

## Seasonal effect on fatty acid composition in phospholipid classes and triacylglycerols of male *Capoeta umbla*

 S. Kaçar<sup>a,✉</sup>,  H. Kayhan Kaya<sup>b</sup> and  M. Başhan<sup>c</sup>

<sup>a</sup>Mardin Artuklu University, Department of Nutrition and Dietetics, Faculty of Health Sciences, 47100 Mardin, Turkey

<sup>b</sup>Dicle University, Faculty of Medicine, Department of Physiology, 21280 Diyarbakır, Turkey

<sup>c</sup>Dicle University, Faculty of Science, Department of Biology, 21280 Diyarbakır, Turkey

✉Corresponding author: semrakacar21@gmail.com

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**SUMMARY:** The seasonal changes in muscle tissue, total lipids and fatty acid composition of phospholipids (PL), triacylglycerol (TAG), and phospholipid classes of male *Capoeta umbla* were investigated in this study. Phosphatidylcholine (PC), phosphatidylethanolamine (PE), phosphatidylinositol (PI), and phosphatidylserine (PS) were identified as the major phospholipids (PLs) in the muscle tissue (PS). Triacylglycerols showed high contents of MUFA, 14:0, 16:1n-7, 18:1n-9, 18:2n-6, and 18:3n-3; while phospholipids presented high contents of AA, DHA, and 18:0. Myristic acid, 16:1n-7, 18:1n-9, monounsaturated fatty acids (MUFA), linoleic acid (18:2n-6), and linolenic acid (18:3n-3) were shown to be present in larger concentrations in TAG than in PL classes. In PL classes, 16:0, 18:0, arachidonic acid (20:4n-6) (AA), eicosapentaenoic acid (EPA) (20:5n-3), docosahexaenoic acid (DHA) (22:6n-3) and polyunsaturated fatty acid (ΣPUFA) were higher than the TAG fraction. The fatty acid composition of total lipid and lipid classes (TAG and PL) were affected by the seasonal variations and lipid fraction.

**KEYWORDS:** *Capoeta umbla*; Phospholipid classes; Seasonal fatty acid composition; Triacylglycerol.

**RESUMEN:** *Efecto estacional sobre la composición de ácidos grasos de fosfolípidos y triacilglicérol de machos de Capoeta umbla.*

En este estudio se investigaron los cambios estacionales en el tejido muscular, la composición total de lípidos y ácidos grasos de fosfolípidos (PL), triacilglicérol (TAG) y clases de fosfolípidos de *Capoeta umbla* machos. Fosfatidilcolina (PC), fosfatidiletanolamina (PE), fosfatidilinositol (PI) y fosfatidilserina (PS) se identificaron como los principales fosfolípidos (PL) en el tejido muscular (PS). Los triacilglicérols tenían un alto contenido de 14:0, de los MUFA 16:1n-7 y 18:1n-9 y de poliinsaturados 18:2n-6 y 18:3n-3, mientras que los fosfolípidos tenían un alto contenido de araquidónico (20:4n-6) (AA), docosahexaenoico (22:6n-3) (DHA) y 18:0. Se demostró que el ácido mirístico, los monoinsaturados (MUFA) 16:1n-7 y 18:1n-9, el ácido linoleico (18:2n-6) y el ácido linolénico (18:3n-3), están presentes en concentraciones superiores en TAG que en los PL. En las diferentes clases de PL, los ácidos 16:0, 18:0, AA, eicosapentaenoico (20:5n-3) (EPA), DHA y la suma de ácidos grasos poliinsaturados (ΣPUFA) fueron más altos que la fracción TAG. La composición de ácidos grasos de los lípidos totales y las clases de lípidos (TAG y PL) se vieron afectados por las variaciones estacionales y la fracción lipídica.

**PALABRAS CLAVE:** *Capoeta umbla*; Clases de fosfolípidos; Composición estacional de ácidos grasos; Triacilglicérols.

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## 1. INTRODUCTION

*Capoeta umbla*, also locally called Siraz or Saribalık, is a species belonging to the cyprinidae family, commonly found in the Euphrates-Tigris river system. *C. umbla* is the most commercially valued fish for the local people.

Fish and other aquatic foods are known to be the main sources of polyunsaturated fatty acids (PUFAs). Therefore, humans get most of their eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) from eating fish. It has been established that DHA is essential for vision and brain development in babies, Fish consumption is related to the amount of DHA in milk, and fish oil reduces sudden death in people with cardiovascular disease, lowers serum triglyceride levels and total cholesterol, increases HDL cholesterol levels, and reduces the risk of breast, colon, lung and prostate cancer (Lu *et al.*, 2021).

It is important to determine the fatty acid composition of the PL and TAG fractions to understand the changes in the fat content of the muscle and to determine the nutritional value of the fish.

The 20-carbon polyunsaturated fatty acids of PL, which are structural components of membranes, play a role as precursor components of eicosanoids, which are biologically and physiologically active molecules. TAGs, which are generally stored in adipose tissue and rich in SFA and MUFA, function as energy reserves (Kaçar *et al.*, 2016).

The most abundant phospholipid in cell membranes is phosphatidylcholine, which accounts for 40-50% of total phospholipids. Phosphatidylethanolamine is the second most abundant phospholipid, accounting for 20-50% of the total. These amounts vary depending on the tissue. For example, the amount of PE in the brain is 45% and the amount in the liver is 20%. Phosphatidylserine is a membrane phospholipid which is low in abundance and accounts for only 2-10% of the total phospholipids. Phosphatidylinositol, sphingomyelin, and cardiolipin phospholipids are minor mammalian membrane components. The phospholipid compositions of different mammalian cells and tissues differ significantly. The lipid composition of fish differs depending on several factors, such as their aquatic environment and the biological, physical and chemical properties of this environment. Seasons, migration, sexual maturity and spawning periods, type of species, dietary

habits and rearing in aquaculture or natural habitats influence the lipid profiles.

