

# Feeding habits of three Batoids in the Levantine Sea (north-eastern Mediterranean Sea) based on stomach content and isotopic data

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*Understanding the diet of marine predators is essential to defining their trophic role in an ecosystem. Elasmobranchs (sharks and batoids) are considered pivotal components of marine food webs, and are often included in the top predator or mesopredator groups. However, in comparison with other Mediterranean areas, research focusing on marine predators inhabiting the Levantine Sea (eastern Mediterranean Sea) is very limited. Here, we examined the feeding habits (diet, trophic width and trophic position) of three endangered batoids (*Gymnura altavela* (Linnaeus, 1758), *Raja asterias* Delaroche, 1809 and *Raja clavata*, Linnaeus, 1758) coexisting in Iskenderun Bay (north-eastern Levantine Sea, Mediterranean Basin) by combining stomach content and stable isotope analyses. The results revealed clear differences in the trophic habits between them. Stomach contents showed differences in the diet between species, showing a clear feeding preference for teleosts in the case of *G. altavela* and a diet composed of fish and crustaceans in the case of *R. asterias* and *R. clavata*. In line with stomach content results, interspecific differences in the isotopic values and trophic levels were found. In particular, *G. altavela* was isotopically segregated from *R. asterias* and *R. clavata*, showing lower isotopic trophic width and higher trophic level. The results of this study provide new insights into the ecological role of these three endangered batoid species in the Levantine Sea and are of crucial importance for management and conservation of these species.*

**Keywords:** Levantine Sea, stable isotopes, trophic ecology, elasmobranchs

Submitted 6 March 2017; accepted 6 April 2017

## INTRODUCTION

Understanding the trophic ecology of a particular species is essential to determining its ecological role in marine ecosystems (Coll *et al.*, 2013; Ferretti *et al.*, 2013). Sharks and batoids are considered important components of marine food webs, often included in the top predator or mesopredator groups, although there are important differences in the diets among species (Cortés, 1999; Young *et al.*, 2015). In fact, the high diversity of feeding strategies makes the ecology of this entire marine group particularly complex to understand (Cortés, 1999). For this reason, the trophic role of these species is often unclear. To unravel this limitation, more trophic studies are essential, as they can help inform conservation strategies for threatened species (Ferretti *et al.*, 2013).

The Mediterranean Sea is considered a global hotspot of elasmobranch diversity, hosting ~7% of all elasmobranchs worldwide (Cavanagh *et al.*, 2007; Dulvy *et al.*, 2014). However, most of the batoids and shark species in the

Mediterranean Sea have declined in abundance and distribution, mainly due to human impacts (Ferretti *et al.*, 2010; Coll *et al.*, 2013). In fact, around 40% of the elasmobranchs are considered threatened in the Mediterranean Sea by regional assessments of the International Union for Conservation of Nature (IUCN) (Abdul Malak *et al.*, 2011; Bradai *et al.*, 2012).

In comparison with other Mediterranean areas, research focusing on elasmobranchs inhabiting the Levantine Sea (eastern Mediterranean Sea) is very limited (Cavanagh *et al.*, 2007), even though these waters host endemic, threatened and rare elasmobranchs. This is the case of the threatened batoids *Gymnura altavela* (spiny butterfly ray), with a vulnerable status, and the Mediterranean endemic *Raja asterias* (Mediterranean starry ray) and *Raja clavata* (thornback ray), both with a near threatened status based on the IUCN Red List (Vooren *et al.*, 2007; Serena *et al.*, 2015; Ellis *et al.*, 2016). In the Levantine Sea, these three batoid fishes are highly impacted by the demersal fisheries operating in coastal and deep-sea waters (Dalyan, 2012; Yeldan *et al.*, 2013; Yemişken *et al.*, 2014).

Regarding their trophic habits, previous studies based on stomach contents conducted in the western and central Mediterranean Sea indicated that these three batoids act as mesopredators in the ecosystem, exploiting a wide variety of

