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BRIEF COMMUNICATION

The occurrence and feeding of a critically endangered shark species, *Oxynotus centrina* in the Sea of Marmara

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Abstract

The angular roughshark, *Oxynotus centrina* (Linnaeus, 1758), is a rarely encountered deep-sea demersal shark species. It is listed as "Critically Endangered" in the IUCN's regional assessment for the Mediterranean. Like other sharks and rays, the angular roughshark is subject to by-catch in demersal trawl and longline fisheries. This study contributes new knowledge on (a) its occurrence, size and sex information; and (b) its feeding ecology in the Sea of Marmara. Data were collected on total length (TL), total weight (TW) and sex from unpublished research survey reports performed intermittently from 1994 to 2020. According to the data, *O. centrina* was mainly recorded from the muddy sand bottom type in the southwest region of the Marmara Sea, which has higher biodiversity of macrobenthic species than other regions. Different methodological approaches were conducted to understand its feeding, such as DNA metabarcoding and isotope analysis to identify its prey spectra and assign a trophic level. Sampled specimens were full of liquid in their stomachs without any discernible visually identifiable prey items. The metabarcoding analyses were also unsuccessful in identifying any prey items. Trophic position calculation based on nitrogen isotope in muscles highlighted that *O. centrina* has the highest trophic position compared to the other sharks and rays in the Sea of Marmara. Although it was found mainly feeding on polychaetes and sipunculids species from previous studies, the $\delta^{15}N$ values made the authors cautious about the possibility of this species feeding on low trophic-level benthic invertebrates. Because the authors could not observe the presence of shark vitellus in the stomachs and could not succeed in DNA identification for prey, this study highlights high $\delta^{15}N$ values similar to top predators for *O. centrina*. The authors conclude that this species strategically feeds mainly on shark eggs to fulfil its nutritional requirements with minimum energy expenditure in line with its slow-moving behaviour and mouth morphology.

KEYWORDS
angular roughshark, feeding strategy, metabarcoding, northeastern Mediterranean, stable isotope

SIGNIFICANCE STATEMENT

The angular roughshark, *Oxynotus centrina* (Linnaeus, 1758), is a rarely encountered deep-sea demersal shark species and it is listed as "critically endangered" in the IUCN's regional assessment for the Mediterranean. The metabarcoding analyses was unsuccessful in identifying any prey items. Trophic position calculation based on nitrogen isotope in muscles highlighted that *O. centrina* has the highest trophic position compared to the other sharks and rays in the Sea of Marmara.

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The angular roughshark, *Oxynotus centrina* (Linnaeus, 1758), is a deep-sea demersal shark species distributed in the eastern Atlantic from Senegal, including the entire Mediterranean Sea aside from the Black Sea (Froese & Pauly, 2022). This species has a very low resilience and hence regeneration time based on its slow-growth and low fecundity viviparous reproduction (Froese & Pauly, 2022). Because it is a rare species, there is only very limited knowledge on its biological and ecological characteristics, especially its population size. Like other sharks and rays, the angular roughshark is caught as by-catch across the Mediterranean in demersal trawl and longline fisheries. *O. centrina* is listed as "critically endangered" in the IUCN's regional assessment for the Mediterranean underlined by its disappearance in the eastern and central regions and rarity in the western region (Soldo & Guallart, 2016). Nonetheless, one very recent study shows the presence of adults and juveniles with notable numbers in the northern Adriatic Sea, which was concluded to host a viable and stable population in the central region (Gajić *et al.*, 2022).

The Sea of Marmara (SoM) is located in the northeastern part of the Mediterranean Sea. It is an enclosed sea connected to the Mediterranean and the Black Sea *via* two narrow straits, Istanbul (Bosphorus) and Çanakkale (Dardanelles) Straits (Figure 1). SoM and its straits are recognized as a biological corridor (Öztürk & Öztürk, 1996) by displaying mostly Black Sea characteristics in its pelagic ecosystem, but also Mediterranean characteristics in the benthic ecosystem. Since 1971, several control measures in fisheries management of SoM have been implemented: (a) temporal industrial fishing bans from 15April to 1 September and (b) a spatial trawling ban in the entire SoM and its straits (Yıldız *et al.*, 2020). Still, beam trawling for the shrimp fishery is allowed in limited areas <50 m in depth along with temporal restrictions (the industrial fishing ban is valid for beam trawling and additional restrictions from 1 to 31 January). The by-catch composition of shrimp fisheries revealed that chondrichthyans comprised 1%–15% of the total catch weight in the SoM (Aslan İhsanoğlu & İşmen, 2020; Bayhan *et al.*, 2006; Bök *et al.*,2011; Daban *et al.*,2021; Zengin & Akyol, 2009). To the best of the authors' knowledge, the first mention of *O. centrina* in the SoM dates back to 1952 in a list of scientific fish names and their dedicated common names in Turkish from the Istanbul University Hydrobiological Research Institute's journal – *Balık ve Balıkçılık*, 1952. The Turkish name given for angular rough shark is "domuz," which translates to pig in English, possibly related to its appearance. Although nearly all demersal research surveys conducted in the SoM listed *O. centrina* in catch compositions, there is still very limited knowledge of its biology, ecology and abundance. A notable contribution on its behaviour was an underwater video provided by Kabasakal (2009) from 30 m depth from the northern coast of the SoM (https://www.youtube.com/watch?v=WlvS8HfeciQandt=5sandab_channel=fishbaseyt).

