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Stable isotope compositions of dominant zooplankton species in relation to environmental factors in the Dardanelles

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Abstract: Samples were collected seasonally from three stations in the Dardanelles between April 2016 and October 2017 to determine the effects of biological and physicochemical properties of the coastal system on the isotopic compositions of the most abundant copepod species, *Acartia clausi* and *Calanus helgolandicus*, and a cladoceran, *Penilia avirostris*. Plankton samples were collected horizontally with a 200-μm-mesh plankton net and isotopic analyses were performed by mass spectrometry after identification to species level. Total zooplankton abundance reached its maximum in spring and summer. The $\delta^{13}C$ values of copepods showed statistically significant differences both between stations and seasons. As a selective feeder, *C. helgolandicus* had the highest values in both $\delta^{13}C$ and $\delta^{15}N$. Being a filter feeder, *P. avirostris* distinctly differed from *C. helgolandicus* and *A. clausi*. Enrichment was observed in $\delta^{15}N$ values of copepods in winter, when the highest values of total suspended solids and chlorophyll-*a* were determined. The results showed that $\delta^{13}C$ values of the copepods were under the influence of environmental and biological factors. Additionally, dietary differences between herbivorous groups and other groups were clearly demonstrated, as well as differences in the diets of filter-feeding and selectively-feeding zooplanktonic species.

Keywords: $\delta^{13}C$; $\delta^{15}N$; zooplankton; environmental variables; Dardanelles

INTRODUCTION

Zooplanktonic organisms have an important role when transferring organic matter from primary producers to consumers at higher trophic levels [1]. There are various factors affecting the stable isotope ratios in marine ecosystems, especially in coastal systems, which are known to be influenced by many physicochemical processes. Physical processes play a major role and result in variations in spatial and temporal variabilities of the marine coastal systems [2]. The lower layer of the trophic level is very sensitive to these changes and the variation in the zooplankton isotopic signatures mostly depends on physical dynamics, river discharges, dust input and other effects [2-6]. The trophic structure of plankton communities can only be described using stable isotopes if the factors influencing variations are considered [2,7-9]. Stable $\delta^{13}C$ and $\delta^{15}N$ isotopes are frequently used to determine these conditions and have an effective role in identifying the carbon sources and trophic position of planktonic organisms in the food web.

Stable isotopes show the transformation and origin of the organic matter [10]. The nitrogen isotope ratio is used to help define the trophic levels of organisms, as $\delta^{15}N$ usually increases by 2.5-4.5‰ (mean 3.4‰) from prey to predator [11,12]. As an increase in $\delta^{13}C$ of 0.4-2‰ only occurs from diet to consumer [12,13], a consumer's carbon isotope composition can give clues about the sources of its diet, particularly in systems with distinct organic matter sources [14]. The relative importance of these sources may differ in space and time, and stable isotope ratios at the base of food webs in the marine environment can be highly variable [15,16].

Although stable isotope determinations are often used on planktonic organisms to understand the ecological structure of the world's oceans and inland waters as well as the marine food web structure, the number of studies conducted in the Mediterranean and the Aegean Sea is low [2,15,17]. Additionally, many of the studies undertaken are among the size fractions of planktonic organisms, while the number of taxonomic group- or species-based studies are not many [10,18].

The present study was conducted in the Strait of the Dardanelles, which is a part of the Turkish Straits system known to have unique flow characteristics, consisting of an upper layer with low salinity from the Black Sea and a lower layer with higher salinity from the Mediterranean [19-22]. As a biological corridor, the Turkish Straits system plays a crucial role in the transport of nutrients, planktonic organisms and fish between the Mediterranean and the Black Sea [23]. In addition, daily changes are observed in nutrient concentrations resulting from the domestic inputs in the region and the pollution load carried by the Turkish Straits System [24,25]. This directly affects the biological diversity of the system. Thus, previous studies in the Dardanelles have generally focused on the density and composition of plankton, the changes of nutrients in the region, and the physiological and hydrobiological characteristics of the Turkish Straits system [24-29]. Stable isotope studies that have been conducted in the region were limited to the isotopic composition of some fish and their feeding habits in close-by rivers and lakes [30,31].