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resources including crustaceans, demersal fish and cephalopods (Cuoco *et al.*, 2005; Romanelli *et al.*, 2007; Valls *et al.*, 2011; Santic *et al.*, 2012; Navarro *et al.*, 2013). Although stomach content methodology permits a high level of taxonomic resolution in the identification of prey, batoids and sharks show a high frequency of empty stomachs. Moreover, the prey species that are often found in the stomachs are those of slower digestion rates, which could cause biases in the diet estimation (Hyslop, 1980). In addition, this conventional method requires a large number of stomachs to quantify diet. This can be difficult to obtain, especially for endangered species. The use of stable isotopes of nitrogen ( $\delta^{15}\text{N}$ ) and carbon ( $\delta^{13}\text{C}$ ) has been used as a complement to stomach content analysis to study the trophic ecology of elasmobranch species (e.g. Shiffman *et al.*, 2012). 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).  $\delta^{15}\text{N}$  values show a predictable increase from one trophic level to the next (Jennings *et al.*, 1997; Layman *et al.*, 2012).  $\delta^{13}\text{C}$  values show little change due to trophic transfer, but are useful indicators of dietary sources of carbon (Layman *et al.*, 2012).

In this study, we investigated the feeding ecology (diet, trophic width and trophic position) of three batoids (*G. altavela*, *R. asterias* and *R. clavata*) coexisting in Iskenderun Bay (north-eastern Levantine Sea, Mediterranean Basin) by combining stomach contents and stable isotope analyses. Our study provides new insights into the ecological role of these three endangered batoid species in the Levantine Sea.

## MATERIALS AND METHODS

### Study area and sampling procedure

The north-east Levantine Sea (Figure 1) has a wide continental shelf and shows high marine productivity influenced by local wind effects, upwelling movements and rich terrestrial nutrient inputs from the Asi River (Polat & Piner, 2002). The area includes a high richness of marine species within the eastern Mediterranean Sea (Bilecenoglu, 2016). The oceanographic conditions, such as the environmental conditions similar to tropical and sub-tropical regions, has promoted the colonization by invasive species from the Red Sea (Bilecenoglu, 2016).

A total of 13 *G. altavela*, 46 *R. asterias* and 26 *R. clavata* individuals were collected between September 2014 and April 2016 at a depth ranging from 33 to 450 m, by commercial trawl vessels in the Iskenderun Bay (Figure 1). All individuals were accidentally captured as by-catch of the fishing fleet. Individuals were taken to the lab in a freezer where body size (disc width length; DW, to the nearest mm) and weight (nearest g) were recorded.

### Stomach content analysis

All prey items presented in the stomachs were identified at the lowest taxonomic levels (copepods, decapods, cephalopods and teleosts). Per cent of number (%N), weight (%W) and frequency of occurrence (%F) of prey items were calculated and these values were utilized for calculating the Index of Relative Importance (IRI) of each prey item ( $\text{IRI} = \%F(\%N + \%W)$ ). The IRI was standardized using the formula:  $\%IRI = (\text{IRI} / \sum \text{IRI}) \times 100$  (Cortés, 1997). The vacuity index ( $v$ ; the

percentage of empty stomachs) and the percentage of fullness of stomachs (Fullness %) were also calculated. Levin's and Pianka's measures were used to determine niche breadth ( $B_i$ ) with the standardized niche breadth ( $B_A$ ), and niche overlap between the three batoid fishes (Colwell & Futuyma, 1971). Prey-specific abundance was calculated according to the following:  $P_i = (\sum S_i / \sum S_t) \times 100$ , where  $P_i$  is the prey-specific abundance of prey  $i$ ,  $S_i$  is the stomach contents (number) including prey  $i$ , and  $S_t$  is the total stomach contents among those individuals with prey in their stomach (Amundsen *et al.*, 1996).

### Stable isotope analysis

We obtained a muscle sample from the pectoral fin of seven spiny butterfly rays, seven Mediterranean starry rays and nine thornback skates. Before stable isotope analysis, we extracted lipid from muscle samples using a chloroform-methanol solution (Kim & Koch, 2011). Samples were subsequently freeze-dried and powdered and 0.28–0.4 mg of each sample was packed into tin capsules. Isotopic analyses were performed at the Stable Isotopes Laboratory at the Estación Biológica de Doñana CSIC (Seville, Spain). Samples were combusted at 1020°C using a continuous flow isotope ratio mass spectrometry system (Thermo Electron) by means of a Flash HT Plus elemental analyser coupled to a Delta-V Advantage isotope ratio mass spectrometer. Stable isotope ratios were expressed in the standard  $\delta$ -notation (‰) relative to Vienna Pee Dee Belemnite ( $\delta^{13}\text{C}$ ) and atmospheric  $\text{N}_2$  ( $\delta^{15}\text{N}$ ). Based on laboratory standards, the measurement error was  $\pm 0.1$  and  $\pm 0.3$  for  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$ , respectively (Cabana & Rasmussen, 1996).

