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Seasonal changes in abundance, lipid and fatty acid composition of *Calanus euxinus* in the South-eastern Black Sea

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ABSTRACT

Seasonal changes in abundance, lipid and fatty acid composition of *Calanus euxinus* Hulsemann, 1991 were analysed monthly during the period from March 2012 to February 2013. The highest abundance of *C. euxinus* was recorded in February (847 ind. m^{-3}) during the sampling period. Female and male *C. euxinus* peaked in February (587 ind. m^3, 169 ind. m^{-3}, respectively). However, copepodites peaked in November (107 ind. m^{-3}). Average total lipid content was determined as percentage (%) and per individual (mg ind^{-1}). It was proportionally highest in February (7.03%) and lowest in September (3.02%). However, average lipid content per individual was highest in February (0.11 mg ind^{-1}) and lowest in September and November (0.04 mg ind^{-1}). Major fatty acids in *C. euxinus* were identified as 16:0, 16:1 n-7, EPA and DHA. ∑SFA, ∑MUFA, ∑PUFA and ∑HUFA were observed to be correlated with temperature. ∑SFA and ∑MUFA increased with the rise in temperature (r^2=0.74, r^2=0.73, $p<0.05$, respectively) whereas ∑PUFA and ∑HUFA increased as temperature decreased (r^2=-0.73, r^2=-0.80, respectively, $p<0.05$). Additionally, while ∑PUFA and ∑HUFA increased (r^2=0.61, r^2=-0.68, respectively, $p<0.05$), ∑MUFA decreased (r^2=-0.68, $p<0.05$) as chlorophyll-a increased. It was observed that the degree of unsaturation increased as temperature decreased. Results of the study revealed that *C. euxinus* has rich lipid content as well as fatty acid composition and it plays an important role in the South-eastern Black Sea ecosystem functionalities especially having key role in energy fluxes to higher trophic levels.

Keywords: Abundance, Black Sea, *Calanus euxinus*, Chlorophyll-a, HUFA, PUFA, Total lipids

Introduction

Fatty acids (FA) are among the most important molecules transferred from plants to animals in the aquatic food web. Certain classes of FA, such as the omega-3 (n-3) highly unsaturated fatty acids (HUFA) are available in limited quantities and very important for herbivorous zooplankton (Muler-Navarra 1995a; Muler-Navarra *et al.*, 2000; Ravet *et al*., 2003). These molecules are also of critical importance for the growth and resistance to disease in juvenile and larval fish (Adams, 1999; Olsen, 1999; Sargent *et al.*, 1999). Docosahexaenoic acid (DHA, 22:6 n-3), eicosapentaenoic acid (EPA, 20:5 n-3) and arachidonic acid (ARA, 20:4 n-6) are essential fatty acids (EFA) for many marine species. Due to competitive interactions between DHA and EPA as well as between EPA and ARA, the importance of considering the relative amounts of DHA, EPA and ARA has been demonstrated simultaneously (McEvoy *et al*., 1998; Estevez *et al*., 1999; Sargent *et al*., 1999).

Information about FA may also help with the interpretation of trophic relationships in aquatic systems (Dalsgard *et al.*, 2003) that occur due to the variations in specific fatty acid compositions of primary producers (Volkman *et al*., 1989; Ahlgren *et al*., 1992). Thus, it is important to know the FA composition of the zooplankton, which is determined by taxonomic affiliation, changed by diet and modified by starvation or temperature (Arts *et al*., 2009). Additionally, the need for accumulation of knowledge about the nutritional importance of FA helps us determine the food quality of the fish species with economic significance since the nutritional impact of FA are conveyed from fish to the human through the food web (Simopoulos, 1999; Arts *et al*., 2001).

Most of the research along the northern regions of the world's oceans focus on copepods (Marshall and Orr, 1972; Mauchline, 1998; Bonnet *et al*., 2005). *Calanus euxinus* Hulsemann, 1991 is one of the most important copepod species involved in the pelagic food chain of the Black Sea (Vinogradov *et al*., 1992). It is a dominant

secondary producer in the Black Sea, accounting for over one-third of the total zooplankton biomass (Vinogradov *et al*., 1992). It has an important role in transferring the organic matter from primary producers to the higher taxa. It is estimated that 14.5 and 9.5% of primary production was consumed by female *C. euxinus* in April and September 1995 in South-western Black Sea, respectively (Besiktepe *et al*., 1998). Another important feature of *C. euxinus* is that a part of the population always remained in the deepest part of the oxygenated zone and formed the majority of the permanent zooplankton biomass just in the suboxic zone.

C. euxinus is also the main food for a variety of commercially important fish species, *e.g.,* sprat and anchovy in the Black Sea (Sirotenko and Danilevsky, 1977; Sirotenko and Sorokalit, 1979; Avsar, 1993). It is also a link between the phytoplankton and many fish species, especially including commercially important fish species (Vinogradov *et al*., 1992, Kovalev *et al*., 1998). One of the most important commercial fishes in the Black Sea region is anchovy (85.64% of the total catch) (TUIK, 2008). Anchovy mainly feed on a variety of phytoplankton and copepod species (*Calanus euxinus*, *Paracalanus parvus*) (Bat and Satilmis, 2010). Additionally, *Calanus* development strongly depends upon seasonal changes in phytoplankton concentration (Ceballos *et al*., 2004; Rey-Rassat *et al*., 2004). Therefore, we used chlorophyll-a (chl-a) concentration as the main criteria of the phytoplankton biomass. The present study attempted to reveal the factors that have an effect on the seasonal variation in fatty acid composition and population structure of *C. euxinus* as well as to study the effects on the change of quality and quantity of the fatty acid composition with respect to chl-a, which is an indicator of phytoplankton biomass.

Materials and methods

Sampling

The study was performed in the southern part of the Black Sea at a coastal station with coordinates 40° 57′ 12″ N, -40° 9` 30″ E (Fig. 1). Each of the individual samplings was made on a single day for every corresponding month between March 2012 and February 2013 aboard KTU's research vessel Yakamoz. The water depth was 250 m. Water samples (2 l) were collected using Nansen bottles on the surface layer. Temperature and salinity were measured with a conductivity-temperature-depth-oxygen CTD profiler (CTD, General Oceanic Idronaut 316). Zooplankton samples were collected with a vertical haul from the depth of the upper border of the anoxic layer with σt = 16.2 (130 m) up to the surface layer with a 200 µm mesh Hydro-Bios net with a mouth diameter of 110 cm.

