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Article *in* Fresenius Environmental Bulletin · February 2018

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THE FEEDING BEHAVIOUR OF *PLEUROBRACHIA PILEUS* (CTENOPHORA: TENTACULATA) IN THE SOUTHEASTERN BLACK SEA: IN RELATION TO AREA AND SEASON

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ABSTRACT

The diet composition of *Pleurobrachia pileus* (Müller, 1776) was studied in five stations (İyidere, Rize, Çayeli, Pazar and Fındıklı) located along the Southeastern Black Sea coast of Turkey. The diet of *P. pileus* was also investigated in relation to seasonal variations. Overall, the diet of *P. pileus* was dominated by prey groups from phytoplankton followed by zooplankton. Also, seasonal diet of *P. pileus* is exclusively dominated by phytoplankton only during autumn and winter periods. Dendrogram, multidimensional scaling plots, ANOSIM and SIMPER analysis indicated an extremely identical diet of *P. pileus* between spring and summer. Autumn and winter have distinct prey assemblages and revealed low diet similarities with the two other seasons. Such trend was not found between different regions indicating a similar diet. The study suggested that *P. pileus* is miscellaneous feeder and its diet composition may evince the availability of different prey groups in the ambient environment. The results obtained from this study would assess not only to describe the diversity of prey species to a certain extent but may also determine interspecific competition for food in the Southeastern Black Sea.

KEYWORDS:
Black Sea, ctenophores, *Pleurobrachia pileus*, predation

INTRODUCTION

Three gelatinous species of the phylum Ctenophora are common in the Black Sea: *Mnemiopsis leidyi*, *Beroe ovata* and *Pleurobrachia pileus* [1, 2, 3, 4]. The *M. leidyi* was invaded to the Black Sea during the early 1980s via ballast water of ships from the north-western Atlantic coastal region which has dramatically affected the population of zooplankton resulting into an abrupt decline in the icthyo- and mesozooplankton abundance of the Black Sea [5, 6]. The swarms/population of *M. leidyi* was controlled once a voracious predator *B. ovata* was introduced in the region [7].

P. pileus has been described as a cosmopolitan species occurring in marine waters of the North-West Atlantic [8, 9], the North-East Atlantic [1, 10, 11] and the Black Sea [2]. Similar to *M. leidyi*, *P. pileus* also fed primarily on copepods (zooplankton) and is considered as a key species of the German Bight ecosystem to control the populations of copepod during maximum abundance regarded as a second trophic control system [12]. However, the presence of *P. pileus* in high densities result in the reduction of zooplankton and after the reduction of zooplankton they may start to consume eggs and larvae of fishes [1, 13, 14] becoming a major competitor with pelagic and larvae fish for zooplankton [15].

Since the diet composition of *P. pileus* mostly depend on the structure of different prey availability and the abundance of the *P. pileus* themselves [16], therefore, their diet composition reflects the ambient food environment [1]. Though it is obvious that *P. pileus* generally fed on copepods however the information on its diet composition in the Black Sea is limited.

In this paper, the diet composition of *P. pileus* was investigated during spring, summer, autumn and winter from five stations along the Southeastern Black Sea. The results of this study will provide a baseline data for future work to assess the proper management of aquatic resources and to estimate the tropic levels in the Black Sea ecosystem.

MATERIALS AND METHODS

Study area. The sampling sites include five offshore stations located two miles from shore. All sampling sites are located in the Southeastern Black Sea (Figure 1). The established five stations for *P. pileus* sampling during spring (May 2014), summer (July 2014), autumn (November 2014) and winter (February 2015) were İyidere (41° 02' 38" N- 40° 21' 46" E), Rize (41° 03' 25" N- 40° 31' 15" E), Çayeli (41° 07' 12" N- 40° 43' 29" E), Pazar (41° 12' 38" N- 40° 53' 07" E) and Fındıklı (41° 18' 21" N- 41° 08' 49" E). Each time the *P. pileus* was sampled from stations. Recep Tayyip Erdoğan University, Faculty of Fisheries research vessel 'R/V RIZESUAR' with an overall length of 12 m (140 HP) was used during *P. pileus* sampling. The vertical temperature of seawater (*in-situ*) was obtained by using SBE 19 CTD probe.