As a measure of trophic width, for each species a Bayesian isotopic ellipse area (SEA) was calculated from the stable isotope values (Jackson *et al.*, 2011). This metric represents a measure of the total amount of the 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 (high values of isotopic standard ellipse areas indicate high trophic width). This metric uses multivariate ellipse-based Bayesian metrics. Bayesian inference techniques allow for robust statistical comparisons between datasets with different sample sizes. Isotopic standard ellipse areas were calculated using the routine Stable Isotope Bayesian Ellipses incorporated in the SIAR library (SIBER, Jackson *et al.*, 2011).

### Trophic position

The trophic position (TP) of each species was estimated by using isotopic values ( $\text{TP}_{\text{SIA}}$ ) and stomach content analysis ( $\text{TP}_{\text{stomach}}$ ).  $\text{TP}_{\text{SIA}}$  was performed according to Zanden & Rasmussen (2001):

$$\text{TP}_{\text{consumer}} = \text{TP}_{\text{basal}} + (\delta^{15}\text{N}_{\text{consumer}} - \delta^{15}\text{N}_{\text{basal}}) / \Delta\delta^{15}\text{N},$$

where  $\delta^{15}\text{N}_{\text{consumer}}$  is the value for each batoid species and  $\delta^{15}\text{N}_{\text{basal}}$  is that of the crab, *Monodaeus couchii* (7.1‰) sampled from the north-eastern Levantine Sea. We used 1.95 for  $\Delta^{15}\text{N}$  values (Hussey *et al.*, 2010), defined as the trophic enrichment factor between organism and diet.

$\text{TP}_{\text{stomach}}$  was calculated using the following equation:  $\text{TL}_j = 1 + \sum n_j - 1 \text{IRI}\% * \text{TP}_i$ , where  $j$  is the predator of prey  $i$ ,  $\text{IRI}\%$  is the fraction of prey  $i$  in the diet of predator  $j$ , and

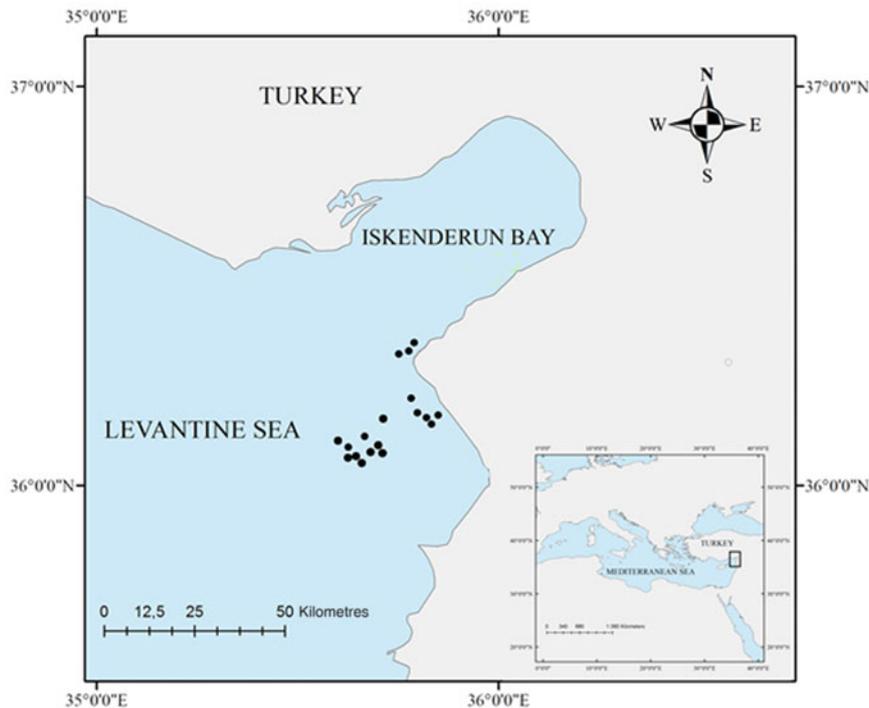


Fig. 1. Study area (North-eastern Levantine Sea, eastern Mediterranean Sea), indicating the sampling locations (black points).

$TL_i$  is the trophic position of prey  $i$  (Cortés, 1999). Trophic positions of prey categories were based on Ebert & Bizzarro (2007).

