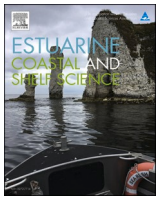
Estuarine, Coastal and Shelf Science 259 (2021) 107448



Contents lists available at ScienceDirect

Estuarine, Coastal and Shelf Science

journal homepage: www.elsevier.com/locate/ecss





Evaluation of the comprehensive feeding strategy and trophic role of overexploited mesopredator species in the Sea of Marmara (northeastern Mediterranean)

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ARTICLE INFO

Keywords:
Demersal sharks
MixSIAR
Predator-prey relationships
Stable isotope analysis
Stomach content analysis
Trophic position

ABSTRACT

Mesopredator species play an essential role in balancing and sustaining ecosystems through their interactions with various species and with their habitats. In ecosystems that have been subjected to overfishing, it is essential to obtain information on the trophic ecology of such predators. Based on this motivation, this study aimed to investigate the feeding strategies and trophic roles of four demersal sharks (e.g., *Mustelus mustelus, Mustelus asterias, Squalus acanthias, and Scyliorhinus canicula*) by analyzing their stomach contents and using stable isotope ($\delta^{13}C$ and $\delta^{15}N$) analysis. These shark species are subject to overfishing, and their landings have decreased by 98% over the last 18 years in the Sea of Marmara. The results demonstrated that interspecific differences in the main prey resources exist among those sharks. *Mustelus mustelus* and *Mustelus asterias* feed mainly on crustaceans, and their diets are very similar. However, *Mustelus asterias* utilizes a specialist feeding strategy while *Mustelus mustelus* utilizes a medium-state generalist strategy. *Squalus acanthias* occupies a high trophic level and mainly feeds on teleosts over both short and long time scales. The probability of isotopic niche overlap of *Scyliorhinus canicula* with the isotopic niches of *S. acanthias* was the highest (70.7%), which indicates that both species exploit similar food resources within the same environment. These results contribute to the knowledge of the ecological role of these highly vulnerable cartilaginous species and may help to improve conservation programs in the Sea of Marmara, a lesser-known part of the northeast Mediterranean.

1. Introduction

Marine ecosystems and, especially, species interactions within them are complex. Many species occupy different trophic levels at different stages of their life cycles, while species at the same trophic level often occupy different habitats and ecological niches that sometimes vary temporally and spatially (Dobson, 2009; FAO, 2014). The removal of keystone species, removal of entire trophic levels and biodiversity loss all contribute to ecosystem instability, which can induce regime shifts since richer biodiversity levels buffer disturbances and support ecosystem regulation (Folke et al., 2004). Demersal elasmobranchs are regarded as keystone species of benthic habitats, as they interact with many diverse groups of organisms (Hattab et al., 2013; Tecchio et al., 2013; Kousteni et al., 2017). Most sharks are competitive carnivores and are categorized as apex or mesopredators. Mesopredator species play the role of apex predators in ecosystems that have lost their apex predators, so their population sizes, distributions, and feeding ecologies directly influence the habitat stability of marine ecosystems (Baum and Worm, 2009). Therefore, understanding the trophic ecology of mesopredator sharks is fundamental for evaluating community structures, as well as for ecosystem management due to their high trophic levels and the subsequent essential role in controlling marine environments (Caut et al., 2013). Investigations of the trophic ecology of aquatic organisms are conventionally based on examinations of predator-prey effects by diet analyses of species. This methodology includes counting, weighting, and estimating the frequency of occurrence of individual prey items to provide insights into their diets (Hyslop, 1980; Cortés, 1997). However, stomach content analysis can be complex due to the digestive physiology of sharks, which includes rapid breakdown of food items (Leake et al., 1975; Cortés et al., 2008; Wieczorek et al., 2018). Stable isotope analysis (SIA) of carbon and nitrogen has become a useful and widespread tool in recent years for studying the trophic ecology of sharks (Shiffman et al., 2012). SIA is based on determining the ratios of heavy to light isotopes (e.g., ^{13}C:^{12}C and ^{15}N:^{14}N) in animal tissues. Animals digest some prey

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https://doi.org/10.1016/j.ecss.2021.107448
Received 3 June 2020; Received in revised form 20 May 2021; Accepted 27 May 2021
Available online 15 June 2021
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items, while the indigestible portions of items accumulate in the animals' tissues (Philips et al., 2014). Stomach content analysis (SCA) provides information on prey items that are consumed over a short time period (e.g., from approximately 1–7 days), while SIA provides an opportunity to gain a longer-term perspective of their diets (Hyslop, 1980; Fry, 2008). Animal tissues may reflect the isotopic signatures of diets, and the duration of this process varies according to the tissue type, which is called the tissue turnover rate (Li et al., 2018). The turnover rates for elasmobranch species were determined to be as long as 8–12 months in white muscles and as short as several weeks to months in the liver (Logan and Lutcavage, 2010). These differences enable the evaluation of individual-based features such as growth, ontogenetic changes, and migrations (Domi et al., 2005). The isotope ratios of carbon (^{13}C) are used to identify carbon sources in marine food webs, while ^{15}N is used to determine the consumer trophic positions and can achieve high-level discrimination between predators and prey (Hussey et al., 2012). The different isotopic signatures of both predators and prey can be used in mixing models to determine the contributions of prey resources to predator diets (Phillips and Gregg, 2003; Phillips et al., 2005).

The Sea of Marmara, which is in the northeastern-most part of the Mediterranean Sea, is a small intercontinental basin that is connected to the Aegean Sea via the Dardanelles Strait and to the Black Sea via the Istanbul (Bosphorus) Strait. Therefore, as a transition zone, the hydrography of the Sea of Marmara consists of both Mediterranean and Black Sea water, and the system has two major currents. The bottom current comes from the saline and oxygenated waters of the Mediterranean and flows northward, while the upper current, which carries less-saline and more nutrient-rich waters, comes from the Black Sea and flows southward (Beşiktepe et al., 1994). Thus, the demersal life in the Sea of Marmara is typical of the Mediterranean, while the pelagic biota mostly reflect the Black Sea ecosystem (Demirel and Gül, 2016), resulting in rich biodiversity (Oğuz and Öztürk, 2011). Sharks, as an export commodity that are not consumed domestically in Turkey, are not the main target of commercial fisheries (Kabasakal, 2016); however, their catch statistics have shown a sharp decline (approximately 98%) between 2000 and 2018 (e.g., 269 t and 3.8 t, respectively; Turkish Statistical Institute [TUIK], 2019). This indicates a high vulnerability of stock collapse and a near-term threat of disappearing completely from the Sea of Marmara (Ulman et al., 2020). Despite their ecological importance as an essential indicator of ecosystem health, knowledge on the biology and ecology of sharks in the Sea of Marmara has received little attention from researchers. Thus, there is no available information regarding their habitat preferences or trophic ecology, aside from records of their occasional presence (Kabasakal, 2016).

In the present study, we investigated the feeding strategies and trophic levels of four demersal shark species: smooth-hound Mustelus mustelus Linnaeus 1758, starry smooth-hound *Mustelus asterias* Cloquet 1819, piked dogfish *Squalus acanthias* Linnaeus 1758 and lesser-spotted dogfish *Scyliorhinus canicula* Linnaeus 1758 in the Sea of Marmara. Specifically, their diets, feeding habits and interspecific interactions were analyzed. Two complementary techniques, stomach content and stable isotope ($\delta^{13}C$ and $\delta^{15}N$) analyses, were conducted to assess the assimilation of the main resources, niche overlaps, and isotopic signatures of these overexploited species in the Sea of Marmara ecosystem.