Here, the authors aim to contribute to the knowledge on *O. centrina* in the SoM on its (a) occurrence, size and sex ratio; (b) stomach contents *via* conventional visual identification and metabarcoding analysis and (c) trophic position by stable isotope analysis. Records of total length (TL), total weight (TW) and sex were collected from research survey reports performed intermittently between 1994 and 2021. They then conducted different methodological approaches to understand its diet. They have used DNA metabarcoding and stable isotope analysis (SIA) to identify its stomach content and trophic position.

O. centrina specimens were recorded as discards during several demersal fishery surveys between 1994 and 2021 performed in the SoM (Figure 1). Specimens in the catch composition were immediately seperated, had sex determined, then measurements of total length (TL, cm) and total weight (TW, g) were taken, before the specimens were released. The length-weight relationship was estimated with the formula: $W = aL^b$ (Froese *et al.*, 2011; Ricker, 1973), and the standard sex ratio was used (male: female).

The stomachs of all specimens were found not containing any prey items but instead were full of liquid. The stomach was removed and fixed in ethanol for metabarcoding analysis. A 50 ml sample of each stomach liquid content was filtered through 0.22 μm filters. Filtrations were performed in triplicates. According to the manufacturer's instructions, DNA was extracted from ethanol-preserved stomach samples and filters using the

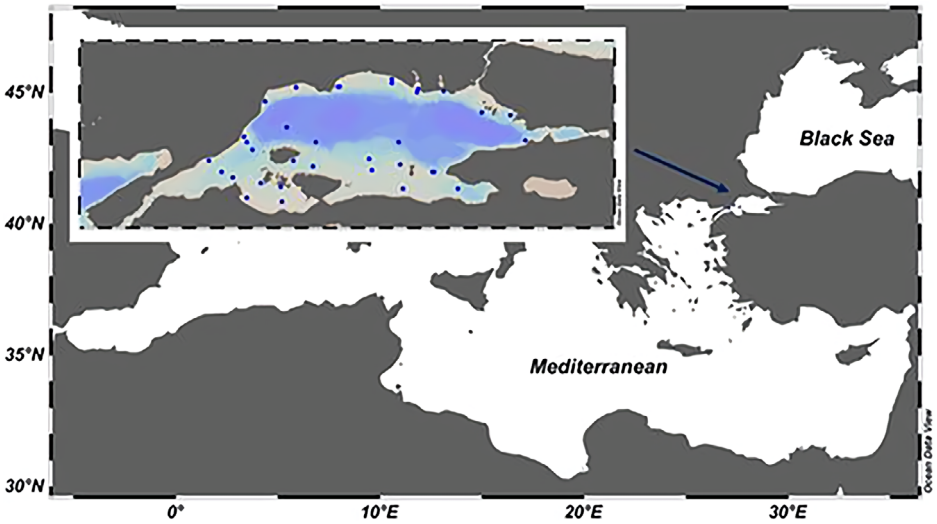


FIGURE 1 Location of the Sea of Marmara and sampling stations during shrimp fishery survey

Microtissue DNA Isolation Kit (AMBRD Laboratories). The quality and quantity of extracted DNA were measured using a Qubit fluorometer (Invitrogen). Negative controls were included in all stages. PCR amplification of a 313 bp COI fragment was performed on each sample (tissues and filters). The mlCOIintF forward primer (5′GGWACWGGWTGAACWGTWTAYCCYCC) and the jgHCO2198 reverse primer (5′TAIACYTCIGGRTGICCRAARAAYCA) were used to amplify both vertebrate and invertebrate sequences (Leray *et al*., 2013). The total volume of the PCR mixture was 20 μl, containing 0.1 μl of 50 μM of each forward and reverse primer, 10 μl of 2X Fast PCR Mix (AMBRD Laboratories), and 1–2 μl of genomic DNA. A touchdown PCR protocol from Leray *et al*. (2013) was applied; 16 initial cycles: denaturation for 10s at 95 °C, annealing for the 30s at 62 °C (−1 °C per cycle) and extension for 60s at 72 °C, followed by 25 cycles with an annealing temperature of 46 °C and a final extension at 72 °C for 7 min. PCR amplification was checked on 1.5% agarose gels. All successful triplicate PCR reactions were pooled into a single product. TruSeq DNA PCR-Free LT Library Prep Kit (Illumina) was used for library preparation according to the manufacturer's instructions. Indexed samples were quantified using a Qubit fluorometer (Invitrogen), and the authors pooled 50 ng of each library. They ultimately normalized the sample to 4 nM and sequenced it on an Illumina MiSeq (Illumina) with a 1% PhiX spike.

The resulting reads were cleaned in the FASTQ filing, and FLASH pair software (Mago & Salzberg, 2011) was used for extending the fragments, which were converted to FASTA files. The reverse complement of the extended reads was combined with the originally extended fragments. Each obtained sequence was manually checked, and the original PCR primers were trimmed. All the duplicates, chimeric sequences and sequences shorter than 250 bp length were discarded. The resulting 313 bp COI fragment sequences were blast searched (BLASTn) in GenBank.

Muscle tissues were sampled for stable carbon and nitrogen isotope analysis. Samples (*c*. 5 g) were frozen at −20 °C until further laboratory analysis. In the laboratory, lipid extraction was performed according to the method specified by Bligh and Dyer (1959) and Hussey *et al*. (2012). Subsequently, the tissues were freeze-dried for approximately 72 h, powdered and then weighed roughly 1–1.5 mg before packing into 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 (Sercon Ltd., Cheshire, UK). Stable isotope ratios of carbon and nitrogen were expressed in delta notation with the equation: $\delta X = ((Rsample/Rstandard) - 1) \times 1000$, 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 Rstandart 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.