### Statistical analysis of stomach content data

Data analysis was performed with multivariate techniques (PERMANOVA). The diets of the three batoid species were analysed using the Bray–Curtis resemblance matrix of  $\log(x + 1)$  transformed, with prey abundance data. The comparison similarities of prey groups among the three batoids were determined by SIMPER and appeared as vector overlay on the principal coordinates analysis (PCO) plot by PRIMER v6 (Clarke & Warwick, 2001). One-way analysis of covariance (ANCOVA) was used to reveal relationships between body size and the fullness index in stomach contents with SPSS 21 software.

## RESULTS

### Stomach content analysis

A total of 85 individual stomachs were analysed belonging to three batoids. We found that 71 of these individuals had food in their stomachs (coefficient of vacuity: 32% for *R. asterias*, 15% for *R. clavata* and 31% for *G. altavela*). We identified 15 different prey species belonging to three different taxonomic groups (Table 1). The minimum average of percentage of fullness was estimated at 14% for the spiny butterfly ray and 43% for the thornback skate and Mediterranean starry ray. There were no significant differences between the fullness index and body size in any of the three batoids ( $P > 0.05$ ).

Although teleosts were the main prey group for all three batoids, we found significant differences in the diet composition (PERMANOVA tests, Pseudo- $F_{2,20} = 28.01$ ;  $P < 0.001$ ).

In particular, pairwise tests indicated that stomach contents differed between *G. altavela* and both *R. asterias* and *R. clavata*. The PCO analysis showed that the horizontal axes explain separation with 57.7% total variation because of the contribution of cephalopods and decapods to the diet of the batoids. The vertical axes explained separation with 29.8% total variation in accordance with the contribution of the teleost species to the diets (Figure 2). While the teleost group was common in all batoids, the main differences among the batoid species were found in decapod and cephalopod groups.

*Champsodon* sp. was a common prey fish species in the stomach of *G. altavela* and *R. clavata*, whereas *Equulites klunzingeri* (IRI = 21.13%) was only found in the stomach of *G. altavela* (Table 2). In addition, *Chlorophthalmus agassizi* was found in the stomachs of both *R. asterias* and *R. clavata*. *Argentina sphyraena* (IRI = 0.2%), *Bregmaceros atlanticus* (IRI = 0.4%) and *Trachurus* sp. (IRI = 0.5%) were identified only in the stomach contents of *R. asterias*. Decapoda was the second main prey group for *R. asterias* and *R. clavata* (IRI = 18.68% and IRI = 44.40%, respectively). Copepods were only found in the stomach of *R. asterias* (IRI = 1.7%). Although it was somewhat common to find cephalopods in the batoid stomachs, they were not represented in a high percentage of the stomach contents (IRI % between 2.15 and 10.64).

Regarding the niche width, *G. altavela* showed lower values ( $B_i$ : 1.1,  $B_A$ : 0.1), followed by *R. asterias* ( $B_i$ : 3.32,  $B_A$ : 0.8) and *R. clavata* ( $B_i$ : 3.25,  $B_A$ : 0.8). Diet overlap was lowest between *G. altavela* and *R. clavata* (0.46), and highest between *R. asterias* and *R. clavata* (0.98). The relationship between prey specific abundance and prey occurrence confirms a specialist feeding strategy on teleosts for *G. altavela* (Figure 3). In contrast, *R. asterias* and *R. clavata* displayed generalist feeding strategies.

**Table 1.** Diet composition of *Gymnura altavela*, *Raja asterias* and *Raja clavata* in the Iskenderun Bay (DW, disc width; TL, trophic level estimated from stomach contents; N, number of stomach; %FO, frequency of occurrence; %N, percentage in number; %W, percentage in mass; %IRI, index of relative importance of prey).