2. Materials and methods

2.1. Sampling

This study was performed seasonally (e.g., in spring, summer, autumn, and winter) between December 2017 and December 2018 at 34 stations that were considered to be representative of the Sea of Marmara (Fig. 1). Sampling followed the protocol of the International Bottom Trawl Survey in the Mediterranean (MEDITS) (MEDITS, 2013), and 151 hauls were obtained by bottom trawling between depths of 25 and 151 m. Bottom trawling was carried out for 30 min at 2.5 knots with commercial fishing vessels.

Shark species were obtained as bycatches during commercial trawling operations (Table 1; Fig. 2) and consisted of *Mustelus mustelus* (n = 28), *Mustelus asterias* (n = 18), *Squalus acanthias* (n = 45), and *Scyliorhinus canicula* (n = 50). The total lengths (TL) and fork lengths (FL) were measured to the nearest 0.1 cm, and the total weights (TW) and stomach weights were measured to the nearest g.

2.2. Stomach content analysis (SCA)

Stomach samples were removed from the bodies and were fixed in a 4% buffered formaldehyde solution. In the laboratory, prey items were

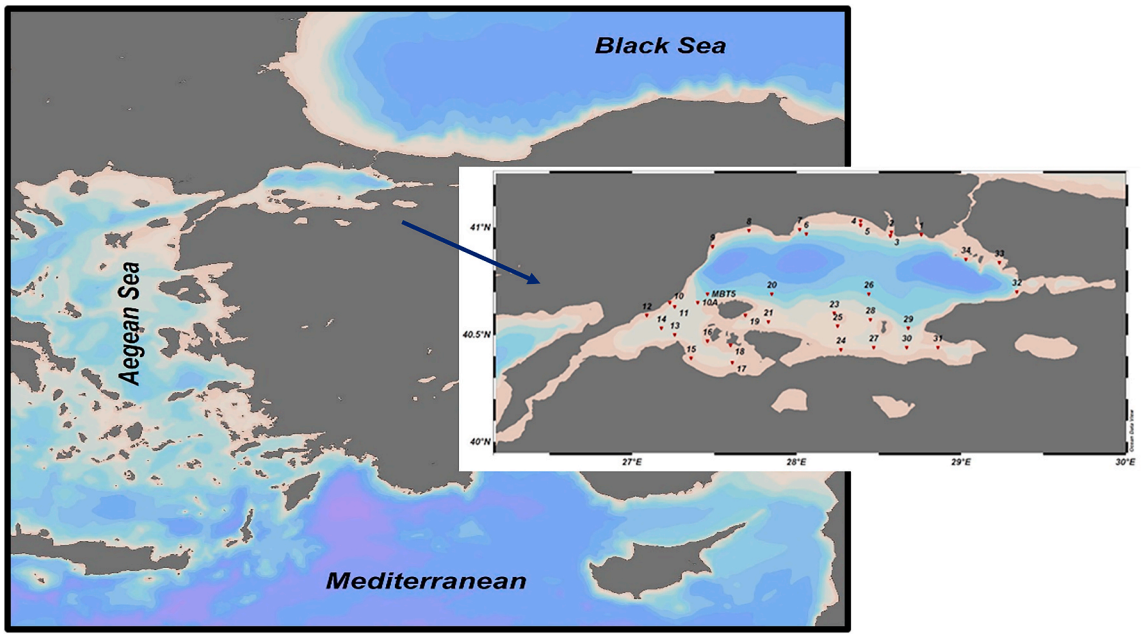


Fig. 1. Sampling stations (indicated by blue dots) and the location of the Sea of Marmara, northeastern Mediterranean. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

Table 1
Overall sampling size (N), average sampling depth (Avg. D.), fork length (FL), total weight (TW) and sample size (n) of each species used in stomach content (SCA) and stable isotope analyses (SIA).

Species	Avg. D. (m)	N	FL (cm)	TW (g)	SCA	SIA	
					n_{Full}	n_{Muscle}	n_{Liver}
Mustelus mustelus	89	28	31.5–130.8	185–9585	27	NA	
Mustelus asterias	151	18	30.0–98.4	115–3585	16	6	NA
Squalus acanthias	101	45	18.1–85.0	30–585	40	10	11
Scyliorhinus canicula	77	50	17.5–71.5	Oct-65	28	6	9

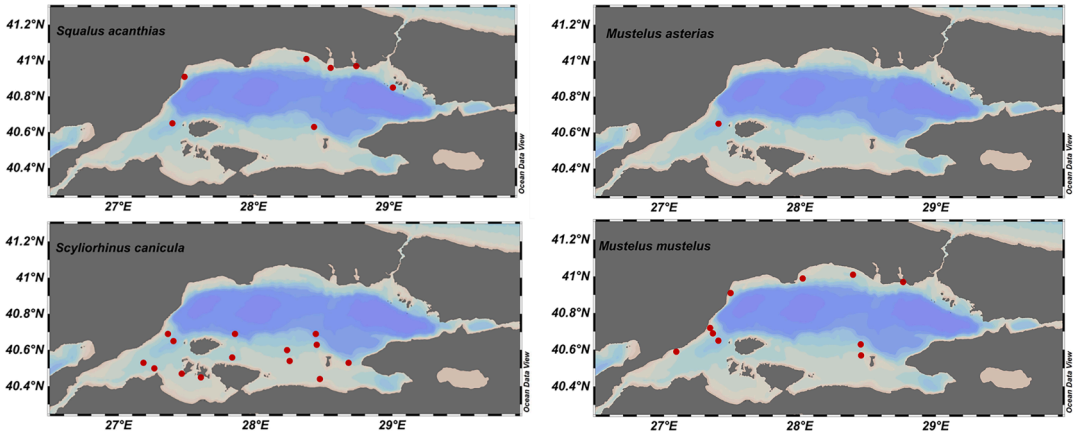


Fig. 2. Sampling locations (indicated by red dots) of four demersal shark species during the sampling period in the Sea of Marmara. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

sorted, weighed and identified to the lowest possible taxonomic level. To check the percentage of empty stomachs, the vacuity index (VI) was used. VI (%) = A*100/B, where A is the total number of empty stomachs and B is the total number of stomachs examined (Hyslop, 1980).

The diet of each shark species was quantified by the numerical percentage (%N), wet weight percentage (%W) of each prey contribution, and the frequency of occurrence (%F) of each prey species in the examined stomachs. The index of relative importance for each prey item (IRI = %F x (%W + %N; Hyslop, 1980)) and the standardized IRI (%IRI) were calculated as follows: %IRI = (IRI/$\sum$ IRI) x 100 (Cortés, 1997).

IRI% is a useful index for evaluating and comparing the feeding characteristics of species. Dominant prey or prey groups in the shark diet accounted for their high IRI% (>50%).

2.3. Stable isotope analysis (SIA)

Carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) stable isotope analyses (SIAs) were performed for three out of the four species due to a tissue sampling problem with the *M. mustelus* species. Additionally, the liver tissues of the *M. asterias* species had unsuccessful lipid extraction during the freeze-drying process, and SIA was only performed on muscle tissue (Table 1). White muscle tissues were extracted from under the dorsal fin (approximately 5 g), and liver tissues were extracted from each individual. The tissues were frozen onboard and were kept frozen until the lipid extraction process. During trawling operations, teleost fish and invertebrates, as potential prey items, were also collected. After the stomach content analyses, selected prey species were transferred for SIA.

