

# Seasonal Changes in Fatty Acid Composition of *Chondrostoma regium* Lipids

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This study examined seasonal variations in fatty acid composition of the phospholipid (PL) fraction, triacylglycerol (TAG) fraction, and phospholipid subclass (phosphatidylcholine, PC; phosphatidylinositol, PI; phosphatidylserine, PS; and phosphatidylethanolamine, PE) of muscle tissue of *Chondrostoma regium*, a freshwater fish inhabiting the Munzur River (Turkey). It was found that the percentages of total monounsaturated fatty acids, myristic acid (C14:0), palmitoleic acid (C16:1n7), oleic acid (C18:1n9), linoleic acid (C18:2n6), and linolenic acid (C18:3n3) were higher in TAG fraction than in the PL fraction. The ratio of total polyunsaturated fatty acids to total saturated fatty acids was 1.44–1.85, the atherogenicity index ranged from 0.36 to 0.46, while the thrombogenicity index was determined to be between 0.17 and 0.21 in total lipids. The *n3/n6* ratio ranged from 6.55 to 10.49. The fatty acid levels of the PL and PL subclasses, TAG, and total lipid were influenced by the season. Throughout the year, palmitic acid (C16:0), C18:1n9, eicosapentaenoic acid (C20:5n3, EPA), and docosahexaenoic acid (C22:6n3, DHA) were the most abundant in PC. In PE, the share of *n3* fatty acids decreased from November to April, and percentages of EPA, DHA and docosapentaenoic acid (C20:4n6), and throughout the year, the share of C18:0 was the highest in November. In the PS fraction, the percentages of C16:1n7 and C18:1n9 were high. In summary, *C. regium* can be deemed an excellent source of nutritionally valuable lipids and recommended for wider use in the human diet.

Keywords: fish lipids, lipid fractions, Munzur River, phospholipids, triacylglycerols

# **INTRODUCTION**

Chondrostoma regium (Heckel, 1843), known as "kababurun" in Turkey, is a member of the Cyprinidae family and is distributed in large river systems such as Tigris, Fırat, Seyhan, Ceyhan and Göksu. It is a planktivorous fish that feeds mainly on Bacillariophyta [Tellioğlu et al., 2004]. It is a species of economic importance and is consumed as food by the local people where it is found.

In addition to being an excellent source of protein, this fish is also rich in lipids with polyunsaturated fatty acids (PUFA), especially *n*3 PUFAs, such as docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA) [Ackman, 2002; Biandolino *et* 

al., 2023; Uysal & Aksoylar, 2005]. These acids are not synthesized in the human body and must be provided with diet. Fish are recommended as an excellent source of dietary n3 fatty acids, which are high-energy nutrients, but also their consumption protects against chronic diseases such as cardiovascular diseases [Caffrey et al., 2023], arthritis [Kostoglou-Athanassiou et al., 2020], respiratory disorders [Lemoine et al., 2019], Alzheimer's disease [Canhada et al., 2018] and cancers [D'Eliseo & Velotti, 2016]. In addition, a balanced ratio of n3 to n6 fatty acids in diet is important for health, e.g., an increase in the n6/n3 ratio increases the risk for obesity [Simopoulos, 2016].

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Fatty acids are components of triacylglycerols and phospholipids. The first of them are storage lipids, the content of which in the fish muscle is significantly correlated with total lipid content [Shirai et al., 2002]. In turn, phospholipids, such as phosphatidylcholine (PC), phosphatidylethanolamine (PE), phosphatidylserine (PS) and phosphatidylinositol (PI) are important structural lipids of the cell membranes [Tocher et al., 2008]. Moreover, PC is a substrate in the synthesis of the neurotransmitter, acetylcholine, and has the function of nourishing the brain and improving intelligence [Li et al., 2015]. In turn, PE plays an important role in membrane fusion, whereas PS improves nerve cell function, regulates nerve impulse transmission, and enhances the brain memory. The main acidic phospholipids are found in platelet membranes and are responsible for the coagulation process.

Knowing the FA composition of fish lipids is important when investigating fish biology, such as nutrition, reproduction, adaptation, growth, and development. In addition, it is thought that it would be useful to know the FA composition of *C. regium*, which is consumed by both local community and also populations from the surrounding provinces. Studies have been conducted on the total fatty acid content of *C. regium* [Cengiz *et al.*, 2010; Kaçar *et al.*, 2018]. However, there are no studies on lipid subclasses. Therefore, the aim of this study was to determine the seasonal changes in the fatty acid composition of phospholipids, triacylglycerols, and phospholipid subclasses of *C. regium* and to estimate the nutritional indices of these lipid fractions.

# **MATERIAL AND METHODS**

# Fish collection

Fish (*C. regium*) were collected from Munzur River at a site located in a deep and rocky valley on the Tunceli-Ovacık road (Turkey), approximately 20 km from the city center. The coordinates of this place were 39°10′44.68″N, 39°27′43.08″E. Fish caught in July, November, January, and April were brought to the Dicle University laboratory (Diyarbakır, Turkey) in a cold environment to prevent them from spoiling. Three sexually mature female fish collected in each season were taken for analysis. Their weights and fork lengths were measured, and results are shown in **Table 1**.

