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Ontogenetic shift in diet and trophic role of *Raja clavata* inferred by stable isotopes and stomach content analysis in the Sea of Marmara

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Abstract

Trophic ecology studies on predator–prey interactions reveal insights into ecological communities and help understand a species' role in the food web by contributing to improved fisheries management and conservation capabilities. Understanding the ecological role of overexploited and endangered predators is essential to deciphering how their feeding behaviour influences food web dynamics. In this study, the authors investigated the feeding behaviour of the common and IUCN-listed Near Threatened (NT) thornback ray *Raja clavata*, using carbon and nitrogen stable isotope and stomach content analysis (SCA). It has recently suffered an 87% decline in reported catches from the Sea of Marmara within the last decade. These results show that thornback ray mainly feeds on teleost species, except in summer, with both methods showing this species changes its diet ontogenetically by SCA. This ontogenetic diet shift was at lengths 40–50 cm by changing group preferences from Crustacea to Teleostei. MixSIAR results showed that both adult and juvenile individuals of *R. clavata* feed mainly on the crustaceans, but the contribution of teleosts represented by *Trachurus* sp. was very low (<15%). The trophic position increased total length and was higher than other batoid species in the Sea of Marmara.

KEYWORDS
dietary shift, elasmobranch, feeding strategy, mesopredator, thornback ray, trophic position

1 | INTRODUCTION

Fish follow a certain trophic structure and have many interactions that shape the community that are unbeknownst to us, and changes to this structure can spread throughout the system, resulting in condition, abundance and even energy flow changes (Baum & Worm, 2009; Bornatowski *et al*., 2014; Heithaus *et al*., 2008; Paine, 1969). As an essential component of predators in the marine ecosystem, batoids are the most diverse elasmobranch group (Aschliman *et al*., 2012), and many of them are currently endangered (Dulvy *et al*., 2014a; Flowers *et al*., 2021). Batoids provide a vital link between upper trophic levels (*e.g*., marine mammals, birds, elasmobranch and teleosts) and lower trophic levels (Vaudo & Heithaus, 2011). Generally, their trophic levels range from 3.4 to 4.5 (Navia *et al*., 2017), with varied diet preferences; thus, their success (or lack thereof) can directly affect both upper and lower associated groups in the food webs (Lotze *et al*., 2011).

Many benthic fish species have showed changes in species' diets throughout their ontogeny (Munä Oz & Ojeda, 1998). Previous studies revealed that body size is directly affecting *R. clavata*'s feeding strategies (Daban *et al*., 2022; Demirhan *et al*., 2007; Yigin & Ismen, 2010) similar to other fish species. Nonetheless, growing body size, mouth dimension and developed swimming capability could increase the ability of prey capture (Karpouzi & Stergiou, 2003). Moreover, changes in the diversity and abundance of prey groups according to the seasons affect food availability. For instance, in seasons with low prey resource abundance, its ecological niche breadth

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may increase to reduce intraspecific competition, whereas diet overlap between species may decrease to minimize interspecific competition (Heng *et al*., 2018). The feeding strategy of a species plays an essential role in its abundance, distribution and competition (Arakaki *et al*., 2014). Significantly, interspecific competition could be altered by the spatial and temporal distribution of the species or its prey preferences. In addition, intraspecific competition could be reduced because of ontogenetic shifts as adults use a much broader ecological niche than juveniles (Dominici-Arosemena & Wolff, 2006).

Stable isotope studies in cartilaginous fish provide a wide range of information, from seasonal feeding and migration pathway patterns to intra- or inter-species resource partitioning and trophic position (Borrell *et al*., 2011; Hussey *et al*., 2014; MacNeil *et al*., 2006; Matich *et al*., 2010). The amount of carbon and nitrogen isotopes in animal tissue reflects the food the organism consumes, absorbs and assimilates into its tissues (Fry, 1988, 2006). Thus, stable isotope analysis (SIA) enables to evaluate long-term dietary characteristics compared to other methods, and it is a useful tool to construct trophic network (Phillips *et al*., 2014; Post, 2002). Because the trophic discrimination of carbon isotopes between prey and predator is small (*c*. 0.6–1.0‰ per trophic level), isotope ratios of carbon ($\delta^{13}C$) are used to describe sources of primary production constituting the base of marine food webs (Hussey *et al*., 2012). The isotope ratios of nitrogen ($\delta^{15}N$) were used to determine relative trophic position as the result of high discrimination between prey and consumer (typically 3.0–3.4‰), because discrimination may change within taxa and consumers' diet (Hussey *et al*., 2012). $\delta^{13}C$ and $\delta^{15}N$ values of liver tissue which has a relatively rapid turnover rate than muscle provide improved information about the short-term feeding behaviours of species (Fry, 2006). In recent years, Bayesian stable isotope mixing models have also become a powerful tool for determining the long-term feeding of individuals by predicting the contribution of preys in predator diets using a Bayesian approach (Moore & Semmens, 2008; Parnell *et al*., 2010; Phillips *et al*., 2014).

Despite the ecological importance of batoid species, they are most often caught as non-target incidental by-catch from bottom trawls, long-lines, gillnets and beam trawls in the Mediterranean and Turkish waters (Coll *et al*., 2013; Dulvy *et al*., 2014). Thornback ray, *Raja clavata*, Linnaeus, 1758, is the most common species of the Batoidea family and elasmobranchs in Turkey and the Mediterranean (Carbonara *et al*., 2020; Kabasakal, 2016) and is widely distributed from the Eastern Antarctic to the Indian Ocean (Froese & Pauly, 2021). According to Turkish fisheries statistics, their reported landings decreased by 87% within the past decade (TUIK, 2019), making them a vulnerable species. Therefore, since 2018, a new regulation was enacted prohibiting their capture in all Turkish waters (Fisheries Regulation Notice No. 2016/35) (Cabbar & Çiğdem Yiğin, 2021). Despite its vulnerable status and the most common batoid species, prior research on this species in the Marmara Sea has only covered stock status and its length–weight relationship with the basic meristic features (Demirel *et al*., 2020; Demirel & Dalkara, 2012; İşmen *et al*., 2018). Nevertheless, trophic ecology and feeding behaviour of species are well described in the predominantly west coast (Barría *et al*., 2015) and east coast (Saglam & Bascinar, 2008; Yemişken *et al*., 2018) parts of the Mediterranean (Daban *et al*., 2022; Demirhan *et al*., 2007; Eronat & Özaydn, 2015; Kadri *et al*., 2014; Šantić *et al*., 2012).

In this study, seasonal and size-based ontogenetic dietary habits of thornback ray were examined in the Sea of Marmara. The authors aim to compare food source utilization and trophic niches at different life stages across seasons and sexes for thornback ray. To understand its feeding habits, both stable carbon (C) and nitrogen (N) isotopes analysis and traditional stomach content analyses were used.