	<i>G. altavela</i> DW = 72.8 ± 8.3 cm, TL = 4.24 (N = 13)				<i>R. asterias</i> DW = 17.3 ± 9.7 cm, TL = 3.91 (N = 46)				<i>R. clavata</i> DW = 30.1 ± 9.8 cm, TL = 3.88 (N = 26)			
	%FO	%N	%W	%IRI	%FO	%N	%W	%IRI	%FO	%N	%W	%IRI
Crustaceans	–	–	–	–	35.00	62.26	32.56	56.02	65.38	62.16	22.93	50.65
Copepods	–	–	–	–	2.50	39.62	0.01	1.67	–	–	–	–
Decapods	–	–	–	–	22.50	18.18	30.99	18.68	61.54	56.76	22.49	44.40
<i>Penaeus japonicus</i>	–	–	–	–	–	–	–	–	3.85	1.35	0.37	0.06
<i>Metapenaeus monoceros</i>	–	–	–	–	–	–	–	–	3.85	8.11	5.81	0.49
<i>Parapenaeus longirostris</i>	–	–	–	–	–	–	–	–	3.85	1.35	1.10	0.09
<i>Alpheus</i> sp.	–	–	–	–	–	–	–	–	19.23	35.14	10.00	7.90
<i>Processa</i> sp.	–	–	–	–	–	–	–	–	3.85	1.35	0.09	0.05
<i>Chlorotocus crassicornis</i>	–	–	–	–	2.50	1.89	0.40	0.10	–	–	–	–
Unidentified Decapod	–	–	–	–	25.00	16.98	10.66	11.67	11.54	9.46	5.90	1.61
Unidentified Crustacea	–	–	–	–	2.50	3.77	1.55	0.22	3.85	1.35	0.39	0.06
Cephalopods	14.29	10.00	25.76	3.61	15.00	11.32	30.70	10.64	19.23	6.76	5.53	2.15
<i>Eledone</i> sp.	–	–	–	–	2.50	1.89	12.92	0.63	–	–	–	–
<i>Illex coidei</i>	14.29	10.00	25.76	3.50	–	–	–	–	–	–	–	–
<i>Sepiola</i> sp.	–	–	–	–	2.50	1.89	0.04	–	3.85	1.35	3.04	0.15
Unidentified cephalopod	–	–	–	–	10.00	7.55	17.73	4.27	15.38	5.41	2.49	1.11
Teleosts	85.71	90.00	74.24	96.50	27.50	22.64	49.17	33.34	61.54	22.97	61.28	47.20
<i>Argentina sphyraena</i>	–	–	–	–	2.50	1.89	2.64	0.19	–	–	–	–
<i>Bregmaceros atlanticus</i>	–	–	–	–	2.50	1.89	8.19	0.43	–	–	–	–
<i>Champsodon</i> sp.	14.29	11.11	8.73	1.94	–	–	–	–	7.69	2.70	22.78	1.78
<i>Chlorophthalmus agassizi</i>	–	–	–	–	7.50	9.43	32.13	5.26	7.69	2.70	3.82	0.46
<i>Equulites klunzingeri</i>	28.57	55.56	52.72	21.21	–	–	–	–	–	–	–	–
<i>Trachurus</i> sp.	–	–	–	–	2.50	1.89	9.80	0.49	–	–	–	–
Unidentified teleosts	42.86	22.22	12.79	10.28	15.00	11.32	16.86	7.13	50.00	17.57	34.68	23.78

**Stable isotope results**

Combined values of stable nitrogen and carbon differed among batoid species ( $\delta^{15}N$ ,  $F_{2,21} = 73.22$ ,  $P < 0.001$ ;  $\delta^{13}C$ ,  $F_{2,21} = 24.38$ ,  $P < 0.001$ ). Specifically, *R. asterias* and *R. clavata* did not differ in their stable nitrogen and carbon values (Tukey post hoc tests, all  $P > 0.05$ ; Table 2, Figure 4) but showed lower values of stable isotopes than *G. altavela* (post hoc test,  $P < 0.05$ ; Table 3, Figure 4). The isotopic

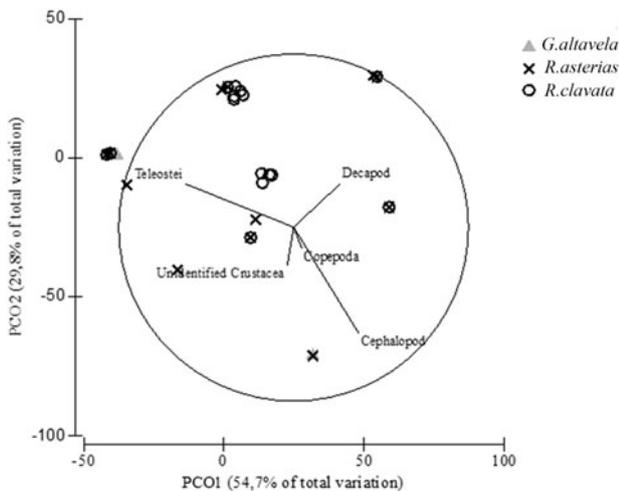
niche width based on the Standard Ellipse Area (SEA) clearly differed between batoid species (Figure 4), with the highest values for the thornback skate (SEA = 0.91‰), followed by *G. altavela* (SEA = 0.91‰) and *R. asterias* (SEA = 0.41‰) (Figure 4).

