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SEASONAL CHANGES IN THE FOOD SPECTRUM AND DAY-TIME RHYTHM OF FEEDING IN RED MULLET *MULLUS BARBATUS* (LINNAEUS, 1758) IN THE SOUTHEAST BLACK SEA

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ABSTRACT

The diet of the red mullet *Mullus barbatus* was studied in the southeast Black Sea region of Turkey during the autumn, winter, summer and spring. In one year, the stomach contents of 760 individuals of *M. barbatus*, a confirmed omnivorous fish species, were examined (April 2017–March 2018), in addition to those of 180 additional individuals examined within a 24-h period (28 April 2018). Among the 14 prey groups identified in the stomachs of red mullet, the predominant one was Bivalvia, followed by Nematoda, Polychaeta, Brachyura and Cumacea. Data analysis revealed significant differences in prey species composition between seasons (ANOSIM, $R = 0.089$, $p < 0.001$). Moreover, the prey groups that constituted the majority of the diet changed significantly with a season. SIMPER analysis revealed that the prey item contributing the most to the differences between seasons was Bivalvia. Microplastic was also found in the samples. Analysis of the daily rhythm diet variation in stomach contents allowed the identification of 8 prey groups, namely Bivalvia, Amphipoda and Cumacea. In 24-hour examinations, feeding began in the first hours of the day, then showed an increase in the following hours and decreased after the evening. The results of this study could be used to describe the diversity of prey species and intraspecific food competition in the Black Sea.

KEYWORDS:

Mullus barbatus, Black sea, Daily feeding, Seasons feeding, Microplastic.

INTRODUCTION

Red mullet (*Mullus barbatus* Linnaeus, 1758) is a common benthic fish species living on sandy and muddy bottoms of the continental shelf at depths as low as 200 m [1]. It is distributed all across the Mediterranean basin, including the Black Sea and the Eastern Atlantic along the European and African coasts [2, 3, 1]. Red mullet is one of

the most important demersal fish of the Black Sea, has a very high value in Turkish fisheries and is one of the most important fish species traditionally fished and consumed in Black Sea countries [4]. Red mullet is also an important component of the Mediterranean demersal resources exploited by bottom trawl and small-scale fisheries [5, 6]. For Turkey, the total catch of these highly valuable goatfishes summed to 4579 tons in 2016 based on statistics from the Turkish Statistical Institute [7]. Feeding activities provide fish with the necessary nutrition and energy to support their maintenance, growth, reproduction and, subsequently, population development [8, 9, 10]. The study of feeding activities and the factors influencing them is thus critically important in our understanding of the dynamics of fish populations. Knowledge of the feeding habits of a fish may play a key role in research on the following ecological issues: prey selection, predator-prey size relationships, distribution of feeding types with latitude, ontogenetic diet shifts, and species invasions [11]. The diet of the red mullet has been well investigated in the Mediterranean Sea [12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27]. However, there is no information about this species in the Black Sea, and this study was planned to fill this gap. This work aims to provide a detailed (monthly and daily) description of the feeding habits of the species *Mullus barbatus* off the Black Sea and to compare them with other areas (e.g. Mediterranean Sea and Atlantic Ocean), which could help provide a basis for understanding trophic levels and interactions in the aquatic food web of the study area. The results of this study can be used in multispecies and ecosystem-based models.

MATERIALS AND METHODS

Fish sampling (annual). A total of 760 red mullet samples were collected monthly using an experimental bottom trawl (12 mm mesh size) between April 2017 and March 2018 off the Rize coast, in the south-eastern Black Sea. Fishing took place within an area defined by the following coordinates: 40°59'29''N/40°19'52''E; 40°59'

57°N/40°18' 50"E; 40°01'32"N/40°22'53"E; 41°02'10"N/40°22'04"E (Fig. 1). The average haul duration was 30 min, and towing speed varied from 2.5 to 3.5 knots between 0-30, 30-60 and 60+ m depth contours. Sampled specimens were fixed in 70% alcohol and transferred to the laboratory. The total length of each individual was measured to 0.1 cm, and wet weight was determined to the nearest 0.01 g after the animal was blotted dry on absorbent paper.