Various investigations into freshwater fish have been carried out, but little information is available on PL and its classes or its TAG fatty acid analysis. (Satar *et al.*, 2012; Kaçar *et al.*, 2016). Studies on phospholipid classes have generally been carried out in marine fish, although no reports have yet been published about the PL classes of *C. umbla*.

The objective of this study was to determine seasonal changes in the FA composition of triacylglycerol (TAG) and phospholipid classes including phosphatidylethanolamine (PE), phosphatidylcholine (PC), phosphatidylinositol (PI) and phosphatidylserine (PS).

## 2. MATERIALS AND METHODS

The study was conducted on *C. umbla*, which were collected in July, November, January and April (39°10'44.68"N, 39°27'43.08"E). The fish were skinned from the area between the dorsal fins and the lateral line of three male and about 5 grams of muscle sample were taken. After determining the wet weights of the muscle samples, they were put into tubes and kept in a chloroform-methanol (2:1v/v) mixture at -80 °C until analysis.

Total lipid extraction was performed according to the method of Bligh and Dyer (1959). Methylation was accomplished by heating with sodium methylate in methanol. Samples containing total muscle lipids were trans-esterified with acidified methanol. The fatty acid composition of the fish was analyzed in triplicate: Auto-oxidation of unsaturated fatty acids was minimized by adding 50 µL of 2% butylated hydroxytoluene in chloroform to each sample during the extraction process, and N<sub>2</sub> was used for the drying process of lipid extracts from tissues.

The method of Vaden *et al.* (2005) was used for the separation of phospholipid classes. Samples containing muscle lipids were transesterified with acidified methanol. The fatty acid methyl esters (FAMES) were extracted with hexane.

### 2.1. Gas chromatography analyses

Fatty acid methyl esters were analyzed by capillary gas chromatography (SHIMADZU GC 2010 PLUS) with a flame ionization detector (FID), and GC solution (version 2.4) software. The flow rates of

compressed air and hydrogen were 400 mL/min and 30 mL/min, respectively. The carrier gas was Helium at a flow rate of 0.5 mL/min. The injection port temperature was 250 °C, the detector temperature 250 °C. One micro-liter was injected through the cold on-column injector. Column (oven) temperature: 170 °C, holding time, 2 minutes; 2 °C/minute to 210 °C, kept constant for 20 minutes; total analysis time: 42 minutes.

A SPSS 16 computer program was used to compare the percentage of fatty acids. A statistical analysis of fatty acid compositions was performed

by analysis of variance (ANOVA) and mean comparison was determined according to Tukey's test. Means were obtained in triplicate and statistically significant differences were reported at ( $P \leq 0.05$ ).

### 3. RESULTS AND DISCUSSION

The captured samples varied between 225 and 290 cm, with weights between 162 and 392 g. Fat content ranged between 1.35% (in November) and 2.46% (in July) (Table 1).

Table 2 shows the seasonal variation in total lipid contents in male *C. umbla*.

TABLE 1. Lengths, weights and lipid contents of *C. umbla*

	July	November	January	April
Length (cm)*	225±11.01	280±12.20	290±10.40	245±11.14
Weight (gr)*	162±8.13	348±14.44	392±15.18	220±9.15
Total lipids (%)*	2.46±0.16a	1.35±0.02b	1.72±0.06c	1.50±0.07bc

\* Means are the average of 3 replicates. Values reported are means ±standard deviation; means followed by different letters in same line are significantly different ( $p < 0.05$ ) according to Tukey's test

TABLE 2. Fatty acid composition in total lipids of the muscle from male *C. umbla* (%)\*

Fatty acids	July	November	January	April
14:0**	1.89±0.06a	2.29±0.06a	4.63±0.19b	2.32±0.12a
15:0	0.21±0.01a	0.27±0.01a	0.27±0.02a	0.22±0.01a
16:0	21.28±1.03a	23.37±1.25a	22.5±1.17a	22.26±1.15a
17:0	0.46±0.02a	0.65±0.03ab	0.38±0.01a	0.82±0.03b
18:0	4.14±0.16a	3.91±0.13a	3.38±0.10a	4.10±0.20a
<b>ΣSFA***</b>	27.98±1.25a	30.49±1.60a	31.16±1.55a	29.72±1.45a
16:1n-7	18.48±0.72a	11.99±0.72b	15.91±0.85ab	17.15±0.63a
18:1n-9	14.2±0.61a	18.11±0.72b	20.07±1.01b	14.66±0.70a
20:1n-9	1.08±0.04a	2.28±0.15b	2.98±0.18b	0.80±0.03a
<b>ΣMUFA</b>	33.76±1.56a	32.38±1.66a	38.96±1.87b	32.61±1.08a
18:2n-6	1.93±0.04a	2.48±0.09b	2.15±0.11b	2.19±0.11b
18:3n-3	6.63±0.30a	5.86±0.23a	3.27±0.15b	5.67±0.22a
20:2n-6	0.14±0.01a	0.20±0.01b	0.16±0.01a	0.20±0.02b
20:3n-6	0.16±0.01a	0.18±0.01a	0.28±0.02b	0.12±0.01a
20:4n-6	1.0±0.05a	0.99±0.03a	1.29±0.06b	1.30±0.05b
20:5n-3	16.1±0.62a	14.94±0.72ab	12.02±0.55b	14.22±0.88ab
22:5n-3	5.46±0.35a	4.88±0.15ab	3.64±0.26b	5.45±0.33a
22:6n-3	6.75±0.28a	7.51±0.34ab	6.99±0.40a	8.43±0.48b
<b>ΣPUFA</b>	38.17±2.04a	37.04±1.71a	29.8±1.02b	37.58±1.80a
<b>Σn-3</b>	34.94±1.65a	33.19±1.45a	25.92±1.30b	33.77±1.57a
<b>Σn-6</b>	3.23±0.10a	3.85±0.12a	3.88±0.20a	3.81±0.19a
<b>n-3/n-6</b>	10.81	8.62	6.68	8.86

\* Means are the averages of 3 replicates. \*\* Values reported are means ±standard deviation; means followed by different letters in the same line are significantly different ( $p < 0.05$ ) according to Tukey's test. \*\*\* **SFA**: saturated fatty acids, **MUFA**: monounsaturated fatty acids, **PUFA**: polyunsaturated fatty acids

The highest saturated fatty acids (SFAs) were found in January (31.16%, prior to reproduction). There were no seasonal changes in total SFA values between July (27.98%) and January (31.16%). The highest concentrations of SFA were 16:0 (21.28-23.37%), and 18:0 (3.38%-4.14%).

