupy higher and medium trophic levels in the food webs, although differences in the trophic preferences exist between species. They are important organisms in marine ecosystems by maintaining the species below them in the food chain and serving as an indicator for ocean health. In comparison to sharks, batoids usually receive less research attention, with very little diet information available. This is true of the speckled ray (*Raja polystigma*), one of the three endemic batoids in Mediterranean waters. Here, by combining analyses of stomach contents and stable isotopes, we examined the trophic ecology (dietary composition and trophic position) of this ray in the north-western Mediterranean Sea. We also compared its trophic niche with the trophic position of other sympatric elasmobranchs present in same marine ecosystem. The results revealed that *R. polystigma* mainly consumes shrimps and to a lesser extent crabs, fin-fish, cephalopods, polychaetes and, surprisingly, small demersal sharks. We also found that *R. polystigma* shows similar trophic position to other crustacean-consumer elasmobranchs such as rays and small demersal sharks. The results of this study provide new insights into the ecological role of this endemic ray species in the Mediterranean Sea.

## 1. Introduction

Identifying the trophic ecology of marine predators is necessary to defining their ecological role in ecosystems (Coll et al., 2013; Ferretti et al., 2013). Based on their trophic habits, sharks and batoids are placed at higher trophic positions within the marine food webs, although differences in the main feeding strategies exist between species (Barría et al., 2015; Wetherbee et al., 2004; Cortés, 1999). However, trophic information for most elasmobranch species, in special for demersal species, is scarce (Cortés, 1999). In comparison to sharks, batoids usually receive less attention in research (Gallagher et al., 2012; Barría et al., 2015); quantitative dietary information is available for few of all existing batoids, and for the majority of these species, this information comes from only a single study (Ebert and Bizzarro, 2007; Jacobsen and Bennett, 2013).

Despite the fact that the Mediterranean Sea hosts a rich fauna of batoids and is considered a hotspot of diversity of elasmobranchs (Malak, 2011; Dulvy et al., 2014), studies focused on describing the trophic habits of batoids are scarce in this basin (e.g. Valls et al., 2011;

Barría et al., 2015). Nevertheless, this region has been described as one of the most dangerous areas for these marine predators (Malak, 2011) due to threats associated with human activities such bottom-trawler and gill net fishing activity (Coll et al., 2013), and more than half of the batoids described in the Mediterranean Sea are in peril (Dulvy et al., 2016). While none of them are the target species of fisheries, many are captured incidentally in the Mediterranean Sea. This risk appears to be greater in the western basin, where human impact associated to fishing mortality is high (Coll et al., 2013). Consequently, there is a necessity to improve the basic ecological knowledge of these marine predators inhabiting this marine basin, including a better understanding of their trophic preferences (Ferretti et al., 2013).

Speckled ray (*Raja polystigma*) is one of the three endemic batoids in the Mediterranean waters (Serena et al., 2005; Dulvy et al., 2016). This species is a small-size demersal batoid present in continental shelf habitats characterized by the presence of sand or mud, mainly at depths between 100 and 400 m (Serena et al., 2005; Ungaro et al., 2015). Although *R. polystigma* is considered as Least Concern by the International Union for Conservation of Nature (IUCN), the information

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available about its basic ecology is very scarce (Ungaro et al., 2015). Its distribution overlaps in areas of intense trawling, and thus it is frequently captured as bycatch in the western Mediterranean (Ungaro et al., 2015). Moreover, fisheries negatively affect the target species of an ecosystem but also non-target species, normally reducing the abundance of large predators but increasing the number of small prey (Daan et al., 2005). Thus, knowing the diet of each species in an ecosystem is of vital importance to evaluate the impact of fishing activities. However, non-target species such as *R. polystigma* are usually not a subject of research due to their lack of economic value (Pope et al., 2000), and the trophic information about *R. polystigma* is currently only based on a few unspecific studies. These studies indicated that the diet of *R. polystigma* is mainly based on crustaceans (Vannucci et al., 2006; Valls et al., 2011), particularly of Natantia decapods (Valls et al., 2011), but teleosts, cephalopods, polychaetes and isopods, among others, have also been recorded in *R. polystigma* stomachs (Vannucci et al., 2006; Valls et al., 2011; Barria et al., 2015).