Fish sampling (daily). A total of 180 red mullet samples were collected in April at 4-hour intervals during the 24-h sampling period using the same experimental bottom trawl (12 mm mesh size) between 13:00 (28 April 2018) and 09:00 (29 April 2018) off the Rize coast, in the south-eastern Black Sea. The area was defined by the following coordinates: 40°59'29"N/ 40°19'52"E, 40°59'57"N/40°18'50"E, 40°01'32"N/40°22'53"E, 41°02'10"N/ 40°22'04"E (Fig. 1). The depth range of the sampling area was from 30 to 40 m. A total of 6 trawl operations were carried out during the 24-h sampling period. The bottom texture in the area was muddy sand. From each haul, 30 specimens were randomly sampled. The total body weight (g) of each fish was measured before dissection for stomach removal. Stomachs were preserved individually in small jars with 10% buffered formaldehyde solution.

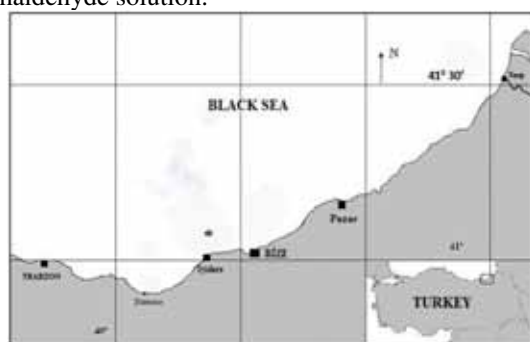


FIGURE 1
Map of the study area

Diet analysis. Stomachs were dissected, and all prey items were identified to the lowest possible taxonomic level after counting and weighing. Sorted prey items were weighed wet to the nearest 0.001 g. The prey group occurrence frequency (F%), prey group numerical frequency (N%), percent prey group weight (W%), relative importance index (IRI) and percent relative importance index (IRI%) (28, 29). were determined as follows:

$$F\% = \frac{n}{N_s} * 100,$$

$$N\% = \frac{n^y}{N_p} * 100$$

$$W\% = \frac{w^y}{W_p} * 100$$

$$IRI = (N\% + W\%). (F\%)$$

$$IRI\% = \frac{IRI}{\sum IRI} * 100$$

where, F% = number of stomachs containing prey n / total number of full stomachs x 100 N_s, N% = number of prey n^y/ total number of prey x 100 N_p, W% = weight of prey w^y / total weight of all prey x 100 W_p.

In the annual samples, stomach fullness was determined visually according to (30) using a scale ranging from 0 to 100% and noted as empty (0%), moderately full (25%), half full (50%), quite full (75%) and very full (100%). One-way analysis of similarity (ANOSIM) (31) was used to determine the differences between seasons in the structure of the stomach contents. The most abundant prey species primarily responsible for an observed difference between seasons were examined using similarity percentages (SIMPER) [31] Multivariate analyses were performed with the software package PRIMER 5.

In order to determine the diel feeding intensity and chronology, fullness index (%FI) values (the percentage ratio of the weight of an individual fish stomach contents to its total body weight) and the percentage of empty stomachs (%ES) were calculated for each sampling time [28, 29].

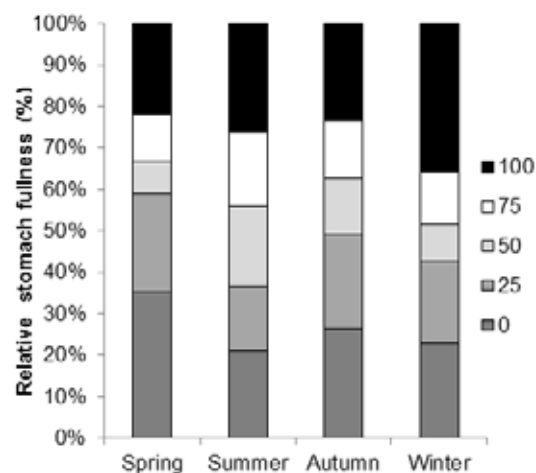


FIGURE 2
Seasonal stomach fullness ratio (empty, 0%; moderately full, 25%; half full, 50%; quite full 75% and very full 100%) of red mullet (*M. barbatus*) sampled from April 2017 to March 2018 from the Black sea

RESULTS

Empty stomachs were found in all seasons throughout the year. The highest proportion of very full stomachs (35.66%) was found in winter, whereas the highest number of empty stomachs (35.26%) was recorded in the spring (Fig. 2).