The trophic position of *O. centrina* (TL_{SIA}) was calculated using the formula provided by Zanden and Rasmussen (2001): $TPconsumer = TPbasal + (\delta^{15}Nconsumer - \delta^{15}Nbasal)/ \Delta\delta15N$, where $\delta^{15}Nconsumer$ is *O. centrina* species, $^{15}N_{basal}$ is that of a bivalve (*Donax turunculus*) ($n = 2$, $\delta^{15}Nmean = 8.42$) that was sampled from the SoM, and Δ is the trophic enrichment factor between organism and diet. Following Hussey *et al*. (2010), $\Delta15N = 2.29$ (diet–tissue discrimination factors for lipid extracted muscle) and TP basal =2 were used. TL of *O. centrina* and previously reported sharks and rays in the SoM (Gül & Demirel, 2020, 2021) and their regional level of IUCN status for the Mediterranean (Soldo & Guallart, 2016) were visualized as a biplot.

A total of 33 O. centrina records were evaluated from the sea survey research data between 1994 and 2021 (Table 1). Specimens ranged from 22.1 and 59 cm in TL and 144 and 3120 g in TW. Most specimens were detected as adult males, whereas females and juveniles were less observed. The sex ratio was 1:0.38 (M:F). The length weight relationship was calculated as follows: $W = 0.02*L^{2.82}$ ($R^2 = 0.84$) for 21 specimens. A total of six *O. centrina* specimens in April–December 2018 and July 2021 were further sampled (Table 1). The length-weight relationship was calculated as follows: $W = 0.02*L^{2.82}$ ($R^2 = 0.84$) for 21 specimens. Of the 33 specimens, 27 of them were immediately released. A total of six specimens, two in April 2018, two in December 2018 and two in July 2021, 88 were highly damaged during fisheries operations and were retained for further examination (Table 1). All six stomachs were observed full of liquid without any solid prey items (Figure 2 and Supporting Information Video S1). According to the results of the metabarcoding analysis, DNA belonging to any prey group could not be barcoded in the stomach of *O. centrina*. Isotopic signatures of *O. centrina* in muscle tissues were found between 13.37 and 14.24 for $\delta^{15}N$ and between 15.96 and 16.98 for $\delta^{13}C$. The trophic position of *O. centrina* using nitrogen isotope was calculated as $TL_{sia} = 4.29$ (Figure 3).

This study showed that *O. centrina* was very rare, but at least one specimen was observed in the catch composition during the demersal research surveys conducted in the SoM. According to accessible survey reports, this species is mainly found in the southwest region, where it encounters the oxygen-rich Mediterranean originating bottom layer water entering from the Dardanelles Strait. The southwest part of SoM also displays higher biodiversity in macrobenthic species (Çınar *et al*., 2021), with mainly a mud and muddy sand bottom type (Algan *et al*., 2004). The demersal research surveys were limited to 150 m depth; thus, this species was rarely observed in the north, where the shelf area is very narrow and abruptly deeper. O. *centrina* has very limited observations in the Sea of Marmara in the past 30 years which may be due to it being ignored by fishers because of its noncommercial status. Also, in line with the results of this study, males are more dominant than females. In addition, the fact that the species may frequent deeper waters and deeper depths are not sampled in both commercial fisheries and scientific surveys may also relate to its low observation rate.

With regard to sex, mainly adult males were observed, but juveniles and females were less frequently recorded. In April 2018, one of two sampled specimens was realized as a gravid female (51 cm in TL).

TABLE 1 Records from 1994 to 2020 for angular rough sharks in the Sea of Marmara

No	Location	Date	Depth (m)	Sex	TL (cm)	TW (g)	Fishing gear	Specimen	Source
1	C	Aug-94	48	M/A	59	3120	Trawling	Released	Gözenç *et al.* (1997)
2	SW	Aug-94	18	M/A	54	1250	Trawling	Released	
3	SW	Aug-94	18	M/A	49	1125	Trawling	Released	
4	SW	Aug-94	18	M/A	50	1045	Trawling	Released	
5	SW	Aug-94	18	M/A	49	1035	Trawling	Released	
6	NW	Aug-94	55	M/A	50	1560	Trawling	Released	
7	SW	Aug-09	53	–	–	900	Trawling	Released	
8	SW	Aug-09	53	–	–	964	Trawling	Released	
9	SW	Aug-09	47	–	–	1050	Trawling	Released	
10	SW	Aug-09	152	–	–	280	Trawling	Released	
11	SW	Dec-09	47	M/A	48.5	1212	Trawling	Released	Demirel and Murat-Dalkara (2012)
12	SW	Dec-09	47	M/A	47.3	1202	Trawling	Released	
13	SW	Dec-09	47	F/J	26.6	218	Trawling	Released	
14	SW	Dec-09	47	F/A	55	2550	Trawling	Released	
15	SW	Dec-10	48	F/A	51	1650	Trawling	Released	
16	SW	Feb-11	47	F/J	22.1	144	Trawling	Released	
17	SW	Apr-18	152	F/A	49	1780	Trawling	Sampled	Gül (2020)
18	E	Apr-18	83	F/A	50	740	Trawling	Sampled	
19	C	Apr-18	170	F/A	51	1655	Trawling	Released	
20	C	Apr-18	170	F/A	52	1060	Trawling	Released	
21	C	Aug-18	170	M/A	50	1110	Trawling	Released	
22	C	Aug-18	170	M/A	47.5	720	Trawling	Released	
23	C	Dec-18	93	M/A	47	980	Trawling	Sampled	
24	C	Dec-18	93	M/A	45.5	1110	Trawling	Sampled	
25	SW	Apr-19	45	M/A	48	1110	Trawling	Released	Demirel *et al.* (2021, 2022)
26	SW	Apr-19	45	M/A	48.2	1200	Trawling	Released	
27	SW	Apr-19	45	M/A	51	1800	Trawling	Released	
28	SW	Jul-19	45	M/A	46	1040	Trawling	Released	
29	SW	Jul-19	45	M/A	49.5	1300	Trawling	Released	
30	SW	Jul-19	45	M/A	45.5	1100	Trawling	Released	
31	SW	Aug-20	138	M/A	46.5	1400	Trawling	Released	
32	E	Jul-21	83	M/A	48	1022	Trawling	Sampled	
33	E	Jul-21	83	M/A	49	1188	Trawling	Sampled	