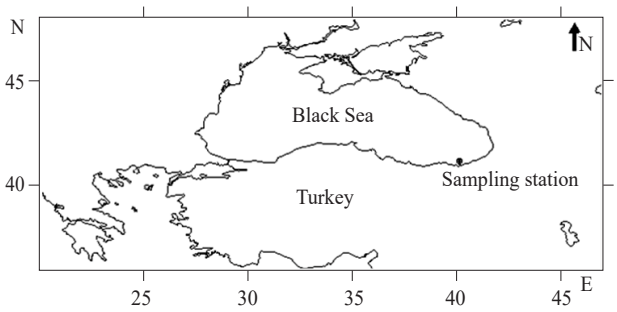

Fig. 1. Sampling station in Camburnu Bay, Trabzon

To determine lipid amount and fatty acid content, samples of copepodites and adults were collected, transferred in to glass vials with screw caps and then stored in -80ºC freezer (NUAIRE, USA) until analysis.

Chlorophyll-a (chl-a) analysis

Seawater samples (2 l) were collected from the surface layer for chl-a assay. Collected samples were filtered through a 0.45 µm cellulose acetate membrane and a few drops of magnesium carbonate solution was added to prevent the acidisation on the filter paper. After the filtering process was completed, the filter papers were folded and placed in 15 ml centrifuge tubes. Then the samples were immediately frozen at -20ºC and kept until analysis. For analysis, 10 ml 90% acetone solution was added and kept in refrgerator at 4ºC for 24 h to ensure transition of chl to acetone. The solution was centrifuged for about 20 min at 5000 rpm and the supernatant solution was considered for determination of chlorophyll pigment. The absorbance values of the samples at 750, 664, 647, 630 nm wavelengths were determined using spectrophotometer (SHIMADZU UV 2100). All the extinction values were corrected for a small turbidity blank by subtracting the 750 nm signal from all the optical densities. Finally, the phytoplankton pigments were estimated as per the following expression of Parsons *et al,* (1984):

$$\text{Chl a} = 11.85\ OD_{664} - 1.54\ OD_{647} - 0.08\ OD_{630}$$

Values obtained from the equations were then multiplied by volume of the extract (ml) and divided by volume of water (L) filtered, to express chlorophyll content in µg l^{-1}.

Population structure of Calanus euxinus

C. euxinus individuals were counted under Olympus BH_2 stereo microscope (USA) during the sampling period. Abundance was calculated as the number of individuals per m^3 (ind. m^{-3}). Quantitative analyses of *C. euxinus* were performed using 3 ml subsamples. Counts were repeated on 4 subsamples (Harris *et al.,*, 2000). Adult copepods

(females and males), copepodites and copepod nauplii were identified to species or genus level (Mauchline *et al*., 1998; Johnson and Allen, 2005).

Total lipid and fatty acid analysis

Lipids were quantitatively extracted from the samples using chloroform/methanol with a mixing ratio of 2:1 (Folch *et al.*, 1957). About 0.5-1.3 g wet zooplankton samples (WW) was used for lipid extraction per replicates. Weight of copepods used was 0.09-1.6 mg ind^{-1} and numbers of copepods ranged between 2600-3500 individuals during the sampling period. Samples were thoroughly mixed on a magnetic stirrer (Wisd WiseStir MS-MP4) for 2 h at room temperature. The homogenate was filtered and the solvent was washed with a solution containing 20% 0.9 N NaCl and stored overnight for further separation in the freezer. On the following day, the separation is expected to yield two phases: the upper phase and the lower phase containing the chloroform and lipids of interest. After discarding the upper phase, chloroform in the lower phase was evaporated under vacuum in a rotary evaporator (IKA-RV 06 ML-US) thereby restoring the lipids. After evaporation of chloroform, the remaining dry lipid was weighed and dry lipid weight (DW) was determined. Total lipids (% and mg ind^{-1}) were calculated per copepod using the equations:

$$\text{Total lipid (\%)} = \frac{\text{Wet weight (WW)}}{\text{Dry lipid weight (DW)}} \times 100$$

Wet weight: Wet zooplankton sample weight (g)

Dry weight: After evaporation of chloroform, the remaining dry lipid weight (g)

$$\text{Average individual weight (mg)} = \frac{\text{Total sample weight (mg)}}{\text{No. of individual in total samples}}$$

$$\text{Total lipid (mg ind}^{-1}\text{)} = \text{Average individual weight (mg)} \times \text{Total lipid (\%)}$$

To determine FAME (fatty acid methyl esters) of total lipids, 2 ml chloroform and 1 ml 0.21 N NaOH in methanol was added in the extracted lipid sample and the solution was mixed for about 2 h on a magnetic stirrer. Then, 0.5-0.7 ml of 0.5 N CH_3COOH in distilled water was added and the mixture was stored in a freezer. Following the removal of upper phase, the liquid portion of lower phase (chloroform) was evaporated, 2 ml hexane was added on the restored dry lipid and the sample was transferred to a vial (Kates, 1986). The FAME were detected by Shimadzu GC-17 ver 3 Gas Chromatograph (GC) (Kyoto, Japan). For this analysis, capillary columns with a length of 25 m, an inner diameter of 0.25 µm and a thickness of 25 µm (Permabond) were used (Macherey-Nagel, Germany). Twenty microlitre sample was used to inject into the GC. During analysis, the column temperature was set to 120-220 ºC using increments of 5ºC per min until 200ºC was reached and 4ºC per min to 220ºC . The column was kept for 8 min at 220ºC and the total time was determined to be 35 min. The injection temperature was set to 240ºC and detector temperature to 280ºC. Nitrogen was used as the carrier gas (Christie, 1990).

Statistical analysis

Data were analysed using STATISTICA 8.0. One-way analysis of variance (ANOVA) was used to test significant differences between the samples. The ANOVA critical significance value "p", was given in the text to indicate the level of difference (Stoline, 1981).