In the present study, samples were collected seasonally from three stations in the Dardanelles. The objectives were: (i) to establish the most abundant zooplankton species in the region, (ii) to determine the seasonal and spatial isotopic compositions of these species, (iii) to reveal the effects of biological and physicochemical properties of the system on the isotopic composition of the abundant species, and (iv) to observe variations in stable isotope signatures of different species in the study area.

MATERIALS AND METHODS

Sampling sites

The study was carried out between April 2016 and August 2017 at three stations in the Dardanelles; however, sampling was not performed during the summer of 2016. The 1st station was located in the coastal zone of the harbor, and the 2nd station is the harbor. The reference station was located 10 km away from the 1st and 2nd stations and chosen far from settlements and terrestrial inputs; it had a depth of about 2-3 m (Supplementary Fig. S1). The 1st and 2nd stations were under the influence of high flow and were exposed to vertical and horizontal currents, whereby they differed from the reference station. The difference between the 1st and the 2nd stations was in the depth, which was 2-3 m for the 1st station and about 30 m for the 2nd. All samplings for all seasons and stations were performed during daytime.

Environmental variables

Environmental variables, i.e. temperature and salinity, were measured *in situ* using an YSI 650 multiple water analysis probe from the surface to about a depth of 2-5 m. Water samples for the measurement of chlorophyll-*a* (chl-*a*) and total suspended solids (TSS) were collected using a 5-L water sampler. For chl-*a*, the samples were filtered using 47-mm GF/F filters by gentle vacuum and then frozen until laboratory analysis. The chl-*a* concentration was determined spectrophotometrically after extraction with 90% acetone [32]. For TSS, the previously weighed and dried GF/C filters were used to filter the water samples; gravimetric analysis was performed according to Clesceri et al. [33].

Zooplankton sampling for abundance

The aim of observing the abundance was to determine the most abundant zooplankton species in the study area. The most abundant zooplankton species were used for stable isotope analyses (SIA). Detecting microzooplankton (<200 μ) abundance was not targeted. Samples for abundance were collected using a 5-L water sampler from the surface at all stations and preserved in 4% buffered formalin (v/v). The samples were settled for two weeks and reduced first to 250 mL and then to 10 mL subsamples. Ten percent or 50% of the samples were counted depending on plankton abundance. Species identification was performed according to Tregouboff et al. and Todd et al. [34,35].

Zooplankton sampling for stable isotope analyses (SIA)

Zooplankton samples for stable $\delta^{13}C$ and $\delta^{15}N$ isotope analyses were collected horizontally from the surface using a 200-μ plankton net at all stations. The most abundant species identified under the microscope that had enough weight for analysis were chosen for SIA. After identifying to the species level, all bulk zooplankton samples were stored at -80°C until isotope analysis in the

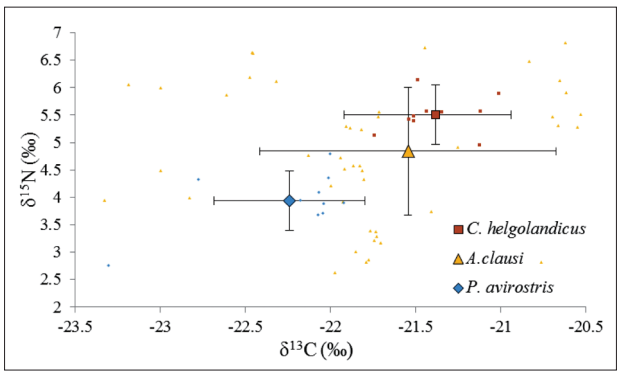

Fig. 1. Scatter plot (mean±SD) of $\delta^{13}C$ and $\delta^{15}N$ values of species detected in study area.