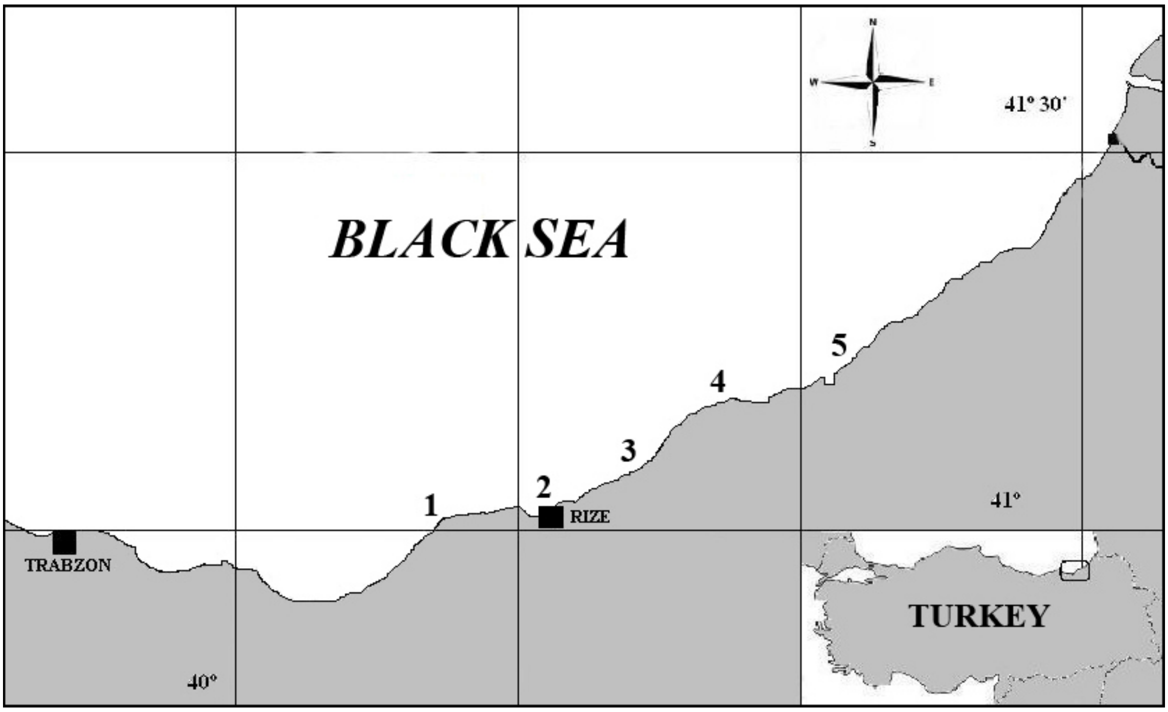

FIGURE 1
Map of the study area showing the five established stations for the collection of *Pleurobrachia pileus*. 1(İyidere) : 41° 02' 38" N- 40° 21' 46" E, 2 (Rize) : 41° 03' 25" N- 40° 31' 15" E, 3 (Çayeli) : 41° 07' 12" N- 40° 43' 29" E, 4 (Pazar) : 41° 12' 38" N- 40° 53' 07" E, 5 (Fındıklı) : 41° 18' 21" N- 41° 08' 49" E.

Sampling and laboratory investigation. *P. pileus* specimens were collected using a plankton net (Hydrobios, 200µm mesh size; vertically between surface and 100 m). They were immediately preserved in 4% formaldehyde [13, 17]. During laboratory investigation, *P. pileus* samples were weighted to the nearest 0.001 g using digital balance and measured to 1.0 mm using electronic digital calliper. The gut contents were then studied under Nikon SMZ1000 stereomicroscope, identified to the lowest possible taxonomic level (mostly genus) and, were counted.

Qualitative dietary analysis. Food composition was expressed as the percentage of prey groups occurrence frequency (*F%*) and percentage of prey groups numerical frequency (*N%*) methods [18, 19]. The *N%* and *F%* were calculated as:

$$F\% = \frac{n}{N_s} \times 100 \quad (1)$$

where, n is number of fish with particular food type, N_s is a total number of fish containing food in their stomach

$$N\% = \frac{n'}{N_p} \times 100 \quad (2)$$

where n^l is the total number of a food group, N_p is the total number of all prey groups.

Statistical analysis. Dendrogram and multidimensional scaling plots (MDS) analysis based on *F%* and *N%* values were used to determine the percentage similarities or dissimilarities among seasons and stations. The logarithmic transformed data were used in MDS. One-way analysis of similarity (ANOSIM) was used to test the null hypothesis of no difference in the diet composition of *P. pileus* among stations and seasons [20]. The typifying prey and discriminating prey for each study area and season were determined using the SIMPER. The SIMPER also determines the average contribution of each prey to the dissimilarity between different study area and seasons. The multivariate function of the PRIMER 6.0 software package and PAST (version 2.14) were used to carry out the statistical tests.