Note: Location indicates C: Central, SW: Southwest, NW: Northwest and E: East for observation of *O. centrina* in catch composition in SoM. Sex indicates M: Male, F: Female, A: Adult, J: Juvenile.

Previous records from the eastern Mediterranean reported that the total length of gravid females ranged between 43 to 56 cm (Başusta *et al.*, 2015) and 69 cm (Megalofonou & Damalas, 2004), and sexual maturity was determined to be 60 cm for male and 65 cm for female in the Tunisian coast (Capapé *et al.*, 1999).

Sampled specimens were full of liquid in their stomachs, devoid of any solid items (Supporting Information Video S1). Further, the metabarcoding analysis did not successfully identify any prey but only found the species' DNA. Trophic position calculation based on nitrogen isotope in muscles highlighted *that O. centrina* has the highest trophic position compared to other sharks and rays in the SoM (Gül & Demirel, 2020, 2021). *O. centrina* feeds on benthic invertebrates, primarily polychaetes (>50%) and less frequently sipunculids, crustaceans and teleosts (Barría *et al.*, 2015; Capapé, 2008). Also, *Scyliorhinus canicula* egg (Barrul & Mate, 2001) and newborn *Raja* sp. (Saïdi, 2002) have been found in their stomachs. Nonetheless, similar to the results of this study, liquid-filled stomachs without visible prey items were also previously reported in the Mediterranean (Dragičević *et al.*, 2009; Megalofonou & Damalas, 2004). Recently an experimental study successfully observed live specimens in aquarium facilities, and its results greatly enhanced the knowledge of feeding preferences. As the study implies, being a suction feeder specializing in worm-like prey, *O. centrina* uses suction feeding to handle elasmobranch egg cases, where after biting through the egg case wall, it

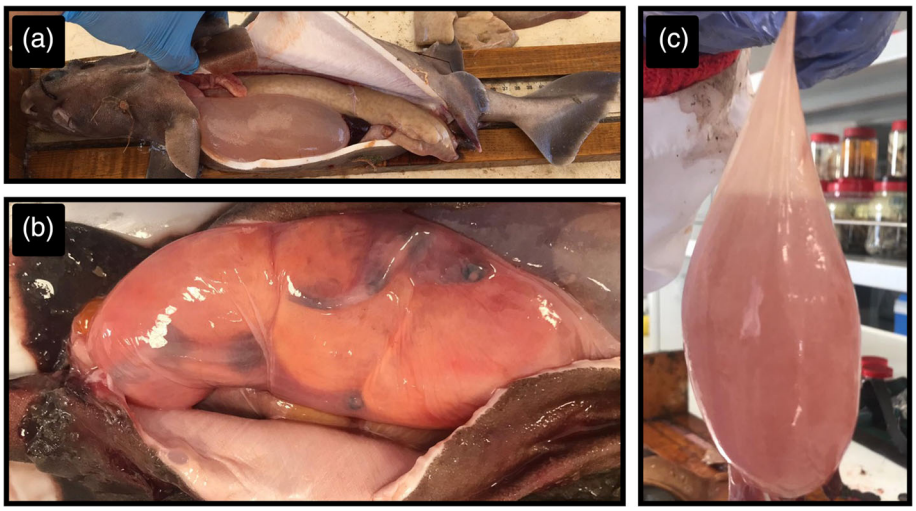


FIGURE 2 Stomach appearance (a) of *Oxynotus centrina*, (b) sampled gravid female and (c) liquid content of stomach

sucks out the yolk and embryo development (Guallart *et al*., 2015). Another species belonging to *Oxynotus* genus, *Oxynotus bruniensis* from the southeast Pacific Ocean, was also reported feeding on an embryonic skate, and an unidentifiable white material (probably vitellus) was observed in its stomach contents (Barrul & Mate, 2001).