# Lipid extraction and fractionation

Five grams of muscle were taken from the region between the lateral line and the dorsal fins of each fish collected in each month. Total lipids were extracted from the tissue with the method of Folch *et al.* [1957] using a mixture of chloroform and methanol (2:1, v/v), and crude extracts were stored at -80°C until analysis. Fractionation of total lipids was performed using thin layer chromatography (TLC). A mixture of petroleum ether, diethyl ether, and acetic acid (80:20:1, v/v/v) was used to run the crude extracts on the plates. The bands corresponding to phospholipid (PL) and triacylglycerol (TAG) fractions established by the standards, were scraped and transferred to tubes. The PL fraction was plated, and the separation of phosphatidylcholine (PC), phosphatidylethanolamine (PE), phosphatidylserine (PS), phosphatidylinositol (PI) was performed using a mixture of chloroform, ethanol, water, and triethylamine (30:35:7:35, v/v/v) [Vaden et al., 2005]. The bands were scraped and individual PL subclasses were washed out with n-hexane.

# Fatty acid analysis

Crude extracts and fractions separated by TLC were heated with methanol (4 mL) and sulfuric acid (4 drops) at 85°C under reflux for 2 h to produce methyl esters of fatty acids. Then, the mixtures were extracted three times with 5 mL of *n*-hexane, and analysis of fatty acid methyl esters was performed using a GC 2010 Plus gas chromatograph with a flame ionization detector (Shimadzu, Kyoto, Japan) and a DB-23 capillary column with (50% cyanopropyl)methylpolysiloxane (30 m  $\times$  0.25 mm i.d.  $\times$  0.25  $\mu$ m film thickness) (J&W Scientific, Folsom, CA, USA). The compressed air and hydrogen were used with the flow rates of 400 mL/min and 30 mL/min, respectively. The flow rate of a carrier gas (helium) was 0.5 mL/min. Temperature of the injection port and the detector were constant at 250°C. The split ratio was 1:20. The oven temperature was set at an initial value of 170°C and held constant for 2 min, and then increased from 170°C to 210°C at a rate of 2°C/min. Total analysis time was 42 min. A mixture of methyl esters of fatty acids (Sigma-Aldrich, Saint Louis, MO, USA) was utilized as a standard in the identification of fatty acids. The GC Solution (version 2.4, Shimadzu) software was utilized to obtain chromatograms of methyl esters of fatty acids and total quantities of fatty acids. Peaks in the chromatogram were identified by comparing the retention times of methyl esters of all fatty acids in the standard. The quantitative values were calculated as percentage of total fatty acids. The samples were examined in triplicate.

#### Nutritional indices calculation

The atherogenic index (AI) and thrombogenic index were (TI) calculated using fish lipid fatty acid data [Biandolino *et al.*, 2023], according to Equations (1) and (2), respectively:

**Table 1.** Weight and length of *Chondrostoma regium* collected in different seasons, and total lipid content of its muscle.

Parameter	July	November	January	April
Length (cm)	25±10 <sup>b</sup>	31±125 <sup>a</sup>	23±10 <sup>b</sup>	24±13 <sup>b</sup>
Weight (g)	162±8.4 <sup>b</sup>	382±14ª	146±4.3 <sup>b</sup>	130±6.5°
Total lipid content (g/100 g)	1.51±0.06 <sup>a</sup>	1.46±0.04 <sup>a</sup>	1.26±0.04 <sup>b</sup>	1.15±0.05 <sup>c</sup>

 $Values \ reported \ are \ means \pm standard \ deviation \ (n=3). \ Means \ followed \ by \ different \ letters \ in the same line \ are \ significantly \ different \ (p<0.05).$ 

$$AI = \frac{4 \times C14:0 + C16:0 + C18:0}{\Sigma PUFA + \Sigma MUFA}$$
 (1)

$$TI = \frac{C14:0 + C16:0 + C18:0}{0.5 \times \Sigma MUFA + 0.5 \times \Sigma n6 + 3 \times \Sigma n3 + \frac{\Sigma n3}{\Sigma n6}}$$
(2)

where:  $\Sigma$ PUFA, total polyunsaturated fatty acids; and  $\Sigma$ MUFA, total monounsaturated fatty acids.

# Statistical analysis

The samples were examined in triplicate. The data were analyzed using one-way analysis of variance (ANOVA), and comparison of means for fish colledted in different seasons was performed using Tukey's test. Differences were determined to be significant at p<0.05. The statistical analyses were carried out using SPSS Statistics 22.0 computer program (IBM, Armonk, NY, USA).

#### **RESULTS AND DISCUSSION**

## Total lipid content

The total lipid content of *C. regium* muscle varied between 1.15 g/100 g (fish caught in April) and 1.51 g/100 g (fish colleted in July) (**Table 1**). These values allow us to consider *C. regium* as a lean fish based on the classification of Ackman [1990], according to which lean fish are those with lipid content less than 2%. In our previous study, we found even a lower total lipid content (0.92%) in female *C. regium* from Atatürk Dam Lake [Kaçar *et al.*, 2018].