laboratory. After drying the frozen bulk samples in the drying oven at 60°C for 24 h, each sample was weighed to 0.150 mg and placed in tin capsules. Measurements were conducted using an Isotope Ratio Mass Spectrometer (Delta V Thermo Finnigan; Thermo, Waltham, USA) in the Akdeniz University Food Safety and Agricultural Research Center. Since acidification can affect nitrogen and carbon isotope values, the samples were not acidified [36-41]. The obtained results are expressed by δ using the following equation:

$$\delta^{13}C \text{ or } \delta^{15}N\ (‰) = (Rsample/Rstandard-1)x1000,$$

where the stable isotope abundance of C and N was expressed as $\delta^{13}C$ or $\delta^{15}N$, $^{13}C/^{12}C$ or $^{15}N/^{14}N$ and was represented by R, with the reference being V-PDB (Vienna Pee Dee Belemnite) and atmospheric N_2 isotope standards.

Statistical analysis

The differences between mean seasonal and spatial groups of stable isotope values of the zooplankton were analyzed by one-way analysis of variances (ANOVAs) (significance level $p<0.05$), followed by Tukey's honestly significant difference (HSD) test for unequal sample sizes for *post hoc* comparisons among regions and seasons. Levene's test was applied for the homogeneity of variances. Pearson's correlation and Spearman's rank correlation analyses were applied to determine the linear relationship between stable $\delta^{13}C$ and $\delta^{15}N$ values vs the environmental variables and abundance of zooplankton. SPSS 18.0 and Minitab 17.1.0 programs were used in the statistical analysis.

RESULTS

Seasonal and spatial variations in environmental variables

The surface temperatures ranged from 7.64 to 24.81°C, following a seasonal progression, and variations were significant between the seasons as expected (Table 1; $p<0.001$). The surface temperature did not significantly differ between the stations. The reference station was significantly different from the 1st and the 2nd stations, having higher values of surface salinity, which varied between 24.20 and 29.80‰ between stations (Table 1; $p<0.001$). No significant differences in salinity values were observed between seasons. The winter values of chl-*a* were significantly higher than in other seasons, with a value at 6.79 µg L^{-1} (Table 1; $p<0.001$). The TSS values ranged between 1.96 and 74.50 mg L^{-1}, with the highest value measured in winter and significantly differing from other seasons, similar to chl-*a* (Table 1; $p<0.001$). There were no significant differences in chl-*a* and TSS among the stations.

Abundance of mesozooplankton

Total mesozooplankton abundance had the highest value in spring 2016 (24.66%), followed by summer 2017 (24.22%) and spring 2017 (18.83%). In winter, when surface temperatures were low, the abundance values decreased (15.25%) when compared to other seasons (Table 2). When evaluating all of the obtained species, the spatial ratios of mesozooplankton total abundance had the highest value in the 2nd station (67.26%), followed by the reference station and the 1st station (Table 2).

At the species level, *A. clausi* was the most dominant species (30.94%) when compared with other species, and with the exception of summer, it was present in the pelagic waters of all stations during the study. *C. helgolandicus*, on the other hand, was observed in summer with a total abundance of 13.00% (Table 2). Cladoceran *P. avirostris* had a total abundance of 14.35% and was mostly present in summer (Table 2). Other species observed in the study included *Noctiluca scintillans* and *Oikopleura dioica* with an abundance of 10.76%. *Copepod nauplii* had an abundance value of 8.07%; it was followed by *Pseudocalanus elongatus*

Table 1. Results of one-way ANOVA and Tukey's HSD *post hoc* tests on seasonal and spatial variations of environmental variables in the Dardanelles.