**Trophic level**

The trophic position estimated from stomach contents (TP<sub>stomach</sub>) varied between 3.88 and 4.24 among the three batoids, with *G. altavela* having a higher value than *R. asterias* and *R. clavata*, which occupied a very similar trophic position. When we estimated the trophic level from nitrogen isotope values, we found that absolute values differed from those estimated by stomach contents, but the relative position of the three studied species remained similar (Table 2).

**DISCUSSION**

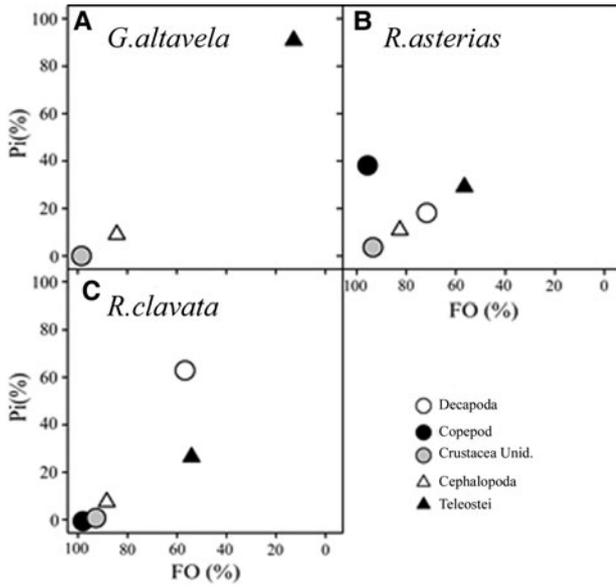
In this study, the trophic ecology of three batoids (*G. altavela*, *R. asterias* and *R. clavata*) inhabiting the Levantine Sea (East



**Fig. 2.** Principal coordinates analysis of stomach contents from *G. altavela*, *R. asterias* and *R. clavata* from the north-eastern Levantine Sea (Mediterranean Sea).

**Table 2.** Sample size (N) and mean and standard deviation of isotopic values and trophic level estimated with  $\delta^{15}N$  values (TL<sub>SIA</sub>) of three batoids in the Iskenderun Bay (north-eastern Mediterranean Sea).

Species	N	$\delta^{13}C$ (‰)	$\delta^{15}N$ (‰)	TL <sub>SIA</sub>
<i>Gymnura altavela</i>	7	-16.44 ± 0.50	11.21 ± 0.52	4.82 ± 0.26
<i>Raja asterias</i>	7	-17.27 ± 0.41	8.81 ± 0.42	2.93 ± 0.22
<i>Raja clavata</i>	9	-17.13 ± 0.42	8.54 ± 0.85	2.74 ± 0.46



**Fig. 3.** Graphical representation of the feeding strategy of *G. altavela* (A), *R. asterias* (B) and *R. clavata* (C) from the north-eastern Levantine Sea (Mediterranean Sea): prey-specific abundance (Pi %) plotted against mean frequency of occurrence (%FO) of the different prey groups.

Mediterranean Sea) was studied by combining stomach contents and isotope analyses. Stomach content results provide a snapshot of the diet of each species, and isotopic values identify the trophic width and trophic level integrating a long-term view (Peterson & Fry, 1987; Kim & Koch, 2011; Navarro *et al.*, 2014). Based on the results of both stomach contents and stable isotopes, we found clear differences in the trophic habits among these three demersal predators.