TABLE 1
The mean length (± standard error) minimum and maximum length of *Pleurobrachia pileus* specimen collected from different season

Season	Body length (cm) Mean ± SE	Min.	Max.	Observations (n)
Spring	0.59 ± 0.03	0.2	1.1	70
Summer	0.41 ± 0.02	0.1	0.7	37
Autumn	0.80 ± 0.05	0.4	1.2	25
Winter	0.95 ± 0.03	0.5	1.5	61

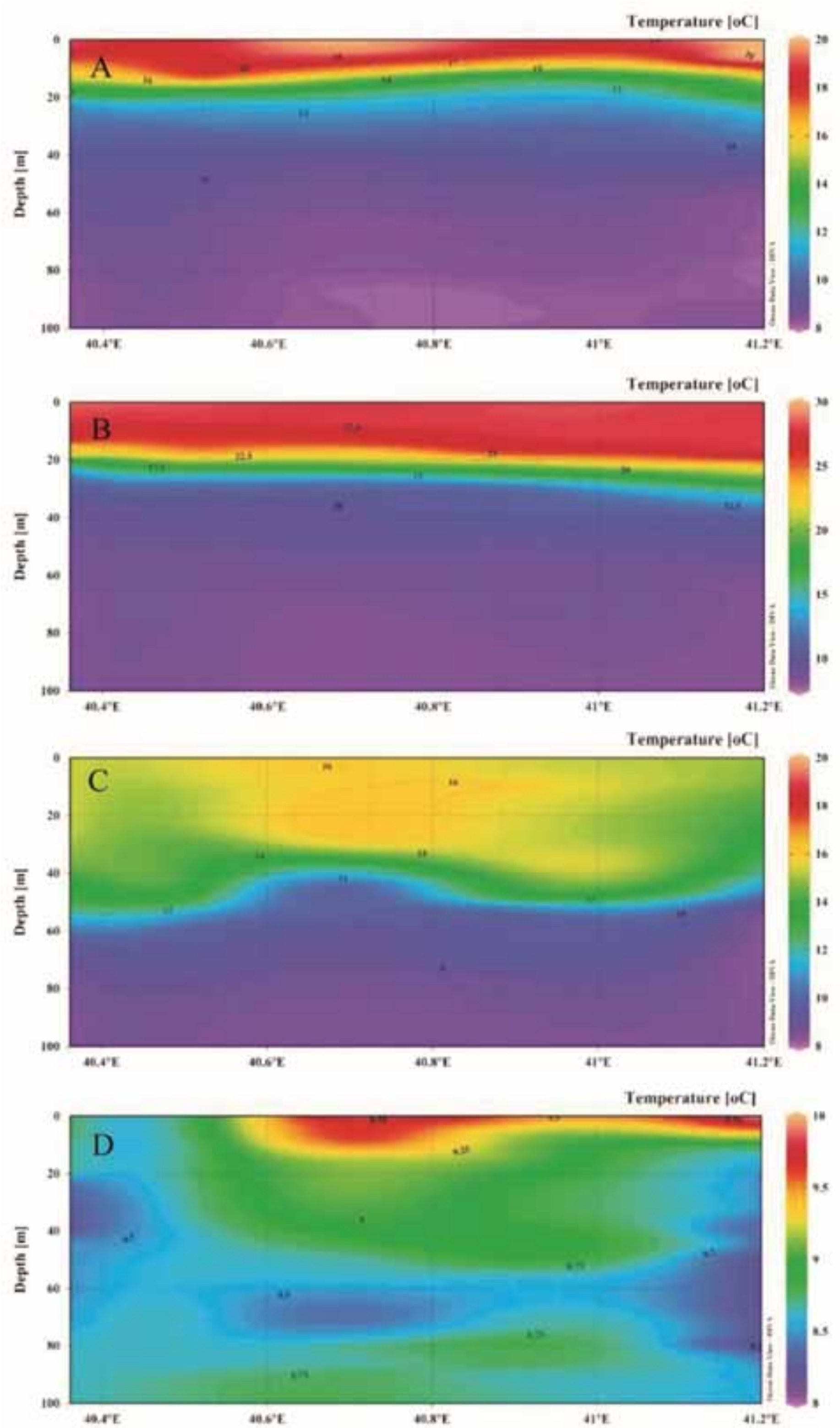

FIGURE 2
The seawater temperature in relation to water depth (A: Spring, B: Summer, C: Autumn, D: Winter)

RESULTS

Figure 2 shows the vertical temperature of seawater in relation to water depth during different seasons. The temperature is mostly constant during winter.

A total of 193 specimens of *P. pileus* were examined during the study (Table 1). Above 80% of *P. pileus* specimens had food in their gut collected from five established stations during different seasons [Figure 3(A) and 3(B)].

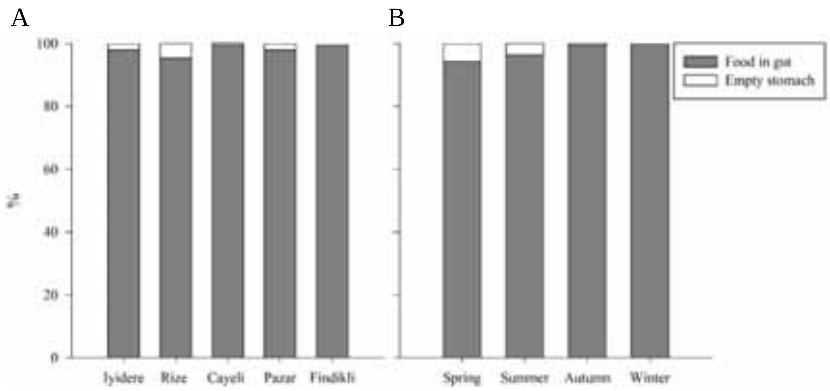

FIGURE 3
The percentage of *Pleurobrachia pileus* specimens with food in gut collected from different study areas (A) and during different season (B).