[1]Var.	***Factor	n	****mean±SD	*p* value	[2]Factor	n	****mean±SD	*p* value
Tepm. (°C)	Spring 2016	9	15.652±2.409[d]	<0.001*	St. 1	15	17.09±6.31	**n.s.
	Autumn 2016	9	20.6122±0.2509[b]		St. 2	15	16.67±6.42	
	Winter 2017	9	7.664±0.895[e]		Ref.	15	18.08±5.20	
	Spring 2017	9	17.650±0.639[c]					
	Summer 2017	9	24.811±0.669[a]					
Salinity (‰)	Spring 2016	9	28.086±1.578	**n.s.	St. 1	15	25.837±1.435[b]	<0.001*
	Autumn 2016	9	26.351±2.287		St. 2	15	24.778±1.442[b]	
	Winter 2017	9	27.02±3.37		Ref.	15	29.877±0.504[a]	
	Spring 2017	9	26.682±2.674					
	Summer 2017	9	26.016±2.380					
Chl-*a* ($\mu g\ L^{-1}$)	Spring 2016	9	3.071±10.617[b]	<0.001*	St. 1	15	2.273±0,563	**n.s.
	Autumn 2016	9	3.239±2.742[a]		St. 2	15	4.03±4.23	
	Winter 2017	9	6.79±4.23[b]		Ref.	15	3.967±2.113	
	Spring 2017	9	1.981±0.423[b]					
	Summer 2017	9	2.0389±0.2512[b]					
TSS ($mg\ L^{-1}$)	Spring 2016	9	3.422±1.259[b]	<0.001*	St. 1	15	8.04±8.34	**n.s.
	Autumn 2016	9	24.0±37.4[b]		St. 2	15	33.7±59.9	
	Winter 2017	9	74.5±57.7[a]		Ref.	15	30.77±29.91	
	Spring 2017	9	1.967±0.529[b]					
	Summer 2017	9	16.88±5.40[b]					

[1]Variable.
[2]St. 1 – 1st station; St. 2 – 2nd station; Ref – Reference station. *Statically significant difference (one-way ANOVA); **statically not significant; ***sampling could not be performed during summer 2016; ****in each line, different letters indicate significantly different means (one-way ANOVA, Tukey's *post hoc* tests at P<0.05) among the seasons and stations ("a" indicated the highest value).

Table 2. Seasonal and spatial abundance percentages (%) of the plankton species collected in the Dardanelles.

Group	Species	[1]Tot. Ind/m^3	[2]Seasonal Abundance (%)						[3]Spatial Abundance (%)			[4]Tot. Ab. Per Spc. (%)
			Sp. 2016	*Sum 2016	Aut. 2016	Win. 2016	Sp. 2017	Sum. 2017	St. 1	St. 2	Ref.	
Copepoda	*Acartia clausi* Giesbrecht, 1889	13800	8.07	-	7.62	8.97	6.28	-	4.93	21.08	4.93	30.94
Cladocera	*Penilia avirostris* Dana, 1849	6400	-	-	0.45	-	0.00	13.90	1.79	12.56	0.00	14.35
Copepoda	*Calanus helgolandicus* Claus, 1863	5800	0.90	-	1.35	3.59	0.45	6.73	1.35	8.97	2.69	13.00
Dinoflagellata	*Noctiluca scintillans* Kofoid & Swezy, 1921	4800	4.93	-	1.35	-	4.48	0.00	0.00	10.76	0.00	10.76
Copepoda	*Oithona nana* Giesbrecht, 1893	4800	3.59	-	3.14	-	2.24	1.79	2.69	5.83	2.24	10.76
Tunicata	*Oikopleura dioica* Fol, 1872	4200	3.14	-	2.24	-	2.24	1.79	1.35	6.28	1.79	9.42
Copepoda	Copepoda nauplii	3600	4.04	-	0.90	-	3.14	-	1.35	0.90	5.83	8.07
Copepoda	*Pseudocalanus elongatus* Boeck, 1865	800	-	-	-	1.79	-	-	0.90	0.90	0.00	1.79
Copepoda	*Paracalanus parvus* Claus, 1863	400	-	-	-	0.90	-	-	0.45	0.00	0.45	0.90
Total Abundance per season / station (%)		-	24.66	0.00	17.0	15.3	18.83	24.22	14.80	67.26	17.9	100.00

[1]Total individuals in m^3; [2]Sp – spring; Sum – summer; Aut – autumn; Win – winter; [3]St. 1 – 1st station; St. 2 – 2nd station; Ref - reference station.
[4]Total abundance per species; *Sampling could not be performed during summer 2016.