2 | MATERIALS AND METHODS

2.1 | Study site and sampling

The Sea of Marmara is located in the north-eastern part of the Mediterranean Sea. It forms a transition zone between the Black Sea and the Aegean Sea, which connects to the greater Mediterranean Sea. The most remarkable feature of the Sea of Marmara is the permanent stratification that separates the Black Sea-originated upper layer water (*c*. 22 salinity) from the saline Mediterranean waters of the bottom layer (*c*. 38.5 salinity) (Beşiktepe *et al*., 1994). Thus, although the pelagic system of the Sea of Marmara primarily reflects the Black Sea, the lower layer and demersal species composition reflects the Mediterranean characteristics; therefore, its productivity and biodiversity are heavily influenced by these two seas (Demirel & Gül, 2016; Oguz & Oztürk, 2011).

In this study, biological samples were collected from the project titled "Determination of Stock Status of Commercial Demersal Fish of the Marmara Sea" (Project No: TAGEM/HAYSUD/2014/05/21) officially conducted by "The Republic of Turkey the Ministry of Agriculture and Forestry." The experimental fishing survey was conducted seasonally at 34 stations, which were chosen to be representative of the Sea of Marmara (Figure 1) between December 2017 and December 2018 (winter–spring–summer–autumn). Trawling was performed following the International Bottom Trawl Survey in the Mediterranean (MEDITS) (MEDITS, 2013) protocol, with 151 hauls between 25 and 151 m. All hauls were positioned at the bottom layer. The trawl net had codend with 200 mesh length with a mesh opening 44 mm; equipped with polyamide cover with 250 mesh length with a mesh opening. Bottom trawling was carried out for 30 min at 2.5 $knots^{-1}$ using a commercial bottom trawl vessel.

2.2 | Stomach content analysis

A total of 98 thornback ray specimens (60 juveniles, 38 adults) were collected from the survey that would have normally been discarded (Table 1). Immediately after the specimens were landed, their total length (L_T, cm; ± 0.1 cm), disc width (DW, cm; ± 0.1 cm) and sex were recorded onboard. The stomachs were then removed and fixed in a 4% buffered formaldehyde solution, and then stored for laboratory processing. At the laboratory, each stomach was weighed, and all prey

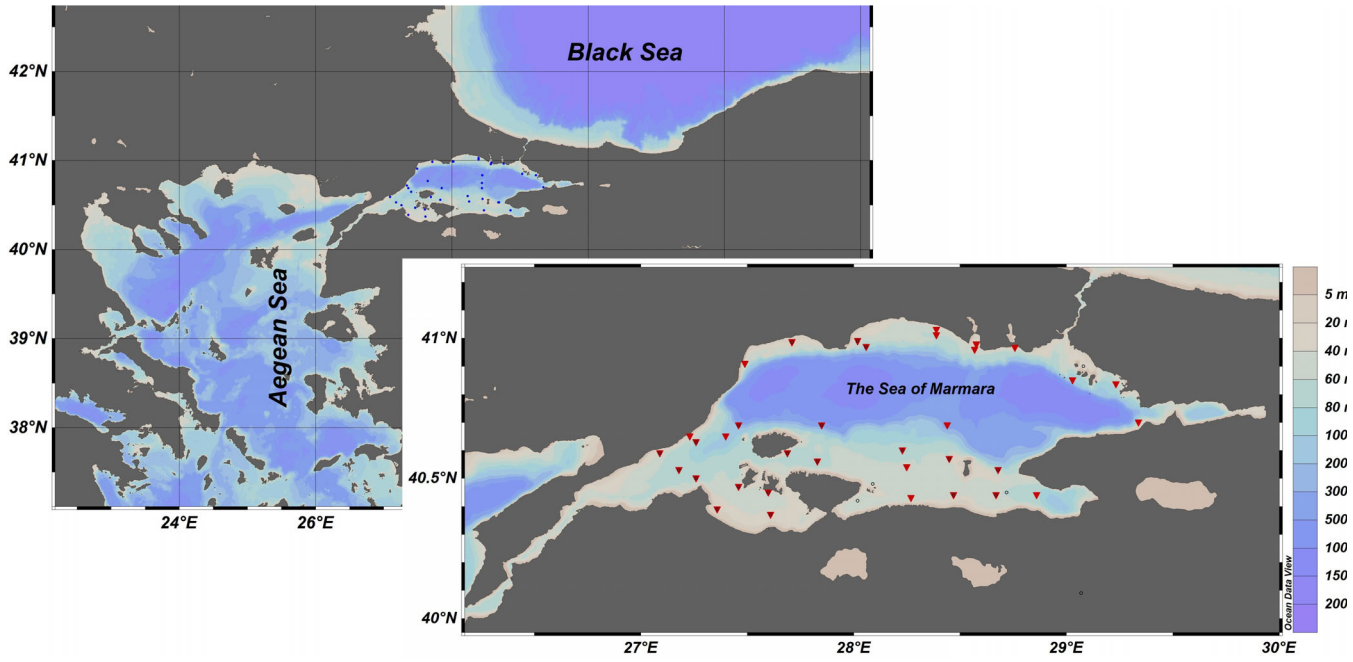


FIGURE 1 The location of the Sea of Marmara (larger panel) and sampling stations (smaller panel)

		SCA		SIA			
	n	Avg. D. (m)	L_T (cm)	Tissue type	*n*	Avg. D. (m)	L_T (cm)
Adult	38	100	61–85.2	Muscle	8	56	61–77
				Liver	9	57	61–78.5
Juvenile	60	58	23–60	Muscle	7	88	37–53.5
				Liver	6	88	37–53.5

TABLE 1 Sample size, total length range (L_T, cm) and average depth (Avg. D., m) of specimens of *Raja clavata* used for stomach content analysis (SCA) and stable isotope analysis (SIA) for this study

items were counted, weighed and identified to the lowest possible taxonomic level. Each empty stomach was recorded also to determine feeding intensity using the vacuity index: VI% = $(A \times 100)/B$, where A is the number of empty stomachs and B is the total number of stomachs analysed (Hyslop, 1980). The percentage of prey number (*N*%), percentage of prey weight (W%) and frequency of occurrence (*F*%) were used to calculate the index of relative importance (IRI) of each prey item using the formula by Hyslop (1980): IRI = (*F*%) × (*W*% + *N* %), and following Cortés (1997), %IRI values calculated: IRI % = (IRI/∑IRI) × 100.

2.3 | Stable isotope analysis

Liver and muscle tissues were sampled for stable carbon and nitrogen isotope analysis to elucidate the difference between adult and juvenile trophic ecology (Table 1). In addition, to advance the understanding of the likely trophic interactions between other demersal species, some common teleost and invertebrate species collected during the trawling operations were also investigated for SIA. The teleost and invertebrate species were not performed lipid extraction because of the low lipid rate by literature knowledge (Post, 2002) and their muscle samples were frozen at −20°C until further analysis.