TABLE 2
The diet composition of *Pleurobrachia pileus* in the Southeastern Black Sea region of Turkey. The contribution of each prey item is given as percentage of prey group numerical frequency (*N %*) and percentage of prey group occurrence frequency (*F%*).

Prey groups	Overall		Spring		Summer		Autumn		Winter	
	%N	*%F*	*%N*	*%F*	*%N*	*%F*	*%N*	*%F*	*%N*	*%F*
PHYTOPLANKTON										
Dinophyceae										
Ceratium fusus	6.60	10.46	0.42	0.53	—	—	61.69	27.16	5.24	13.90
Ceratium furca	1.21	4.04	—	—	—	—	5.19	12.35	1.29	5.08
Ceratium tripos	2.84	7.11	—	—	—	—	2.60	8.64	3.52	11.76
Noctiluca scintilans	0.45	2.51	3.22	7.37	0.45	2.78	—	—	0.03	0.53
Protoperidinum sp.	0.75	2.93	—	—	—	—	3.90	7.41	0.77	4.01
Alexandrium sp.	0.05	0.42	—	—	—	—	0.65	2.47	0.03	0.27
Dinophysis sp.	0.03	0.28	—	—	—	—	0.65	2.47	—	—
Prorocentrum sp.	0.03	0.28	—	—	—	—	—	—	0.03	0.53
Bacillariophyceae										
Chaetoceros sp.	0.04	0.14	0.31	0.53	—	—	—	—	—	—
Navicula sp.	0.04	0.28	0.31	1.05	—	—	—	—	—	—
Proboscia sp.	5.66	0.14	—	—	—	—	—	—	7.28	0.27
Pseudanitzchia delicatisima	55.91	6.83	—	—	—	—	—	—	71.90	13.10
Rhizosolenia sp.	0.93	3.49	—	—	—	—	—	—	1.20	6.68
Thallassionema nitzschioides	0.05	0.42	—	—	—	—	—	—	0.07	0.80
PROTOZOA										
Stenosemella nivalis	0.01	0.14	—	—	—	—	—	—	0.02	0.27
ZOOPLANKTON										
Isopoda										
Isopot larvae	0.05	0.56	0.42	2.11	—	—	—	—	—	—
Copepoda										
Acartia sp.	0.22	1.95	—	—	—	—	1.95	6.17	0.18	2.41
Acartia sp. naupli	0.06	0.70	—	—	—	—	—	—	0.08	1.34
Calanus sp.	2.74	5.16	12.98	10.53	14.93	16.67	6.17	4.94	0.02	0.27
Calanus sp. copopodit	0.35	2.51	—	—	—	—	0.97	2.47	0.40	4.28
Oithona sp.	0.08	0.42	—	—	1.13	2.78	0.32	1.23		
Chaetognatha										
Sagitta setosa	0.27	1.39	1.66	3.68	1.13	4.17	—	—	—	—
Appendicularian										
Oikopleura sp.	0.65	2.37	4.05	5.26	1.13	2.78	—	—	0.10	1.34
Nemertea										
Nemertea sp.	0.09	0.70	—	—	—	—	—	—	0.12	1.34
Bivalvia										
Mytilus sp. veliger	0.90	5.72	4.26	13.16	4.98	15.28	—	—	0.10	1.34
Micrometazoan egg-larvae			—	—	—	—	—	—		
Jellyfish egg	0.62	1.95	—	—	—	—	—	—	0.80	3.74
Jellyfish planula	1.47	3.35	10.18	10.00	2.49	5.56	—	—	0.07	0.27
Polychaete larvae	0.26	1.67	1.66	4.74	0.23	1.39	—	—	0.05	0.53
Shrimp larvae	0.01	0.14	0.10	0.52	—	—	—	—		
Fish egg	4.76	6.97	24.92	14.21	21.04	16.67	0.32	1.23	0.55	2.67
Microplastic							—	—		
Particle plastic	10.91	17.85	35.51	26.32	52.49	31.94	15.58	23.45	3.64	9.63
Fiber	0.30	1.95	—	—	—	—	—	—	0.38	3.74
Other										
Fecal pellet	1.65	5.16	—	—	—	—	—	—	2.13	9.89

Overall diet composition. The overall contribution of each prey item to the total bulk of the diet of *P. pileus* is summarized in Table 2. The diet of *P. pileus* is mainly composed of phytoplankton and zooplankton. On the basis of *N%* and *F%* the most predominate prey group was phytoplankton that constituted to the maximum portion of diet followed by zooplankton. Moreover, microplastic was also found in the gut of *P. pileus*.