Similar to the unsuccessful metabarcoding results of this study, the only other metabarcoding study on *O. bruniensis* reported that stomachs filled with liquid were found, with no other prey items being identifiable (Finucci *et al*., 2016). DNA metabarcoding has been increasingly utilized to study fish diet composition (Casey *et al*., 2019). Because species-diagnostic DNA fragments can be discovered even after digestion, PCR-based molecular analysis of gut content is a powerful and more accurate methodology to determine a predator's diet (Symondson, 2002). Experiments showed that even very small prey items such as fish eggs or larvae in stomach samples could be successfully identified by metabarcoding studies even after 48 h of their last feeding (Traugott *et al*., 2021). Thus, the authors conclude that the last feeding of sampled specimens was likely longer than 48 h. Based on feeding studies, intermittent feeding rather than continuous one is recognized for many shark species (Wetherbee *et al*., 2004). For example, *Squalus acantias* feed only each 10 to 16 days after filling their stomachs. Also, the feeding frequency of juvenile *Carcharhinus plumbeus* and *Negaprion brevirostris* is estimated to be longer than 36 h (Wetherbee & Cortes, 2004). Infrequent feeding and fasting period in sharks is evaluated as an energy-conserving mechanism. The duration of fasting periods is associated with the species' moving behaviour or activity level (Papastamatiou & Lowe, 2005). As a slow-moving species (Kabasakal, 2009) and metabarcoding results show, *O. centrina* adults may have a fasting period of more than 48 h.

After Barría *et al*. (2015), this study provides the second stable isotope results of *O. centrina* for the Mediterranean Sea. The authors found its isotopic signatures 13.76 ± 0.39 for $\delta^{15}N$ and − 16.77 ± 0.43 for $\delta^{13}C$, which was almost one trophic position (3.4 ‰) higher for $\delta^{15}N$ (10.92 ± 0.49) and slightly lower for $\delta^{13}C$ (−17.31 ± 0.84) than the study provided for the western Mediterranean (Barría *et al*., 2015). Nonetheless, the trophic position of *O. centrina* was calculated pretty similar for both regions (TL_{SIA} = 4.57 and 4.29 for the west Mediterranean and SoM, respectively). The differences between nitrogen values, indicating higher values in SoM, were reported in previous studies for other species, including sharks, rays, teleosts and benthic invertebrates (Gül & Demirel, 2021, 2022). Trophic position estimation using stable isotope is strictly related to the preference of suitable basal $\delta^{15}N$, namely the isotopic baseline of given organisms. Post (2002) recommended that an isotopic baseline be chosen from long-lived primary consumers, such as snails and mussels. Thus, in this study, the authors used $\delta^{15}N$ of *D. turunculus* (8.42) as an isotopic baseline obtained during the same sampling operations. Barría *et al*. (2015) reported that they used $\delta^{15}N$ basal of the bivalve *Pecten jacobaeus* (5.90 ± 0.21), which has a $\delta^{15}N$ value significantly lower than our bivalve species. Thus, the authors concluded that there were differences in nitrogen values among different regions that may be related to different productivity levels or eutrophic characteristics of the waters. Furthermore, a remarkable point in stable isotope results for both west Mediterranean and SoM indicates a very high level of $\delta^{15}N$ for *O. centrina* compared with the obvious piscivorous species *Torpedo marmorata* ($\delta^{15}N$ = 11.43 ± 0.70 by Barría *et al*., 2015, and 13.08 ± 0.61 by Gül & Demirel, 2020), and *Squalus acanthias* ($\delta^{15}N$ = 10.66 ± 0.47 by Barría *et al*., 2015, and 13.33 ± 0.45 by Gül and Demirel, 2021). Animal tissue is enriched in $\delta\ ^{15}N$ relative to food resources. Through top predators, the concentration of ^{15}N gradually increases (Cabana & Rasmussen, 1994). Thus, stepwise enrichment in $\delta\ ^{15}N$ is related to increasing trophic position, reaching its maxima in top predators (Oelbermann & Scheu, 2002).

Considering its known targeted feeding preference of mainly polychaetes and sipunculids, the concentration of *O. centrina* ^{15}N was much higher than that obtained from such very low-level benthic invertebrates. Also, studies of trophic levels based on stomach content (SCA) determined its TL_{SCA} as 3.1 (Barría *et al*., 2015; Froese & Pauly, 2022). As Guallart *et al*. (2015) explain well, the presence of vitellus in the stomach contents of *Oxynotus* spp. could not be recognized in previous studies and evaluated as digested remains or amorphous unidentifiable material. Although the authors of this study could not observe the presence of

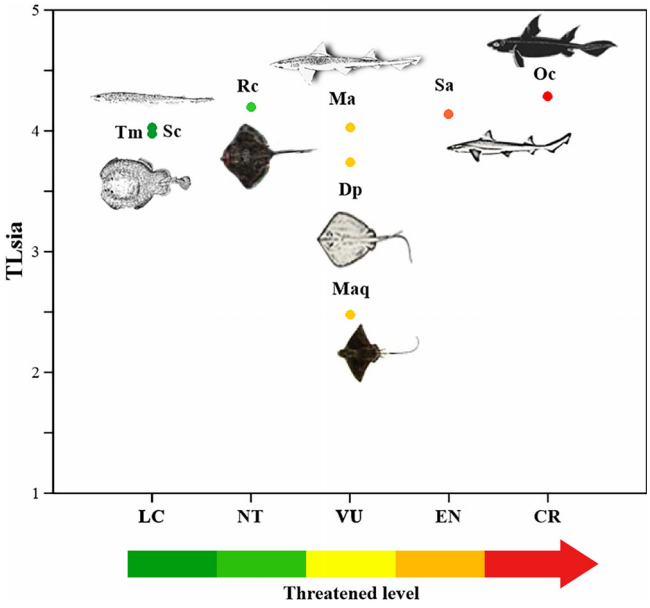


FIGURE 3 Biplot of the calculated TL based on δ^{15}N in *Oxynotus centrina* and previously reported sharks and rays in the Sea of Marmara (SoM; Gül & Demirel, 2020, 2021, 2022) and their regional level of IUCN status for Mediterranean (Soldo & Guallart, 2016). Ox: *Oxynotus centrina*, Sa: *Squalus acanthias*, Ma: *Mustelus asterias*, Dp: *Dasyatis pastinaca*, Maq: *Myliobatis aquila*, Rc: *Raja clavata*, Sc: *Scyliorhinus canicula*, Tm: *Torpedo marmorata*; Threatened levels in IUCN Red List categories, LC: Least concern, NT: Nearly threatened, VU: Vulnerable, En: Endangered, CR: Critically endangered