Results and discussion

Hydrography

Vertical profiles of the water salinity (g l^{-1}) and temperature obtained during the sampling are shown in Fig. 2 and 3. Over the sampling period, sea surface temperatures fluctuated from 8.84ºC in March to 27.75ºC in August and a seasonal thermocline was observed to start at a depth of 20 m in April. Water temperature at a depth of 40 m decreased to 6.8ºC and the cold intermediate layer (CIL) was observed. CIL is a well-known feature of the Black Sea thermohaline structure (Oguz *et al*., 1992, Oguz *et al*., 1999). CIL, marked by temperatures

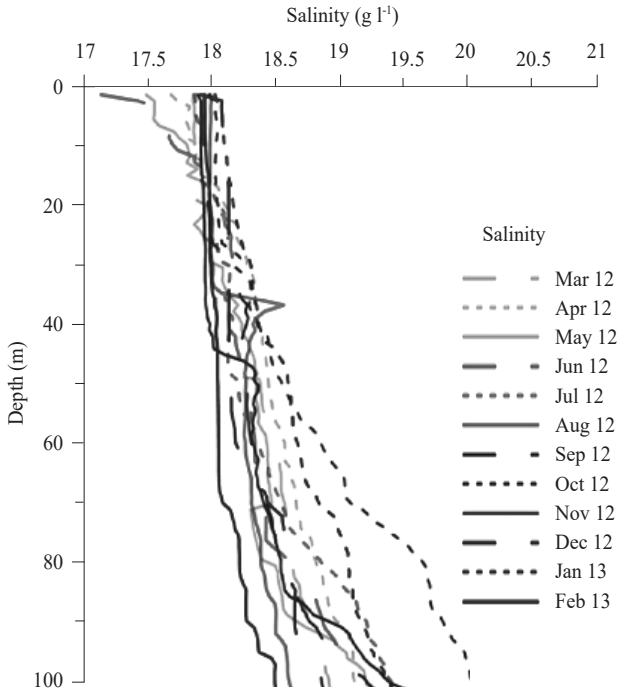

Fig. 2. Vertical distribution of salinity (g l^{-1}) at sampling station during the sampling period

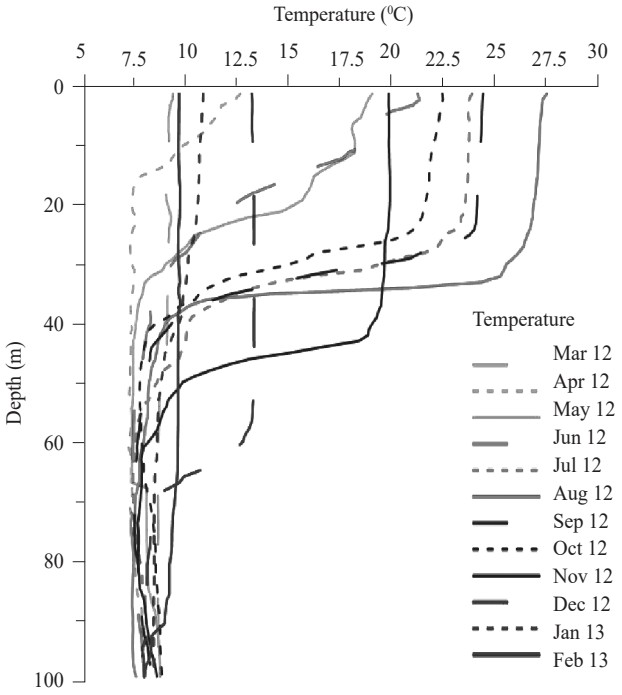

Fig. 3. Vertical distribution of temperature (ºC) at sampling station during the sampling period

lower than 8ºC, is a unique water mass of the upper layer thermohaline structure. It is formed by the convective processes associated with the winter cooling of surface waters (Tomazin, 1985; Ovchinnikov and Popov, 1987). In the surface layer, the lowest salinity was observed at 17.14 g l^{-1} in June, indicating that runoff and/or rainfall influenced water salinity, whereas the highest salinity was measured at 17.96 g l^{-1} in August. Additionally, salinity increased depending on the depth and at 100 m it ranged from 18.70 g l^{-1} in August to 20.1 g l^{-1} in October. The CTD profiles (salinity, temperature) from March and April are significantly different from those of the rest of the year. Bottom water of the Black Sea is more saline than the surface water since the denser water of the Mediterranean reaches the Black Sea through the straits (Latif *et al*., 1991).

Chl-a concentration

The mean integrated concentration of chl-a during sampling season was 1.08 µg l^{-1}. Chl-a was lowest in April (0.54 µg l^{-1}) and the highest in December (1.82 µg l^{-1}). While chl-a was 1.41 µg l^{-1} in March, it decreased sharply in April and increased again to 1.65 µg l^{-1} in May. In June, the amount of chl-a was 1.16 µg l^{-1} and it exhibited a homogeneous structure from July upto November. This suggested that spring peak occurs later in the spring (May; 1.65 µg l^{-1}) whereas autumn peak is in November (Fig. 4).

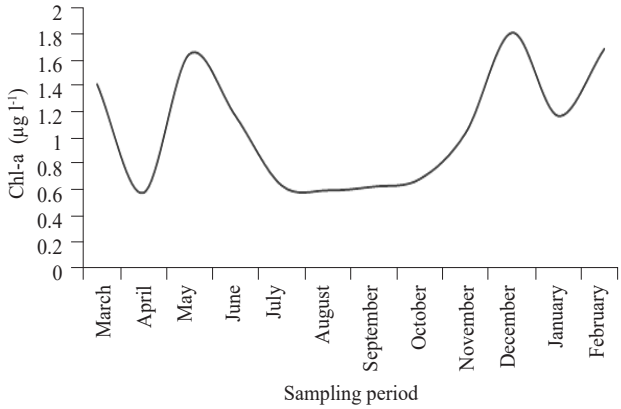

Fig. 4. Chl-a concentration during sampling period

Population structure of C. euxinus

The highest abundance for *C. euxinus* was recorded in February (847 ind. m^{-3}) and the least abundance in March (37 ind. m^{-3}). Seasonally *C. euxinus* reached the most average total abundance in winter (1063 ind. m^{-3}) and the least abundance was in spring (256 ind. m^{-3}) (Fig. 5). Female *C. euxinus* peaked in February (587 ind. m^{-3})during the sampling period. It was 70% of the total abundance in February. Female *C. euxinus* was the least abundant in June (13 ind. m^{-3}). Similarly, male *C. euxinus* peaked in February (169 ind. m^{-3}) and it was 20% of the total abundance in February. Male *C. euxinus* was least abundant with same value in March and August (5 ind. m^{-3}). However, copepodites reached the highest abundance in November (107 ind m^{-3}) and were the least abundant in March (14 ind. m^{-3}) (Fig. 6).