From the freshly collected *R. clavata* samples, muscle and liver samples (*c.* 5 g each) were partitioned onboard and then immediately frozen at −20°C until further laboratory analysis. As the high lipid ratio in the tissues of elasmobranchs may cause depletion in the $\delta^{13}C$ values of species (Carlisle *et al.*, 2017; Kim & Koch, 2012; Post, 2002), lipid extraction was performed for both liver and muscle tissue according to the method specified by Bligh and Dyer (1959) and Hussey *et al.* (2012), and the authors did not remove urea from the tissues. Subsequently, the tissues were freeze-dried for *c.* 72 h, powdered and then weighed in the range of roughly 1–1.5 mg before packing in a tin capsule. The analysis of ^{13}C and ^{15}N was executed at the Stable Isotope Facility at the University of California, Davis (UC Davis SIF). The SIA analysis (^{13}C:^{12}C and ^{15}N:^{14}N) was performed using a PDZ EuroLevinpa ANCA-GSL elemental analyser interfaced with a PDZ Europa 20–20 isotope ratio mass spectrometer. Stable isotope ratios of carbon and nitrogen were expressed in delta notation with the equation: $\delta X = ((\text{Rsample}/\text{Rstandard}) - 1) \times 1000$, where X is the heavy isotope, Rsample is the ratio of heavy to light isotope in the sample (*e.g.*, ^{13}C:^{12}C) and Rstandard is the ratio of heavy to light

isotope in the reference standard. The long-term standard deviations, as characterized by analytical precisions, were accepted as 0.2‰ for $\delta^{13}C$ and 0.3‰ for $\delta^{15}N$. In addition, quality control of the sample analyses was tested by several laboratory reference materials, such as alfalfa flour, nylon 6, bovine liver and enriched alanine at UC Davis SIF.

2.4 | Data analysis

The authors performed permutational multivariate analysis of variance (PERMANOVA) on the Bray–Curtis similarity matrices and 999 permutations analysis to determine statistical differences in the diet preferences according to the factors of sex, size groups (juvenile and adult), depth (0–50, 50–100, >100 m) and season (winter–spring–summer–autumn) using the number of individuals (biomass W) obtained from the stomach contents of each prey species. Biomass data were fourth-root transformed to reduce the influence of common prey taxa. Analysis was performed using PRIMER 7 software (v.7.0; PRIMER).

Because there are no reproduction studies of thornback ray in the Sea of Marmara, the length groups of the species were determined by maturity length (L_{50}) revealed by Krstulović Sifner *et al.* (2009) from the northern Adriatic Sea. In line with the study result, the authors separated individuals <60 cm as juveniles and >60 cm as adults.

A Bayesian isotope mixing model, MixSIAR (Stock & Semmens, 2016), was used to estimate the potential contributions of each prey group to the diets of juvenile and adult *R. clavata* based on the variations in $\delta^{15}N$ and $\delta^{13}C$ values between tissues (Stock & Semmens, 2016; Stock *et al.*, 2018; R Core Team, 2020). The dominant fish and invertebrates which were obtained in the catch composition during the sampling campaign were selected as potential prey groups. The discrimination factors were obtained from Caut *et al.* (2013) (*e.g.*, *Parapenaeus longirostris*: $\Delta^{15}N = 0.23 \pm 0.16$, $\Delta^{13}C = 0.44 \pm 0.12$; *Liocarcinus depurator*: $\Delta^{15}N = 2.77 \pm 0.33$, $\Delta^{13}C = 1.05 \pm 0.07$; *Trachurus* sp.: $\Delta^{15}N = 1.81 \pm 0.14$, $\Delta^{13}C = 1.49 \pm 0.1$; and *Loligo* sp. and Bivalvia: $\Delta^{15}N = 2.49 \pm 0.26$, $\Delta^{13}C = -0.46 \pm 0.26$). Gelman-Rubin and Geweke's diagnostic tests confirmed that the models converged (Gelman diagnostic revealed <1.05 for 65 variables, Geweke diagnostic revealed less than 5%, 14 of 65 variables, were outside of ±1.96).

The diet breadth (B) was calculated using Levins' index (Levins, 1968), and then Hulbert's formula was used to be Levin's standardized niche breadth (Hurlbert, 1978). Niche breadth was evaluated as follows, low (0–0. 39), intermediate (0.4–0.6) and high (0.61–1) (Grossman & Hart, 1986). The diet overlap between juvenile and adult individuals was estimated by Pianka's formula (Pianka, 1973). The resulting Pianka overlap was ranked between 0 and 1, using the same values as the niche breadth, except with a value of 1 indicating complete overlap. The number of prey items (*N*) was used as the factor in both Levin and Pianka formulas.

The trophic positions of juveniles and adults were calculated using both stomach content data (TPsca) and stable isotope data (TPsia). TPsca was performed using Trophlab (Froese & Pauly, 2021), whereas TPsıa was performed by Zanden and Rasmussen (2001): TPconsumer = TP basal + (δ^{15}Nconsumer – δ^{15}N basal)/$\Delta\delta^{15}N$, where δ^{15}Nconsumer is thornback ray species, ^{15}Nbasal is that of a bivalve (*Donax turunculus*) ($n = 2$, δ^{15}Nmean = 8.42) that was sampled from the Sea of Marmara and Δ is the trophic enrichment factor between organism and diet. Following Hussey *et al.* (2010), $\Delta^{15}N = 2.29$ [diet–tissue discrimination factors (DTDF) for lipid extracted muscle] and TP basal = 2 were used.