The muscle and liver samples were primarily subjected to lipid extraction. The lipid extraction process is recommended, especially for shark species, because lipids may deplete $\delta^{13}C$ in animal tissues (Post, 2002; Kim and Koch, 2012; Carlisle et al., 2017). Lipid extraction was performed by following the protocol described by Bligh and Dyer (1959) and Hussey et al. (2012). Following lipid extraction, the samples were frozen at −20 °C for one night and were then freeze-dried for 3 day at approximately −65 °C. All samples were powdered, weighed, packed in tin capsules (1.1–1.5 mg of each sample) and transferred for ^{13}C and ^{15}N isotope analyses at the Stable Isotope Facility at the University of California, Davis (UC Davis SIF). The isotope ratios of the samples were measured on a PDZ EuroLevinpa ANCA-GSL elemental analyzer that was interfaced with a PDZ Europa 20-20 isotope ratio mass spectrometer. The stable isotope ratios of carbon and nitrogen were expressed in conventional δ notation (‰) as $\delta^{13}C$ and $\delta^{15}N = [(R_{sample}/R_{standard}) - 1]$ x 1000, where R_{sample} indicated the ratio of heavy to light isotopes (^{13}C:^{12}C and ^{15}N:^{14}N); $R_{standard}$ was the international standard in which Vienna Pee Dee Belemnite was used for carbon and atmospheric N_2 was used for nitrogen. The long-term standard deviations, as characterized by analytical precisions, were accepted as 0.2‰ for $\delta^{13}C$ and 0.3‰ for $\delta^{15}N$. Additionally, 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.

To avoid any potential lipid-related biases in the $\delta^{13}C$ determinations, the SIA results were checked for lipid contents based on the C:N ratios. Low values (e.g., C:N < 3.5; Post et al., 2007) were found in four shark species (e.g., *S. canicula* with 3.3 ± 0.5, *S. acanthias* 3.4 ± 0.4, and *M. asterias* 3.3 ± 0.3). Therefore, no mathematical corrections were applied to their $\delta^{13}C$ values.

2.4. Isotope mixing model

A Bayesian isotope mixing model, MixSIAR (Stock and Semmens, 2016), was used to estimate the potential contributions of each prey group to the shark diets based on the variations in $\delta^{15}N$ and $\delta^{13}C$ values. The MixSIAR model also allows users to create graphical outputs while running the code in R software (Stock and Semmens, 2016; Stock et al., 2018; R Core Team, 2020). In this study, potential prey items were selected based on their importance in the SCAs. These species accounted for the representatives of prey groups, such as *Trachurus* sp. for Teleostei, *Parapeneus longirostris* and *Liocarnicus depurator* for Crustacea, and *Loligo* sp. for Cephalopoda. The discrimination factors were obtained from Caut et al. (2013) (e.g., Shrimp: $\Delta^{15}N = 0.23 \pm 0.16$, $\Delta^{13}C = 0.44$

± 0.12; Crab: $\Delta^{15}N = 2.77 \pm 0.33$, $\Delta^{13}C = 1.05 \pm 0.07$; Teleost: $\Delta^{15}N = 1.81 \pm 0.14$, $\Delta\delta^{13}C = 1.49 \pm 0.1$; and Cephalopods: $\Delta^{15}N = 2.49 \pm 0.26$, $\Delta^{13}C = -0.46 \pm 0.26$). Gelman-Rubin and Geweke's diagnostic tests confirmed that the models converged.

2.5. Trophic niches

To understand the trophic niche characteristics of shark species, different calculations, such as niche breadth by SCA results, niche size by SIA results, and niche overlap by both SCA and SIA results, were conducted.

Niche breadth or niche size is also known as the Levin's index (Colwell and Futuyma, 1971). According to Levin (1968), niche breadth (B) can be calculated as $B = 1/\Sigma p_j^2$, where p_j is the proportion of each food category consumed. Hurlbert (1978) proposed a standardization to express the B ranges between 0 and 1, which was later called Levin's standardized niche breadth (B_A) and was estimated as follows: $B_A = \frac{B-1}{n-1}$. The index was categorized as high (>0.6), moderate, (0.4–0.6) and low (<0.4) (Novakowski et al., 2008).

Pianka's index (Pianka, 1973) was used to estimate the amount of dietary overlap within shark species. This index ranges between 0 and 1, where 0 indicates an absence of overlap, and 1 indicates complete overlap. To better understand the overlap level within species, the values were categorized as low (0–0. 39), intermediate (0.4–0.6) and high (0.61–1) (Grossman, 1986). The EcoSimR package was used to calculate niche overlap levels within species and to test the calculated values against null models (Gotelli and Ellison, 2015; R Development Core R Core Team, 2020). Null models ran simulations by using 1000 Monte Carlo randomizations and estimated the expected overlap levels. The differences between expected overlap and observed overlap can be evaluated as resource partitioning or sharing between species. The observed niche overlap levels can be expressed relative to those expected by chance using the standardized effect size (SES) to correct for differences among species in their variations of simulated overlap values (Gotelli and Ellison, 2015).

The isotopic niche breadth, as the standard ellipse corrected area (SEAc, ‰^{2}, adjusted for small sample size), was calculated by using the stable isotope Bayesian ellipses (SIBER) method in the SIAR package (Jackson et al., 2011; Parnell et al., 2008; R Core Team, 2020). SEAc is a metric that provides a measure of the isotopic niche of each individual. In this study, biplots of SEAc that used the $\delta^{15}N$ and $\delta^{13}C$ values of three shark species were generated to determine the extent of their trophic diversity. An SEAc biplot was also considered for potential isotopic niche overlap among species to consider further pairwise comparisons. Thus, to estimate the probability of isotopic niche overlap among species, the NicheROVER package (Swanson et al., 2015; R Core Team, 2020) was used. The niche region was defined as a given 95% probability region in multivariate space, which was then calculated as the probability of an individual from species A occupying the isotopic niche region of species B (Swanson et al., 2015). The estimated pairwise niche overlap among shark species was considered to represent "high niche overlap" if the median overlap was greater than 50% between two species.

2.6. Trophic positions

The trophic level (TL) of each species was calculated using the SCA results according to the following formula (Cortés, 1999): $TLSCA = 1 + \sum_{j=1}^{k} Pj^{*}TLj$ where TL_{SCA} is the trophic level of the consumer, Pj is the proportion of each prey category in the stomach (W %), and TLj is the trophic level of each prey category j, which was obtained from Ebert and Bizzarro (2007).

The trophic position (TP) of each species was estimated based on its nitrogen isotopic signature according to the following equation (Zanden and Rasmussen, 2001): $TP_{consumer} = TP_{basal} + (\delta^{15}N_{consumer} - \delta^{15}N_{basal})/\Delta^{15}N$, where $\delta^{15}N_{consumer}$ is the value for each shark species, $\delta^{15}N_{basal}$ is that of a bivalve (*Donax turunculus*) (n = 2, $\delta^{15}N_{mean} = 8.40 \pm 0.33$) that was sampled from the Sea of Marmara, and Δ is the trophic enrichment factor between organisms and diets. Following Hussey et al. (2010), $\Delta^{15}N = 2.29$ (diet–tissue discrimination factors for lipid extracted muscle) and $TP_{basal} = 2$ were used.