Total lipids

Table 1 represents the seasonal variations in total lipids of *Calanus euxinus* (wet weight, WW) in terms of per individual (mg ind^{-1}) and percentage (%) during

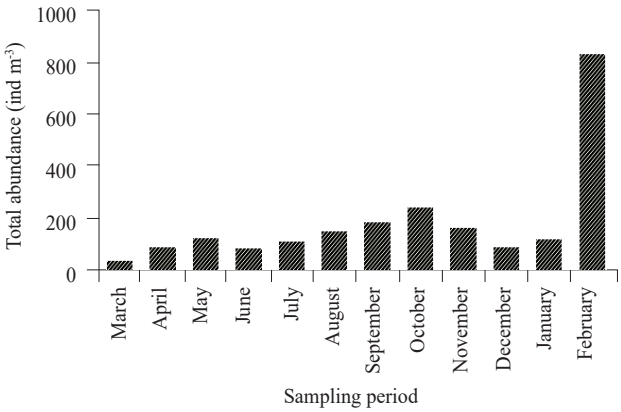

Fig. 5. Total abundance of *C. euxinus* during the sampling period

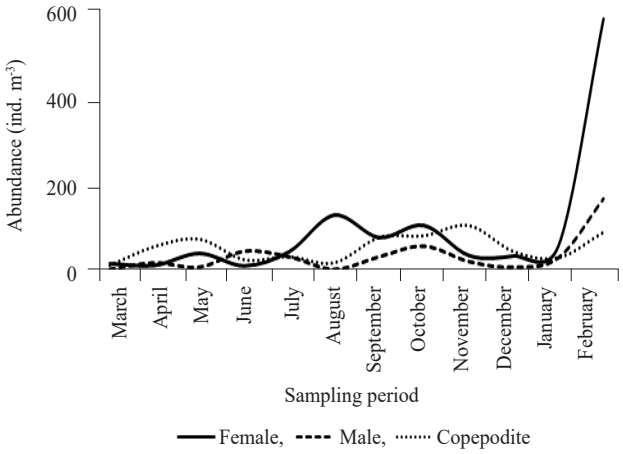

Fig. 6. Abundance of *Calanus euxinus* male, female and copepodites during the sampling period

behenic acid (22:0). The highest values of behenic acid and myristic acid were measured in November (7.61 and 13.30% respectively) while palmitic acid was highest in April (12.34%). Additionally, the lowest values of behenic acid and myristic acid were observed in April (2.84% and 3.34 % respectively) while palmitic acid was lowest in July (9.48%). Total monounsaturated fatty acids (∑MUFA) ranged from 16.39-29.95% during the sampling period, with the highest ∑MUFA in September and the lowest in March. The main components of ∑MUFA were palmitoleic acid (16:1 n-7) and oleic acid (18:1 n-9*c*). Palmitoleic acid levels ranged from 3.34 to 9.85%. The highest values of palmitoleic acid and oleic acid were recorded in September (9.85% and 5.93%, respectively) while the lowest figure was observed in March (3.34% and 3.07%, respectively). The total polyunsaturated fatty acids

Table 1. Total lipid (% and mg ind^{-1}) content of *Calanus euxinus* during the sampling period

Total lipid	March	April	May	June	July	August
%	5.61± 0.31^{abc}	4.43 ± 0.35^{abc}	5.50±1.68^{abc}	4.37±1.76^{abc}	6.84± 2.90^{a}	6.26±1.27^{ac}
mg ind.$^{-1}$	0.09±0.01^{d}	0.06 ±0.01^{b}	0.05±0.02^{ab}	0.07± 0.03^{c}	0.09±0.04^{d}	0.09±0.02^{d}
Total lipid	September	October	November	December	January	February
%	3.02 ±0.36^{b}	5.72 ±0.42^{abc}	3.24±0.22^{bc}	4.80± 1.31^{abc}	5.10±0.13^{abc}	7.03±2.04^{a}
mg ind^{-1}	0.04± 0.01^{a}	0.09±0.01^{d}	0.04±0.01^{d}	0.07±0.02^{c}	0.08 ±0.01^{cd}	0.11±0.03^{e}

(n = 3), values are means± standard deviation
Means followed by different letters and letter groups in the same row are significantly different ($p<0.05$)

the sampling period which spans from March 2012 to February 2013. The percentage of total lipids changed depending on the sampling period. Total lipids by percentage and total lipids by mg ind^{-1} showed a significant linear relationship (total lipid by mg ind^{-1} = 0.02, total lipid by % = 0.01, $r^2 = 0.78$, $p<0.05$). The average levels of total lipids by percentage during March, April, May, June, October, December and January were found to be close to each other whereas the levels in July and September differed from the other months ($p<0.05$). Moreover, total lipids (both by % and mg ind^{-1}) reached the highest level in February (7.03%, 0.11 mg ind^{-1}), total lipids by percentage was at its lowest level in September (at 3.02%) and the total lipids by mg ind^{-1} were lowest in September and November (0.04 mg ind^{-1}).

Fatty acid profile

Table 2 represents the changes in FA profile (percent of total FA) of *Calanus euxinus* during the sampling period (March 2012-February 2013). The total saturated fatty acids (∑SFA) contributed 23.47-37.71% of the total FA, the lowest value of which was obtained in March and the highest in November. The main component was palmitic acid (16:0) followed by myristic acid (14:0) and (∑PUFA) fluctuated within a wide range. The highest value for ∑PUFA was detected in March at 60.14%, while the lowest level was measured in November at 33.91%. EPA and DHA were the two PUFA detected mainly regardless of the sampling period. Other fatty acids that are important in aquatic ecosystems are highly unsaturated fatty acids (HUFA). The highest value of ∑HUFA was recorded in March (57.73%) while palmitic acid (16:0) was highest in April (12.34%) and lowest in July (9.48%).

Marine zooplankton are a very diverse group in the world's oceans with numerous taxa of high abundance and biomass. Many of these zooplankton species, in particular the dominating copepods, are able to accumulate large reserves of energy-rich lipids (Lee *et al*., 1971b; Lee and Hirota, 1973). These accumulated amounts of lipids are the major pathways for marine food webs (Dalsgaard *et al*., 2003). *C. euxinus* is a dominant component of the zooplankton biomass in the Rim Current system of the Black Sea (Svetlichny *et al*., 2009). It has adapted to the low salinity of the Black Sea waters (18 to 20‰). Preadults and adults undergo diel vertical migration within the aerobic layer (0 to 150 m)

Table 2. Fatty acid composition in *Calanus euxinus* during the sampling period (% total defined FAME)