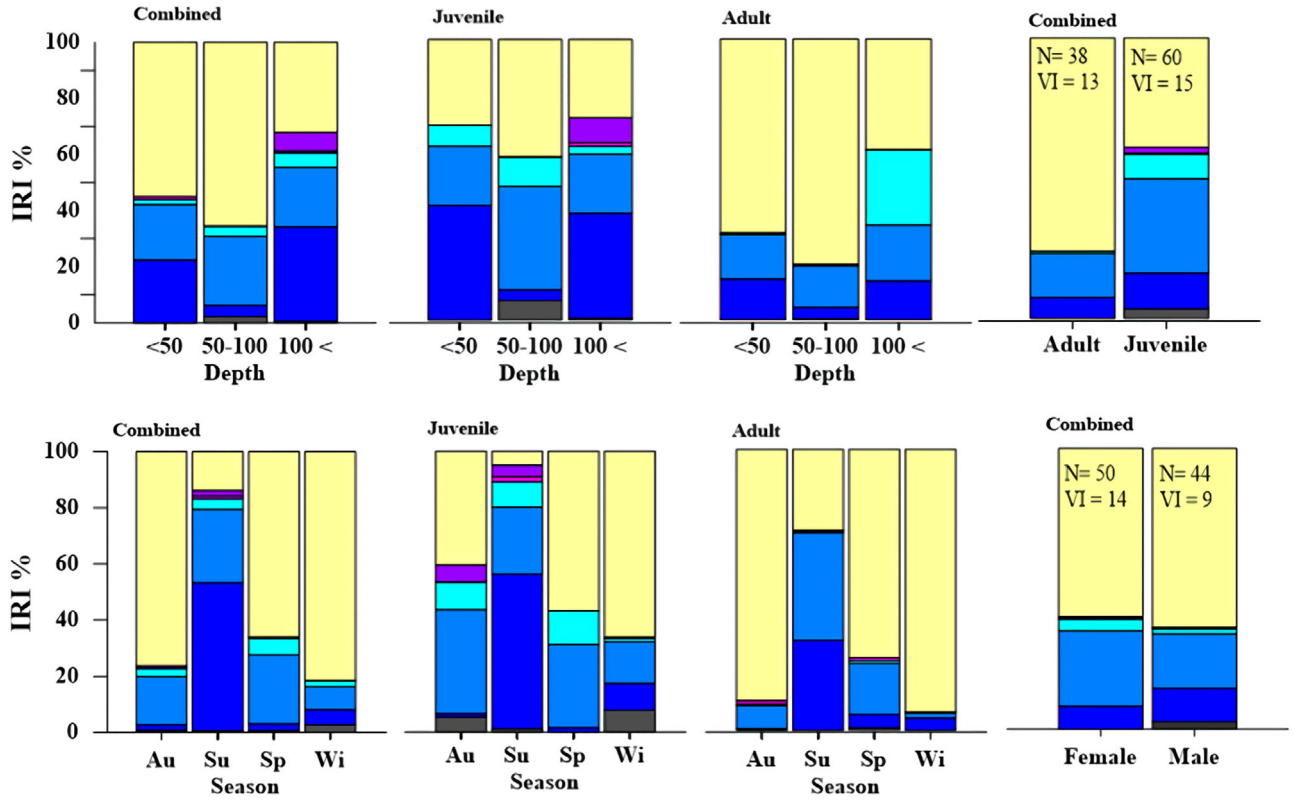
Following Levene's test to check homogeneity of variances, the Kruskal–Wallis test was used to test the differences in isotopic values ($\delta^{13}C$ and $\delta^{15}N$) among juveniles and adults of individuals (significance level $\alpha = 0.05$). Linear regression analysis of $\delta^{15}N$ and $\delta^{13}C$ against the total lengths of juvenile and adult specimens was used to assess significance levels of size-based changes using IBM SPSS Statistics (v.12.0; IBM Corp.).

3 | RESULTS

3.1 | General dietary preferences

A total of 98 stomachs were studied for stomach content analysis (SCA). Overall, 14 specimens had empty stomachs (VI = 14.2%), and the VI of females was higher than males (VI = 14% and 9%, respectively), whereas juveniles in spring had the highest VI value (Figure 2). According to the SCA results, Teleostei was identified as the dominant prey group with 60.1 IRI %, followed by Crustacea with 37.8 IRI %. The Teleostei group was comprised of six demersal and one bentho-pelagic species, *Trachurus* sp. For species level and of the identified preys, *Trachurus* sp. was the dominant teleost species, and *P. longirostris* was the dominant crustacean species. Lastly, Mollusca and Nematoda were determined as rare prey groups for the thornback ray (Figure 2; Table 2). The PERMANOVA analysis revealed the feeding characteristics of the two size groups and seasons to be statistically significant (Pseudo-$F = 1.855$ $P < 0.05$ and Pseudo $F = 3.7837$ $P < 0.001$, respectively), whereas the factors sex and depth were not significant (Pseudo-$F = 1.1879$ $P > 0.05$ and Pseudo-$F = 0.82901$ $P > 0.05$, respectively). The pair-wise comparison test indicated feeding differentiated between autumn and summer ($P < 0.01$).

In summer, the stomach content of thornback ray consisted mainly of Crustaceans, which were dominated by Brachyura (IRI 82.7%), but in adults, Caridea (IRI 38%) was higher than Brachyura (IRI 32%). Interestingly, Caridea was dominant in other seasons, aside from winter for adults. In winter, Teleostei dominated the prey items in juveniles and adults (IRI 66.1%, IRI 93.6% respectively) and combined individuals. In addition, the Mollusca group, which has a negligible contribution, was higher in summer than other seasons (Figure 2).



Depth

	< 50 m		50 -100 m		< 100 m	
	N	VI %	N	VI %	N	VI %
Combined	29	14	56	14.2	15	13.3
Adult	11	9	24	4.16	2	0
Juvenile	4	25	30	13	27	14.8

Season

	Autum		Summer		Spring		Winter	
	N	VI %	N	VI %	N	VI %	N	VI %
Combined	18	0	34	17.6	25	24	21	14
Adult	8	0	11	1.1	11	19	7	14
Juvenile	10	0	23	17.3	14	29	14	14

FIGURE 2 IRI % values of thornback ray by seasons (Au: autumn; Sp: spring; Su: summer; Wi: winter), and other factors of depth, sex and size (*N*: number of stomach samples; VI %: vacuity index). Nematoda, Caridea, Cephalopoda, Teleostei, Brachyura, Other Crustacean and Bivalvia

3.2 | Ontogenetic feeding shifts

The L_T of sampled specimens ranged from 23 to 85 cm (mean $L_T = 52.8 \pm 1.23$), and the DWs ranged from 16.5 cm to 54 cm (mean DW = 38.2 ± 0.78 cm). In order to better understand the relationship between ontogeny and the feeding behaviour of the species, individuals were divided into six different length groups using 10 cm intervals (Figure 3). Size-based diet preferences were apparent by differences in prey groups from the smallest juveniles to adults (Figure 3). Whereas the smallest individuals (sizes I and II) primarily fed on prey belonging to the Crustacea group, those of larger sizes (sizes V and VI) generally fed on Teleostei. Moreover, Mollusca was found as prey items only in juvenile diets and consisted of Bivalvia for size II and Cephalopoda for size III (Figure 3). The ontogenetic dietary shift was slightly revealed at size III (40–50 cm) by changing group preferences from Crustacea to Teleostei. Teleostei became the dominant prey group at size IV (IRI = 60%, Figure 4).

3.3 | Niche breadth and diet overlap

The Levin standardized niche breadth (B*a*) values were determined at 0.41 for the overall population, whereas clear differences were found between adults and juveniles (B*a* = 0.34 and 0.69, respectively). Levin's niche breadth was found to gradually decrease with an increase in L_T. According to the classification of niche breadth size, small individuals (sizes I and II) were found to be generalist feeders, whereas adults exhibited a specialist feeding strategy. Pianka's index for diet overlap indicated high overlap between size I and size II, and also the largest individuals (size VI) had significant overlap (>0.60) with other size classes (Table 3).