Overall diet composition. All stomach contents of red mullet contained Bivalvia (N% = 30.46), Amphipoda (N% = 2.07), Isopoda (N% = 0.87), Cumacea (N% = 13.16), Gastropoda (N% = 3.98), Brachyura (N% = 2.31), Decapoda (N% = 2.17), Teleostei (N% = 0.15), Polychaeta (N% = 4.77), Nematoda (N% = 38.42), Mysidacea (N% = 0.80), Tanaidacea (N% = 0.15), Echinodermata (N% = 0.56) and microplastic (N% = 0.12). According to the percent relative importance index, IRI%, red mullet generally feed on three prey groups: Bivalvia (IRI% = 68.69), Nematoda (IRI% = 14.59) and Polychaeta (IRI% = 5.36) (Table 1).

Seasonal prey variations were observed in the diet of red mullet. The IRI% of food items of red mullet is shown in Table 2. It was clear that Bivalvia was the most important food item in all seasons and showed the highest percentage during the summer, with an average of 70.8%. The prey groups Cumacea, Brachyura and Decapoda followed Bivalvia. In addition, microplastic was found in the samples.

According to the ANOSIM, the global R value was 0.089 [an R value between 0 and 1 indicates

that all the most similar samples are within the same groups [32] and the significance level of the sample statistics was 0.001. SIMPER analysis (Table 3) was performed on the number of species found in stomachs in all seasons. The average dissimilarity between spring and summer was 73.57% and showed that the groups Bivalvia and Nematoda were good indicators of spring and Cumacea and Brachyura, of summer. The average dissimilarity between spring and autumn was 69.14% and showed that the groups Bivalvia, Brachyura and Polychaeta were good indicators of spring and Nematoda, of autumn. The average dissimilarity between Summer and Autumn was 67.91% and showed that the groups Bivalvia, Cumacea and Brachyura were good indicators of summer and Nematoda, of Autumn. The average dissimilarity between spring and winter was 69.01% and showed that the groups Bivalvia and Amphipoda were good indicators of spring and Nematoda and Polychaeta, of winter. The average dissimilarity between summer and winter was 72.85% and showed that the groups.

TABLE 1

Overall distribution of prey group occurrence frequency (F%), numerical frequency (N%), weight (W%) and relative importance (IRI%) for the prey groups observed in red mullet stomach.

Species	n	F%	N%	W%	IRI%
Bivalvia	1783	60.75	30.46	41.67	68.69
Amphipoda	121	9.32	2.07	2.60	0.68
Isopoda	51	4.66	0.87	0.11	0.07
Cumacea	770	16.49	13.16	0.27	3.47
Gastropoda	233	11.29	3.98	1.99	1.06
Brachyura	135	11.65	2.31	23.04	4.63
Decapoda	127	10.75	2.17	5.25	1.25
Teleostei	9	1.25	0.15	4.03	0.08
Polychaeta	279	15.41	4.77	17.40	5.36
Nematoda	2249	22.58	38.42	2.80	14.59
Mysidacea	47	3.23	0.80	0.11	0.05
Tanaidacea	9	0.72	0.15	0.04	0.00
Echinodermata	33	3.58	0.56	0.69	0.07
Microplastic	7	1.08	0.12	0.00	0.00

TABLE 2

Seasonal distribution of prey group occurrence frequency (F%), numerical frequency (N%), weight (W%) and relative importance index (IRI%) values observed in red mullet stomach.