Fatty acids	March	April	May	June	July	August
10:0	0.71±0.06^{abc}	1.04±0.01^{b}	-	0.86±0.31^{ab}	0.71±0.08^{abc}	0.79±0.02^{abc}
12:0	-	-	-	-	-	-
14:0	3.34±0.02^{f}	7.47±1.10^{a}	10.34±0.16^{ce}	10.80±0.84^{cd}	12.85±0.26^{b}	10.67±0.51^{cd}
15:0	0.61±0.13^{f}	-	1.40±0.10^{ce}	1.11±0.09^{ad}	1.39±0.07^{bce}	1.23±0.10^{abc}
16:0	12.00±0.37^{ab}	12.34±0.01^{b}	11.04±0.13^{abcd}	10.61±0.80^{acd}	9.48±0.30^{d}	11.60±0.94^{abc}
17:0	1.26±0.21^{d}	-	0.77±0.08^{bc}	0.37±0.06^{a}	0.99±0.03^{c}	0.60±0.05^{ab}
18:0	2.71±0.33^{f}	2.52±0.05^{ef}	1.63±0.01^{abcd}	1.89±0.19^{cde}	1.28±0.12^{abcd}	1.10±0.23^{ab}
20:0	-	-	-	-	-	1.35±0.28^{ab}
21:0	-	-	-	-	-	-
22:0	2.84±0.10^{f}	4.63±0.26^{de}	5.22±0.24^{abe}	7.09±0.,61^{c}	7.50±0.13^{c}	4.15±0.02^{d}
24:0	-	-	-	-	-	-
∑SFA	23.47±0.41^{g}	28.00±0.90^{d}	30.40±0.43^{abd}	32.73±1.04^{ace}	34.20±0.43^{ce}	31.49±0.38^{abc}
14:1	-	-	-	-	-	-
15:1	-	1.25±0.01^{cd}	1.06±0.08^{abc}	1.03±0.04^{d}	1.34±0.08^{ab}	0.91±0.07^{abc}
16:1 n-7	3.34±0.37^{b}	9.79±0.02^{a}	5.11±0.31^{cde}	5.59±0.69^{de}	7.52±0.40^{f}	9.34±0.77^{ag}
17: 1	0.57±0.09^{a}	1.26±0.03^{b}	0.86±0.05^{ab}	0.90±0.02^{ab}	0.99±0.03^{ab}	0.89±0.15^{ab}
18:1 n-9*t*	-	-	-	-	-	1.01±0.02^{ab}
18:1 n-9*c*	3.07±0.29^{c}	4.26±0.24^{ad}	4.23±0.03^{ad}	5.15±0.08^{a}	5.51±0.05^{be}	4.81±0.20^{ef}
18:1 n-7	0.68±0.14^{a}	0.90±0.01^{b}	0.59±0.01^{a}	-	-	-
20:1 n-9	1.98±0.02^{b}	2.81±0.44^{bd}	2.92±0.04^{bde}	4.28±0.63^{ac}	4.41±0.28^{ac}	4.02±0.63^{ae}
20:1 n-X	4.64±0.12^{abcd}	4.16±0.12^{acd}	5.03±0.07^{abd}	5.27±0.63^{ab}	5.88±0.06^{b}	4.17±0.63^{acd}
22:1 n-9	1.31±0.05^{d}	0.90±0.01^{ac}	0.86±0.06^{abc}	0.79±0.01^{abc}	0.85±0.02^{abc}	0.59±0.01^{bc}
24:1	0.80±0.03^{d}	-	0.49±0.01^{a}	-	-	-
∑MUFA	16.39±0.42^{e}	25.33±0.71^{bc}	21.15±0.55^{a}	23.00±1.80^{ab}	26.50±0.81^{abc}	25.74±2.29^{bc}
18:2 n-6*t*	-	-	-	0.48±0.02^{c}	0.43±0.02^{b}	-
18:2 n-6*c*	1.78±0.02^{bc}	2.02±0.04^{cd}	2.43±0.02^{ad}	2.38±0.10^{ad}	2.66±0.19^{a}	2.54±0.04^{a}
18:3 n-3*c*	0.63±0.11^{c}	1.19±0.02^{a}	1.46±0.07^{b}	1.25±0.07^{a}	1.48±0.06^{b}	1.49±0.06^{b}
18:3n-6	-	-	0.34±0.01^{c}	0.69±0.25^{b}	0.58±0.11^{bd}	-
20:2 n-6	-	-	0.45±0.08^{a}	0.48±0.03^{ab}	0.54±0.02^{ab}	-
20:3 n-6	-	-	-	-	-	-
20:4 n-6	-	-	-	-	-	-
20:5 n-3 (EPA)	18.05±0.12^{e}	16.70±0.19^{a}	15.92±0.09^{a}	15.66±0.39^{ac}	15.72±0.30^{ac}	16.41±0.10^{a}
22:5 n-3	0.67±0.04^{a}	-	0.73±0.04^{a}	0.77±0.04^{a}	1.01±0.22^{b}	0.61±0.02^{a}
22:6 n-3 (DHA)	39.01±0.68^{f}	26.76±0.03^{b}	27.12±0.92^{b}	22.56±1.34^{cd}	16.88±0.31^{a}	21.72±1.99^{c}
∑PUFA	60.14±0.69^{f}	46.67±0.21^{ac}	48.45±0.94^{a}	44.27±1.48^{ce}	39.30±0.44^{b}	42.77±1.99^{e}
∑HUFA	57.73±0.65^{e}	43.46±0.70^{a}	44.22±1.02^{a}	39.47±1.73^{d}	34.15±0.10^{b}	38.74±2.08^{d}
DHA/EPA	2.16±0.05^{f}	1.60±0.02^{ab}	1.70±0.05^{b}	1.44±0.05^{aef}	1.07±0.02^{c}	1.32±0.11^{def}
	September	October	November	December	January	February
10:0	0.40±0.04^{cd}	0.52±0.08^{ac}	0.60±0.02^{abc}	0.86±0.18^{ab}	0.81±0.21^{abc}	1.98±0.30^{e}
12:0	-	0.37±0.04^{a}	0.38±0.03^{a}	-	-	-
14:0	12.20±0.11^{bd}	12.89±0.34^{b}	13.30±0.78^{b}	8.70±0.56^{a}	7.73±1.10^{a}	7.99±0.25^{a}
15:0	1.34±0.04^{bce}	1.49±0.01^{e}	1.19±0.01^{abd}	1.19±0.05^{abc}	1.07±0,09^{ad}	0.98±0.02^{d}
16:0	9.98±0.32^{cd}	10.85±0.11^{abcd}	10.99±0.62^{abcd}	11.64±0.13^{abcd}	11.28±0.41^{abc}	11.86±1.22^{ab}
17:0	-	0.43±0.06^{a}	0.39±0.04^{a}	-	0.47±0.05^{a}	0.77±0.17^{bc}
18:0	0.88±0.08^{a}	1.22±0.01^{abc}	1.24±0.07^{abc}	1.83±0.02^{bcde}	1.36±0.20^{abcd}	2.01±0.78^{abcd}
20:0	1.99±0.02^{d}	-	1.58±0.04^{b}	1.26±0.07^{a}	0.89±0.05^{c}	-
21:0	0.40±0.02^{a}	-	-	-	-	-
22:0	5.86±0.09^{ab}	7.20±0.01^{c}	7.61±0.30^{c}	4.93±0.24^{ade}	6.04±0.88^{b}	5.91±0.14^{ab}
24:0	-	0.22±0.04^{b}	0.43±0.^{0}2a	-	-	0.49±0.06^{a}
∑SFA	33.05±0.46^{ace}	35.19±0.51^{ef}	37.71±0.36^{f}	30.41±0.76^{abd}	29.65±1.31^{bd}	31.99±2.54^{abc}

Table 2 cont.....