Monounsaturated fatty acids (MUFAs) in the muscle tissue increased in January (38.96%, prior to reproduction) and decreased in November (32.38%, post reproduction). The 16:1n-7 ratio was high in July (18.48%) and decreased in November (11.99%). In July, the amount of 18:1n-9 reached its lowest value (14.20%). 18:2n-6, which is one of the n-6 components which are important and essential fatty acids for fish, 18:3n-3 from the n-3 and arachidonic acid (ARA), another important n-6, were detected at a low rate. Among them, 18:2n-6 was found to be 2.48% in November, 18:3n-3 to be 6.63% in July, ARA to be 1.30% in April.

The concentration of PUFA varied seasonally from 29.80% (in January, prior to reproduction) to 38.17% (in July, reproduction). The amount of n-3 PUFAs is much higher than n-6 PUFAs.

In this study, the n-3/n-6 ratios vary for muscle tissue according to season, and are lower in January (6.68), increasing up to 10.81 in July.

The fatty acid results of *C. umbla* male from the Munzur River are compatible with those obtained from *C. umbla* (Yılmaz *et al.*, 1995, Aras *et al.*, 2009) and other freshwater fish previously taken from different water sources (Uysal *et al.*, 2008; Cengiz *et al.*, 2012; Çakmak *et al.*, 2012; Satar *et al.*, 2012; Fallah *et al.*, 2013; Kayhan *et al.*, 2015; Emre *et al.*, 2015; Emre *et al.*, 2017; Emre *et al.*, 2018; Emre *et al.*, 2020). The major fatty acids identified in the fish were 16:0, 18:1n-9, DHA and EPA in all seasons. Linoleic, 18:3n-3, 20:3n-6 and AA were also detected in a much lower percentage.

The levels of SFA, MUFA, and PUFA in fish are not constant and change according to a number of variables. Unsaturated fatty acids (PUFA and MUFA) are often more prevalent than saturated ones in cold water fish. PUFA is less prevalent in tropical species. While PUFAs play a structural role in organs, saturated fatty acids and MUFAs typically serve as energy sources. We can infer that the water source may be the primary factor in this (Kayhan *et al.*, 2015). Fish living in cold water, like the Munzur River, are predicted to have higher PUFA ratios. This

finding increases the nutritional value of the fish living here. Since total SFA increases the amount of cholesterol in the blood, having more of these components creates negative health effects and reduces the nutritional value of fish.

Because palmitic acid is essential for fatty acid metabolism, nutrients have no effect on the amount of this component, which has the highest percentage of saturated fatty acids. The saturated 16:0 and 18:0, as well as the monounsaturated 16:1n-7 and 18:1n-9, are not essential and can be obtained through food or synthesized from carbohydrates and amino acids. The amounts of these components, which are generally stored and serve to meet energy needs, depend on the organisms that make up the nutrient. It has been seen that the ratio of 16:1n-7 from the monounsaturated FA is higher in some fish. According to Ackman (1989), this feature is unique to freshwater fish. The ratio of 16:1n-7 found in our study was significantly higher than the rates found in *C. umbla* from Hazar Lake (Yılmaz *et al.*, 1996), Tuzla Stream, and Tercan Dam Lake (Aras *et al.*, 2009). This finding demonstrated that the fatty acid composition of fish is affected by the water source. We believe that fish nutrients are responsible for this effect. Some phytoplankton, zooplankton, and other microorganisms in the water were found to be high in 16:1n-7. Palmitoleic acid serves important nutritional and biological functions because this component is essential for fat synthesis and storage, as well as signaling pathways between organs, cell differentiation, and proliferation. In our analysis, the percentage of 18:2n-6 in muscle total lipids was lower than in other studies (Yılmaz *et al.*, 1996; Aras *et al.*, 2009) on the same type of fish, while the percentage of 18:3n-3 acid was higher. Fish consume essential fatty acids such as 18:2n-6 and 18:3n-3, which they cannot synthesize, and use these components as precursors in the formation of twenty-carbon PUFAs. Analyses show that the ratio of 18:2n-6 and 18:3n-3 acids are generally low in fish (Aggelousis and Lazos, 1991).

In our study, the percentage of 18:2n-6 in the total muscle lipid was lower than in previous studies on the same type of fish (Yılmaz *et al.*, 1996; Aras *et al.*, 2009), while the percentage of 18:3n-3 acid was higher. Henderson and Tocher (1987) reported in their study that the 20:3n-6 ratio in many freshwater fish was 1.5%. This component, which could not be determined in previous studies on *C. umbla*

(Yılmaz *et al.*, 1996, Aras *et al.*, 2009), was found at a rate of less than 1% in the fish species examined in our study. The amount of ARA is high because the solubility of oxygen in hot water is low. Because arachidonic acid is a precursor to eicosanoids, which are vasoconstrictors and stimulate platelet aggregation, only small amounts should be found in fish oils. ARA was found to be lower in the fish species whose total muscle lipids were examined in our study than in the same type of fish previously supplied from other water sources. This could be due to the fact that the Munzur River is colder because the ARA ratio is higher in fish living in warm water environments. The low ARA ratio in Munzur River fish increases the nutritional value of these fish. EPA and DHA were the major fatty acids identified as n-3 PUFAs

in the muscle. Similar results have been reported in *Capoeta capoeta* (Çakmak *et al.*, 2012), in *Capoeta damascina* (Fallah *et al.*, 2013) and for *Capoeta angorae* (Emre *et al.*, 2015). EPA and DHA, which are important n-3 components, have preventive effects on many diseases including cardiovascular disease and cancer. Moreover, EPA is the precursor to eicosanoids, which, unlike ARA, is vasodilator and prevents erythrocyte aggregation. We can say that the fish species we used in our study are rich in EPA and DHA which are n-3 fatty acids.