Stomach contents revealed that the diet of *G. altavela* was mainly composed of fish prey, a result that agrees with the very few studies conducted previously in this species in Mediterranean waters (Table 3; Neifar *et al.*, 2002; Psomadakis *et al.*, 2008; Barria *et al.*, 2015). This indicates that this species is a predator with clear preferences for fish. Although *R. asterias* and *R. clavata* also included fish in their diet, crustaceans were important prey as well for these species, contributing to the diet in the same proportion as fish. These results contrast with those from other locations in the Mediterranean, where the diet of these two rajidae species were composed mainly by crustaceans (Kabasakal, 2001; Vannucci *et al.*, 2006; Valls *et al.*, 2011; Navarro *et al.*, 2013; Eronat & Özyaydin, 2015; Fatimetou & Younes, 2016). For example, Navarro *et al.* (2013) found that crabs were the dominant prey for *R. asterias* in the western Mediterranean Sea. In the Ligurian Sea and Tyrrhenian Sea, similar results were found with *R. asterias*. *Goneplax rhomboides* and *Liocarcinus* sp. were reported mostly in stomach content of *R. asterias* from shallow water (Cuoco *et al.*, 2005; Romanelli *et al.*, 2007). Yeldan (2005) showed that crustacean species were the main prey in the diet of *R. asterias* along the east coast of the Iskenderun Bay (North Levantine Sea). The current study differs from Yeldan (2005) in its sampling area. Yeldan (2005) sampled the individuals in coastal waters, where the availability of crustaceans is high. Our samples of *Raja* spp. were captured mostly from deeper waters. Discrepancies in the diet of *R. clavata* between our study and those carried out previously are probably due to geographic and depth differences reported for this batoid (Kabasakal, 2001; Vannucci, 2006; Valls *et al.*, 2011; Eronat & Özyaydin, 2015). For instance, Eronat & Özyaydin (2015) indicated the dominant occurrence of crustaceans in the diet of *R. clavata* between 120 and 350 m in the Aegean Sea, while Valls *et al.* (2011) showed that the contribution of teleosts was much more relevant for this species in

**Table 3.** Main prey groups in the diet of *Gymnura altavela*, *Raja asterias* and *Raja clavata* from the Mediterranean Sea. NW, north-western; SC, south-central; C, central; W, western; SE, south-east.

Species	Region	Main prey group	Reference
<i>G. altavela</i>	NE	Teleosts	Present study
<i>G. altavela</i>	NW	Teleosts, Crustaceans	Barria <i>et al.</i> (2015)
<i>G. altavela</i>	C	Teleosts	Psomadakis <i>et al.</i> (2008)
<i>G. altavela</i>	C	Teleosts	Neifar <i>et al.</i> (2002)
<i>R. asterias</i>	NE	Crustaceans, Teleosts	Present study
<i>R. asterias</i>	NW	Crustaceans, Teleosts	Fatimetou & Younes (2016)
<i>R. asterias</i>	NW	Crustaceans	Navarro <i>et al.</i> (2013)
<i>R. asterias</i>	C	Crustaceans	Romanelli <i>et al.</i> (2007)
<i>R. asterias</i>	C	Crustaceans	Cuoco <i>et al.</i> (2005)
<i>R. asterias</i>	C	Crustaceans	Serena <i>et al.</i> (2005)
<i>R. asterias</i>	SC	Crustaceans, Teleosts	Capapé & Quignard (1977)
<i>R. clavata</i>	NE	Crustaceans, Teleosts	Present study
<i>R. clavata</i>	NW	Crustaceans, Teleosts	Barria <i>et al.</i> (2015)
<i>R. clavata</i>	E	Crustacea, Teleosts	Eronat & Özyaydin (2015)
<i>R. clavata</i>	NW	Crustaceans, Teleosts	Valls <i>et al.</i> (2011)
<i>R. clavata</i>	C	Crustaceans, Teleosts	Santic <i>et al.</i> (2012)
<i>R. clavata</i>	E	Crustaceans, Teleosts	Yığın & İşmen (2010)
<i>R. clavata</i>	E	Crustaceans, Teleosts	Yeldan <i>et al.</i> (2008)
<i>R. clavata</i>	E	Crustaceans, Teleosts	Kabasakal (2001)
<i>R. clavata</i>	E	Cephalopods	Kabasakal (2002)
<i>R. clavata</i>	C	Crustaceans, Cephalopods, Teleosts	Bello (1997)
<i>R. clavata</i>	C	Crustaceans, Teleosts	Azouz & Capape (1971)
<i>R. clavata</i>	C	Crustaceans, Cephalopods, Teleosts	Capapé (1975)
<i>R. clavata</i>	C	Crustaceans, Teleosts	Jardas (1972)

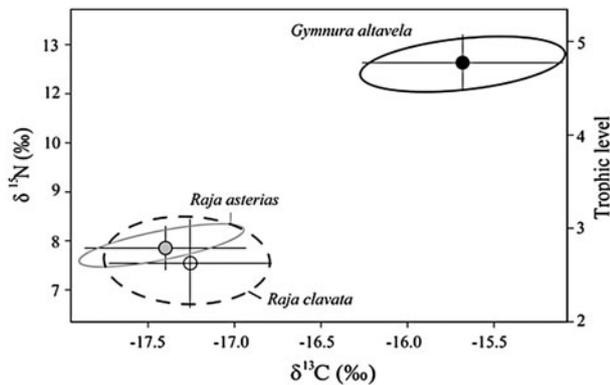


Fig. 4. Mean and standard deviation of  $\delta^{13}\text{C}$ ,  $\delta^{15}\text{N}$  and trophic level values of *G. altavela*, *R. asterias* and *R. clavata* from the north-eastern Levantine Sea (Mediterranean Sea). The Bayesian standard ellipse areas are also indicated.

deeper waters. In our study, the relative contribution of crustaceans and teleosts was nearly the same.