2.7. Data analysis

The stomach contents were tested to determine whether diet compositions differed among species. A one-way permutational multivariate analysis of variance (PERMANOVA) to a matrix of prey items (based on the number of individuals) using Bray-Curtis similarity matrices with 9999 permutations was applied. If significant results were found, pairwise comparisons were carried out. To identify the relationships among predator and prey species, principal component analysis (PCA) was performed based on the cumulative numbers of each prey type (N) found in the shark stomachs. Principal components were extracted by using the correlations among cumulative prey numbers as variables. PCA plots were used to visualize the trophic relationships among samples with highly correlated (Pearson correlation>0.5) prey groups. In this study,

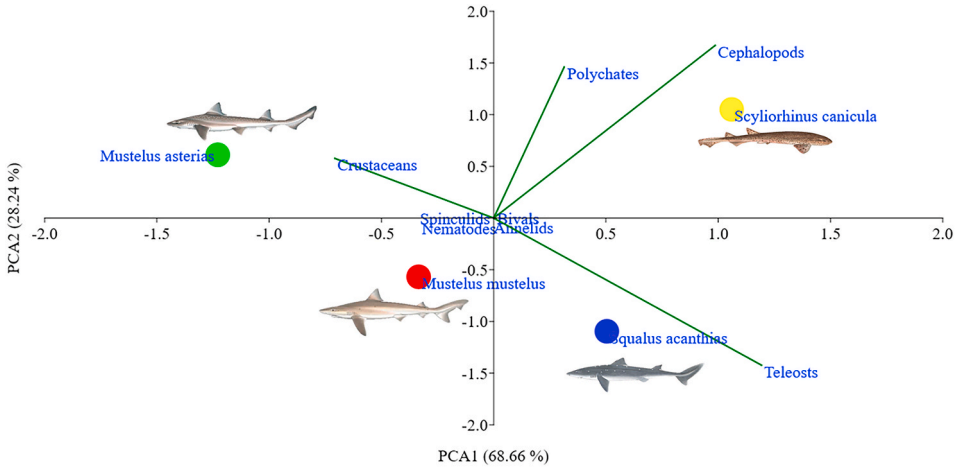


Fig. 3. Results of principal component analysis (PCA) derived from stomach content compositions based on the number of prey species (N) in four demersal sharks (*Squalus acanthias*, blue dot; *Scyliorhinus canicula*, yellow dot; *Mustelus asterias*, green dot; and *Mustelus mustelus*, red dot). Significant group variables are indicated by green lines. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

Table 2
Diet compositions of four shark species in the Sea of Marmara, northeastern Mediterranean (%N, percentage in number; %W, percentage in weight; %FO, frequency of occurrence; and %IRI, index of relative importance of each prey item).

Prey	*Mustelus mustelus*				*Mustelus asterias*				*Squalus acanthias*				*Scyliorhinus canicula*			
	%N	%W	%FO	%IRI	%N	%W	%FO	%IRI	%N	%W	%FO	%IRI	%N	%W	%FO	%IRI
Nematodes																
Unidentified nematodes	5.52	0.04	16.00	1.28	4.63	0.00	12.50	0.53	1.20	0.00	2.50	0.06	–	–	–	–
Sipuncula																
Unidentified sipuncula	–	–	–	–	–	–	–	–	22.16	0.14	5.00	2.38	–	–	–	–
Annelid									10.18	0.22	12.50	2.77	–	–	–	–
Unidentified Annelida	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Polychaeta																
Sternaspis scutata	–	–	–	–	–	–	–	–	–	–	–	–	1.61	0.15	3.57	0.13
Unidentified polychaeta	0.69	0.00	4.00	0.04	–	–	–	–	1.20	0.02	2.50	0.06	6.45	4.17	10.71	2.30
Bivalves																
Unidentified bivalvia	0.69	0.05	4.00	0.04	–	–	–	–	–	–	–	–	–	–	–	–
Crustaceans																
Decapods																
Parapenaeus longirostris	19.31	25.37	52.00	33.38	43.52	85.74	75.00	88.48	9.58	10.01	25.00	10.44	1.61	3.81	3.57	0.39
Liocarnicus depurator	32.41	44.35	48.00	52.93	6.48	9.37	37.50	5.43					1.61	2.78	3.57	0.32
Dendrobranchiata	–	–	–	–	–	–	–	–	4.19	1.25	10.00	1.16	17.74	5.10	21.43	9.88
Unidentified crustaceans	–	–	–	–	–	–	–	–	1.20	0.17	2.50	0.07	9.68	2.10	17.86	4.25
Unidentified decapods	0.69	0.17	4.00	0.05	1.85	0.33	12.50	0.25	1.20	0.15	5.00	0.14	8.06	7.08	17.86	5.46
Isopods																
Unidentified isopods	–	–	–	–	–	–	–	–	–	–	–	–	1.61	1.17	3.57	0.20
Stomatopods																
Squilla mantis	0.17	1.53	4.00	0.10	0.93	3.47	6.25	0.25	0.60	1.04	2.50	0.09	–	–	–	–
Cephalopods																
Unidentified cephalopods	13.79	0.35	8.00	1.63	40.74	0.11	12.50	4.66	1.20	1.03	5.00	0.24	16.13	21.28	32.14	24.29
Teleosts																
Trachurus sp.	15.86	22.41	16.00	8.80	1.85	1.67	12.50	0.40	15.57	41.47	27.50	33.43	3.23	8.36	7.14	1.67
Engraulis encrasicolus	8.97	5.58	8.00	1.67	–	–	–	–	6.59	8.64	12.50	4.06	1.61	4.39	3.57	0.43
Gobius niger	–	–	–	–	–	–	–	–	2.40	6.74	2.50	0.49				
Unidentified teleosts	1.38	0.14	4.00	0.09	–	–	–	–	26.35	29.46	37.50	44.61	30.65	39.61	35.71	50.68

the diet results of sharks could not be tested according to season or region due to the low sampling sizes, which would preclude reliable analyses. Multivariate analyses were performed with PRIMER7 + PERMANOVA software (v.7.0; PRIMER-E).

By following Levene's test to check the homogeneity of variances, one-way ANOVA or the Kruskal–Wallis test was used to test the differences in isotopic values (e.g., $\delta^{13}C$ and $\delta^{15}N$) among shark species at the $\alpha = 0.05$ significance level. If the results were found to be significant, then post hoc analyses (Tukey HSD; Wilcoxon) were applied to determine the specific differences among species. Linear regression analyses of the isotopic values (e.g., $\delta^{13}C$ and $\delta^{15}N$) against the fork lengths (FL, cm) of shark species were used to determine the significance levels of size-based trophic position changes in each species. Univariate analyses were performed by using IBM SPSS Statistics (v.12.0; IBM Corp.).

3. Results

3.1. Evaluation of diet preferences based on SCA

A total of 111 stomachs from 141 shark specimens were analyzed (Table 1). The vacuity index was calculated as 5.5% for *M. mustelus*, 12% for *M. asterias*, 11.1% for *S. acanthias,* and 44% for *S. canicula.*

The PERMANOVA results indicated significant differences among the diet compositions of the four species ($F_{3,18} = 7.357$, $p < 0.001$). According to the pairwise comparisons, the stomach contents were significantly different between *S. acanthias* and *S. canicula* ($p = 0.0012$), between *S. acanthias* and *M. mustelus* ($p = 0.0006$), between *S. acanthias* and *M. asterias* ($p = 0.0006$), between *S. canicula* and *M. mustelus* ($p = 0.0006$), and between *S. canicula* and *M. asterias* ($p = 0.0006$) but were not significantly different between *M. mustelus* and *M. asterias* ($p = 0.092$).