### 3.1. Fatty acid composition of the PL fraction

Table 3 shows the PL fatty acid content of male *C. umbla* muscle tissue. The ratio of 16:0 decreased

TABLE 3. Fatty acid composition in the phospholipid fraction of the muscle from male *C. umbla* (%)\*

Fatty acids	July	November	January	April
14:0**	1.09±0.06a	0.83±0.02ab	0.65±0.02b	0.89±0.03ab
15:0	0.16±0.01a	0.09±0.01b	0.21±0.02c	0.15±0.02a
16:0	19.82±0.91a	23.97±1.13b	21.32±1.20a	21.34±1.13a
17:0	0.68±0.03a	1.54±0.05b	0.50±0.02a	0.77±0.03a
18:0	8.36±0.40a	9.16±0.43a	5.73±0.26b	6.79±0.30b
<b>ΣSFA***</b>	30.11±1.57a	35.59±1.70b	28.41±1.36a	29.94±1.47a
16:1n-7	6.46±0.29a	3.98±0.20b	5.80±0.27a	4.09±0.19b
18:1n-9	12.9±0.58a	12.72±0.67a	12.95±0.55a	8.92±0.41b
20:1n-9	1.27±0.06a	1.60±0.06a	1.62±0.05a	0.60±0.02b
<b>ΣMUFA</b>	20.63±1.10a	18.3±0.86a	20.37±1.02a	13.61±0.75b
18:2n-6	1.16±0.05a	1.47±0.06b	1.60±0.06b	1.04±0.04a
18:3n-3	2.01±0.10a	2.59±0.13a	1.57±0.07b	1.10±0.06b
20:2n-6	0.14±0.01a	0.23±0.02b	0.17±0.01a	0.14±0.01a
20:3n-6	0.25±0.01a	0.25±0.02a	0.33±0.02b	0.16±0.01c
20:4n-6	2.12±0.12a	2.22±0.15a	3.54±0.17b	3.69±0.14b
20:5n-3	18.8±0.75a	18.45±0.93a	19.23±0.83a	23.3±1.20b
22:5n-3	9.62±2.02a	7.14±2.11a	6.92±1.80a	7.53±2.16a
22:6n-3	15.09±0.80a	13.7±0.65a	17.77±0.76b	19.41±0.90b
<b>ΣPUFA</b>	49.19±2.33a	46.05±2.34b	51.13±2.77a	56.37±2.83c
<b>Σn-3</b>	45.52±2.72a	41.88±1.94b	45.49±2.18a	51.34±2.62c
<b>Σn-6</b>	3.67±0.13a	4.17±0.20a	5.64±0.28b	5.03±0.20b
<b>n-3/n-6</b>	12.40	10.04	8.06	10.20

\* Means are the average of 3 replicates. \*\* Values reported are means ±standard deviation; means followed by different letters in the same line are significantly different ( $p < 0.05$ ) according to Tukey's test. \*\*\* **SFA**: saturated fatty acids, **MUFA**: monounsaturated fatty acids, **PUFA**: polyunsaturated fatty acids

to a minimum level in July (reproduction period, 19.82%). The highest  $\Sigma$ SFA contents were found in November (35.59%). The  $\Sigma$ SFA amounts decreased prior to the reproduction season (January) in *C. umbla*. Total MUFAs were lowest in April (13.61%). 18:1 n-9 was the most abundant fatty acid in all seasons and ranged from 8.92 to 12.95%. Throughout the year, the PUFAs accounted for 46.05–56.37% of the PL fatty acid composition. The most abundant n-3 PUFA was EPA (18.45-23.3%). This was followed by DHA (13.7-19.41%) and then 22:5n-3 (6.92-9.62%) and n-3/n-6 ratios were found to be 8.06-12.40.

The major fatty acids were in accordance with those obtained from other freshwater fish (Satar *et al.*, 2012; Görgün *et al.*, 2014, Kayhan *et al.*, 2015): 16:0 in SFA, 18:1n-9 in MUFA, and EPA and DHA among PUFAs were found in the PL fraction. Another important finding was that the rate of stearic acid was found to be higher than total lipids. This is an expected result because, despite being a saturated component, 18:0 accumulates more in the PL fraction.

Henderson and Tocher (1987) found that the n-3/n-6 ratio in freshwater fish PL was between 1.6 and 2.0. The most important reason for the high n-3/n-6 ratio, which is used to determine the nutritional value of fish, in the species we studied is that EPA, one of the important n-3 components, was very high, while n-6 fatty acids were very low.

In response to a decrease in ambient temperature, poichlothermic organisms, such as fish, increase the degree of unsaturation of the fatty acids in their membrane lipids. This is an adaptation developed to keep the membranes fluid.

Henderson and Tocher (1987) reported that if the ambient temperature decreases, the degree of unsaturation in fatty acids will increase, and in Farkas *et al.* (1980) the amount of unsaturated fatty acids in the PL fraction will increase. These components with increasing percentages can be monounsaturated or polyunsaturated.

### 3.2. Fatty acid composition of the TAG fraction

In the muscle TAG fraction, saturated fatty acids 16:0, monounsaturated 16:1n-7 and 18:1n-9, polyunsaturated EPA and DHA were the most abundant fatty acids in the percentage distribution.

The results show that the percentage of FAs varied according to season, ranging from 29.40

to 33.58% SFAs; 22.22% to 30.83% MUFAs, and 38.3% to 48.33% PUFAs.

n-3/n-6 ratios were found to be 8.53 (January) - 9.37 (July) (Table 4).