Prey Groups	Spring				Summer				Autumn				Winter			
	F%	N%	W%	IRI%	F%	N%	W%	IRI%	F%	N%	W%	IRI%	F%	N%	W%	IRI%
Bivalvia	47.0	42.8	25.2	64.1	63.1	34.9	57.2	70.8	63.7	31.2	39.3	70.3	69.4	20.3	31.0	39.7
Amphipoda	13.4	4.4	13.8	4.9	11.9	2.9	0.6	0.5	6.7	1.4	1.1	0.3	4.1	0.5	1.0	0.1
Isopoda	5.2	0.7	0.4	0.1	2.4	0.3	0.1	0.0	9.6	2.0	0.1	0.3	1.7	0.9	0.0	0.0
Cumacea	8.2	3.2	0.3	0.6	36.3	38.3	0.6	17.2	5.9	1.6	0.0	0.2	9.9	1.6	0.1	0.2
Gastropoda	12.7	4.2	6.2	2.6	11.9	4.0	0.6	0.7	6.7	7.3	2.9	1.1	14.0	2.3	0.5	0.4
Brachyura	9.7	2.8	32.5	6.9	14.9	3.0	29.2	5.8	13.3	3.0	25.1	5.9	7.4	1.1	5.9	0.6
Decapoda	3.7	0.6	0.1	0.1	22.6	5.6	7.4	3.6	10.4	1.6	5.0	1.1	2.5	0.2	5.2	0.2
Teleostei	2.2	0.4	0.4	0.0	0.6	0.1	1.8	0.0	1.5	0.3	11.6	0.3	0.8	0.1	0.0	0.0
Polychaeta	16.4	6.5	15.4	7.2	3.6	0.5	1.0	0.1	9.6	4.7	10.3	2.3	37.2	7.6	52.0	24.7
Nematoda	18.7	32.2	2.9	13.2	8.9	8.3	1.4	1.0	23.0	46.5	4.5	18.4	45.5	64.0	2.6	33.7
Mysidacea	1.5	0.5	0.4	0.0	7.7	2.2	0.1	0.2	0.0	0.0	0.0	0.0	2.5	0.3	0.0	0.0
Tanaidacea	2.2	0.7	0.3	0.0	0.0	0.0	0.0	0.0	0.7	0.1	0.0	0.0	0.0	0.0	0.0	0.0
Echinodermata	3.7	0.9	2.2	0.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	12.4	1.2	1.8	0.4
Microplastic	0.7	0.1	0.0	0.0	0.0	0.0	0.0	0.0	2.2	0.4	0.0	0.0	1.7	0.1	0.0	0.0

TABLE 3

Results of SIMPER analysis: Groups contribution to average dissimilarity between seasons.

Spring-Summer		Average dissimilarity = 73.57					
Groups	Av.Abund	Av.Abund	Av.Diss	Diss/SD	Contrib%	Cum.%	
Bivalvia	4.73	4	18.56	1.16	25.23	25.23	
Cumacea	0.35	4.37	11.46	0.79	15.57	40.81	
Nematoda	3.56	0.95	9.61	0.63	13.06	53.87	
Brachyura	0.31	0.33	7.39	0.5	10.05	63.92	
Spring-Autumn		Average dissimilarity = 69.14					
Groups	Av.Abund	Av.Abund	Av.Diss	Diss/SD	Contrib%	Cum.%	
Bivalvia	4.73	2.86	18.81	1.19	27.2	27.2	
Nematoda	3.56	4.27	13.07	0.79	18.9	46.11	
Brachyura	0.31	0.28	7.38	0.52	10.67	56.78	
Polychaeta	0.72	0.43	7.15	0.57	10.35	67.12	
Summer-Autumn		Average dissimilarity = 67.91					
Groups	Av.Abund	Av.Abund	Av.Diss	Diss/SD	Contrib%	Cum.%	
Bivalvia	4	2.86	17.79	1.16	26.2	26.2	
Cumacea	4.37	0.15	11.28	0.77	16.61	42.8	
Nematoda	0.95	4.27	9.95	0.67	14.64	57.45	
Brachyura	0.33	0.28	7.64	0.53	11.25	68.7	
Spring-Winter		Average dissimilarity = 69.01					
Groups	Av.Abund	Av.Abund	Av.Diss	Diss/SD	Contrib%	Cum.%	
Bivalvia	4.73	3.72	17.04	1.21	24.69	24.69	
Nematoda	3.56	12.36	16.99	1.03	24.61	49.31	
Polychaeta	0.72	1.48	10.8	0.83	15.65	64.96	
Amphipoda	0.48	0.09	5.53	0.44	8.01	72.97	
Summer-Winter		Average dissimilarity = 72.85					
Groups	Av.Abund	Av.Abund	Av.Diss	Diss/SD	Contrib%	Cum.%	
Bivalvia	4	3.72	16.75	1.22	22.99	22.99	
Nematoda	0.95	12.36	16.24	1.02	22.3	45.29	
Cumacea	4.37	0.31	10.8	0.78	14.82	60.11	
Polychaeta	0.06	1.48	8.62	0.72	11.83	71.94	
Autumn-Winter		Average dissimilarity = 64.70					
Groups	Av.Abund	Av.Abund	Av.Diss	Diss/SD	Contrib%	Cum.%	
Nematoda	4.27	12.36	16.88	1.05	26.09	26.09	
Bivalvia	2.86	3.72	16.76	1.23	25.9	51.99	
Polychaeta	0.43	1.48	9.63	0.78	14.88	66.87	
Brachyura	0.28	0.21	5.41	0.46	8.36	75.23	