Table 3. Results of one-way ANOVA and Tukey's HSD *post hoc* tests on seasonal and spatial stable isotope ratios (±SD) of Copepods.

[1]Var.	***[2]Factor	n	****mean±SD	p value	[3]Factor	n	****mean±SD	p value
$\delta^{13}C$ (‰)	Sp. 2016	23	-20.9830±0.6830[a]	<0.001*	St. 1	14	-21.8936±0.3501[b]	<0.001*
	Aut. 2016	8	-21.7090±1.2360[abc]		St. 2	26	-21.8600±0.5720[b]	
	Win. 2017	8	-22.4950±0.5180[c]		Ref.	23	-20.9100±0.8700[a]	
	Sp. 2017	14	-21.8357±0.2037[bc]					
	Sum. 2017	10	-21.3830±0.2304[ab]					
$\delta^{15}N$ (‰)	Sp. 2016	23	4.933±1.152[bc]	<0.001*	St. 1	14	4.544±1.219	**n.s.
	Aut. 2016	8	4.261±0.887[c]		St. 2	26	5.018±0.958	
	Win. 2017	8	6.271±0.337[a]		Ref.	23	5.115±1.173	
	Sp. 2017	14	4.211±0.899[c]					
	Sum. 2017	10	5.508±0.337[ab]					

[1]Variable.
[2]Sp – spring; Sum – Summer; Aut – autumn; Win – winter; [3]St. 1 – 1st station; St. 2 – 2nd station; Ref – reference station.
*Statically significant difference (one-way ANOVA); **statically not significant; ***sampling could not be performed during summer 2016;
****in each line, different letters indicate significantly different means (one-way ANOVA, Tukey's *post hoc* tests at P<0.05) among the seasons and stations ("a" indicates the highest value).

and *Paracalanus parvus* with total abundance values of 1.79% and 0.90%, respectively (Table 2). Therefore, the three most abundant species, *A. clausi*, *C. helgolandicus* and *P. avirostris*, were chosen for the study.

Stable isotope compositions of the zooplankton in the study area

The $\delta^{13}C$ values of the copepods *A. clausi* and *C. helgolandicus* showed statistically significant differences among stations and seasons. Further, $\delta^{13}C$ of the copepods had a maximum value in spring 2016 (-20.983±0.683‰) while the lowest value was observed in winter 2017 (-22.4950±0.5180‰) with statistically significant differences (Table 3; p<0.001). Among stations, the $\delta^{13}C$ values of the copepods *A. clausi and C. helgolandicus* significantly differed in the reference station when compared to the other stations (Table 3; p<0.001).

Seasonal differences of total $\delta^{15}N$ values of copepods were also statistically significant (Table 3). The highest value was observed in winter 2017 (6.271±0.337‰), while the lowest value was measured in spring 2017 (4.211±0.899‰). At all three stations, the total $\delta^{15}N$ values of the copepods ranged between 4.544±1.219 and 5.115±1.173‰, showing no significant differences; however, when comparing the stations in each season, significant differences were observed in winter 2017. In winter, copepod total $\delta^{15}N$ values in the 2nd station were significantly higher than in the other stations (Table 4; p=0.002).

Table 4. Results of one-way ANOVA and Tukey's HSD *post hoc* tests on spatial $\delta^{15}N$ values (±SD) of Copepods in Winter 2017.

Variable	Factor	n	**mean±SD	p value
$\delta^{15}N$	1st station	3	6.050±0.178[b]	*0.002
	2nd station	3	6.660±0.053[a]	
	Reference St.	2	6.020±0.042[b]	

*Statically significant difference (one-way ANOVA); **different letters indicate significantly different means (one-way ANOVA, Tukey's *post hoc* tests at P<0.05) among the stations ("a" indicate the highest value).