The PCA of the cumulative prey compositions (N) of shark diets reflected that the first (PC1) and second (PC2) components explained 97.1% of the total variance. PC1 accounted for 68.7% and crustaceans, cephalopods and teleosts contributed to the separation of *M. asterias*, *S. acanthias* and *S. canicula* along this axis. PC2 accounted for 18.2% and the main prey groups that contributed to this were polychaetes and cephalopods (Fig. 3).

Crustacea was the dominant prey group in the *M. mustelus* and *M. asterias* diets (86.5% and 94.4% IRI, respectively), while teleosts were the dominant prey for *S. acanthias* and *S. canicula* (82.5% and 52.79% IRI, respectively). The deep-water rose shrimp, *Parapenaeus longirostris,* was the dominant Crustacea species, with 88.5% IRI in the stomach contents of *M. asterias*, while the harbor crab, *Liocarcinus depurator,* was the dominant species for *M. mustelus* (52.9% IRI). Although *S. canicula* feeds mainly on teleosts, cephalopods are also an essential part of its diet (24.3% IRI; Table 2).

Table 3
Isotopic values of sharks and their prey from the Sea of Marmara, northeastern Mediterranean.

Species	Muscle		Liver	
	$\delta^{13}C_{mean}$ ‰	$\delta^{15}N_{mean}$ ‰	$\delta^{13}C_{mean}$ ‰	$\delta^{15}N_{mean}$ ‰
Mustelus asterias	−17.72 ± 0.29	13.07 ± 0.49	–	–
Squalus acanthias	−18.30 ± 0.21	13.33 ± 0.45	−18.75 ± 0.98	12.23 ± 0.50
Scyliorhinus canicula	−18.38 ± 0.33	12.95 ± 0.39	−18.64 ± 0.55	11.13 ± 0.54
Parapenaeus longirostris	−19.29 ± 0.14	9.82 ± 0.33	–	–
Liocarcinus depurator	−18.85 ± 0.39	11.3 ± 0.49	–	–
Loligo sp.	−19.87 ± 0.06	12.37 ± 0.25	–	–
Trachurus sp.	−20.16 ± 0.43	11.81 ± 0.78	–	–

3.2. Isotopic results of three shark species

In this study, *Mustelus mustelus* was not included in the SIA analysis, and the trophic signatures of *M. asterias* individuals were analyzed based only on muscle tissue (Table 3). The $\delta^{15}N$ values showed no significant differences among the three species (one-way ANOVA $F_{2,20} = 1.56$, $p = 0.23$). The $\delta^{13}C$ values differed significantly ($H_2 = 7.94$, $p < 0.05$) between *S. acanthias* and *M. asterias* ($p < 0.05$) according to a pairwise comparison. In particular, *S. acanthias* showed the highest $\delta^{15}N$ value, while the lowest $\delta^{15}N$ and highest $\delta^{13}C$ values were found for *S. canicula*

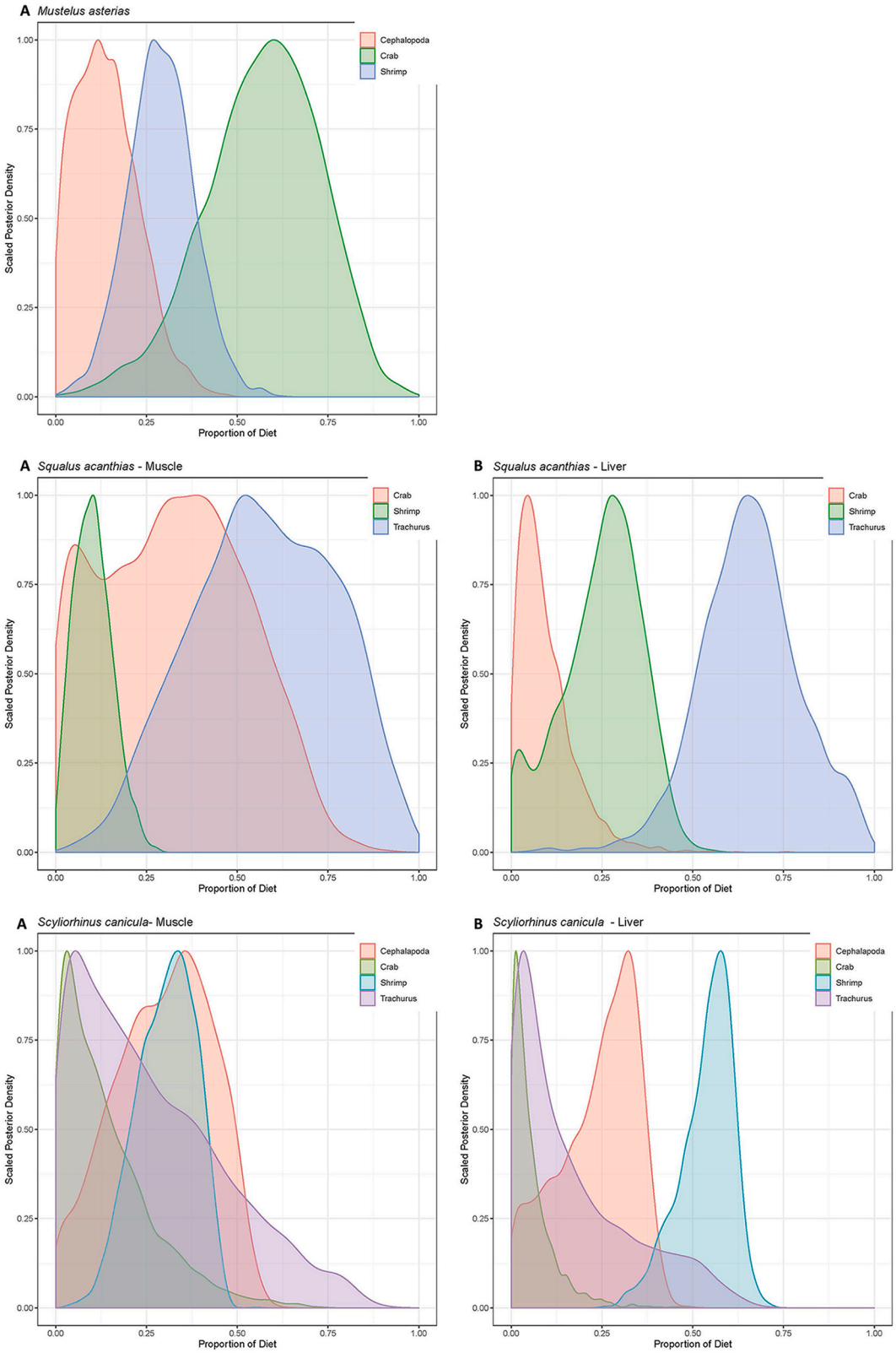


Fig. 4. Proportional contributions of prey items in the diets of *Mustelus asterias*, *Squalus acanthias*, and *Scyliorhinus canicula,* as by a Bayesian isotopic mixing model (A-muscle tissue, B- liver tissue).

(Table 3). The $\delta^{15}N_{liver}$ values were found to be significantly different between *S. acanthias* and *S. canicula* ($t_{18} = 4.65$, $p < 0.001$), while the $\delta^{13}C_{liver}$ values were not ($t_{18} = 0.56$, $p = 0.58$).

The $\delta^{15}N$ values showed a positive relationship with fork length (FL) in *S. acanthias* (Mean FL = 60 cm; $r^2 = 0.70$, $p < 0.05$; $\delta^{15}N = 0.016$*FL + 12.35) but not in the other two species. No relationship was found between the $\delta^{13}C$ values and FLs for any species.