vitellus in the stomachs and could not succeed in DNA identification for prey, this study highlights high δ^{15}N values similar to top predators for *O. centrina*. Limited other studies compared δ^{15}N of animal eggs such as *Larus argentatus* (Hebert *et al.*, 1999) and *Caretta caretta* (Kaufman *et al.*, 2014a, 2014b) with their female maternal enriched δ^{15}N in eggs; based on this study's results and these studies, the authors suggest that *O. centrina* mainly preys on shark eggs as a strategy to fulfil its nutritional requirements with minimum energy expenditure in line with its slow-moving behaviour and mouth morphology.

Of late, stable isotope analysis has become an important tool for feeding ecology studies (Phillips *et al.*, 2014). The authors concluded that their results showed that SIA is a handy tool for species suffering from long-term fasting periods. When considering the long-term fasting period and stock status of this species, the technique of non-lethal approaches by stable isotopes (*e.g.*, blood sample) is appropriate to determine the trophic ecology of the species. Also, Bayesian mixing models will be very effective in pointing out species' diet consumption in further detailed studies.

Although SoM is suffering from various ecosystem pressures such as industrial pollution, organic matter enrichment, high urbanization, habitat loss and overfishing, demersal species may have benefitted from the regulations on the permanent demersal trawling ban and annual industrial fishing ban during the summer season. The occurrence of *O. centrina* in the SoM should be considered an essential example of the necessity and benefit of strict control measures in fisheries management.

AUTHOR CONTRIBUTION

We declared that all persons who meet authorship criteria are listed as authors, and all authors certify that they have participated sufficiently in the work to take public responsibility for the content, including participation in the concept, design, analysis, writing or revision of the manuscript. Conception and design of study: GG, ND; Acquisition and analysis of data: GG, BY and ND; Drafting the manuscript: GG, ND; Revising the manuscript critically for important intellectual content: GG, BY and ND.

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CONFLICT OF 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.

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REFERENCES

Algan, O., & Balkıs, N. (2004). Çağatay, M.N. (2004). The sources of metal contents in the shelf sediments from the Marmara Sea, Turkey. *Environmental Geology*, *46*, 932–950. https://doi.org/10.1007/s00254-004-1104-2.

Aslan İhsanoğlu, M., & İşmen, A. (2020). Marmara denizi biyoçeşitliliği ve Derinsu pembe karidesi av miktarı. *Aquatic Research*, *3*(2), 85–97. https://doi.org/10.3153/AR20008.

Balık ve Balıkçılık. (1952). *Türkiye Deniz Balıklarının Sözlüğü*. İstanbul: Istanbul University Hydrobiological Research Institute Publications.

Barría, C., Coll, M., & Navarro, J. (2015). Unravelling the ecological role and trophic relationships of uncommon and threatened elasmobranchs in the western Mediterranean Sea. *Marine Ecology Progress Series*, *539*, 225–240. https://doi.org/10.3354/meps11494.

Barrul, J., & Mate, I. (2001). First confirmed record of angular rough-shark *Oxynotus centrina* (Linnaeus, 1758) predation on shark egg case of small-spotted catshark *Scyliorhinus canicula* (Linnaeus, 1758) in Mediterranean waters. *Annales, Series Historia Naturalis*, *11*(1), 23–28.

Başusta, N., Turan, C., & Başusta, A. (2015). New records of gravid female and adult male of the angular rough shark, *Oxynotus centrina* (Oxynotidae) from the northeastern Mediterranean. *Journal of the Black Sea/Mediterranean Environment*, *21*(1), 92–95.

Bayhan, Y. K., Çiçek, E., Ünlüer, T., & Akkaya, M. (2006). Catch and by-catch composition of the shrimp fishery by beam trawl in the south-eastern Marmara Sea. *Ege Journal of Fisheries and Aquatic Sciences*, *23*(3), 277–283.

Bligh, E. G., & Dyer, W. J. (1959). A rapid method of total lipid extraction and purification. *Canadian Journal of Biochemistry and Physiology*, *37*(8), 911–917.

Bök, T. D., Göktürk, D., & Kahraman, A. E. (2011). Bycatch in 36 and 40 mm PA Turkish twin rigged beam trawl codends. *African Journal of Biotechnology*, *10*, 7294–7302.

Cabana, G., & Rasmussen, J. B. (1994). Modelling food chain structure and contaminant bioaccumulation using stable nitrogen isotopes. *Nature*, *372*(6503), 255–257.

Capapé, C., Seck, A. A., & Quignard, J.P. (1999). Aspects of the reproductive biology of the angular rough shark, *Oxynotus centrina* (Oxynotidae). *Cybium*, *23*(3), 259–271.

Capapé, C. (2008). Diet of the angular rough shark *Oxynotus centrina* (Chondrichthyes: Oxynotidae) off the Languedocian coast (southern France, North-Western Mediterranean). *Vie et Milieu*, *58*, 57–61.