The study of the diet of marine predators is commonly determined through the analysis of stomach contents (Hyslop, 1980). While this methodology allows high levels of taxonomic prey resolution, batoids often have empty stomachs and the prey present in the stomachs are often skewed towards those that are difficult to digest (Hyslop, 1980; Vaudo and Heithaus, 2011; Kim et al., 2012). In addition, stomach content analyses require large sample sizes to quantify long-term dietary patterns (Hyslop, 1980; Cortés, 1999), which are difficult to achieve for most species of elasmobranchs (Stergiou and Karpouzi, 2002; MacNeil et al., 2005). The analysis of stable isotopes of nitrogen ( $\delta^{15}\text{N}$ ) and carbon ( $\delta^{13}\text{C}$ ), and isotopic mixing models have been used as complementary tools to investigate time-integrated dietary records of marine predators (Shiffman et al., 2012; Kim et al., 2012; Layman et al., 2012). For example, stable isotopic values in elasmobranch muscle reflect long-term dietary information ( $\sim 1$  year; MacNeil et al., 2005; Logan and Lutcavage, 2010). The combination of stomach content and isotopic analyses is valuable for a better understanding of the trophic ecology of marine organisms (Shiffman et al., 2012).

In the present study, we examined the trophic ecology of *R. polystigma* in the western Mediterranean Sea using a combined approach of stomach contents and stable isotopes (nitrogen and carbon isotopic values) in muscle tissue. We then compared trophic niche and trophic level with published trophic information on other sympatric batoids present in the ecosystem. Our study provides new information about the ecological role of *R. polystigma* within the marine ecosystems, offering new data on how this demersal predator exploits available resources.

## 2. Material and methods

### 2.1. Study area and sampling procedures

This study was carried out in the north-western Mediterranean Sea (Fig. 1). The Mediterranean Sea is considered as an oligotrophic sea due mainly to the loss of nutrient-enriched waters to the Atlantic through the Gibraltar Strait (Estrada, 1996). However, the north-western Mediterranean waters are characterized by the presence of the Liguro-Provençal-Catalan Current that follows the continental shelf towards the west and south according to the geostrophic equilibrium. This, combined with the winter disruption of the thermocline and the river discharge of the Ebro and other rivers, causes a huge input of nutrients to the superficial layers of the Catalan Sea (Salat, 1995; Estrada, 1996).

Between 2015 and 2018, a total of 47 mature *R. polystigma* individuals were captured opportunistically as by-catch during commercial fishing operations of a hake trawl fishery at 125–145 m depth in a fishing management area of 51 km<sup>2</sup> (LLUS project; Institut de Ciències del Mar – CSIC; Fig. 1). After collection, all individuals were frozen at  $-20^\circ\text{C}$  until their dissection. In the laboratory, from each collected individual, we measured the body weight ( $\pm 1$  g), the total body length

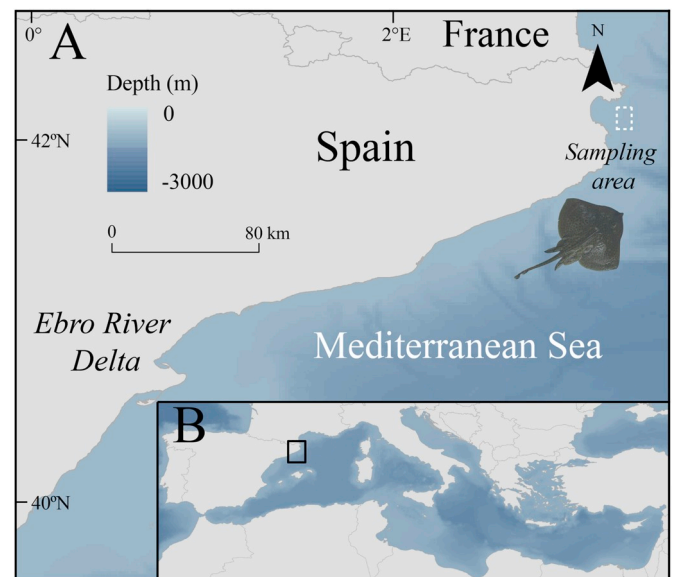


Fig. 1. Study area and sampling location (white square) in the Catalan Sea, north-western Mediterranean. A picture of *Raja polystigma* is also displayed (C. Barria).