3.3. Isotopic mixing model

The Bayesian isotopic mixing model outputs revealed that the potential prey contributions for *M. asterias* consisted of two crustaceans (e.g., 57% - *L. depurator* and 29% - *P. longirostris* Fig. 4). The isotopic values of *M. asterias* livers were not available. For *S. acanthias*, the potential prey contributions differed in the secondary resources between tissues. The main resources were teleosts (57%); crustaceans (32%, *L. depurator*) for muscle; teleosts (66%); and crustaceans (25%, *P. longirostris*) for liver (Fig. 4). The potential prey contributions for *S. canicula* differed depending on whether muscle or liver tissues were examined. The model suggested that the main resources, based on the mean percentages, were crustaceans (30%), cephalopods (30%), and teleosts (26%) for muscle and crustaceans (54%, *P. longirostris*) and cephalopods (23%) for liver.

Table 4
Pianka niche overlap and null model results among the species. Observed Index is Pianka's index. The SES values for nonsignificant probabilities were usually between −2.0 and + 2.0.

Species	Observed Index	Simulated Index	SES
Mustelus mustelus x Mustelus asterias	0.62	0.20	**2.20***
Mustelus mustelus x Squalus acanthias	0.30	0.29	0.03
Mustelus mustelus x Scyliorhinus canicula	0.22	0.28	−0.33
Squalus acanthias x Mustelus asterias	0.22	0.03	−0.11
Squalus acanthias x Scyliorhinus canicula	0.53	0.31	1.42
Scyliorhinus canicula x Mustelus asterias	0.30	0.20	0.52

3.4. Niche overlap and trophic levels

The Levin standardized niche breadth (B_A) values showed medium niche breadths in terms of prey category consumption for all shark species ($0.4 < B_A < 0.6$). Among the species, *M. asterias* presented the lowest value ($B_A = 0.29$), while other species showed similar values that were between 0.41 and 0.43.

Based on stomach content data, high dietary overlap was observed between *M. mustelus* and *M. asterias* (Pianka index > 0.60). This was also confirmed by the significant standardized effect size (SES) from the null model outputs between *M. mustelus* and *M. asterias* (Table 4).

Considering the isotopic niche width that was determined by the SEAc values for the three shark species, *S. canicula* was broader than the other two sharks, and the SEAc biplot showed high overlap between *S. canicula* and *S. acanthias* (Fig. 5). Isotopic niche overlaps were found with the highest probability (70.7%) of overlap between *S. canicula* and *S. acanthias*, while *M. asterias* had the lowest overlap with the other two species (Table 5). The niche overlap results, as determined by SCA and SIA, were found to be comparable to each other.

The trophic levels that were estimated using stomach content analysis (TL_{SCA}) and isotopic values (TL_{SIA}) were similar for *S. acanthias* ($TL_{SCA} = 4.47$, $TL_{SIA} = 4.15 \pm 0.37$) and for *S. canicula* ($TL_{SCA} = 4.24$, $TL_{SIA} = 3.98 \pm 0.53$). For *M. asterias*, the TLs were significantly different for the two estimates, and TL_{SIA} was clearly higher ($TL_{SCA} = 3.31$, $TL_{SIA} = 4.03 \pm 0.25$). For *M. mustelus*, the TLs were only estimated from SCA ($TL_{SCA} = 3.76$). Overall, *S. acanthias* exhibited the highest trophic level.

4. Discussion

To our knowledge, this study presents the first comprehensive assessment of the feeding ecology and trophic roles of four demersal shark species by using stomach contents and stable isotope ($\delta^{13}C$ and $\delta^{15}N$) analyses. According to our results, four shark species exhibited clear differences in their feeding strategies. Given the recent massive declines that have been reported in shark catches in the Sea of Marmara (~98%), sharks are an overexploited group that warrants effective conservation efforts and strategies for more responsible management (Kabasakal, 2016). Due to their impoverished stocks, sample sizes that were large enough to determine their feeding strategies from SCA alone were not collected. However, the Bayesian mixing model, when integrated with the SCA results, was used to sufficiently increase the

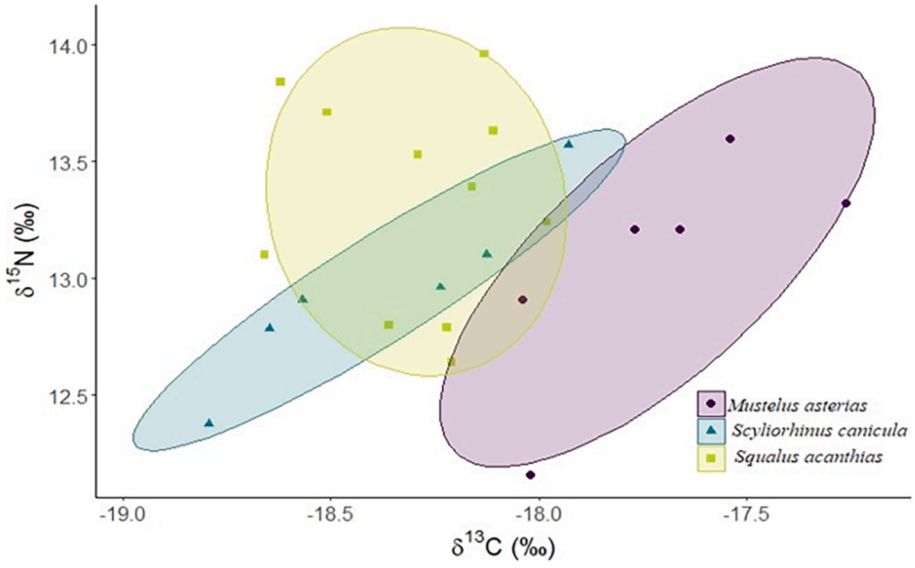


Fig. 5. Biplots of the 95% standard isotopic ellipses (SEAc) of *M. asterias* (purple, black circle); *S. canicula* (blue, black triangle); and *S. acanthias* (yellow, black square) in the Sea of Marmara. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

Table 5
Isotopic niche overlaps of three shark species in the Sea of Marmara. Value increases that are shown as light to dark green colors are probabilities (%) of the isotopic niche of species.

Species A	Species B		
	Mustelus asterias	*Squalus acanthias*	*Scyliorhinus canicula*
Mustelus asterias		23.5	7.38
Squalus acanthias	20.6		42.9
Scyliorhinus canicula	17.3	70.7	

knowledge of the respective feeding ecology features of the investigated species.