	September	October	November	December	January	February
14:1	0.45±0.03^{b}	0.32±0.01^{a}	0.59±0.01^{c}	-	-	-
15:1	1.05±0.05^{abc}	1.09±0.04^{bc}	0.84±0.02^{a}	-	-	0.99±0.23^{ab}
16:1 n-7	9.85±1.05^{a}	8.04±0.40^{fg}	9.42±0.32^{ag}	4.66±0.33^{bcd}	3.59±0.11^{bc}	6.70±0.83^{ef}
17: 1	1.06±0.02^{ab}	1.08±0.04^{ab}	1.32±0.59^{b}	0.57±0.03^{a}	0.91±0.02^{ab}	1.07±0.39^{ab}
18:1 n-9*t*	0.92±0.06^{a}	-	0.79±0.03^{d}	1.00±0.07^{ab}	1.13±0.04^{c}	1.07±0.10^{bc}
18:1 n-9*c*	5.01±0.13^{bc}	5.74±0.21^{f}	4.67±0.16^{abd}	4.12±0.07^{a}	3.22±0.02^{c}	3.09±0.30^{c}
18:1 n-7	-	-	-	-	-	-
20:1 n-9	5.38±0.17^{cf}	4.04±0.13^{a}	5.67±0.05^{f}	3.61±0.45^{ade}	4.49±0.31^{ac}	2.43±0.54^{b}
20:1 n-X	5.12±0.03^{ab}	4.42±0.02^{acd}	3.41±0.08^{c}	5.25±0.27^{ab}	5.88±0.75^{b}	3.73±1.08^{cd}
22:1 n-9	0.58±0.08^{b}	0.86±0.01^{abc}	0.89±0.02^{abc}	0.96±0.28^{a}	0.85±0.22^{abc}	1.05±0.06^{ad}
24:1	0.53±0.01^{ac}	0.58±0.01^{ac}	0.78±0.02^{d}	0.61±0.06^{c}	0.51±0.06^{a}	0.48±0.06^{a}
∑MUFA	29.95±1.05^{d}	26.27±0.12^{abc}	28.38±0.36^{cd}	20.78±0.90^{a}	20.58±1.01^{a}	20.61±2.91^{a}
18:2 n-6*t*	-	0.20±0.02^{a}	-	-	-	0.97±0.42^{b}
18:2 n-6*c*	2.64±0.08^{a}	2.49±0.02^{a}	1.93±0.03^{bc}	1.53±0.08^{b}	1.57±0.07^{b}	1.55±0.04^{e}
18:3 n-3c	1.62±0.02^{b}	1.19±0.01^{a}	1.16±0.05^{a}	1.15±0.05^{a}	1.26±0.11^{a}	0.74±0.04^{c}
18:3 n-6	0.39±0.01^{cd}	0.64±0.03^{b}	0.34±0.01^{cd}	-	-	0.57±0.04^{b}
20:2 n-6	-	0.65±0.07^{de}	-	-	0.72±0.03^{e}	0.43±0.04^{cd}
20:3 n-6	-	0.21±0.03^{a}	-	-	-	-
20:4 n-6	0.37±0.02^{a}	0.33±0.03^{a}	0.53±0.04^{b}	-	0.37±0.02^{a}	0.59±0.01^{c}
20:5 n-3 (EPA)	14.64±0.33^{bc}	14.07±0.15^{b}	11.31±0.10^{d}	14.22±0.74^{b}	14.64±1.06^{bc}	16.48±0.24^{ca}
22:5 n-3	0.59±0.04^{a}	0.63±0.09^{a}	0.56±0.07^{a}	-	0.64±0.05^{a}	0.72±0.03^{a}
22:6 n-3 (DHA)	16.75±0.36^{a}	18.21±0.69^{a}	18.08±0.34^{a}	31.91±1.43^{c}	30,57±1.12^{c}	25.35±1.11^{bd}
∑PUFA	37.00±0.73^{bd}	38.64±0.61^{b}	33.91±0.48^{d}	48.81±1.32^{a}	49.77±2.03^{a}	47.40±1.15^{ac}
∑HUFA	32.35±0.72^{bc}	34.11±0.63^{b}	30.47±0.42^{c}	46.13±1.25^{a}	46.94±2.11^{a}	43.57±1.40^{a}
DHA/EPA	1.14±0.01^{cd}	1.29±0.04^{cde}	1.60±0.02^{ab}	2.24±0.10^{f}	2.09±0.13^{f}	1.54±0.05^{abf}

Means followed by different letters and letter groups in the same row are significantly different ($p<0.05$, n=3), values are means±SD
MUFA - Monounsaturated fatty acids; PUFA - Polyunsaturated fatty acids; HUFA - Highly unsaturated fatty acids; SFA - Saturated fatty acids