Although their rates varied in all periods,  $\Sigma$ PUFA was determined the highest in terms of mean values,  $\Sigma$ SFA followed it and  $\Sigma$ MUFA was determined to be the lowest. It was an interesting finding that  $\Sigma$ PUFA had the highest percentage in the muscle TAG of fish, although they had storage lipids. The reason why there is a large amount of PUFA in the TAG fraction is that the water source is very clean, the oxygen content is high, as well as the fact that the nutrients are rich in EPA and DHA. Dominant PUFA, such as EPA and DHA, is either synthesized from the 18:3n-3 acid obtained from food or obtained directly from the food.

In previous studies, it was observed that the ratio of 16:0 was mostly found in the saturated fatty acids in the muscle TAG fraction of fish, (Kozlova and Khotimchenko, 2000; Görgün *et al.*, 2013).

One of the highest fatty acids in the TAG fraction of fish tissues is 16:1n-7. The ratio of this component in fish collected from the same water source was discovered to be quite different. It was proposed that this component, which is supplied by both food and synthesis, is found in high concentrations in the neutral lipids of freshwater fish.

The high ratio of 18:1n-9 and 16:1n-7 in the TAG fraction, in particular, indicates that these components are used more for energy purposes.

The percentage of polyunsaturated dominant components, EPA and DHA, in the fish muscle TAG fraction varied among species (Görgün *et al.*, 2014; Satar *et al.*, 2012; Kaçar *et al.*, 2016). The percentage of EPA and DHA, which are important n-3 components, was higher in *C. umbla* collected from the Munzur River than in many freshwater fish (Satar *et al.*, 2012; Kaçar *et al.*, 2016).

Henderson and Tocher, (1987) stated that in the TAG fraction of fish, MUFA is the highest, followed by SFA and with PUFA as the least. Many studies have found this same order (Kozlova and Khotimchenko, 2000; Görgün *et al.*, 2013; Görgün *et al.*, 2014).

The reason for the presence of MUFA being the highest and PUFA being the lowest in the TAG fraction of fish is that this fraction is rich in monounsaturated fatty acids such as 18:1n-9 and 16:1n-7 and poor in polyunsaturated fatty acids such as EPA and DHA.

**TABLE 4.** Fatty acid composition in triacylglycerol fraction of the muscle from male *C. umbla* (%)\*

Fatty acids	July	November	January	April
14:0**	1.35±0.07a	2.82±0.12b	1.01±0.08a	2.34±0.15b
15:0	0.16±0.01a	0.27±0.02b	0.27±0.02b	0.24±0.02b
16:0	25.46±1.21a	24.72±1.17a	22.14±1.38a	23.85±1.42a
17:0	0.61±0.03a	0.74±0.03a	0.74±0.03a	0.43±0.02b
18:0	6.0±0.30a	2.67±0.19b	5.24±0.26a	4.48±0.20ab
<b>ΣSFA***</b>	33.58±1.57a	31.22±1.58ab	29.4±1.51b	31.34±1.99ab
16:1n-7	14.28±0.87a	15.2±0.68a	9.85±0.57b	11.6±0.65b
18:1n-9	12.71±0.67a	13.92±0.63a	10.83±0.54b	11.23±0.57ab
20:1n-9	1.03±0.05a	1.71±0.11b	1.54±0.06b	0.62±0.03c
<b>ΣMUFA</b>	28.02±1.10a	30.83±1.52a	22.22±1.08b	23.45±1.27b
18:2n-6	1.90±0.03a	2.30±0.03b	1.61±0.02c	1.94±0.03a
18:3n-3	5.38±0.25a	13.39±0.32b	2.20±0.13c	3.40±0.15c
20:2n-6	0.15±0.01a	0.17±0.02a	0.13±0.01a	0.18±0.02a
20:3n-6	0.21±0.02a	0.20±0.01a	0.30±0.02b	0.21±0.02a
20:4n-6	1.43±0.06a	1.07±0.06a	3.03±0.14b	2.07±0.10ab
20:5n-3	17.09±0.78a	10.49±0.57b	19.63±0.98a	22.08±1.18c
22:5n-3	5.91±0.35a	3.56±0.13b	6.45±0.30a	5.85±0.27a
22:6n-3	6.23±0.28a	6.70±0.31a	14.98±0.62b	9.39±0.42ab
<b>ΣPUFA</b>	38.3±1.82a	37.88±1.69a	48.33±2.24b	45.12±2.12b
<b>Σn-3</b>	34.61±1.52a	34.14±1.78a	43.26±2.28b	40.72±2.04b
<b>Σn-6</b>	3.69±0.19a	3.74±0.12a	5.07±0.24b	4.4±0.17ab
<b>n-3/n-6</b>	9.37	9.12	8.53	9.25

\* Means are the averages of 3 replicates. \*\* Values reported are means ±standard deviation; means followed by different letters in the same line are significantly different ( $p < 0.05$ ) according to Tukey's test. \*\*\* **SFA**: saturated fatty acids, **MUFA**: monounsaturated fatty acids, **PUFA**: polyunsaturated fatty acids

In our study, unlike other fish species in two seasons, the reason why the TAG fraction had the highest PUFA and the lowest MUFA was that the fish were very rich in EPA (10.49-22.08%) and DHA (6.23-14.98%).

In our study, the n-3/n-6 ratio was significantly higher than in other freshwater fish (Satar *et al.*, 2012; Görgün *et al.*, 2013; Görgün *et al.*, 2014). The most important reason for this is that EPA and DHA, the dominant n-3 components in fish, are relatively high in comparison to other fish, while 18:2n-6, ARA, and 20:3n-6 acids, which are among the n-6 fatty acids, are relatively low.

### 3.3. Fatty acid composition of PL classes

In the PC fraction, among the saturated FA, 16:0 was found to be the highest (19.66-29.63%), and

EPA from the unsaturated FA was found to be 18.66-26.32%, and DHA to be 13.06-19.13%.