Table 5. Results of one-way ANOVA and Tukey's HSD *post hoc* tests on stable isotope ratios (±SD) of abundant species detected in study area throughout the year.

Variable	Factor	n	**mean±SD	p value
$\delta^{13}C$ (‰)	A. clausi	53	-21.546±0.8690[a]	*0.023
	C. helgolandicus	10	-21.383±0.2304[a]	
	P. avirostris	10	-22.242±0.4460[b]	
$\delta^{15}N$ (‰)	A. clausi	53	4.843±1.167[a]	*0.004
	C. helgolandicus	10	5.508±0.337[a]	
	P. avirostris	10	3.942±0.538[b]	

*Statically significant difference (one-way ANOVA); **different letters indicate significantly different means (one-way ANOVA, Tukey's *post hoc* tests at P<0.05) among the species ("a" indicates the highest value).

Table 6. Spearman's correlation results between $\delta^{13}C$ and $\delta^{15}N$ values of Copepods vs. environmental variables and abundance of zooplankton in study area (n=8).

		Temperature	Salinity	Chl-a	TSS
$\delta^{13}C$	**r**	0.518	-0.218	0.118	0.018
	***p* value**	0.102	0.519	0.729	0.958
$\delta^{15}N$	**r**	-0.464	-0.118	0.600	0.673*
	***p* value**	0.151	0.729	0.051	0.023

*Correlation is significant at the 0.05 level.

When species-specific isotopic compositions were considered, as a selective feeder *C. helgolandicus* had the highest values in both $\delta^{13}C$ and $\delta^{15}N$ (Table 5). Being a filter feeder, the $\delta^{13}C$ and $\delta^{15}N$ values of *P. avirostris* were significantly different from both *C. helgolandicus* and *A. clausi* (Table 5; p=0.023, p=0.004, respectively).

DISCUSSION

Spatial variations in stable isotope composition of zooplankton showed significant differences between the stations. The total mean $\delta^{13}C$ values of the copepods in the 1st and the 2nd stations, which are known to be exposed to intense currents between the Black Sea and the Mediterranean Sea, and known to have a high plankton density [42], were lower than in the reference station. On the other hand, the 2nd station had the highest total zooplankton abundance. It is known that isotopic values depend on the characteristics of zooplankton communities [39,43]. Therefore, the high abundance in zooplankton could be one of the reasons for the depletion in $\delta^{13}C$ values of zooplankton. Furthermore, environmental and biological conditions can change depending on the horizontal and vertical flows as well as the vertical migration of zooplankton and phytoplankton in marine systems [44,45]. This migration and the changes in environmental conditions affect the feeding habits of the species living in the epipelagic zone. Therefore, when these factors were compared between the sampling stations, the feeding habits of zooplankton found in the 1st and the 2nd stations were expected to be different from those at the reference station since the origins of carbon sources and other nutrients varied. Thus, both the flow and depth at the 1st and the 2nd stations, as well as the biological properties of the environment, altered the $\delta^{13}C$ values. Previous studies of zooplankton isotopic composition showed that environmental factors, especially terrestrial inflows, as well as biological parameters such as primary production, affected $\delta^{13}C$ values [39,46] as we observed in this present study. Furthermore, the $\delta^{13}C$ values of the copepods obtained in this study also coincided with studies conducted in different regions of the Mediterranean [10,15,46].

The $\delta^{15}N$ values obtained in the Mediterranean and Aegean are lower than those observed in open seas and ocean waters because primary production in the Mediterranean and the Aegean has low $\delta^{15}N$ values [17,47-49]. Except in winter, the $\delta^{15}N$ values obtained for all zooplankton groups (both copepods and cladocerans) in the present study were consistent with the results obtained in previous studies in the Mediterranean [2,15,50]. The highest value of $\delta^{15}N$ for copepods was observed in winter when the highest values of TSS and chl-*a* were estimated. This suggests that high densities of terrestrial and nutrient inputs after heavy rains during winter sampling were one of the reasons for the enrichment in $\delta^{15}N$. Indeed, a positive correlation between the $\delta^{15}N$ values of copepods and TSS supports this idea. Furthermore, winter values of copepod $\delta^{15}N$ were similar to the values obtained in ocean waters [48] where nitrate was the main nitrogen supply for primary production [17]. Besides, copepod $\delta^{15}N$ values were significantly higher at the 2nd station than at other stations in winter, indicating that primary producers had high $\delta^{15}N$ values during the study period. The high chl-*a* content in winter sampling supports this. The total $\delta^{15}N$ values for copepods and the chl-*a* values showed close correlation, indicating a linear relationship between the two variables.