( $\pm 0.1$  cm), the anal length ( $\pm 0.1$  cm), and the disk width ( $\pm 0.1$  cm). The stomach and a small portion of dorsal muscle (without skin or cartilage) were extracted from each specimen and stored at  $-20^\circ\text{C}$  until analysis. All collected individuals were mature based on their total body length ( $>40$ – $53$  cm was considered mature; see Serena et al., 2005; Ungaro et al., 2015).

### 2.2. Stomach content analysis

At the laboratory, each stomach was weighed and its contents were extracted. Prey items found in each stomach were separated and identified to the lowest taxonomic level possible and weighed. We did not consider prey items found in the mouth to avoid a potential bias derived from the possible capture in the trawling net. Whenever fragments were observed, the number of individuals was registered as the smallest number so as not to overestimate the occurrence of prey items. Teleost prey were identified by combining visual identification directly or by their otoliths (Tuset et al., 2008). Crustacean prey were identified using the reference guide of Zarquiey-Alvarez (1968).

To assess the importance of different prey in the diet, we calculated three trophic metrics: %FO (frequency of occurrence), %N (contribution by number of each prey) and %W (wet weight of each prey). Moreover, the %IRI (percentage of the index of relative importance; Pinkas, 1971; Cortés, 1997) of each prey *i* in the diet of *R. polystigma* was also calculated:

$$\%IRI_i = \%FO_i \cdot (\%N_i + \%W_i) \quad (1)$$

All calculations were based on the number of non-empty stomachs. The vacuity index, %V = percentage of empty stomachs, was also calculated.

### 2.3. Stable isotope analysis

Muscle samples were dried, powdered, urea extracted and 0.28–0.33 mg of each sample was packed into tin capsules. Isotopic analyses were performed at the Laboratory of Stable Isotopes of the Estación Biológica de Doñana (LIE-EBD, Sevilla, Spain). Encapsulated samples 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). Urea content was removed from the muscle samples. The C:N ratio of all tissues was always lower than 3.5‰, and hence, no correction of the  $\delta^{13}\text{C}$  values was required to account for the presence of lipids in muscle samples (Logan et al., 2008).

#### 2.4. Comparison of the trophic position with other sympatric batoid species

To understand the ecological role of *R. polystigma* in relation to other batoid species in the ecosystem we compared its trophic position (based on the isotopic values) with published isotopic information of Myliobatiformes (*Gymnura altavela*, *Myliobatis aquila*, *Mobula mobular*), Rajiformes (*Dipturus oxyrinchus*, *Leucoraja naevus*, *Raja asterias*, *Raja clavata*, *Raja montagui*, *Raja polystigma*) and Torpediniformes (*Torpedo marmorata*, *Torpedo nobiliana*, *Torpedo torpedo*) present in the study area (from Barría et al., 2015).

#### 2.5. Statistical analyses

Stomach content differences (%W) were analysed applying semi-parametric permutation multivariate analyses of variance tests (PERMANOVA tests). Also, we compared the stable isotopic values between sexes by using Student's T tests.