It was observed that the ratio of 16:0, and accordingly ΣSFA, decreased in July. It was determined that 18:1n-9 from the monounsaturated FA and ΣMUFA, due to this component, decreased in the months representing spring and EPA, DHA and ΣPUFA only slightly increased compared to other months in the same period. The n-3/n-6 ratios were found to be 8.85-10.10 (Table 5).

16:0 was found to be dominant, as in previous studies (Medina *et al.*, 1995; Kızmaz, 2015).

The rate of EPA from twenty-carbon polyunsaturated FA was found to be significantly higher than in previous studies (Lin *et al.*, 2012; Kızmaz, 2015). As previously stated, these Munzur River fish are extremely high in EPA.

**TABLE 5.** Fatty acid composition in phosphatidylcholine of the muscle from male *C. umbla* (%)\*

Fatty acids	July	November	January	April
14:0**	0.28±0.01a	0.44±0.02ab	0.68±0.02b	0.76±0.03b
15:0	0.14±0.01a	0.20±0.01b	0.20±0.02b	0.14±0.01a
16:0	19.66±0.92a	28.69±1.22b	29.63±1.34b	23.92±0.65ab
17:0	0.55±0.03a	0.97±0.05b	0.77±0.03ab	0.66±0.03ab
18:0	3.17±0.13a	2.89±0.15a	2.35±0.19b	2.17±0.10b
<b>ΣSFA***</b>	23.8±1.08a	33.19±1.57b	33.63±1.62b	27.65±1.40a
16:1n-7	4.72±0.23a	4.04±0.24a	5.90±0.27b	4.29±0.21a
18:1n-9	11.94±0.55a	14.06±0.68a	12.09±0.69a	8.48±0.52b
20:1n-9	1.23±0.06a	1.32±0.05a	1.39±0.10a	0.75±0.03b
<b>ΣMUFA</b>	17.89±0.80a	19.42±0.92a	19.38±0.89a	13.52±0.63b
18:2n-6	2.91±0.13a	2.14±0.09a	1.54±0.09b	1.49±0.10b
18:3n-3	1.32±0.06a	3.34±0.14b	1.22±0.11a	1.53±0.10a
20:2n-6	0.21±0.01a	0.19±0.02a	0.14±0.01b	0.18±0.02a
20:3n-6	0.26±0.02a	0.55±0.03b	0.44±0.02b	0.10±0.01c
20:4n-6	2.04±0.14a	1.71±0.08a	2.64±0.10a	3.52±0.15b
20:5n-3	26.32±1.33a	18.66±0.77b	23.29±1.10a	26.11±1.41a
22:5n-3	8.35±0.44a	5.29±0.32ab	4.56±0.25b	6.68±0.36ab
22:6n-3	16.81±0.73a	15.45±0.62a	13.06±0.67b	19.13±0.85c
<b>ΣPUFA</b>	58.22±2.82a	47.33±2.84b	46.89±2.59b	58.74±2.91a
<b>Σn-3</b>	52.8±2.53a	42.74±2.15b	42.13±2.24b	53.45±2.71a
<b>Σn-6</b>	5.42±0.30a	4.59±0.22a	4.76±0.25a	5.29±0.26a
<b>n-3/n-6</b>	9.74	9.31	8.85	10.10

\* Means are the averages of 3 replicates. \*\* Values reported are means ±standard deviation; means followed by different letters in the same line are significantly different ( $p < 0.05$ ) according to Tukey's test. \*\*\* **SFA**: saturated fatty acids, **MUFA**: monounsaturated fatty acids, **PUFA**: polyunsaturated fatty acids

The DHA levels in sea fish were found to be higher than in our study (Medina *et al.*, 1995; Takama *et al.*, 1999). The high content of *C. umbla* with EPA increased the n-3/n-6 ratio in these fish.

In the PC fraction, 16:0 from the saturated FA, 18:1n-9 from the monounsaturated FA, EPA and DHA from the polyunsaturated FA were determined as major components. They were found to be dominant in previously studied sea fish (Takama *et al.*, 1999) and in *Alburnus tarichi* (Kızmaz, 2015).

Previous research has found that PUFA is the most abundant and MUFA is the least abundant. (Lin *et al.*, 2012; Kızmaz, 2015). In our study, we obtained the same results.

Season, environment, nutritional fatty acids, and temperature have all been reported to influence the phospholipid fatty acid composition.

In the PE fatty acid composition, 16:0 from the saturated FA as in the PC fraction, 18:1n-9 from the monounsaturated FA and EPA and DHA from the polyunsaturated FA were determined as dominant. However, these components were found at different ratios from the PC fraction.

The levels of 16:0, 18:1n-9, EPA, DHA, ΣSFA, ΣMUFA and ΣPUFA in the male muscles were found in the range of 7.15 to 22.66%, 5.38 to 14.64%, 12.80 to 18.81%, 17.16 to 27.53%, 12.93 to 36.12%, 9.94 to 20.54%, 43.27 to 69.14%, respectively (Table 6).

In their studies, Medina *et al.* (1995) and Nadcisa *et al.* (2001) identified 18:0 as the dominant saturated fatty acid. In our study, however, the predominant saturated component was 16:0.

In terms of EPA and DHA composition, the results were similar to previous studies (Lie *et al.*, 1992; Medina *et al.*, 1995; Nadcisa *et al.*, 2001).