TABLE 2 Diet composition of adult and juvenile thornback ray specimens in the Sea of Marmara, northeastern Mediterranean

	Overall IRI ($n = 98$)				Juvenile <60 cm ($n = 60$)				Adult >60 cm ($n = 38$)			
Prey groups	**%N**	**%W**	**%FO**	**%IRI**	**%N**	**%W**	**%FO**	**%IRI**	**%N**	**%W**	**%FO**	**%IRI**
Nematoda												
Undefined Nematoda	6.71	0.07	7.14	1.20	12.03	0.24	9.80	3.74	2.16	0.00	3.03	0.11
Crustacea												
Parapenaeus longirostris	11.66	12.94	26.19	16.01	12.78	17.39	21.57	20.23	11.51	11.20	33.33	12.48
Alpehus glaber	2.47	0.54	7.14	0.53	1.50	0.37	3.92	0.23	3.60	0.60	12.12	0.84
Liocarnicus depurator	3.18	4.10	3.57	0.65	3.76	6.45	1.96	0.62	2.88	3.18	6.06	0.61
Goneplax rhomboides	8.13	2.95	9.52	2.62	12.78	3.15	9.80	4.85	4.32	2.86	9.09	1.08
Undefined. Caridea	12.72	2.93	17.86	6.95	13.53	6.66	23.53	14.76	12.95	1.47	9.09	2.16
Undefined Brachyura	8.13	6.16	21.43	7.61	8.27	4.32	19.61	7.67	8.63	6.88	24.24	6.20
Undefined Decapoda	0.71	0.03	2.38	0.04	0.75	0.03	1.96	0.05	0.72	0.03	3.03	0.04
Undefined Crustacea	6.01	1.82	17.86	3.47	9.77	3.84	11.76	4.98	2.88	1.03	12.12	0.78
Mollusca												
Undef. Bivalvia	3.53	0.39	4.76	0.46	7.52	1.40	7.84	2.17	0.00	0.00	0.00	0.00
Cephalopoda												
Undefined Cephalapoda	1.41	1.25	4.76	0.31	1.50	2.45	3.92	0.48	1.44	0.78	6.06	0.22
Undefined Mollusca	0.35	0.00	1.19	0.01	0.75	0.01	1.96	0.05	0.00	0.00	0.00	0.00
Teleostei												
Trachurus sp.	11.31	27.76	22.62	21.97	8.27	32.07	15.69	19.67	15.11	26.08	33.33	22.64
Mullus sp.	0.35	3.02	1.19	0.10	0.00	0.00	0.00	0.00	0.72	4.20	3.03	0.25
Undefined Gobiidae	0.35	0.42	1.19	0.02	0.00	0.00	0.00	0.00	0.72	0.59	3.03	0.07
Lesueurigobius friesii	0.71	0.85	1.19	0.05	0.00	0.00	0.00	0.00	1.44	1.18	3.03	0.13
Gobius niger	0.35	1.58	1.19	0.06	0.00	0.00	0.00	0.00	0.72	2.19	3.03	0.15
Serranus hepatus	1.41	4.84	3.57	0.55	0.00	0.00	0.00	0.00	2.88	6.73	9.09	1.44
Merluccius merluccius	0.35	1.96	1.19	0.07	0.00	0.00	0.00	0.00	0.72	2.73	3.03	0.17
Merlangius merlangus	0.35	2.83	1.19	0.09	0.00	0.00	0.00	0.00	0.72	3.94	3.03	0.23
Undefined Teleostei	19.79	23.57	34.52	37.21	12.03	21.61	19.61	20.50	28.78	24.34	57.58	50.42

Note: %FO: frequency of occurrence; %IRI: index of relative importance of each prey item; %N: percentage in number; %W: percentage in weight.

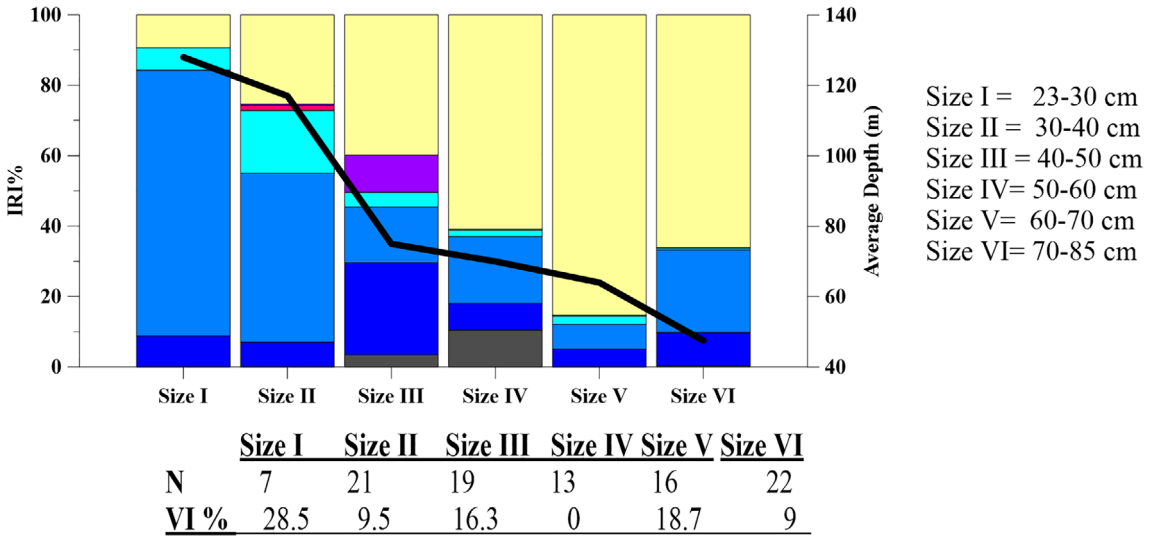


FIGURE 3 IRI % values of different size classes and average sampling depths across size classes (*N*: number of stomach samples; VI %: vacuity index). Nematoda, Caridea, Bivalvia, Teleostei, Brachyura, Other Crustacean and Cephalopoda

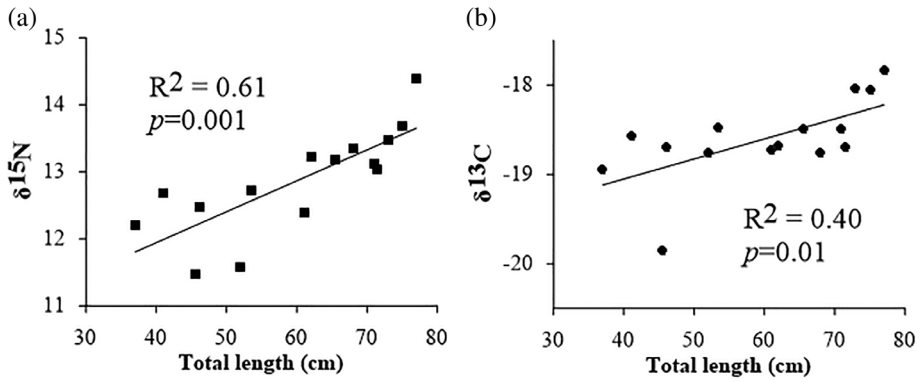


FIGURE 4 Relationship between total length and (a) $\delta^{15}N$ and (b) $\delta^{13}C$ values of muscle tissues