To estimate the diet composition of *R. polystigma* based on isotopic values, MixSIAR Bayesian isotopic mixing model was applied (Stock and Semmens, 2016). Models were run with a generalist type prior, 3 Markov chain Monte Carlo (MCMC) chains of 300,000 draws and a burn-in of 200,000 draws. The convergence of models was checked using both Gelman-Rubin and Geweke diagnostics (Stock and Semmens, 2016; Stock et al., 2018). To run the MixSIAR model, potential preys were selected in accordance with the information obtained from the stomach content analysis, namely Natantia ( $\delta^{15}\text{N}$ :  $7.82 \pm 0.66$ ;  $\delta^{13}\text{C}$ :  $19.39 \pm 0.90$ ), Reptantia ( $\delta^{15}\text{N}$ :  $7.18 \pm 0.52$ ;  $\delta^{13}\text{C}$ :  $16.08 \pm 1.80$ ) and teleosts ( $\delta^{15}\text{N}$ :  $8.61 \pm 0.93$ ;  $\delta^{13}\text{C}$ :  $19.50 \pm 0.80$ ). Isotopic values of potential prey used were obtained from an isotopic library containing 128 species captured in the study area (IsoLibrary Database; Institut de Ciències del Mar CSIC; Barría et al., 2015). ANOVA and Tukey post-hoc tests indicated that these three prey groups differed in their  $\delta^{15}\text{N}$  values (teleosts showed higher  $\delta^{15}\text{N}$  values than Natantia and Reptantia;  $F = 3.54$ ,  $p < 0.001$ ) and  $\delta^{13}\text{C}$  values (Reptantia showed higher  $\delta^{13}\text{C}$  values than Natantia and teleosts;  $F = -2.72$ ,  $p < 0.001$ ).

The accurate use of isotopic mixing models is always limited by the correct knowledge and application of the diet tissue discrimination factors of  $\delta^{15}\text{N}$  ( $\Delta^{15}\text{N}$ ) and  $\delta^{13}\text{C}$  ( $\Delta^{13}\text{C}$ ) and (see Bond and Diamond, 2011). Different diet tissue discrimination factors have been previously used in other studies dealing with elasmobranch muscle (e.g. Hussey et al., 2010; Tilley et al., 2013). Here, because no specific discrimination factor for *R. polystigma* is available, we applied the combination of different diet tissue discrimination factors used in other elasmobranchs to obtain an overall picture of the potential prey contributions as a result of varying DTDF (Tilley et al., 2013; Albo-Puigserver et al., 2015). Specifically, we used three combinations of diet-tissue-discrimination factor values of  $\Delta^{15}\text{N} = 1.95 \pm 0.26\text{‰}$  and  $\Delta^{13}\text{C} = 0.49 \pm 0.32\text{‰}$  (Hussey et al., 2010),  $\Delta^{15}\text{N} = 3.39 \pm 3.03\text{‰}$  and  $\Delta^{13}\text{C} = -0.22 \pm 2.33\text{‰}$  (Tilley et al., 2013) and  $\Delta^{15}\text{N} = 2.29 \pm 0.22\text{‰}$  and  $\Delta^{13}\text{C} = 0.90 \pm 0.33\text{‰}$  (Hussey et al., 2010).

### 3. Results

#### 3.1. Diet composition based on stomach contents

All of the 47 stomachs analysed presented contents; hence, the vacuity index was 0%. The results of the stomach content analysis showed that the diet of *R. polystigma* was similar between sexes (Tables 2 and 3; PERMANOVA tests, pseudo- $F = 3.43$ ,  $p = 0.002$ ; pairwise tests, pseudo- $T = 1.08$ ,  $p = 0.11$ ). Specifically, based on the stomach content results the diet of this ray was mainly based on decapods of the suborder Natantia, although many other prey from different taxonomic groups were also found (Table 2). Within the group Natantia, the presence of *Solenocera membranacea* and especially *Processa* sp. were particularly important, although the highest %IRI was obtained from unidentified Natantia individuals (Table 2). The suborder Reptantia was also important because of the number of species found (*Goneplax rhomboides*, *Liocarcinus depurator* and *Monodaeus couchii*), yet the weight and number of individuals were lower than prey of the group Natantia (Table 2). We also found species of teleosts (*Argentina sphyraena*, *Merluccius merluccius* and *Trisopterus minutus*), polychaetes (*Aphrodita* sp.) and a cephalopod (*Illex coindetii*). The presence of small embryos of the demersal shark *Scyliorhinus canicula*, found in two different stomachs (see SM1). Many stomachs also contained material of anthropogenic origin (Table 2).

#### 3.2. Stable isotopes

We did not find isotopic differences ( $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$ ) between males and females (Table 1). Isotopic mixing models showed that the diet of *R. polystigma* is primarily composed of the Natantia crustaceans (mean = 86–97%), followed by Reptantia crustaceans (mean = 5–10%) and teleosts (mean = 2–4%; Fig. 2).