Food items in relation to season and area. ***Phytoplankton.*** The winter season encompasses a wide variety of phytoplankton that contributed to the total diet bulk of *P. pileus* (11 prey items) whereas only a single phytoplankton species was observed in the gut content of *P. pileus* during summer (Table 2).

On the basis of stations, the numbers of different phytoplankton species were 8-12 and maximum variety was found in the gut content of *P. pileus* collected from Rize (Table 3).

Zooplankton. Similar to phytoplankton, a wide variety of zooplankton was found during winter (11 prey items) whereas during autumn only 5 prey items belonging to zooplankton was observed in the gut of *P. pileus* (Table 2).

The gut content of *P. pileus* collected from Rize had a wide variety of zooplankton with 13 different prey items (Table 3). The variety of different zooplankton decreased in the study areas of Pazar and Fındıklı (10 prey items).

TABLE 3
The diet composition of *Pleurobrachia pileus* in relation to different study areas.

	Study area									
	İyidere		Rize		Çayeli		Pazar		Fındıklı	
Prey groups	***N%***	***F%***	***N%***	***F%***	***N%***	***F%***	***N%***	***F%***	***N%***	***F%***
PHYTOPLANKTON										
Dinophyceae										
Ceratium fusus	12.92	10	5.64	10.96	13.16	12.27	2.08	10.09	2.78	7.98
Ceratium furca	0.32	2.94	2.00	2.05	8.08	7.36	0.27	4.59	0.23	2.45
Ceratium tripos	4.56	7.65	2.11	6.16	6.02	6.75	0.91	7.34	1.45	6.13
Noctiluca scintilans	0.13	1.18	0.07	1.37	0.19	1.23	0.64	6.42	0.98	3.07
Protoperidinum sp.	0.51	2.94	0.64	3.42	0.66	1.23	0.05	0.92	1.39	4.91
Alexandrium sp.			0.14	2.05						
Dinophysis sp.	0.06	0.59			0.09	0.61				
Prorocentrum sp.			0.04	0.68			0.05	0.92		
Bacillariophyceae										
Chaetoceros sp.									0.17	0.61
Navicula sp.			0.04	0.68					0.12	0.61
Proboscia sp.			15.57	0.68						
Pseudanitzchia delicatisima	47.18	8.24	53.73	6.16	46.15	5.52	79.93	9.17	62.34	4.91
Rhizosolenia sp.	1.33	3.53	0.82	4.11	0.66	1.84	0.59	3.67	0.58	3.68
Thallassionema nitzschioides	0.06	0.59	0.11	1.37						
PROTOZOA										
Stenosemella nivalis			0.04	0.68						
ZOOPLANKTON										
Isopoda										
Isopot larvae	0.06	0.59			0.09	0.61	0.11	1.83		
Copepoda										
Acartia sp.	0.63	4.12	0.18	3.42	0.19	1.23				
Acartia sp. naupli	0.13	1.18	0.04	0.68	0	0	0.05	0.92	0.05	
Calanus sp.	2.28	3.53	0.93	4.11	1.60	2.45	2.40	6.42	0.37	
Calanus sp. copopodit	0.25	1.76	0.21	2.74	1.32	5.52	0.16	1.83	0.11	
Oithona sp.			0.04	0.68						
Chaetognatha										
Sagitta setosa			0.07	0.68	0.47	1.23	0.32	2.75	0.46	2.45
Appendicularian										
Oikopleura sp.	0.82	4.12	0.04	0.68	0.47	1.84	1.66	5.50		
Nemertea										
Nemertea sp.	0.38	2.35	0.04	0.68						
Bivalvia										
Mytilus sp. veliger	1.27	5.88	0.25	2.05	0.56	2.45	0.43	6.42	1.62	10.43
Micrometazoan egg-larvae										
Jellyfish egg	0.44	1.76	1.04	2.74	0.66	2.45			0.29	1.84
Jellyfish planula	0.44	1.18	0.14	0.68	2.16	21.47	0.16	0.92	5.45	9.82
Polychaete larvae	0.44	2.35	0.11	1.37	0.19	0.61	0.32	2.75	0.12	1.23
Shrimp larvae									0.06	0.61
Fish egg	3.10	4.71	6.39	10.27	2.44	4.29	3.15	7.34	5.21	7.36
OTHER										
Particle plastic	19.63	19.41	8.78	22.60	8.27	7.98	5.82	13.76	10.78	20.86
Fiber	0.44	2.35	0.29	2.74	0.38	1.84	0.16	1.83	0.06	0.61
Fecal pellet	2.60	7.06	0.54	3.42	6.20	9.20	0.75	4.59		

N %, percentage of prey group numerical frequency; *F%*, percentage of prey group occurrence frequency

TABLE 4
One-way ANOSIM results of the gut content for *Pleurobrachia pileus* in relaiton to different season periods (*R* values and significance level *p*). SIMPER results showed that the average dissimilarities (%) and the four species contributing most to the dissimilarity and their contribution ratios to the average dissimilarities between seasons.