TABLE 3 Pianka's diet overlap and Levin niche breadth (BA) by size classes

	Size I	Size II	Size III	Size IV	Size V	Size VI
Size I		0.91	0.34	0.29	0.47	0.64
Size II			0.43	0.38	0.58	0.71
Size II				0.52	0.5	0.61
Size IV					0.24	0.68
Size V						0.72
Levin niche BA	0.71	0.66	0.6	0.54	0.27	0.44

TABLE 4 Isotopic values of thornback ray together with other species sampled during fisheries operation in the Sea of Marmara, northeastern Mediterranean

	Muscle					Liver				
Species	***n***	**$\delta^{13}C$ mean ‰**	**S.D.**	**$\delta^{15}N$ mean ‰**	**S.D.**	***n***	**$\delta^{13}C$ mean ‰**	**S.D.**	**$\delta^{15}N$ mean ‰**	**S.D.**
Juvenile *Raja clavata*	7	−18.82	0.44	12.33	0.49	6	−18.5	0.41	11.3	0.39
Adult *R. clavata*	8	−18.41	0.34	13.33	0.53	9	−18.23	0.41	12.02	0.50
Lophius budegassa	6	−18.55	0.1	14.34	0.36					
Trachurus sp.	4	−20.16	0.43	11.81	0.78					
Mullus sp.	1	−19.08	-	10.67	-					
Serranus hepatus	3	−18.93	0.43	12.8	0.58					
Parapenaeus longirostris	5	−19.29	0.14	9.82	0.33					
Liocarnicus depurator	3	−18.85	0.39	11.3	0.49					
Loligo sp.	4	−19.87	0.06	12.3	0.25					
Donax turunculus	2	−20.28	0.1	8.42	0.08					
Brissopsis lyrifera	3	−14.12	1.86	4.97	1.17					

3.4 | $\delta^{13}C$ and $\delta^{15}N$ analysis

The $\delta^{15}N$ values for juveniles and adult were significantly different for both muscle and liver tissues (Kruskal–Wallis, $P < 0.05$), but not significant for $\delta^{13}C$ despite very close to significance level ($P = 0.06$). While depletion in $\delta^{13}C$ was found in juvenile muscles, the highest ^{15}N was found in adult muscles (Table 4). The relationship between L_T (cm) and stable isotope values was significant and positive for both $\delta^{13}C$ and $\delta^{15}N$ (Figure 4).

The results of MixSIAR model revealed that the potential prey contributions for juveniles consisted of shrimps and bivalves by different rates between tissues (41.8% and 29.5% in muscle and 24.8% and 26.8% in liver respectively; Figure 5). For *R. clavata* adults prey contributions were found clearly different that juveniles as the main resources were crabs (26.2%) and cephalopods (24.2%) for muscle; shrimps (36.7%); and crabs (20.3%) for liver. Contribution rate of the teleosts represented by *Trachurus* sp. to the diet of *R. clavata* was determined below 15% for both sizes in both tissues (Figure 5).

3.5 | Trophic position and ecological role of thornback ray in the Sea of Marmara

The trophic level assigned to thornback ray using nitrogen isotope (TPsia) was 4.15 for adults and 3.71 for juveniles. The trophic level by

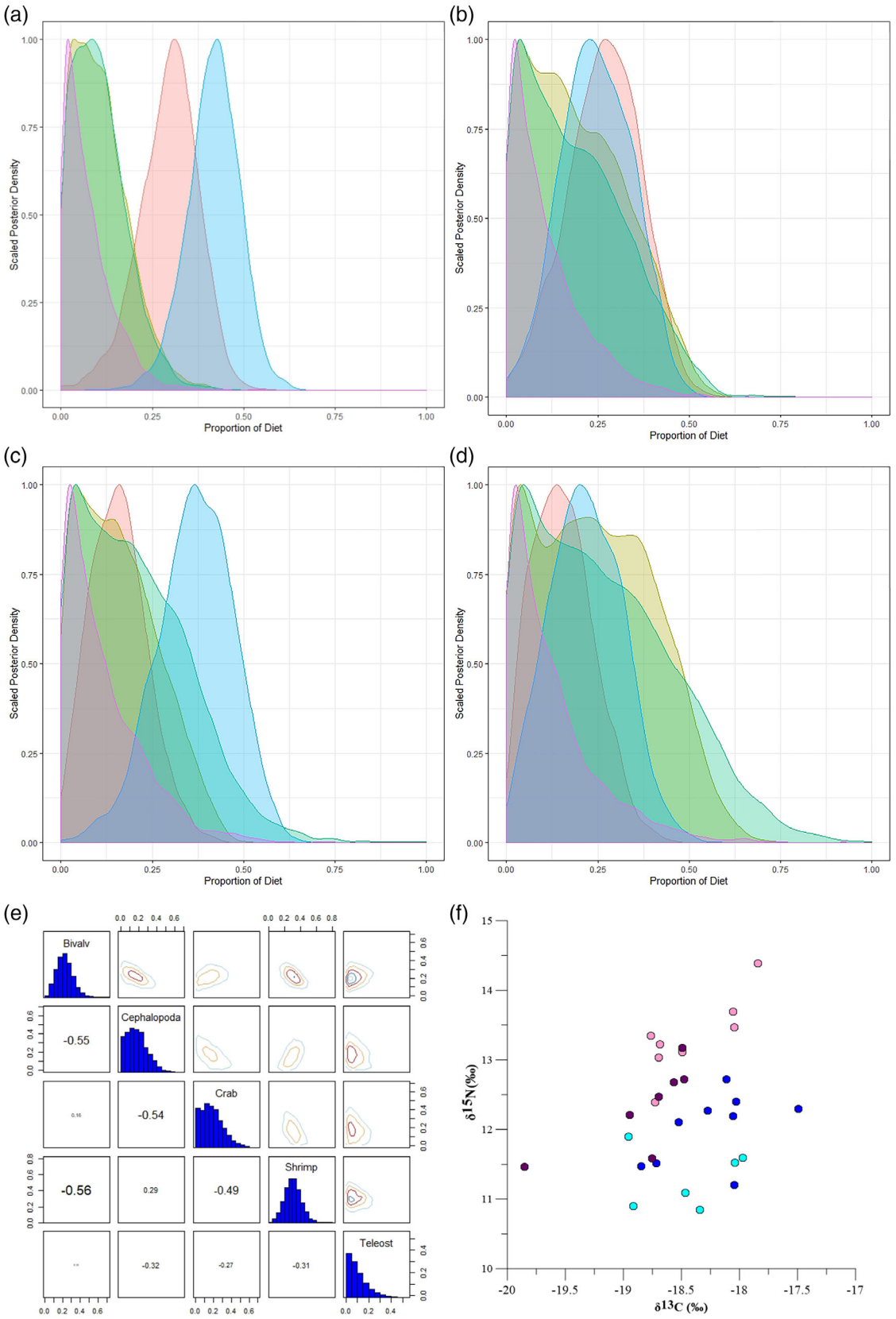


FIGURE 5 Proportional contributions of prey items in the diets of *Raja clavata* according to size and tissue by Bayesian isotopic mixing model; (a) adult muscle, (b) adult liver, (c) juvenile liver, (d) juvenile muscle, with (e) isospace plots of potential prey and (f) isotopic signatures by size and tissue. (a–d): Bivalv, Cephalopod, Crab, Shrimp, Teleost. (f): Adult liver, Adult muscle, Juvenil muscle and Juvenil liver