#### 3.3. Comparison of the trophic position with other sympatric batoid species

When comparing the isotopic values of *R. polystigma* with those of other sympatric batoid species in the study area, we found that *R. polystigma* has similar isotopic values to other batoids such as *L. naevus* or *Dipturus oxyrinchus* but with a higher trophic position based on their  $\delta^{15}\text{N}$  values (Fig. 3). Nevertheless, the trophic position based on the  $\delta^{15}\text{N}$  values of *R. polystigma* is lower than those of batoids from the family Torpedinidae such as *Torpedo torpedo* (Fig. 3).

### 4. Discussion

Determining the biological and ecological characteristics of marine species, and concretely of vulnerable and endangered species, is of vital importance to understand their trophic or ecological role in the environment. Batoids can play significant ecological roles in the marine food webs due to their abundance and species diversity (Gallagher et al., 2012; Barría et al., 2015). In the case of *R. polystigma*, the few studies conducted on this species and the probable confusion with other batoid species such as *R. montagui* (Serena et al., 2005) contribute to the

**Table 1**

Mean  $\pm$  standard deviation of the different morphometric measurements and stable isotopic values of males and females of *Raja polystigma* from the north-western Mediterranean Sea.

Measure	Males (n = 26)	Females (n = 21)
Total Weight (g)	807.29 $\pm$ 211.63	1023.81 $\pm$ 380.26
Total Length (cm)	52.55 $\pm$ 3.93	54.54 $\pm$ 5.78
Anal Length (cm)	22.68 $\pm$ 1.93	24.49 $\pm$ 2.98
Disk Length (cm)	26.38 $\pm$ 2.06	27.83 $\pm$ 3.06
Disk Width (cm)	33.07 $\pm$ 2.45	35.43 $\pm$ 3.67
$\delta^{15}\text{N}$ (‰)	10.27 $\pm$ 0.45	10.12 $\pm$ 0.37
$\delta^{13}\text{C}$ (‰)	-17.05 $\pm$ 0.36	-17.01 $\pm$ 0.31

Table 2

Diet composition of *Raja polystigma* expressed in frequency of occurrence (%FO), frequency in number (%N), wet weight (%W) and Index of Relative Importance in percentage (%IRI). N = 47 individuals.

PREY	%F	%N	%W	%IRI
POLYCHAETA				0.01
<i>Aphrodita</i> sp.	2.13	0.41	0.06	0.01
CEPHALOPODA				0.07
<i>Illex coindetii</i>	2.13	0.41	2.90	0.07
NATANTIA				97.27
<i>Processa</i> sp.	40.43	25.00	19.00	18.01
<i>Solenocera membranacea</i>	23.40	6.15	9.30	3.66
Unidentified	80.85	57.79	34.54	75.60
REPTANTIA				0.47
<i>Goneplax rhomboides</i>	2.13	0.41	0.71	0.02
<i>Liocarcinus depurator</i>	4.26	0.82	0.20	0.04
<i>Monodaeus couchii</i>	2.13	0.41	0.78	0.03
Unidentified	10.64	2.05	1.40	0.37
TELEOSTEI				1.79
<i>Argentina sphyraena</i>	2.13	0.41	12.81	0.28
<i>Merluccius merluccius</i>	8.51	2.05	15.13	1.48
<i>Trisopterus minutus</i>	2.13	0.41	0.81	0.03
CHONDRICHTHYES				0.26
<i>Scyliorhinus canicula</i> (embryos)	6.38	2.46	1.59	0.26
OTHERS				0.13
Anthropogenic content	6.38	1.23	0.78	0.13

Table 3

Percentage of the index of relative importance (%IRI) of the main taxonomic groups in the diet of *Raja polystigma* expressed in %F, %N, %W and %IRI based on the sex of the individuals (N males = 26; N females = 21).