up to the OMZ. Temperature changes within the range of 6.5-22°C during warm seasons within the layer (Besiktepe *et al*., 1998; Besiktepe *et al*., 2005) but they live at 5 to 8.5°C in winter and spring (Sazhina, 1987). Svetlichny *et al.* (2000) showed that total metabolism of *C. euxinus* occupying a deep daytime habitat decreased nearly 8 fold. The ability to decrease energy expenditure is of great importance especially during summer season when chl-a concentration reduces and averages at 0.22 mg m^{-3} (Yunev *et al*., 2002). Almost same results were obtained during the present study and average chl-a concentration reduced in summer season (June, July, August) (0.78 mg m^{-3}) and autumn (September, October, November) (0.76 mg m^{-3}) during the sampling period. It increased in spring (March, April, May) (1.20 mg m^{-3}) and winter (December, February, January) (1.56 mg m^{-3}). In this study, *C. euxinus* reached the highest average total abundance in winter (December, February, January) (1063 ind m^{-3}) when chl-a was the highest. In spring (March, April, May), the abundance of *C. euxinus* was the least (256 ind m^{-3}) when chl-a was second highest during the sampling period. Results of statistical analyses showed that the correlation between total abundance of *C. euxinus* and chl-a, temperature and salinity was not significant (r^2=-0.11, r^2= 0.44 and r^2= -0.04 respectively, $p<0.05$). In addition, total female and male *C. euxinus* abundance peaked in February (847, 587 and 169 ind. m^{-3} respectively) during the sampling period. However, copepodites reached the most abundance in November (107 ind. m^{-3}). Ustun (2005) indicated that *C. euxinus* peaked in July 2002 (1.800 ind. m^{-2}) and in February 2003 (5.200 ind. m^{-2}) at coastal station in Sinop Bay of the Black Sea. Yildiz and Feyzioglu (2014) reported that *Calanus euxinus* reached the highest abundance in December 1999 (18.750 ind. m^{-2}), May 2000 (30.890 ind. m^{-2}), April 2001 (52.690 ind. m^{-2}), February 2002 (20.714 ind. m^{-2}), May 2005 and 2006 (15.869 ind. m^{-2} and 10.868 ind. m^{-2}) at coastal station (Trabzon) in the South-eastern Black Sea. Our results are consistent with that of Ustun (2005) and Yıldız and Feyzioglu (2014). However, it is probable that there are several differences in relation to change of environmental conditions, different sampling periods and stations.

During all seasons, *C. euxinus* is able to accumulate large amounts of lipids (especially wax esters) in the body (Yuneva *et al*., 1997, 1999). In the present study, during the sampling period, *C. euxinus* contained the highest amount of total lipids in February (7.03%) and

the lowest in September (3.02%). However, seasonally, the average amount of total lipids was higher in summer (June, July, August) (5.82%) than the other seasons during the present study. Seasonally, the lowest average reading was observed in autumn (September, October, November) (3.99%). Svetlichny *et al.* (2006) showed that *C. euxinus* in deep layers of the Black Sea was dominated by pre-diapause and diapausing postmoult copepodite stage V with small sexually undifferentiated gonads and mean lipid content of 14.1% of body volume in summer. Additionally, total lipid in terms of mg ind^{-1} was the highest in February (0.11 mg ind^{-1}). Seasonally, the total lipid was highest in winter (December, February, January) (0.086 mg ind^{-1}) and summer (0.083 mg ind^{-1}) at almost the same level. The results presented in Katnerr *et al.* (1994) are nearly consistent with the present study. It was indicated that the lipid content of Atlantic copepods was higher in summer than the other seasons. Moreno *et al*. (1979) reported that the lipid content of *Paracalanus parvus* along the Mar Del Plata coastline, south of Buenos Aires was 2% in July (corresponding to the winter season in the northern hemisphere) and increased in September (corresponding to the spring season in the northern hemisphere) to 6.8%. The average amount of total lipids of *C. euxinus* was 14.1±6.0% at a different station at the South-eastern Black Sea and this proportion decreased to 7.7±5.1% in female *C. euxinus* living in deeper waters, as reported by Svetlich *et al.* (2006).

Yuneva *et al.* (1999) reported on total lipids of female *C. euxinus* at the Southern Black Sea (September 1996) and indicated that the average amount of total lipids of *C. euxinus* in cyclonic regions (101.9 µgind^{-1}) was greater than that from anticyclonic regions (58.8 µg ind^{-1}). It was pointed out that lipid content (wax esters, triacylglycerols) of female *C. euxinus* was correlated with chl-a (r^2=0.92, $p<0.05$). In the present study, the correlation between lipid content (4.87% and 0.07 mg ind^{-1}) and chl-a concentration was not significant (r^2=0.16, r^2=0.08, $p<0.05$ respectively). The correlation between lipid (% and mg ind^{-1}) and chl-a was not significant (r^2=0.16 and r^2= 0.08 respectively, $p<0.05$). Additionally, the correlation between lipid (% and mg ind^{-1}) and temperature was not significant (r^2=0.04 and r^2= 0.01 respectively, $p<0.05$). Similarly, the correlation between lipid (% and mg ind^{-1}) and salinity was also not significant (r^2=-0.29 and r^2=-0.02 respectively, $p<0.05$). Yuneva *et al.* (1999) indicated that the level of lipid accumulation in *Calanus* depends on the concentration and composition of the phytoplankton consumed. However, in the present study, it was thought that rather than the composition and concentration of the phytoplankton consumed, differences in location and timeframe of the sampling may be a factor in the level of lipid accumulation. Black Sea phytoplankton abundance, biomass, and species composition continuously varies depending on seasonal and regional changes (Eker-Develi and Kideys, 2003).

Calanoid copepods have a special importance in the marine food web since they are the most intensive group of zooplankton (Morris, 1971; Ackman *et al*., 1974; Lee *et al*., 1971a). Therefore, the large (2-10 mm), lipid-rich *Calanus* species have been studied extensively. In early literature, *Paracalanus parvus* in particular is reported to contain abundant amounts of fatty acids 16:0, 16:1, 20:5 n-3 and 22:6 n-3 as compared to the other marine copepods (Ackman and Hooper, 1970; Morris, 1971; Lee *et al*., 1971a; Ackman *et al*., 1974;). In the present study, ∑SFA varied from 23.47% to 37.71% (March-November), ∑MUFA varied from 16.39% to 29.95% (March-September) and ∑PUFA varied from 33.91 to 60.14% (November-March) in *C. euxinus*. It was observed that 16:0, 16:1 n-7, 18:1 n-9, 20:5 n-3 and 22:6 n-3 were the major fatty acids in *C. euxinus* and the fatty acid composition and content changed continuously depending on the season and the month. This change in fatty acid composition due to temperature is thought to be an adaptation to optimise phase behaviour that is crucial for proper membrane functioning (Hazel and Williams, 1990; Hochachka and Somero, 2002).

Hagen *et al*. (1993) reported that the major fatty acids in summer were 20:1 n-9, 20:5 n-3, 22:6 n-3, 18:4 n-3, 22:1 n-11 and 16:1 n-7 in *Calanus acutus*, 22.1 n-11, 22:1 n-9, 16:0, 20:5 n-3 and 22:6 n-3 in *Calanus propinguus* collected in the Antarctic Weddell Sea in late winter-early spring (October-November). Kattner *et al*. (1994) suggested that 22:1 n-9 and 22:1 n-11 long chains were the most important fatty acids and created up to 50% ∑FAME in *Calanus propinquus*. They also indicated that MUFA (18:1 n-9 and 16:1 n-7) reached high proportions but PUFA is the dominant fatty acid in *Rhincalanus gigas*. Kattner *et al*. (1981) measured the fatty acid content of *Pseudocalanus elongatus* and *Calanus finmarchicus*. They found that in *P. elongates* SFA was 54.1±6.4%, MUFA was 31.0±7.4% and PUFA 14.8±4.9%, and in *C. finmarchicus* SFA was 52.2±7.6%, MUFA 19.1±2.5% and PUFA 28.7±10.1%. As shown, major fatty acids vary in different *Calanus* species, however 20:5 n-3 and 22:6 n-3 are the main fatty acids and they comprise a large proportion of the PUFA of nauplii and copepodite stages of copepods in the North-western Mediterranean (Kattner *et al*., 1994). Moreover, it should be noted that food supply is the most important determinant of fatty acid profile changes of copepods as reported by Escribano and Perez (2010).