Casey, J. M., Meyer, C. P., Morat, F., Brandl, S. J., Planes, S., & Parravicini, V. (2019). Reconstructing hyperdiverse food webs: Gut content metabarcoding as a tool to disentangle trophic interactions on coral reefs. *Methods in Ecology and Evolution*, *10*(8), 1157–1170. https://doi.org/10.1111/2041-210X.13206.

Çınar, M. E., Bilecenoğlu, M., Yokeş, M. B., & Güçlüsoy, H. (2021). *Pinna nobilis* in the South Marmara Islands (sea of Marmara); it still remains uninfected by the epidemic and acts as egg laying substratum for an alien invader. *Mediterranean Marine Science*, *22*(1), 161–168. https://doi.org/10.12681/Mms.25289.

Daban, B., İşmen, A., Şirin, M., Yığın, C. Ç., & Arslan İhsanoğlu, M. (2021). Analysis of demersal fish fauna off the sea of Marmara, Turkey. *COMU Journal of Marine Science Fish*, *4*(1), 20–31. https://doi.org/10.46384/jmsf.912403.

Demirel, N., Gül, G., Bedikoğlu, D. (2022). Population structure of European hake in the sea of Marmara. Final report. Istanbul University scientific research unit (project no: FDP-2020-36673).

Demirel, N., Gül, G., Dursun, F., Bedikoğlu, D., Keskin, E. (2021). Feeding ecology and trophic level of European hake in the sea of Marmara. Final report. Istanbul University scientific research unit (project no: FBA-2018-30845).

Demirel, N., & Murat-Dalkara, E. (2012). Weight-length relationships of 28 fish species in the sea of Marmara. *Turkish Journal of Zoology*, *36*(6), 785–791.

Dragičević, B., Dulcic, J., & Capapé, C. (2009). Capture of a rare shark, *Oxynotus centrina* (Oxynotidae), in the eastern Adriatic Sea. *Journal of Applied Ichthyology*, *25*(Suppl. 1), 56–59.

Finucci, B., Bustamante, C., Jones, E. G., & Dunn, M. R. (2016). Reproductive biology and feeding habits of the prickly dogfish Oxynotus bruniensis. *Journal of Fish Biology*, *89*, 2326–2344. https://doi.org/10.1111/jfb.13116.

Froese, R., & Pauly, D. (Eds.). (2022). *FishBase*. World Wide Web electronic publication www.fishbase.org, version (02/2022).

Froese, R., Tsikliras, A. C., & Stergiou, K. I. (2011). Editorial note on weight-length relations of fishes. *Acta Ichthyologica et Piscatoria*, *41*(4), 261–263.

Gajić, A. A., Lelo, S., Joksimović, A., Pešić, A., Tomanić, J., Beširović, H., & Dragičević, B. (2022). Contemporary records of the rare and critically endangered angular rough shark, *Oxynotus centrina* (L innaeus, 1758), from the eastern Adriatic Sea. *Journal of Fish Biology*, *100*(1), 329–334. https://doi.org/10.1111/jfb.14932.

Gözenç, S., Kurter, A., Mater, K.A., Bilecik, N., Adatepe, F.M., Uysal, A., Orhon, S., Kıratlı, N., Gümüş, E., Demirel, S., Yüksek, A., Orhon, V., Çimen, N., Yıldırım, V., Dilek, M., Kamil, Y. and K. Harun (1997). Stok determination of some commercial demersal fish in the Sea of Marmara. Final report, TÜBİTAK DEBAG-13/G.

Guallart, J., García-Salinas, P., Ahuir-Baraja, A. E., Guimerans, M., Ellis, J. R., & Roche, M. (2015). Angular roughshark *Oxynotus centrina* (Squaliformes: Oxynotidae) in captivity feeding exclusively on elasmobranch eggs: An overlooked feeding niche or a matter of individual taste? *Journal of Fish Biology*, *87*(4), 1072–1079.

Gül G (2020). Feeding habits and trophic levels of elasmobranch species in the sea of Marmara. Ph D, dissertation, Istanbul University, Turkey, 111 p.

Gül, G., & Demirel, N. (2020). Trophic interactions of uncommon batoid species in the sea of Marmara. *J. Black Sea/Mediterranean Environment*, *26*, 294–309.

Gül, G., & Demirel, N. (2021). Evaluation of the comprehensive feeding strategy and trophic role of overexploited mesopredator species in the sea of Marmara (northeastern Mediterranean). *Estuarine, Coastal and Shelf Science*, *259*, 107448.

Gül, G., & Demirel, N. (2022). Ontogenetic shift in diet and trophic role of *Raja clavata* inferred by stable isotopes and stomach content analysis in the sea of Marmara. *Journal of fish Biology*, *101*(3), 559–571. https://doi.org/10.1111/jfb.15123

Hebert, C. E., Shutt, J. L., Hobson, K. A., & Weseloh, D. V. C. (1999). Spatial and temporal differences in the diet of Great Lakes herring gulls (*Larus argentatus*): Evidence from stable isotope analysis. *Canadian Journal of Fisheries and Aquatic Sciences*, *56*, 323–338.

Hussey, N. E., MacNeil, M. A., & Fisk, A. T. (2010). The requirement for accurate diet-tissue discrimination factors for interpreting stable isotopes in sharks. *Hydrobiologia*, *654*(1), 1–5.