PREY	Males (n = 26)	Females (n = 21)
Cephalopoda	0.35	0.03
Natantia	95.51	97.44
Reptantia	0.73	0.50
Teleostei	2.80	1.70
Chondrichthyes	0.55	0.11
Anthropogenic content	0.06	0.21

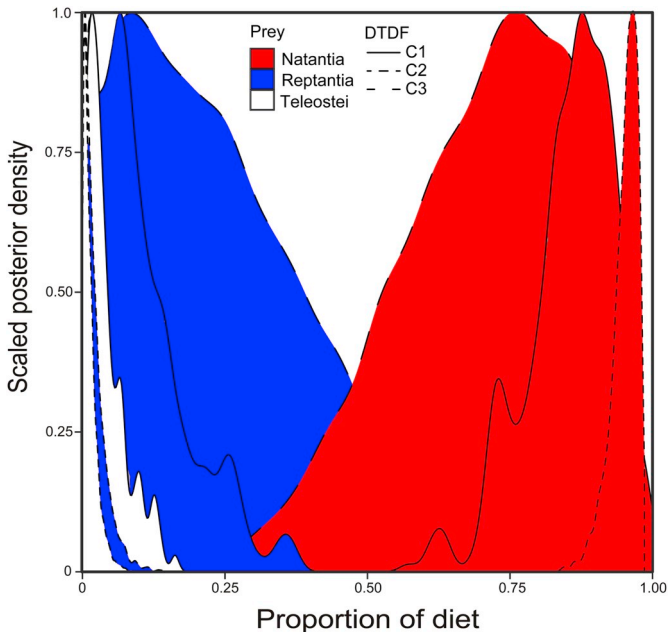


Fig. 2. Results of the MixSIAR model showing estimated prey group contributions to the diet of *Raja polystigma* using three combinations of diet-tissue discrimination factors DTDF (C1:  $\Delta^{15}\text{N} = 1.95 \pm 0.26\text{‰}$ ,  $\Delta^{13}\text{C} = 0.49 \pm 0.32\text{‰}$ , Hussey et al., 2010; C2:  $\Delta^{15}\text{N} = 3.39 \pm 3.03\text{‰}$ ,  $\Delta^{13}\text{C} = -0.22 \pm 2.33\text{‰}$ , Tilley et al., 2013; C3:  $\Delta^{15}\text{N} = 2.29 \pm 0.22\text{‰}$ ,  $\Delta^{13}\text{C} = 0.90 \pm 0.33\text{‰}$ ).

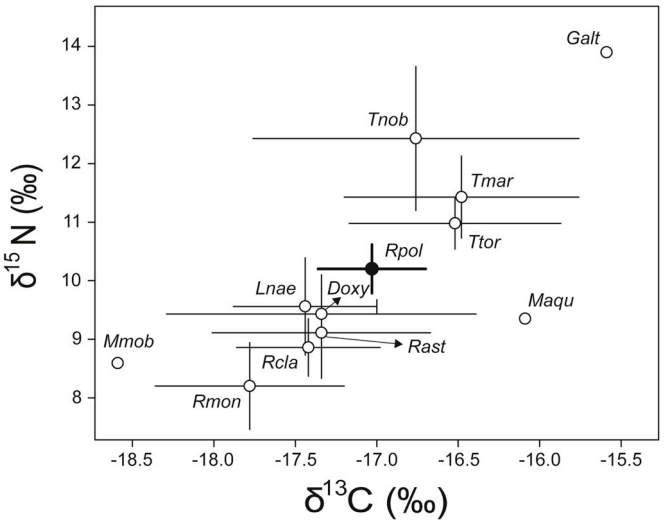


Fig. 3. Mean and standard deviation of  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  values for the batoid community of the north-western Mediterranean: Myliobatiformes (Galt: *Gymnura altavela*, Maqu: *Myliobatis aquila*, Mmob: *Mobula mobular*), Rajiformes (Doxy: *Dipturus oxyrinchus*, Lnae: *Leucoraja naevus*, Rast: *Raja asterias*, Rclla: *Raja clavata*, Rmon: *Raja montagui*, Rpol: *Raja polystigma*) and Torpediniformes (Tmar: *Torpedo marmorata*, Tnob: *Torpedo nobiliana*, Ttor: *Torpedo torpedo*). Isotopic data from all batoid species except *R. polystigma* was extracted from Barría et al. (2015).

scarcity of available information about basic aspects of *R. polystigma* ecology. For this reason, the information presented in this paper could be useful to complement the little existing data on this species, endemic to the Mediterranean Sea.