TABLE 4

Distribution of prey group occurrence frequency (F%), numerical frequency (N%), weight (W%) and relative importance (IRI%) for the prey groups observed in red mullet stomach during 24-h period

Hours	%	Bivalvia	Amphipoda	Cumacea	Gastropoda	Decapoda	Polychaeta	Nematoda	Tanaidacea
13:00	F%	63.64	0.00	0.00	36.36	18.18	63.64	18.18	0.00
	N%	14.47	0.00	0.00	5.26	3.95	73.68	2.63	0.00
	W%	4.89	0.00	0.00	1.04	54.48	39.54	0.05	0.00
	IRI%	12.60	0.00	0.00	2.34	10.86	73.69	0.50	0.00
17:00	F%	50.00	0.00	0.00	25.00	0.00	12.50	0.00	0.00
	N%	50.00	0.00	0.00	25.00	0.00	25.00	0.00	0.00
	W%	67.74	0.00	0.00	11.18	0.00	21.08	0.00	0.00
	IRI%	79.91	0.00	0.00	12.28	0.00	7.82	0.00	0.00
21:00	F%	27.27	0.00	0.00	9.09	18.18	45.45	18.18	0.00
	N%	20.83	0.00	0.00	4.17	8.33	58.33	8.33	0.00
	W%	27.65	0.00	0.00	0.29	3.58	67.91	0.57	0.00
	IRI%	17.68	0.00	0.00	0.54	2.90	76.72	2.17	0.00
01:00	F%	77.78	0.00	0.00	11.11	0.00	66.67	22.22	0.00
	N%	36.11	0.00	0.00	5.56	0.00	47.22	11.11	0.00
	W%	52.96	0.00	0.00	2.07	0.00	44.30	0.67	0.00
	IRI%	51.79	0.00	0.00	0.63	0.00	45.62	1.96	0.00
05:00	F%	92.59	0.00	3.70	40.74	3.70	22.22	25.93	3.70
	N%	76.01	0.00	1.01	10.47	1.01	6.42	4.73	0.34
	W%	63.67	0.00	0.08	10.32	0.25	25.47	0.20	0.01
	IRI%	88.42	0.00	0.03	5.79	0.03	4.84	0.87	0.01
09:00	F%	68.18	9.09	4.55	27.27	18.18	31.82	4.55	9.09
	N%	51.63	1.63	0.54	9.24	3.26	30.43	0.54	2.72
	W%	21.58	1.22	0.03	3.78	36.92	35.89	0.03	0.56
	IRI%	60.52	0.31	0.03	4.31	8.86	25.58	0.03	0.36

Bivalvia and Cumacea were good discriminators of summer and Nematoda and Polychaeta, of winter. The average dissimilarity between autumn and winter was 64.70% and showed that the group Brachyura was a good discriminator of autumn and Nematoda, Bivalvia and Polychaeta, of winter.

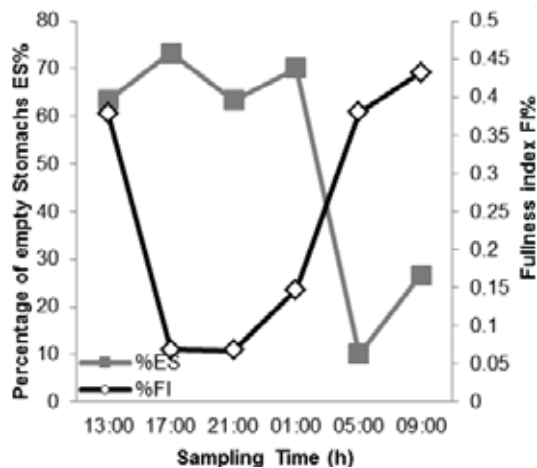


FIGURE 3

Variations in feeding intensity of red mullet during a 24-h sampling period

Daily diet composition. Feeding activity started early in the day (01:00) and increased with the sunrise. It reached the maximum level in the subsequent hours. There was a decrease in feeding activity in the afternoon, towards sunset (Fig. 3).