Season	One-way ANOSIM		SIMPER							
	R value	*p* value	Average Dissimilarity %	Discriminating species 1	Contr. (%)	Discriminating species 2	Contr. (%)	Discriminating species 3	Contr. (%)
Spring-Autumn	0.2459	0.0002	81.95	*Ceratium fusus*	50.32	Particle plastic	31.63		
Spring-Winter	0.4609	0.0001	94.04	*P. delicatisima*	61.05	Particle plastic	18.18	*Ceratium fusus*	13.27
Summer-Autumn	0.3545	0.0001	85.9	*Ceratium fusus*	48.08	Particle plastic	37.82		
Summer-Winter	0.5303	0.0001	93.65	*P. delicatisima*	60.04	Particle plastic	19.21	*Ceratium fusus*	12.86
Autumn-Winter	0.3471	0.0001	85.29	*P. delicatisima*	56.92	*Ceratium fusus*	18.35	Particle plastic	8.50

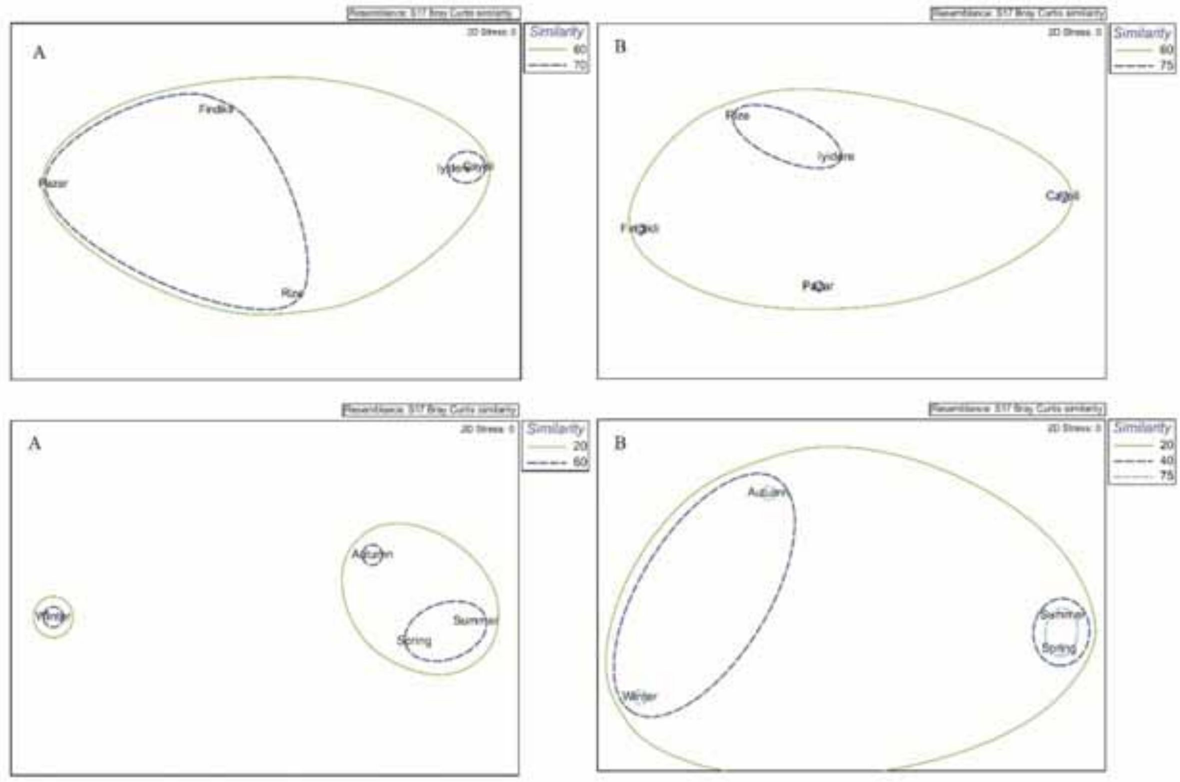

FIGURE 4
Multi-dimensional scaling (MDS) plot of similarity for the diet of *Pleurobrachia pileus* among different study areas and seasons based on: (A) percentage of prey groups numerical frequency *N%* and (B) percentage of prey groups occurrence frequency *F%*. The logarithmic transformed data of *N%* and *F%* were used in MDS.

Dendrogram, MDS, ANOSIM and SIMPER analysis. Diet variation due to seasons. Dendrogram and MDS based on either *N%* or *F%* values showed that spring and summer group together with high similarities (>80%) indicating extremely similar diet (Figure 4 and 5 for season). While the autumn and winter grouped separately from the spring and summer with low similarities indicating differences between the diets at these two periods. Based on *N%*, the diet composition of *P. pileus* in winter showed extremely different diet; shared only 8.0% similarity with spring, summer and autumn. The result of SIMPER analysis indicated that the dissimilarity among different seasons were primarily due to *Pseudo-nitzschia delicatissima, Ceratium fusus* and particle plastic (Table 4).