When the stable isotope ratios of most abundant species were evaluated, we found that *C. helgolandicus* and *A. clausi* consistently differed from *P. avirostris*. The total mean $\delta^{13}C$ values for both *A. clausi* and *C. helgolandicus* were prominently higher than for *P. avirostris*, which could be an indication that *C. helgolandicus* and *A. clausi* behaved differently with regard to their food choices as compared to the cladoceran *P. avirostris*. Being selective feeders, both *C. helgolandicus* and *A. clausi* prefer to feed on high-carbon-content phytoplankton species [51-53], which supports the results above. *P. avirostris* is typically a warm-water species and is known to make significant contributions to zooplankton abundance in the region, particularly in summer [26,44]. It is a filter feeder, preferring not only algae but also bacteria and nanoflagellates as food sources [54-56]. In the present study, *P. avirostris* was the most abundant in the 2nd station and in summer in warm waters, when the chl-*a* content had the lowest value. Considering that chl-*a* is an indicator of primary production, it can be concluded that depletion in $\delta^{13}C$ values of *P. avirostris* was related to the reduction of primary production; previous studies showed that it can be found in warm waters with low

primary production, which supports the findings of this study [10,26,55,57].

Similar to $\delta^{13}C$ values, the $\delta^{15}N$ values of *A. clausi* and *C. helgolandicus* significantly differed from *P. avirostris*. Filter feeders, which have the lowest $\delta^{15}N$ values, are accepted to be in the lower layer of the food web [46]. Indeed, *P. avirostris*, with the lowest $\delta^{15}N$ values in this study, was also located below the two copepod species, *A. clausi* and *C. helgolandicus*. Additionally, although there were no significant differences between the $\delta^{15}N$ values of *A. clausi* and *C. helgolandicus*, the $\delta^{15}N$ values of *C. helgolandicus* were higher than the $\delta^{15}N$ values of *A. clausi*. This may indicate that *C. helgolandicus* can be considered closer to the upper layer of the trophic level than *A. clausi*. As a result, when examining the food web structure of the Dardanelles, *C. helgolandicus* and *A. clausi* can be considered to be closer to the higher trophic level than *P. avirostris*.

CONCLUSIONS

Although the sampling stations were close to each other, the stable isotope signatures of primary consumers varied, being under the influence of seasonal changes as well as environmental and biological factors. The observed significant variations in stable isotope signatures of abundant zooplankton species between seasons were expected because of the differences in available food sources depending on seasonal changes. However, significant differences in the stable isotope signatures of dominant zooplankton among the stations during the same sampling period indicated that food sources varied in different regions of the coastal system in the Dardanelles; this may be helpful in planning further studies about the food web of the system. Additionally, the study showed that stable isotope signatures of copepods, which are known as selective feeders, differed from filter feeders with high values of stable carbon and nitrogen isotopes, indicating that selective feeders were closer to a higher trophic level than filter feeders. Furthermore, seasonal and spatial $\delta^{13}C$ and $\delta^{15}N$ values of different zooplankton groups in the Dardanelles were consistent with previous studies in nearby regions [1,2,15,17,46] and no high deviation was detected. This was the first study of the isotopic composition of zooplanktonic organisms in the area. Because the study was performed at the species level, it differs from previous studies that were conducted in the Mediterranean and the Aegean. We believe that it provides an important reference for future work in coastal areas of these systems.

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Supplementary Data

Supplementary Fig. S1.
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