The daily rhythm in the variation of stomach content analysis allowed the identification of 8 prey groups, namely Bivalvia, Amphipoda, Cumacea, Gastropoda, Decapoda, Polychaeta, Nematoda and Tanaidacea. The following percent relative importance indices (IRI%) were obtained: at 13:00, Polychaeta IRI% = 73.69; at 17:00, Bivalvia IRI% = 79.91; at 21:00, Polychaeta IRI% = 76.72; at 01:00, Bivalvia IRI% = 51.79; at 05:00, Bivalvia IRI% = 88.42 and at 09:00, Bivalvia IRI% = 60.52 (Table 4).

DISCUSSION

Overall diet composition. Red mullet is a carnivorous fish that scoops up the substratum to detect and feed on a wide range of benthic invertebrates, primarily polychaetes but also crustaceans and molluscs [25, 33, 34, 35, 36, 37]. The diet of red mullet in the Black Sea differs from that of red mullet in the Mediterranean Sea and Atlantic Ocean. This reveals that the presence of Bivalvia in the diet of red mullet predominates in the Black Sea. In the other areas (Mediterranean, Atlantic Ocean and the Aegean Sea), the dominant groups in the stomach contents were reported as Polychaeta [13, 26, 37, 12, 23, 19, 20] and Amphipoda [14]. [27], reported the primary diet group of red mullet

as Decapoda in the Mediterranean. Differences in diet of red mullet are generally not important as they could be the results of the areas sampled and/or the biological environment. According to previous studies, the morphological characteristics and foraging behaviour of red mullet accounted for both prey type selection and feeding patterns [25, 35]. In our study and in the study of [27], the Nematoda prey group was reported. Also, some groups reported in other studies [37, 20, 23, 27] were not reported in our study. Marine plastic litter is slowly broken up by mechanical, chemical and photolytic degradation processes and then fragmented into small sizes. This plastic litter floats on the sea or sinks to the bottom [38]. [39] reported that demersal fish species contain higher levels of microplastics in their stomach contents than pelagic fish species. Microplastic was found in *M. barbatus*, one of the demersal fish species in the study.

Diet composition according to season. In this study the group Bivalvia was determined as the dominant group in all seasons (spring IRI% 64.1, summer IRI% 70.8, autumn IRI% 70.3 and winter IRI% 39.7) (Table 3). [27] and [13] reported the Polychaete group as dominant in all seasons for the same species in the Mediterranean. In several other studies, it was reported that the main feeding group varied between seasons. [14], reported differences between the seasons: in autumn Copepoda was more abundant, whereas in spring Amphipoda was more abundant. In addition, Copepoda was found in abundance in winter and autumn. Amphipoda abundance was higher in summer than in other seasons. Amphipoda and Cumacea were abundant in spring and winter. [23], reported that although the dominant group was Polychaeta in February, May and July, the Bivalvia group was dominant in September. Moreover, in November, Polychaeta and Bivalvia were similar in importance. [19] reported differences between the seasons, but in the spring, there is a higher abundance of food compared with other seasons. Our study also showed differences between the seasons. Such differences in the diet of *M. barbatus* between seasons might be caused by the presence of certain food groups in the environment during a specific season [40].

Diet composition in relation to time of day.

Feeding begins in the first hours of the day, then shows an increase in subsequent hours and decreases after the evening. [12, 19] conducted daily rhythm studies in the Aegean Sea and obtained similar results to ours. In two studies, feeding was reported to be intense during the morning, decreasing at night. However, they reported the main group as Polychaeta.

CONCLUSION

Most of the previous studies cited in this manuscript reported Polychaeta as the predominant prey item in the diet of red mullet. However, according to the present study, Polychaeta were not the predominant prey item in the diet of red mullet in the Black Sea. To confirm this, further research is required to investigate the feeding habits of other predators in the Black Sea that consume Polychaeta. In addition, studies on the abundance of Polychaeta in the Black Sea are almost negligible; therefore, it is important to investigate this issue. In the present study, we reported plastic pollution in commercial fishes from the Black Sea. This study provides an important contribution to the knowledge and understanding of plastic occurrence in these commercial fish, given their importance in Black Sea catches and human consumption. The presence of plastic in other commercial fish species has important consequences for human health. The results of this study could be used for ecosystem-based management of *M. barbatus* and to describe the diversity of prey and interspecific food competition in the Black Sea.

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