Diet variation due to study area. The dendrogram and MDS based on *N%* values showed high percentage of similarity among all study areas indicating a similar diet (Figure 4A and 5A for areas). This was confirmed by ANOSIM, that show low R values (0.12 – 0.18) showing low differentiation between different study areas (Table 5). However dendrogram based on *F%* values revealed that four different stations (İyidere, Rize, Pazar and Fındıklı) showed high percentage of similarity indicating a similar diet, while the Çayeli station showed lower percentage of similarity with the other stations indicating a different diet (Figure 5B). The MDS based on *F%* showed high percentage of similarity between Rize and İyidere (see Figure 4B for different area). The SIMPER analysis indicated that the dissimilarity between different study areas was primarily due to *P. delicatisima*, plastic particle, *C. fusus* and fish egg (Table 5).

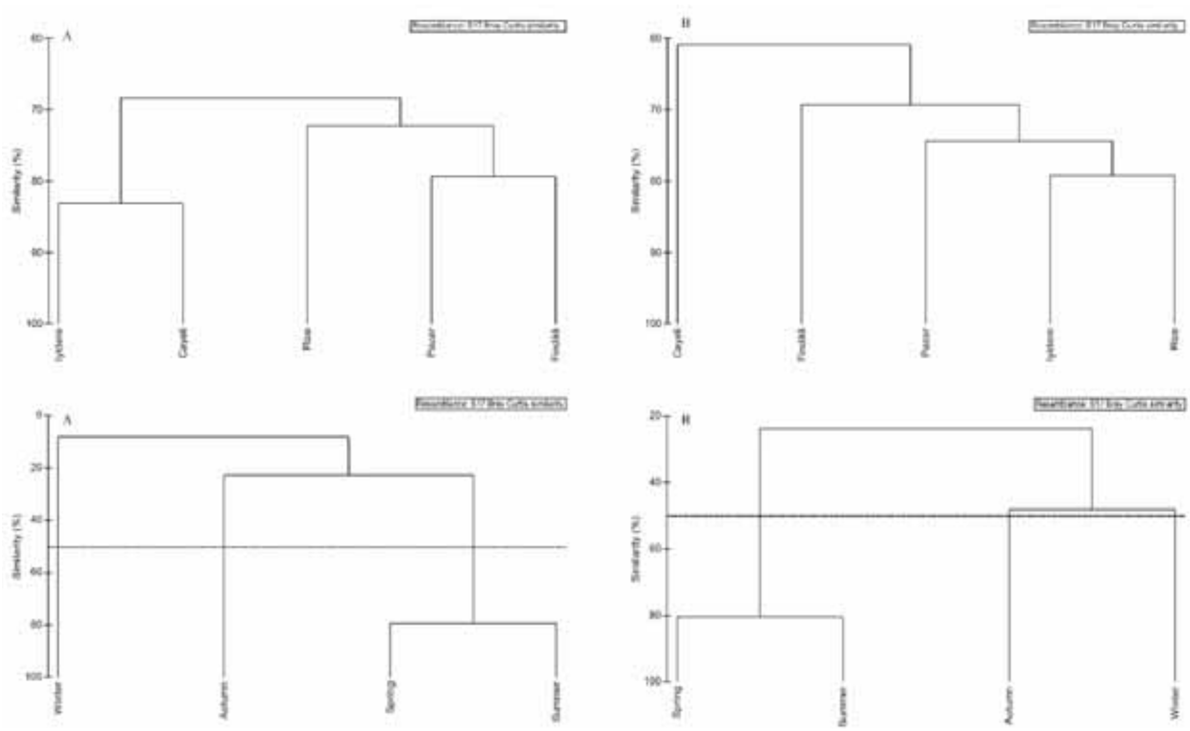

FIGURE 5
Dendrogram of diet similarities of *Pleurobrachia pileus* among different study areas and seasons based on: (A) percentage of prey groups numerical frequency *N%* and (B) percentage of prey groups occurrence frequency *F%*.

TABLE 5
One-way ANOSIM results of the gut content for *Pleurobrachia pileus* in relation to different study areas. SIMPER results showed the average dissimilarities (%) and the contribution of different food items to the dissimilarities.