The existence of interspecific differences in teeth morphology could explain differences in the diet (McEachran & Capapé, 1984; Jacobsen & Bennett, 2013). The presence of crushing teeth plates in the two *Raja* spp. probably confers a greater capacity to crush the carapace of crustaceans, whereas the cuspidate teeth of *G. altavela* facilitate the capture of fish (Vannucci *et al.*, 2006; Motta & Huber, 2012; Ellis *et al.*, 2016). Based on the principle of competitive exclusion, we expect that competing predators coexisting in the same waters segregate their exploitation of trophic resources (e.g. Papastamatiou *et al.*, 2006; Follesa *et al.*, 2010; Albo-Puigserver *et al.*, 2015). For this reason, the three batoids partially segregate their main trophic resources as a mechanism that allows coexistence in the demersal habitat.

As expected from the stomach content results, interspecific differences in the isotopic values and trophic levels were found. In particular, *G. altavela* was isotopically segregated from *R. asterias* and *R. clavata*, showing a lower isotopic trophic width and higher trophic level. The trophic width estimated from SEAs was larger for *G. altavela* and *R. clavata* in comparison to *R. asterias*. Distribution of *R. clavata* shows variety from shallow to deep water in the area. This could be a result of the more generalized feeding strategy of *R. clavata*. On the other hand, previous studies on the feeding ecology of *R. asterias* show its specialized feeding strategy on crustacean species (Barriá *et al.*, 2015). The diversity richness of the coastal area in which *G. altavela* is mainly distributed (Emre Yemisen, unpublished data) probably explains the high trophic width of this species. Based on the trophic position of the species, both methodologies (stomach contents and isotopic values) revealed that *G. altavela* was at a higher position than the other two species. This pattern was previously found within demersal food webs in the western Mediterranean Sea where *G. altavela* shows a higher trophic position than coexisting batoids (Valls *et al.*, 2011; Barriá *et al.*, 2015), probably related to its large body size.

Although we expected a similar estimation of trophic position using stable isotope analysis (SIA) and stomach contents, we found differences between the methods in both Rajidae species. The estimation of trophic level from stable isotopes was lower than from stomach contents. Differences between  $\text{TP}_{\text{SIA}}$  and  $\text{TP}_{\text{stomach}}$  would be expected considering that the estimated trophic levels from isotopic data are vulnerable to

the basic assumption of which basal sources are used (Olin *et al.*, 2013). Discrepancies between the methodologies ( $\text{TP}_{\text{SIA}}$  and  $\text{TP}_{\text{stomach}}$ ) revealed the need for caution when values of trophic levels are compared (Albo-Puigserver *et al.*, 2015). However, differences observed in the trophic position between the two methods in this study might be explained by long-term and short-term prey preference differences of Rajidae species in the region. When resources are restricted in the ecosystem, sometimes species may adapt and change their feeding behaviour after a while in the area. Although stomach content results have shown teleost and shrimp preferences in feeding behaviour, prey availability may not be sustainable on the same prey.

In conclusion, this study presents new information regarding the feeding ecology of three endangered batoids (*G. altavela*, *R. asterias* and *R. clavata*) in the Levantine Sea. The results indicate differences in the diet between species, showing a clear feeding preference for teleosts in the case of *G. altavela* and a diet composed of fish and crustaceans in the case of *R. asterias* and *R. clavata*. These results can be used by managers to conduct an appropriate assessment and inform conservation strategies for these species.

## ACKNOWLEDGEMENTS

We thank Suna Tüzün, Onur Gonual and Mert Kesiktas for their help during the sampling and laboratory process and special thanks to Susana Carrasco during the stable isotope analysis at Laboratory of Estación Biológica de Doñana CSIC (Seville, Spain).

## FINANCIAL SUPPORT

This study was partially funded by Istanbul University (project no: 42822) and TUBITAK 2214A (PhD student international scholarships programme).

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