**TABLE 6.** Fatty acid composition in phosphatidylethanolamine of the muscle from male *C. umbla* (%)\*

Fatty acids	July	November	January	April
14:0**	0.49±0.02a	0.24±0.01b	1.51±0.06c	0.79±0.03ac
15:0	0.03±0.01a	0.04±0.01a	0.27±0.02b	0.09±0.01c
16:0	7.15±0.35a	15.37±0.74b	22.66±1.08c	15.49±0.70b
17:0	0.62±0.03a	1.39±0.06b	0.74±0.03a	1.15±0.05b
18:0	4.64±0.21a	9.64±0.40b	10.94±0.51b	8.92±0.47b
<b>ΣSFA***</b>	12.93±0.64a	26.68±1.25b	36.12±1.69c	26.44±1.24b
16:1n-7	3.49±0.17a	2.55±0.11a	4.37±0.21b	3.06±0.15a
18:1n-9	12.32±0.59a	10.61±0.54a	14.64±0.62a	5.38±0.27b
20:1n-9	2.03±0.10a	1.74±0.08b	1.53±0.06b	1.50±0.05b
<b>ΣMUFA</b>	17.84±0.88a	14.9±0.67b	20.54±0.98c	9.94±0.87d
18:2n-6	1.76±0.06a	1.78±0.05a	1.46±0.06ab	1.18±0.03b
18:3n-3	1.12±0.06a	1.53±0.04b	0.93±0.03a	1.43±0.06b
20:2n-6	0.20±0.01a	0.17±0.01a	0.31±0.02b	0.21±0.01a
20:3n-6	0.33±0.02a	0.30±0.01a	0.31±0.02a	0.32±0.02a
20:4n-6	3.30±0.16a	2.69±0.13a	3.09±0.13a	3.86±0.17a
20:5n-3	18.81±0.76a	17.69±0.69a	12.8±0.60b	18.79±0.92a
22:5n-3	16.09±0.74a	11.44±0.55b	7.21±0.30c	10.2±0.49b
22:6n-3	27.53±1.30a	22.72±1.02b	17.16±0.91c	23.18±1.11b
<b>ΣPUFA</b>	69.14±3.36a	58.32±2.81b	43.27±2.23c	59.17±2.88b
<b>Σn-3</b>	63.55±3.12a	53.38±2.60b	38.1±1.79c	53.6±2.57b
<b>Σn-6</b>	5.59±0.27a	4.94±0.21a	5.17±0.28a	5.57±0.25a
<b>n-3/n-6</b>	11.36	10.80	7.36	9.62

\* Means are the average of 3 replicates. \*\* Values reported are means ±standard deviation; means followed by different letters in same line are significantly different ( $p < 0.05$ ) according to Tukey's test. \*\*\* **SFA**: saturated fatty acids, **MUFA**: monounsaturated fatty acids, **PUFA**: polyunsaturated fatty acids

In other studies, the PUFA content was found to be the highest and MUFA to be the lowest, which is similar to our findings (Lin *et al.*, 2012; Kızmaz, 2015).

As previously stated, n-6 components were relatively low in the fish used in this study, while n-3 polyunsaturated components were excessively high, so the n-3/n-6 ratio was found to be higher than in previous studies (Lin *et al.*, 2012; Kızmaz, 2015).

It was found that 16:0 and ΣSFA ratios increased in January, EPA and ΣPUFA increased in July, but its percentage and n-3/n-6 ratio decreased in January. According to our findings, the amount of muscle PE fatty acid in fish increased and decreased depending on the season.

In the PI fraction, 16:0 and 18:0 from the saturated fatty acids, 18:1n-9 from the monounsaturated,

and ARA, EPA and DHA from the polyunsaturated FA were determined in greater abundance in the percentage distribution (Table 7).

Similar to previous studies (Lie *et al.*, 1992; Medina *et al.*, 1995; Kızmaz 2015), dominant components in PI were found in this study. When compared to the PC and PE classes, the major saturated fatty acid in the PI class was not 16:0, but 18:0, as in other fish species. All these data showed that it was 18:0 in the *sn*-1 position in the PI class.

Another finding from our study was that the ARA ratio found in the PI fraction was significantly higher than the value found in the PC and PE classes. Previously, similar results were discovered in other fish species. Therefore, it was reported that the PI was rich in 18:0 from the saturated and in ARA from the polyunsaturated.

TABLE 7. Fatty acid composition in phosphatidylinositol of the muscle from male *C. umbla* (%)\*

Fatty acids	July	November	January	April
14:0**	0.49±0.02a	0.46±0.03a	0.24±0.01b	0.32±0.01ab
15:0	0.04±0.01a	0.05±0.01a	0.66±0.05b	0.12±0.02c
16:0	8.18±0.34a	8.06±0.29a	8.79±0.32a	11.02±0.56b
17:0	0.59±0.03a	1.34±0.05b	0.74±0.03a	1.45±0.04b
18:0	20.14±1.12a	28.98±1.40a	36.71±1.78b	35.02±1.77b
<b>ΣSFA***</b>	29.44±1.27a	38.89±1.95ab	47.14±2.36b	47.93±2.23b
16:1n-7	4.20±0.18a	1.42±0.05b	0.94±0.04b	1.46±0.05b
18:1n-9	12.77±0.59a	7.92±0.37b	6.25±0.31b	6.24±0.25b
20:1n-9	1.14±0.04a	0.77±0.03b	0.67±0.03b	0.66±0.04b
<b>ΣMUFA</b>	18.11±0.78a	10.11±0.50b	7.86±0.41b	8.36±0.47b
18:2n-6	0.86±0.04a	0.73±0.03a	0.28±0.01b	0.99±0.04a
18:3n-3	1.01±0.05a	0.48±0.02b	0.20±0.01c	0.33±0.02bc
20:2n-6	0.19±0.01a	0.51±0.02b	0.13±0.01a	0.19±0.01a
20:3n-6	3.95±0.14a	0.63±0.03b	0.20±0.01c	0.55±0.02b
20:4n-6	5.03±0.25a	10.54±0.39b	10.1±0.43b	9.47±0.50b
20:5n-3	19.6±1.05a	20.11±1.11b	13.5±0.69c	16.13±0.85ac
22:5n-3	8.35±0.43a	6.48±0.28a	6.49±0.33a	5.28±0.24b
22:6n-3	13.38±0.61a	11.44±0.53b	14.02±0.67a	10.7±0.50b
<b>ΣPUFA</b>	52.37±2.63a	50.92±2.53a	44.92±2.14b	43.64±2.19b
<b>Σn-3</b>	42.34±2.06a	38.51±1.84ab	34.21±1.71b	32.44±1.62b
<b>Σn-6</b>	10.03±0.55a	12.41±0.61a	10.71±0.67a	11.2±0.53a
<b>n-3/n-6</b>	4.22	3.10	3.19	2.89

\* Means are the averages of 3 replicates. \*\* Values reported are means ±standard deviation; means followed by different letters in the same line are significantly different ( $p < 0.05$ ) according to Tukey's test. \*\*\* **SFA:** saturated fatty acids, **MUFA:** monounsaturated fatty acids, **PUFA:** polyunsaturated fatty acids

The reason for the high percentage of ΣSFA in the PI of fish is that this class is rich in the saturated component 18:0. The n-3/n-6 ratio we determined in the PI class of fish in our study was lower than the PC and PE classes.