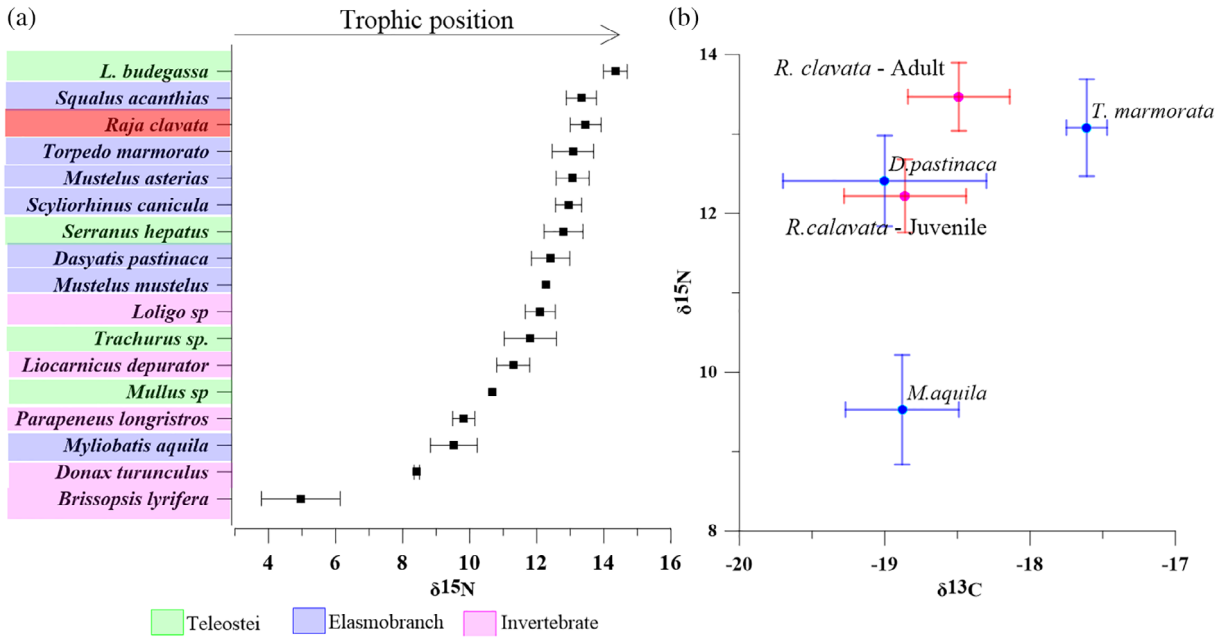


FIGURE 6 (a) Trophic position of *Raja clavata* and some associated benthic species collected during the sampling campaigns. (b) Biplot of the isotopic signatures of *R. clavata* and other batoid species in the Sea of Marmara (Gül & Demirel, 2020, 2021). Teleostei, Elasmobranch and Invertebrate

SCA was TLsca 4.09 ± 0.79 for the total population, 4.27 ± 0.75 for adults and 4.11 ± 0.9 for juveniles. The TPsia results were compatible with the TLsca results for adults, but were lower than TLsca for juveniles.

To understand the ecological role of *R. clavata*, the trophic structure of frequently observed species from the ecological groups Teleostei, Elasmobranchii and Invertebrata from both these trawl operations and published data for the Sea of Marmara (Gül & Demirel, 2020; Gül & Demirel, 2021) was plotted according to their trophic position estimated by δ^{15}N values (Figure 6a). Previously published data for stable isotope results were compatible by following the same protocol including lipid extractions. The results showed *Lophius budegassa* from the Teleostei group to have the highest δ^{15}N, followed by the elasmobranch species *Squalus acanthias*, whereas the lowest δ^{15}N value was a benthic invertebrate *Brissopsis lyrifera*. Two crustacean species, *L. depurator* and *P. longirostris*, were placed in different TP because *L. depurator* is higher enriched δ^{15}N, whereas *Loligo* sp. was enriched with higher δ^{15}N than some batoid species with a value of 12.3. Isotopic signatures of thornback ray were plotted to compare their TP with other Batoidea species from the Sea of Marmara (Figure 5b). Enriched δ^{15}N values of adult thornback ray were found in comparison to other batoids such as *Dasyatis pastinaca*, *Torpedo marmorata* and *Myliobatis aquila* (Figure 6b).

4 | DISCUSSION

Rays fulfil many ecological functions, such as nutrient fixing and predator and prey relationships, directly influencing the associated species (Flowers *et al*., 2021). The results of this study provide the first information on the trophic ecology of the thornback ray through SCA and SIA in the Sea of Marmara. The results of trophic position and isotopic signatures of *R. clavata* were also compared with other benthic and sympatric batoid species in the Sea of Marmara.

The VI was found as a low percentage which agrees with previous studies from the Mediterranean Sea (Kadri *et al*., 2014; Yemişken *et al*., 2018). Although the VI depends on the feeding behaviour of species, the sampling protocol, namely fishing gear, has also a notable effect on which samples *via* bottom trawling also used in this study were reported with higher VI than passive fishing gears such as longline or hook and line (Morato *et al*., 2003; Saglam & Bascinar, 2008).

The SCA results of this study showed the ontogenetic shift in prey groups by size; whereas juveniles feed on mainly crustacean prey items, adults prefer mainly teleosts. Previous studies in the Mediterranean and the Black Sea also reported that their main prey items were primarily crustaceans, followed by teleosts (Daban *et al*., 2022; Eronat & Özaydn, 2015; Saglam & Bascinar, 2008; Šantić *et al*., 2012; Yemişken *et al*., 2018). In the southern Black Sea, Demirhan *et al*. (2007) reported that its main prey item was European anchovy (*Engraulis encrasicolus*), especially in the winter period along with the increase in pelagic fish abundance because of southward migration. There are very few records showing pelagic fish in its diet, but demersal fish preys were frequently recorded (*e.g*., *Mullus* sp., *Merlangius merlangus*; Saglam & Bascinar, 2008). In contrast with previous studies, the results of this study showed that among the identified teleost species *Trachurus* sp. had higher contribution, whereas it was not reported in Levantine Sea (Yemişken *et al*., 2018), and reported very low in the Aegean Sea (0.06% IRI, Daban *et al*., 2022; 0.26% IRI, Eronat &

Özaydn, 2015). The authors evaluated the differences in fish prey contribution likely changes in diet preferences according to prey availability (Anthony *et al*., 2000; Pinnegar *et al*., 2003). Regarding PERMANOVA results, significant differences in the sampled stomachs from different depths were mainly caused by the changes in the contribution of *Trachurus* sp. which decreased to 0.65 IRI % below 100 m depth. In contrast with previous studies (Muto *et al*., 2001; San Martin *et al*., 2006; Šantić *et al*., 2012), the authors found high seasonality as crustaceans was the main prey during summer, but no differences between sex in feeding preferences as hypothesized in previous studies (Farias *et al*., 2006; Morato *et al*., 2003; Saglam & Bascinar, 2008).