Hussey, N. E., MacNeil, M. A., Olin, J. A., McMeans, B. C., Kinney, M. J., Chapman, D. D., & Fisk, A. T. (2012). Stable isotopes and elasmobranchs: Tissue types, methods, applications and assumptions. *Journal of Fish Biology*, *80*(5), 1449–1484.

Kabasakal, H. (2009). Scientific note observations on a rare shark, *Oxynotus centrina* (Chondrichthyes: Oxynotidae), in the sea of Marmara (North-Western Turkey). *Pan-American Journal of Aquatic Sciences*, *4*(4), 609–612.

Kaufman, T. J., Pajuelo, M., Bjorndal, K. A., Bolten, A. B., Pfaller, J. B., Williams, K. L., & Vander Zanden, H. B. (2014a). Mother–egg stable isotope conversions and effects of lipid extraction and ethanol preservation on loggerhead eggs. *Conservation physiology*, *2*(1), cou049.

Kaufman, T. J., Pajuelo, M., Bjorndal, K. A., Bolten, A. B., Pfaller, J. B., Williams, K. L., & Vander Zanden, H. B. (2014b). Mother–egg stable isotope conversions and effects of lipid extraction and ethanol preservation on loggerhead eggs, conservation. *Physiology*, *2*, cou049. https://doi.org/10.1093/conphys/cou049.

Leray, M., Yang, J. Y., Meyer, C. P., Mills, S. C., Agudelo, N., Ranwez, V., … Machida, R. J. (2013). A new versatile primer set targeting a short fragment of the mitochondrial COI region for metabarcoding metazoan diversity: Application for characterizing coral reef fish gut contents. *BMC Frontiers in Zoology*, *10*, 34. https://doi.org/10.1186/1742-9994-10-34.

Mago, T., & Salzberg, S. L. (2011). FLASH:Fast length adjustment of short reads to improve genome assemblies. *Bioinformatics*, *27*, 2957–2963.

Megalofonou, P., & Damalas, D. (2004). Morphological and biological characteristics of a gravid angular rough shark (*Oxynotus centrina*) and its embryos from the eastern Mediterranean Sea. *Cybium*, *28*, 105–110.

Oelbermann, K., & Scheu, S. (2002). Stable isotope enrichment (delta super (15) N and delta super (13) C) in a generalist predator (*Pardosa lugubris*, Araneae: Lycosidae): Effects of prey quality. *Oecologia*, *130*(3), 337–344.

Öztürk, B., & Öztürk, A. A. (1996). On the biology of the Turkish straits system. *Journal of Bulletin Institute of Oceanography Special Issue*, *17*, 205–221.

Papastamatiou, Y. P., & Lowe, C. G. (2005). Variations in gastric acid secretion during periods of fasting between two species of shark. *Comparative Biochemistry and Physiology Part A: Molecular and Integrative Physiology*, *141*(2), 210–214.

Phillips, D. L., Inger, R., Bearhop, S., Jackson, A. L., Moore, J. W., Parnell, A. C., … Ward, E. J. (2014). Best practices for use of stable isotope mixing

models in food-web studies. *Canadian Journal of Zoology*, *92*(10), 823–835.
Post, D. M. (2002). Using stable isotopes to estimate trophic position: Models, methods, and assumptions. *Ecology*, *83*(3), 703–718.
Ricker, W. E. (1973). Linear regressions in fishery research. *Journal of the fisheries board of Canada*, *30*(3), 409–434.
Saïdi B (2002). Contribution à l'étude éco-biologique des élasmobranches pleurotrêmes de la région du golfe de Gabès. Ph D, dissertation. University of Sfax, Tunisia, 95 p.
Soldo, A., Guallart, J. (2016). *Oxynotus centrina* (errata version published in 2016). The IUCN Red List of Threatened Species 2016: e. T63141A97834254. Accessed on February 10, 2022.
Symondson, W. O. C. (2002). Molecular identification of prey in predator diets. *Molecular Ecology*, *11*, 627–641. https://doi.org/10.1046/j.1365-294x.2002.01471.x.
Traugott, M., Thalinger, B., Wallinger, C., & Sint, D. (2021). Fish as predators and prey: DNA-based assessment of their role in food webs. *Journal of Fish Biology*, *98*, 367–382. https://doi.org/10.1111/jfb.14400.
Wetherbee, B. M., & Cortes, E. (2004). Food consumption and feeding habits. In J. C. Carrier, J. A. Musick, & M. R. Heithaus (Eds.), *Biology of sharks and their relatives* (p. 596). Florida, USA: CRC Press, ISBN 0-8493-1514-X.
Wetherbee, B. M., Cortés, E., & Bizzarro, J. J. (2004). Food consumption and feeding habits. *Biology of sharks and their relatives*, 225–246.
Yıldız, T., Ulman, A., & Demirel, N. (2020). A comparison of market landings during fish spawning seasons to better understand the effectiveness of the temporal fishery closure in Turkey. *Ocean and Coastal Management*, *198*, 105353. https://doi.org/10.1016/j.ocecoaman.2020.105353.
Zanden, M. J. V., & Rasmussen, J. B. (2001). Variation in δ15N and δ13C trophic fractionation: Implications for aquatic food web studies. *Limnology and Oceanography*, *46*(8), 2061–2066.
Zengin, M., & Akyol, O. (2009). Description of by-catch species from the coastal shrimp beam trawl fishery in Turkey. *Journal of Appied. Ichthyology*, *25*, 211–214.

SUPPORTING INFORMATION

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