With the combination of two complementary techniques, stomach content analysis and stable isotope analysis, we have described the diet of *R. polystigma*. Stomach content analysis provides short-term trophic information whereas stable isotopes allow us to obtain long-term information, although the taxonomic resolution is lower (Hyslop, 1980; Shiffman et al., 2012). Both methodologies indicate that the diet of *R. polystigma* is primarily composed of Natantia crustaceans. This reconfirms the results obtained in previous studies (Valls et al., 2011; Barría et al., 2015), albeit in this study the contribution of Natantia to the diet of *R. polystigma* showed higher IRI% values. The presence of *Processa* sp. and *Solenocera membranacea* in the diet suggests that *R. polystigma* capture these prey from their dens in the sediment. These two crustacean species inhabit soft bottoms also occupied by *R. polystigma* and are notably abundant in the Catalan Sea, with maximum abundances at depths of 200–400 m (Abelló et al., 1988). Thus, *R. polystigma*, which is distributed at these depths, would have easy access to these resources. The next groups in terms of importance in the diet are the Reptantia crustaceans and teleosts. Many of the teleosts found in the stomachs of *R. polystigma* were only partial specimens due to digestive activity, and in some cases we only found otoliths; thus, the importance in weight of this group may be slightly higher. In much lower proportions, we obtained other prey such as polychaetes and cephalopods, but the most surprising prey was the embryos of the demersal shark *Scyliorhinus canicula* (see SM1). This is the first time that a chondrichthyan has been described in the diet of *R. polystigma*. The fact that this species was found in two different stomachs would indicate that it is not an isolated event. *S. canicula* is the most abundant demersal shark in the Mediterranean Sea (Compagno, 1984; Navarro et al., 2013; Barría et al., 2018). Moreover, this is an oviparous species that lays eggs year-round in soft bottoms (Compagno, 1984) frequented by *R. polystigma*. All of these characteristics suggest that embryos of *S. canicula* could be an easy-access prey for *R. polystigma*.

Knowing the trophic niche of elasmobranchs is useful to understand their ecological role in relation to other species in the marine ecosystem



(Cortés, 1999; Barría et al., 2015). By comparing the trophic niche of *R. polystigma* with those of other sympatric batoid species of the Mediterranean, we observed that this ray showed similar values than other Mediterranean batoids with a crustacean-based diet habits. However, in the present study the isotopic values of this ray is different than the results published previously in the study area, in which *R. polystigma* showed similar values than *Mobula mobular* (Barría et al., 2015). This is probably because the individuals in the current study had a greater total mean length, and consequently larger mouths. In fact, three of the individuals captured in this study exceeded the maximum total length of 60 cm described previously for *R. polystigma* (Serena et al., 2005; Ungaro et al., 2015), reaching 63.1 cm. Larger sizes were detected in females, as many of them had larger gonads or even capsules. As previously noted in other batoid species, an increase in corporal size provokes changes in the contribution of different prey species or different size of the prey to the diet (White et al., 2004), consequently altering the trophic niche.

In conclusion, this study provides new information about the trophic ecology of *R. polystigma* in the north-western Mediterranean Sea and complements the knowledge described in previous studies. The two methodologies used indicated the great importance of Natantia crustaceans to the diet of *R. polystigma* and the lack of feeding differences between sexes. Furthermore, this study confirms the great benefits of combining two complementary methodologies like stomach content and stable isotope analyses with the purpose of studying the trophic ecology of batoids and other chondrichthyan. We strongly recommend further studies about the trophic ecology of *R. polystigma* in other Mediterranean areas and with immature individuals to analyse potential differences, due to the fact that batoids occupy several habitats throughout their life, generating variations in their ecological role depending on location, sex, size and availability of prey.

#### Declaration of competing interest

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

#### CRediT authorship contribution statement

**Ethan Coll-Calvo:** Data curation, Formal analysis, Writing - original draft, Writing - review & editing. **Claudio Barría:** Conceptualization, Methodology, Writing - review & editing. **Laura Recasens:** Conceptualization, Methodology, Writing - review & editing. **Joan Navarro:** Conceptualization, Methodology, Writing - review & editing.

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#### Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.marenvres.2020.104932>.

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