Stations/ Location	One-way ANOSIM		SIMPER								
	R value	*p* value	Average Dissimilarity %	Discriminating species 1	(%)	Discriminating species 2	(%)	Discriminating species 3	(%)	Discriminating species 4	(%)
İyidere - Rize	0.1602	0.0001	86.23	*P. delicatisima*	36.12	Particle plastic	33.95	*Ceratium fusus*	21,3	Fish egg	7.44
İyidere - Pazar	0.1602	0.0001	84.53	*P. delicatisima*	45.52	Particle plastic	31.45	Fish egg	9,77	*Ceratium fusus*	9.69
İyidere - Fındıklı	0.0308	0.0466	78.63	*P. delicatisima*	38.32	Particle plastic	33.01	*Ceratium fusus*	15,34	Fish egg	13.33
Rize – Çayeli	0.1481	0.0001	88.25	*P. delicatisima*	32.80	Particle plastic	31.24	*Ceratium fusus*	21,22	Fish egg	14.74
Rize – Pazar	0.1265	0.0005	85.11	*P. delicatisima*	41.15	Particle plastic	30	Fish egg	17,47	*Ceratium fusus*	11.39
Çayeli - Fındıklı	0.1829	0.0001	87.55	*P. delicatisima*	34.06	Particle plastic	28.92	*Ceratium fusus*	19,95	Fish egg	17.08
Pazar - Fındıklı	0.1500	0.0002	84.43	*P. delicatisima*	42.71	Particle plastic	29.25	Fish egg	19,57	*Ceratium fusus*	8.46

DISCUSSION

The overall diet of *P. pileus* was dominated by phytoplankton. In relation to different seasons, the diet of *P. pileus* during autumn and winter was mainly dominated by phytoplankton followed by zooplankton, however the diet of *P. pileus* during spring and summer periods were mainly composed of zooplankton. Thus the results reaffirmed the opportunistic feeding habits of *P. pileus* and no prey type preferences suggest that *P. pileus* is miscellaneous feeder eat anything encountered that falls within the parameters of prey. This result is concur with a number of similar investigations [1, 13, 21, 22, 23, 24]

The contribution of fish eggs was found to be high during spring and summer periods whereas its contribution to the total diet bulk of *P. pileus* decreased abruptly and showed comparatively little importance during autumn and winter periods. The *Pleurobrachia* collected from Scottish sea during 1967 has also reported fish eggs only in spring period [1].

Differences between the food items taken by *P. pileus* at five different stations do not differ greatly from each other. The dominate food items of *P. pileus* was found to be phytoplankton in all five study areas.

Up to 20% of *P. pileus* sampled during spring and summer periods had empty guts that gradually decreased till autumn (only 4% empty gut) and winter (no empty gut). Yip [13] also reported a similar gradually decreased in the percentage of empty gut from May to November.

Both overall diet composition and the diet of *P. pileus* in relation to stations showed that the phytoplankton was the dominate prey group in the diet of *P. pileus* in the Southeastern Black Sea. According to earlier studies, different prey group was found to be the dominate prey group in the diet of *P. pileus* at different places. For example, the research work published during 1948, 1962, 1970 and 1971 showed that the dominate prey group in the total diet bulk of *P. pileus* in the Black Sea included fish larvae, *Sagitta* spp., copepods and larvae of benthic organisms while the dominate prey group in the diet of *P. pileus* collected from Hawaiian waters, northern North Sea and Galway Bay was salp, *Oikopleura longicauda* and barnacle nauplii (for Hawaiian waters), Crustaceans, especially copepods and the cladoceran *Evadne* sp. (for northern North Sea and Galway Bay) [cited in 13].

CONCLUSIONS

In conclusion, most of the studies have reported that zooplankton as the main prey items in the diet of *P. pileus*, however, in the Southeastern Black Sea the dominant prey groups in the diet of *P. pileus* is phytoplankton (overall diet). As the diet of *P. pileus* actually shows the availability of different prey groups in the ambient environment and thus the zooplankton fauna of the Black Sea seems to be limited that could possibly cause the collapse of whole ecosystem in near future just similar to the 1980s era during which the icthyo- and mesozooplankton abundance/production of the Black Sea was badly affected due to the shortage of zooplankton. The Black Sea contributes to a larger portion of the total Turkish fisheries yield and according to a recent report published by the Turkish Statistical Institute (TÜİK) the production of fishing industry of Turkey has been gradually shrinking since 2011 with a decrease of around 21.5% with respect to the previous year [25]. The results reported from Mazlum et al. [4] and present study showed that the population of zooplankton in the Southeastern Black Sea is limited compare to other regions and the inadequate of zooplankton especially copepods might be the possible reason in the recent reduction of Turkey fisheries production.

The Southeastern Black sea is reported as one of the hotspot of microplastic pollution [26]. In this study, the presence of microplastics in the diet of *P. pileus* shows bioavailability of these particles for marine biota.

It is believed that the results of this study will provide a baseline data for future work to understand the tropic levels that will assess the proper management of aquatic resources in the Black Sea.

ACKNOWLEDGEMENTS

This study was founded by Recep Tayyip Erdogan University Scientific Research Projects Unit (Project Number: 2013.103.03.2).

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Received: **24.05.2017**
Accepted: **02.12.2017**

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