Two congeneric species, *M. mustelus* and *M. asterias,* have sparse distributions in the Sea of Marmara (Kabasakal and Karhan, 2015) as well as in the Mediterranean Sea (Schembri et al., 2003) and are frequently misidentified due to their similar morphometry (Farrell et al., 2009). *Mustelus* species are known as bottom dwellers; these sharks have molariform teeth to enable crushing of hard-shelled invertebrates such as crustaceans (Compagno, 1984; McCully Phillips et al., 2020). Our results are in line with previous studies showing that Crustacea was the main prey group in the *Mustelus* spp. diet (Jardas et al., 2007; Filiz, 2009; Di Lorenzo et al., 2020). Crustaceans are reported to have lower energetic contents with slower digestion rates than cephalopods and teleosts (Heupel and Bennett, 1998; McCully Phillips et al., 2020). This also supports the low vacuity index (VI) for both *Mustelus* species examined in the present study. At the species level, *L. depurator* was the main prey for *M. mustelus*, which was similar to the findings of previous studies (Gračan et al., 2014; Yemişken et al., 2019). This species exhibited a medium-state niche breadth that used a broader range of resources than *M. asterias.* The SCA results and the mixing model by SIA demonstrated that the primary prey group of *M. asterias* was also crustaceans. However, for the probabilities of the prey contributions were different at the species level between SIAs and SCAs, crabs (*L. depurator*) provided the highest contribution to the mixing model outputs (57%), while shrimp (*P. longirostris*) was found to be the main prey in the stomach (IRI% 88). This might lead to the conclusion that the mixing model underestimated the shrimp contribution due to a bias from low sampling sizes. However, other reasons, such as shifting prey choice, could be due to the high level of fishery exploitation of shrimp, which is a commercially important demersal resource in the Sea of Marmara (Demirel et al., 2020). Despite very limited knowledge on the feeding ecology of sharks, two notable studies reported crustaceans as the dominant prey group in the British Isles (Ellis et al., 1996) and in the Atlantic Ocean (McCully Phillips et al., 2020; IRI% 97.4 and IRI% 98.4, respectively). *M. asterias* exhibited the narrowest niche breadth, which indicated a limited number of resources in its diet compared to other species. This was supported by its lower $\delta^{13}C$ values that reflected smaller variations in exploited carbon sources. Nevertheless, based on SCA, the amount of dietary overlap between the two *Mustelus* species was found to be significant (PI > 0.60; SES>2.00). Therefore, these two species exploited the same resources, but differences in the importance of particular crustaceans indicated segregation in their feeding strategies. This may also be the reason for the differences in sampling depths and locations of the two species. *M. asterias* individuals were found in only one location (151 m) in every season, and *M. mustelus* individuals were sampled in various shallower locations during the study period. Although building a distribution pattern by season and region was not possible due to the small sampling size, it is likely that due to their similar feeding habits, these two species prefer different areas and depths to decrease potential competition by partitioning their main resources. It has been confirmed by observations that shark species with similar diets do not occur together (Papastamatiou et al., 2006); however, ontogeny, season or prey availability can greatly affect their strategies (Macpherson, 1980; Barría et al., 2018).

Squalus acanthias had a diverse diet based on SCA, which was composed of 14 different taxa belonging to 7 taxonomic groups. Teleosts were most abundant, according to the numerical (50% N) and relative importance indices (82% IRI). The proportions of teleost contributions were high in both muscle and liver tissues (e.g., 57% and 76%, respectively). Based on stomach contents and isotopic results, it was evident that teleosts were the main prey items in its short (SCA), medium (liver), and long (muscle) term diets, which leads to the conclusion that *S. acanthias* is a piscivorous predator. Our results were mostly compatible with previously reported results (Avşar, 2001; Demirhan et al., 2007), but cephalopods were negligible, while several studies reported them to be the second-most common prey group (Alonso et al., 2002; Barria et al., 2015; Gračan et al., 2017). These observations can be related to either region-based benthic diversity (e.g., habitat types and species composition) or low sample sizes. In contrast with other studies (Gračan et al., 2017; Demirhan et al., 2007), *S. acanthias* was found to be a medium-state generalist feeder ($0.40<B_A<0.60$) that exclusively consumed teleosts. A noteworthy study by Ellis et al. (1996) concluded that niche breadths became narrower in terms of resource variations by body size ($B_A = 0.31$ and 0.11 for <60 cm and >79 cm, respectively). In our study, most individuals were smaller than 60 cm in total length and showed niche breadths that were similar to those given by Ellis et al. (1996). Additionally, a strong and positive relationship was found between body size and $\delta^{15}N$ value, which likely indicated enrichment by a shift of prey selection through higher TL. Our findings were also supported by others and indicated ontogenetic changes in diet (Gračan et al., 2017) and a significant size-TL relationship (Barría et al., 2015). Due to changes in morphometry and increasing energy demands, animal body sizes can be considered as a proxy of trophic level (Romanuk et al., 2011); however, it is also likely to find no relationship in each elasmobranch diet study (Albo-Puigserver et al., 2016; Barría et al., 2018).

Scyliorhinus canicula had a diet consisting of 12 different taxa belonging to 5 taxonomic groups, according to the stomach content data. Teleosts were the most abundant, according to numerical analysis (35% N) and relative importance index (52% IRI). In contrast, the mixing model results showed that shrimp and cephalopods were equally important in the muscle tissue results, while the shrimp contribution was found to be quite high in the liver results (54%). The proportion of the teleost contribution was much lower in the liver. According to previous studies, different results for the main prey group have been reported, such as crustaceans (Valls et al., 2011), specifically shrimp (Barría et al., 2018), cephalopods (Karachle and Stergiou, 2010; Yemişken et al., 2019), and teleosts (Kousteni et al., 2017; Bengil et al.,

2019). In addition to those three prey groups, polychaetes (Western Mediterranean, Valls et al., 2011; Barría et al., 2018) and tunicates as soft bodied filter feeders (Irish coasts; Wieczorek et al., 2018) were also reported to provide important contributions to its diet. While tunicates were not detected in our study, polychaetes were found in very low proportions and therefore were not selected for SIA. The underrepresentation of polychaetes or other soft-bodied invertebrates, such as annelids and tunicates, might be related to their rapid digestion and evacuation (Heupel and Bennett, 1998) because the vacuity index was quite high (44%) in *S. canicula*. Based on the available stomach data, *S. canicula* was found to be a medium-state generalist feeder ($B_A = 0.43$) but with broader niche breadth than the other species. Isotopic niches that were determined by SEAc suggested that *S. canicula* and *S. acanthias* tended to exploit food resources with similar carbon sources but from different trophic positions. This was supported by the fact that the fish and benthic invertebrates consumed by *S. canicula* were diverse and in lower TLs, which was exhibited in a somewhat wide range of $\delta^{13}C$ values. The probability of isotopic niche overlap of *S. canicula* with the isotopic niches of *S. acanthias* was highest (70.7%). This indicated that *S. canicula* individuals exploited similar food resources within the same environment as *S. acanthias* individuals. However, according to the sampling data, these two species were rarely found together in the same haul during the study period. *S. canicula* individuals were sampled only in the southern part of the study during spring and summer, while *S. acanthias* individuals were sampled in the northern part during autumn and winter. This fact is well defined in ecological studies as the competitive exclusion principle, where two species cannot occupy the same ecological niche, but one species must slightly alter its niche to reduce the competition for prey (Hardin, 1960). We cannot determine this kind of niche alteration with the present data, but other explanations might be seasonal diet shifts and potential migrations (Logan and Lutcavage, 2010) to either deeper waters (Barría et al., 2018) or other areas (Ellis et al., 1996) because significant differences were found in the $\delta^{15}N$ values between tissues, in which the liver exhibited lower values for both species.