Goncalves *et al.* (2012) observed fatty acid content of different zooplankton species in the waters of the Western

Atlantic off the coast of Portugal and reported that the dominant unsaturated fatty acids of *Acartia tonsa* were 14:0, 16:0 and 18:0. Fatty acid content of *A. clausi* and *A. tonsa* had the same predominant MUFA and SFA and *A. clausi* also had 17:0 (14.95%) in the spring. Goncalves *et al*. (2012) also recorded the highest DHA value in summer (13.28%) and showed that EPA was observed in all seasons except for autumn. The group also indicated that zooplankton species had higher FA concentrations in winter and spring than in summer and autumn. In the present study, the fatty acid composition of *C. euxinus* was predominantly ∑SFA (average of 31.52%) and ∑PUFA (average of 43.01%) during the sampling period and the average ∑MUFA was 23.72%. *C. euxinus* had 17:0 during all sampling periods except in April, September and December, although the amount (17:0) was very low (average of 0.50%).

Fatty acid groups (∑SFA, ∑MUFA, ∑PUFA, ∑HUFA) were correlated with temperature. While the amount of ∑SFA and ∑MUFA was directly related to temperature (r^2=0.74 and r^2=0.73 respectively, $p<0.05$), the amounts of ∑PUFA and ∑HUFA were inversely related to temperature (r^2=-0.73 and r^2=-0.80 respectively, $p<0.05$). The amount of ∑PUFA and ∑HUFA increased (r^2=0.61 and r^2=-0.68 respectively, $p<0.05$), but the ∑MUFA decreased (r^2=-0.68, $p<0.05$) while chl-a increased. The degree of unsaturation increased as temperature decreased. Jeffries (1970) emphasised that the degree of unsaturation decreases from winter to summer. On the other hand, the correlation between chl-a (as an indicator of phytoplankton biomass) and fatty acid groups was significant with the exception of the ∑SFA. It was determined that there is a negative correlation (r^2=-0.75) between ΣMUFA and chlorophyll-a and the ΣPUFA (r^2=0.61) and DHA/EPA ratio (r^2=0.60) and a positive correlation between DHA (r^2=0.61) and EPA (**r^2**=0.63, $p<0.05$). In particular, there is a strong relationship between the EPA and the chl-a concentration and chl-a concentrations are associated with diatoms (Budge *et al*., 2014). Ozdemir and Ak (2012) have reported that dinoflagellates were the most abundant in April and June and the diatoms in October and March in the South-eastern Black Sea (Trabzon Coast). In this study, it was determined that EPA in *C. euxinus* (as an indicator of the presence of diatoms) had the highest value in March (18.5%) and increased in spring. This is thought to be a result of the abundance of diatom species at that time. Diatoms represent 85% of the total phytoplankton in the Black Sea in October (Eker-Develi *et al*., 1999). However, EPA and DHA were seasonally at its average lowest level in autumn (13.34%; 17.68%, respectively). The lowest value of EPA was in November (11.31%) and DHA was the lowest in September (16.75%) during the sampling period. Also, at this time, chl-a had the lowest value (0.76 µg l^{-1}). This shows that the phytoplankton, specifically the diatoms and flagellate phytoplankton were the lowest at this time. However, it should be noted that phytoplankton species composition, growth and distribution are controlled by such environmental factors as time-dependent changes of temperature, salinity, density, light intensity and nutrient supply (Uysal, 2002). Therefore, it is likely that there is a difference in abundance of phytoplankton from year to year.

The proportion of DHA was observed to be higher than the proportion of EPA during all sampling period. DHA/EPA varied from 1.07 to 2.24 (July-December). Seasonally, average DHA/EPA was the highest in winter (1.96) and the lowest in spring (1.82). This suggests that the high levels of DHA/EPA during spring and winter in *C. euxinus*, a herbivore, depends on the high phytoplankton concentration since chl-a (which is an indicator of phytoplankton biomass) was highest in winter (1.56 µg l^{-1}) and spring (1.20 µg l^{-1}). DHA/EPA is a biomarker for carnivorous species (Goncalves *et al*., 2012). Therefore, the DHA/EPA values of *C. euxinus* can affect fish species that feed on them from larval stages to adult stages in many ways.

Zooplankton forms an essential link between primary producers and higher trophic levels (Dalsgard *et al*., 2003) and *C. euxinus* is one of the most important pelagic copepods in the food chain of the Black Sea (Vinogradov *et al*., 1992). Therefore, the biochemical role of *C. euxinus* in the Eastern Black Sea is highly significant. *C. euxinus* has a rich lipid content as well as fatty acid composition and it is a vital link between phytoplankton and commercial fish species (Vinogradov *et al.,* 1992; Kovalev *et al*., 1998). Results of the study shows that seasonality plays an important role on *C. euxinus* in the South-eastern Black Sea ecosystem functions, in particular in energy fluxes to higher trophic levels. Lipid content and fatty acid profile of *C. euxinus* is extremely important in the sustainability of Black Sea anchovy that has an important place in Turkey's fisheries. Because, among the lipids, certain essential fatty acids are considered to be important determinants of ecosystem health and stability (Parrish, 2013). Also, fatty acids provide versatile signatures that are being increasingly employed to delineate the transfer of dietary material through marine and terrestrial food webs (Parrish *et al*., 2015). *C. euxinus* is one of the most important food source for anchovy in the Black Sea ecosystem. In this way, it was thought to affect higher trophic levels including anchovy from larval stages to adult stages in many ways such as recruitment, reproduction, growth and survival. Additionally, fatty acid composition of *C. euxinus* is significantly affected by temperature; the degree of unsaturation is inversely proportional to the temperature.

If global warming continues, this will have a severe impact on cold water species such as *C. euxinus* and cause losses in the energy transfer in the South-eastern Black Sea food chain, thereby eventually affecting humans.

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