The high rate of ARA, one of the major n-6 components, is responsible for the low rate of PI subclass in fish.

The ratio of 16:0 acid from the saturated components increased in April along with 18:0, and due to these fatty acids, ΣSFA increased in January and April, 18:1n-9 and ΣMUFA increased in July, ARA and EPA increased in November, DHA increased in January, and the n-3/n-6 ratio increased in July, compared to other periods.

The highest ratio of 16:0 from dominant saturated components was 21.95%, 18:0, 21.01%, 18:1n-9,

16.98%, EPA 9.12%, DHA 10.96%, ΣSFA 47.29%, ΣMUFA 25.22%, ΣPUFA 30.90%, n-3/n-6 4.93 (Table 8).

According to the previous study, PS had the highest percentage of SFA among the classes, and this class contained 16:0 and 18:0 acids in similar proportions, as well as DHA in low proportions. (Medina *et al.*, 1995).

Similar results were found in this study in terms of 16:0 and 18:0 values (16:0 18.67-21.95%, 18:0 15.89-21.01%). Moreover, in our study, the rate of DHA was found to be lower than the PC and PE classes, similar to the previous study (Medina *et al.*, 1995). The percentage of 18:1n-9 increased in April, and EPA and ΣPUFA increased in July.

TABLE 8. Fatty acid composition in phosphatidylserine of the muscle from male *C. umbla* (%)\*

Fatty acids	July	November	January	April
14:0**	2.67±0.06a	1.96±0.07b	1.85±0.04b	2.22±0.10ab
15:0	0.22±0.01a	0.25±0.01a	0.24±0.02a	0.20±0.01a
16:0	20.0±1.04a	20.04±1.10a	18.67±0.81a	21.95±0.94a
17:0	1.55±0.06a	1.49±0.05a	1.30±0.08a	1.91±0.06a
18:0	19.37±0.92a	15.89±0.77b	18.61±0.85a	21.01±1.11a
<b>ΣSFA***</b>	43.81±2.13a	39.63±1.91b	40.67±2.07b	47.29±2.27c
16:1n-7	9.68±0.57a	5.37±0.35b	7.77±0.38ab	6.97±0.30ab
18:1n-9	14.78±0.71a	15.25±0.87a	15.97±0.68a	16.98±0.70a
20:1n-9	0.76±0.04a	1.10±0.06ab	1.16±0.04b	0.92±0.04ab
<b>ΣMUFA</b>	25.22±1.32a	21.72±1.14b	24.9±1.20a	24.87±1.17a
18:2n-6	1.37±0.03a	1.95±0.06b	2.08±0.06b	1.11±0.05a
18:3n-3	0.76±0.03a	1.28±0.05b	1.06±0.05b	0.50±0.04a
20:2n-6	0.23±0.01a	0.53±0.03b	0.25±0.01a	0.67±0.04b
20:3n-6	0.27±0.01a	0.53±0.03b	0.33±0.01a	0.10±0.01a
20:4n-6	3.34±0.13a	2.20±0.10b	3.60±0.16a	3.15±0.11a
20:5n-3	9.12±0.44a	8.62±0.35a	4.42±0.17b	6.26±0.23ab
22:5n-3	5.31±0.28a	5.01±0.20a	7.17±0.37a	6.45±0.30a
22:6n-3	10.5±0.52a	9.26±0.47a	10.96±0.63a	9.52±0.50a
<b>ΣPUFA</b>	30.9±1.40a	29.38±1.26a	29.87±1.33a	27.76±1.30a
<b>Σn-3</b>	25.69±1.13a	24.17±1.19a	23.61±1.10a	22.73±1.66a
<b>Σn-6</b>	5.21±0.26a	5.21±0.18a	6.26±0.23a	5.03±0.27a
<b>n-3/n-6</b>	4.93	4.63	3.77	4.51

\* Means are the average of 3 replicates. \*\* Values reported are means ±standard deviation; means followed by different letters in the same line are significantly different ( $p < 0.05$ ) according to Tukey's test. \*\*\* **SFA**: saturated fatty acids, **MUFA**: monounsaturated fatty acids, **PUFA**: polyunsaturated fatty acids

## CONCLUSIONS

The highest n-3/n-6 ratio found in the fish's total muscle lipid was 10.81. The n-3/n-6 ratio is used to assess the quality of fish oils. The fact that these values are significantly higher than all other freshwater fish studied in Turkey to date indicates that the nutritional value of these Munzur River fish species is very high. The PL and TAG fatty acid compositions of the fish were found to be different. Monounsaturated fatty acids such as 16:1n-7, 18:1n-9, and thus MUFA and 18:2n-6, 18:3n-3 were found to be more abundant in the triacylglycerols. In PL, unsaturated fatty acids such as 18:0, 20:3n-6, ARA and DHA from the saturated FA were higher. TAG and PL fatty acid compositions were found to vary depending on the season, breeding period, and temperature. Furthermore, it was discovered

that different PL classes had different fatty acid compositions based on their biochemical functions.

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## Disclosure statement

No potential conflict of interest was reported by the author(s).

## Ethical approval

All applicable national guidelines for the care and use of animals were followed.

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