The Mediterranean Sea is one of the most compromised marine ecosystems worldwide (Costello et al., 2010). Overfishing and ecological extinction of species usually precede ecosystem collapses (Jackson et al., 2001) and highlight a much greater danger of ecosystem collapse now than in the past (Sala et al., 1998) because the simplified food webs have reduced resilience. There is only a partial understanding of how the loss of predatory species will affect the Mediterranean marine ecosystem (Stevens et al., 2000). The northeastern-most point of the Mediterranean, the Sea of Marmara, which has been deprived of large predators since the 1970s, suffers from a buildup of gradual stressors, overexploitation and depletion (Oğuz, 2017; Demirel et al., 2020), eutrophication, heavy industrialization and habitat degradation (Ediger et al., 2016; Topçu et al., 2019). Mesopredator species play an essential role in balancing and sustaining ecosystems via their interactions with various species and habitats. Therefore, in ecosystems that have been subjected to several stressors, it is essential to obtain information regarding the trophic ecology of such predators. Our results showed similar TLs for *S. canicula* and *S. acanthias* by using both SCA and SIA estimates but showed significant differences in TLs for *M. asterias*. For this species, TL_{SIA} was much higher than TL_{SCA}, and as a primary crustacean consumer in both the SCA and SIA results, a lower trophic level estimation is expected. Isotopic trophic position estimates are highly sensitive to which trophic enrichment factor ($\Delta^{15}N$) and basal source ($\delta^{15}N_{basal}$) are used (Post, 2002); therefore, we used mussel (*Donax turunculus*) as an appropriate baseline species based on a recommendation by Post (2002). However, in our SCA analysis, the main prey of *M. asterias* was crustaceans, which comprise a very diverse group with species at different trophic levels and which was apparently underestimated in our SCA. Compared to the very limited literature, fine-scale taxonomic studies using SCA (Ellis et al., 1996; McCully Phillips et al., 2020) have estimated that TL_{SCA} was very similar to our TL_{SIA} results. Here, our case is a typical example that demonstrates a limitation of stomach content analysis but also shows the importance of using different methodologies for precise results. As a piscivorous species, *S. acanthias* showed the highest TLs, with values that were similar to those from other studies (Gračan et al., 2014; Barría et al., 2015; Froese and Pauly, 2021). For *S. canicula*, different TL results are reported in the literature with similar results to ours (Karachle and Stergiou, 2017; Barría et al., 2018) and there also studies that show much lower trophic positions (Yemişken et al., 2019; Froese and Pauly, 2021).

In contrast with the various reported isotopic signatures of three shark species in the Mediterranean Sea (Barría et al., 2015, 2018; Valls et al., 2017; Yemişken et al., 2019), the $\delta^{15}N$ values were higher for these species in the Sea of Marmara (Fig. 6). Despite these results, the isotopic trophic positions that were estimated by these enriched $\delta^{15}N$ values were compatible with those of other studies and were not higher. Again, compared to other studies, we noticed that potential prey species had higher $\delta^{15}N$ values (Table 6). Although we do not have enough information to explain this enrichment, we can speculate that the long-standing eutrophication due to the high urbanization and industrialization levels (Ediger et al., 2016) has affected every compartment from primary producers to secondary consumers in the Sea of Marmara ecosystem. For example, Umbricht et al. (2018) stated that eutrophication increases the $\delta^{15}N$ values of macrophytes living in

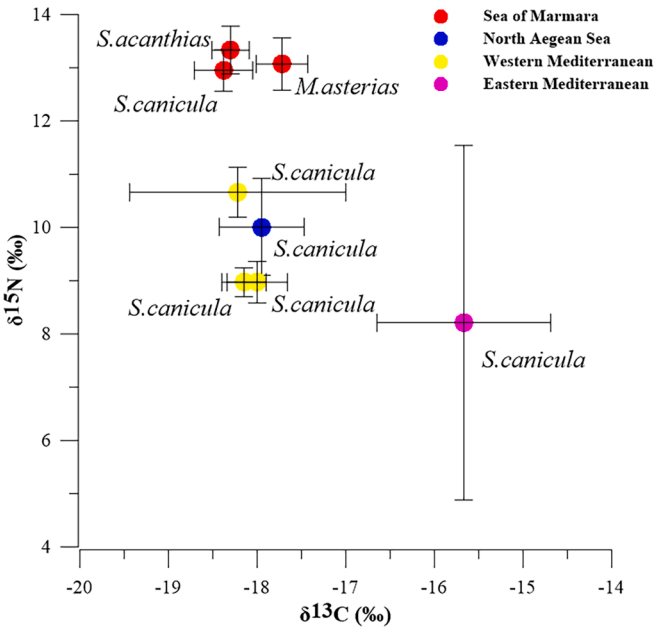


Fig. 6. Biplots of the mean isotopic values ($\delta^{13}C$ and $\delta^{15}N$ with ±standard deviation) of three demersal shark species, *Squalus acanthias*, *Scyliorhinus canicula*, and *Mustelus* asterias, in the different regions; red - the Sea of Marmara (present study); yellow - western Mediterranean (Barría et al., 2015, 2018; Valls et al., 2017); and blue - northern Aegean Sea (Yemişken et al., 2019). (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

Table 6
Comparison of the $\delta^{15}N_{muscle}$ values from various locations of the main prey species.

Region	$\delta^{15}N_{muscle}$ (‰) ±SD						Reference
	Liocarcinus depurator	Parapenaeus longirostris	Loligo vulgaris	Trachurus mediterraneus	Trachurus trachurus	Trachurus sp.	
Northeastern Atlantic (North Sea)	11.83 ± 0.69		14.63 ± 0.23		12.88 ± 0.46		Caut et al. (2013)
Northeastern Atlantic (Bay of Biscay)	11.70 ± 0.7[a]		13.90 ± 1.30[b]		11.40 ± 0.90[a] 12.00 ± 1.20[b] 12.40 ± 0.70[c]		[a]Spitz et al., 2013;[b]Chouvelon et al., 2014;[c]Lassalle et al., 2014
Western Mediterranean (Gulf of Lion, Catalan Sea)	8.30 ± 0.48	8.16 ± 0.40	11.16 ± 0.38	10.82 ± 0.23	9.13 ± 0.19		Barría et al. (2015)
Northwestern Mediterranean (Ebro River Delta)			11.20 ± 0.8	10.30 ± 0.50	9.20 ± 0.4		Albo-Puigserver et al. (2016)
Central Mediterranean (Adriatic Sea)		6.20 ± 0.2		9.20 ± 0.6	8.20 ± 0.8		Zorica et al. (2021)
Black Sea (Danube Delta)				13.50 ± 0.2			Bănaru and Harmelin-Vivien (2009)
Northeastern Mediterranean (The Sea of Marmara)	11.30 ± 0.49	9.82 ± 0.33	12.37 ± 0.25			11.81 ± 0.78	Present study

the region and, accordingly, may affect other species in the food web. In addition, Ofukany et al. (2014) reported that under eutrophic pressure, increased planktonic production could encourage fish species to find high-quality prey with enriched $\delta^{15}N$ values.

In conclusion, this study presents new information regarding the feeding ecology of four demersal shark species in the Sea of Marmara, which is a lesser-known part of the Mediterranean Sea. Trophic analyses using two different methods and related models indicate that interspecific differences in the main prey resources exist among those sharks. Our results can contribute to the knowledge of the ecological role of highly vulnerable cartilaginous species and help to improve conservation programs in the Sea of Marmara.

Funding

This study was funded by the Scientific Research Projects Coordination Unit of Istanbul University [Project number: FDK-2018-30841 and Project number: MAB-2021-37457] and the General Directorate of Agriculture Research and Policies of the Republic of Turkey Ministry of Agriculture and Forestry [Project number: HAYSUD/2014/05/01].

Author contribution

Güzin Gül, Nazli Demirel: Conceptualization, Methodology, **Nazli Demirel:** Supervision, Project administration. **Güzin Gül:** Writing-Original draft preparation. **Güzin Gül, Nazli Demirel:** Writing-Reviewing and Editing.

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.

Acknowledgments

The present study is a part of a Ph.D. dissertation entitled 'Feeding habits and trophic levels of Elasmobranch species in the Sea of Marmara' by Güzin GÜL.

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