The ontogenetic shift of thornback ray was also revealed by previous studies in line with our results of juveniles preferring crustaceans and the contribution of teleost preys increased by growth (Farias *et al*., 2006, 2012; Saglam & Bascinar, 2008; Daban *et al*., 2022). This study detected the ontogenetic shift to occur between 40 and 60 cm L_T, and specimens between 40 and 50 cm L_T had a more balanced diet according to the IRI % and Levin niche width results. Saglam and Bascinar (2008) also noted an ontogenetic shift to occur at 45 cm L_T in the Black Sea.

Thornback ray is a generalist predator (Mulas *et al*., 2010; Šantić *et al*., 2012; Yemişken *et al*., 2018) with an intermediate niche breadth (between 0.40 and 0.60). Nonetheless, its niche breadth was found to be narrower in Sea of Marmara, compared to the eastern Mediterranean (Levin's index: 0.80). The trophic level TLsca was previously reported between 3.20 and 4.14 (Daban *et al*., 2022; Ebert & Bizzarro, 2008; Ellis *et al*., 1996; Morato *et al*., 2003; Yemişken *et al*., 2018) which were lower than the results of this study (TLsca = 4.21). Dietary overlap between relatively smaller size groups (size I and size II with 0.91% Pianka index) is related to similar niche breadths, and prey preferences according to similar depth distribution. In addition, because of the enhanced prey capture capability, the largest size class (VI) had dietary overlap between all length groups which was reported previously (Saglam & Bascinar, 2008). In case of sizes III and IV, their lower dietary overlap was most likely attributable having more balanced diets.

The SIA results of this study showed an increase in δ^{15}N and δ^{13}C values with length, as juveniles feed on benthic invertebrates, mainly shrimps and bivalves, whereas adults prefer crabs and cephalopods. A positive linear relationship between the size and ^{15}N and ^{13}C values may support the SCA results of this study for ontogenetic shift. Although the sampling depths of juveniles and adults were different, diet overlap was very low first two sizes (sizes I and II) and the rest (sizes III, IV and, V). δ^{13}C values were not statistically significant between juveniles and adults, may indicate similar habitat preferences, and should further be investigated. Prey contributions were found different between tissues, such as remarkable differences were found in shrimp contribution, which were found higher in liver tissues than muscles. On the contrary, significant increase in the contribution of crabs and cephalopods in muscle tissues was detected. Those remarkable differences between tissues may indicate that *R. clavata* diet changes seasonally because of food availability. The author's SIA results were different in prey contributions than the author's SCA results. The MixSIAR results showed that both adult and juvenile individuals of *R. clavata* feed mainly on the crustaceans, but the contribution of teleosts represented by *Trachurus* sp. was very low (<15%), which the authors estimated its contribution 20% and 33% in SCA for juveniles and adults, respectively. Nonetheless, similar differences between SCA and SIA were also previously reported, and the SIA results of this study were in line with Barría *et al*. (2015) showing that crustaceans (shrimps and crabs) were the main prey group and teleost contribution was very low in the *R. clavata* diet. Still, there are limitations in our SIA practice in terms of samples size adequacy and isotope values for diverse preys, specifically for teleost which only represented by *Trachurus* sp. In addition, SIA has some strict requirements in terms of knowledge on DTDF while performing more elaborate analysis, which these kinds of background knowledge of the required DTDF values were missing for the Sea of Marmara. DTDF is both species-specific and tissue-specific, requiring laboratory experiments following the comparison of field data (*i.e*., Post 2002; Hussey *et al*., 2010; Caut *et al*., 2013; Li *et al*., 2018; Kadye *et al*., 2020).

According to previous studies, the authors found that thornback ray had the highest trophic position among batoid species in the Sea of Marmara, followed by piscivorous *T. marmorata* and *D. pastinaca*, *Myliobatis aquilla*, with the latter two usually feed on benthic invertebrates (Gül & Demirel, 2020). *D. pastinaca* and juvenile *R. clavata* occupy similar trophic positions because of the invertebrate dominance in their diets (Gül & Demirel, 2020). Among the other common benthic species, a teleost species *L. budegassa* had the highest δ^{15}N values compared to another piscivorous mesopredator species *S. acanthias* (Gül & Demirel, 2021). Another teleost species *Trachurus* sp. which was reported mainly fed on mesozooplankton species (Rumolo *et al*., 2017) and found as main prey item of thornback ray in this study had δ ^{15}N values of 11.8‰ (1.1 ‰ lower than thornback ray adults). In addition, the enrichment has occurred at 0.9 ‰ *L. depurator* between thornback ray juvenile specimens. In general, the enrichment of δ^{15}N values preys to predators in elasmobranch was determined as 3.4 ‰ (Post, 2002). Nonetheless, the authors could not evaluate lower δ^{15}N difference between predator and prey because of lack of stable isotope studies belonging to basal sources or zooplankton groups, thus the inadequacy of the proper food chain in the Sea of Marmara. It should be considered that comprehensive studies are necessary for investigating the effects of environmental conditions on the δ^{15}N values to determine the groups that suppress the system and dominate the food chain in the highly eutrophic Sea of Marmara.

5 | CONCLUSION

In this study, the authors showed that the thornback ray has the highest trophic level among batoids and it is an essential member of mesopredator species. Moreover, Thornback ray is consumed by larger sharks, marine mammals, other batoid species and teleosts (Flowers *et al*., 2021). Although predation risk affects species' behaviour and population structure, it is not prominent as the Sea of Marmara has

gradually lost most of its large marine predators since the 1980s (Demirel *et al*., 2020). Aside from predation and interspecific competition of other batoid species, the high fishing effort and intraspecific competition may be a factor for its population decrease in the Sea of Marmara. In addition, to the interaction and overlap between size groups, future studies should be designed by either better sampling design or novel approaches such as metabarcoding studies for stomach content. Considering the vulnerable population status of the species, stable isotopes that are non-lethal and can be interpreted with a relatively small number of samples by SCA may provide more effective results without further harming the delicate population of this species.

AUTHOR CONTRIBUTIONS

G.G. and N.D.: conceptualization, methodology, data analysis, writing original draft, reviewing and editing. N.D.: supervision and project administration.

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