The total lipid content of *C. regium* muscle was determined to be significantly (p<0.05) higher in the pre-reproductive period (January) than in the reproductive period (April) (**Table 1**). According to research [Rasoarahona *et al.*, 2008], muscle tissue's lipid content decreases during the reproductive stage, which is consistent with our study findings. The content of storage lipids changes throughout the breeding and feeding periods, as the total lipids have been reported to increase in the winter but decrease in the summer months [Di Lena *et al.*, 2016; Guerra *et al.*, 2022]. In the present study, the highest total lipid content was found in the fish from summer (**Table 1**). Later, it was determined that the total lipid content of *C. regium* decreased towards April, which is the breeding season.

# ■ Fatty acid composition of total lipids

Fatty acid composition of total lipids of *C. regium* collected in different months is shown in **Table 2**. Total saturated fatty acids ( $\Sigma$ SFA) and palmitic acid (main SFA), were found at the lowest levels (26.03% and 18.16% of total FAs, respectively) in April, *i.e.*, in the middle of *C. regium* breeding period. These values started to increase and reached the highest level (32.36% and 24.25% of total FAs for  $\Sigma$ SFA and C16:0, respectively) in January, which was the pre-breeding period. The contribution of total monounsaturated fatty acids ( $\Sigma$ MUFA) and C16:1n7 in total FAs of *C. regium* lipids was low before fish breeding (January) and increased during the breeding period.

The fatty acid with the highest percentage among SFAs was C16:0 (**Table 2**). C18:0 and C14:0 acids were found in smaller amounts. In turn, C18:1*n*9 and C16:1*n*7 acids were found to be dominant among MUFAs, while EPA and DHA were found to be dominant among polyunsaturated fatty acids. The C18:2*n*6, C18:3*n*3, C20:2*n*6, C20:3*n*6, C20:4*n*6 and C22:5*n*3 acids were also identified, but their share in MUFAs of total lipids was lower. The *n*3/*n*6 ratio of total lipids, which is a significant indicator in determining the nutritional value of fish lipids for humans, was found to be between 6.55 to 10.49.

The share of total polyunsaturated fatty acids in FAs of *C. regium* lipids was higher than that reported in literature for other freshwater fish species [Bušová *et al.*, 2020; Emre *et al.*, 2020; Łuczyńska *et al.*, 2012; Tommonaro *et al.*, 2023]. This could be primarily due to water sources. It was expected that fish in cold waters, such as Munzur River, would have greater PUFA levels. Freshwater fish in temperate and warm climates contain less *n*6 fatty acids, because plankton, which fish feed on, inhibits the synthesis of unsaturated fatty acids with a low melting point at a higher temperature, whereas cold and deep-sea fish contain more fatty acids, which melt at a lower temperature [Sushchic *et al.*, 2018].

Previous studies have reported that the content of C16:0 was high in total lipids of *C. regium* caught in Atatürk Dam Lake [Kaçar *et al.*, 2018] and Tigris River [Cengiz *et al.*, 2010]. A similar result was obtained in the present study. Our finding regarding the percentage of C16:1*n*7 in total lipids of *C. regium* was also consistent with literature data [Cengiz *et al.*, 2010; Kaçar *et al.*, 2018].

Fish obtain fatty acids, such as C18:2n6 and C18:3n3, which they cannot synthesize, from their food and use these fatty acids as precursors for the production of other PUFAs. Moreover, fish that generally feed on zooplankton are rich in C18:2n6 and C20:4n6 [Parzanini et al., 2020]. However, in this study, these fatty acids were determined to be present in total lipids of C. regium, feeding on plankton-based diet, in low percentages. This seems to be specific to this species because previous studies have also shown that C. regium lipids contained low levels of C18:2n6 and C18:3n3 [Cengiz et al., 2010; Kaçar et al., 2018]. In the total lipid fraction of C. regium muscle, the percentage of DHA and particularly EPA was found to be higher than those in many other freshwater fish [Bušová et al., 2020; Emre et al., 2020; Haliloğlu et al., 2004; Tommonaro et al., 2023] and in the same species from other fishing locations [Cengiz et al., 2010; Kaçar et al., 2018]. Similarly, the n3/n6 ratio of fatty acids of C. regium total lipids was substantially higher than in many freshwater fish (Sander lucioperca, Pseudophoxinus fahrettini, Capoeta mauricii) [Emre et al., 2020; Uysal & Aksoylar, 2005], and in C. regium from other rivers [Cengiz et al., 2010; Kaçar et al., 2018]. The quality of fish lipids is usually assessed using several indices including, apart from n3/n6 ratio, also  $\Sigma PUFA/\Sigma SFA$  ratio, Al and TI based on fatty acid composition [Biandolino et al., 2023].

The  $\Sigma$ PUFA/ $\Sigma$ SFA ratio in a human diet is recommended to be above 0.45, whereas Al and Tl of foods are recommended to be less than 1.0 and 0.5, respectively [Woloszyn *et al.*, 2020].

Table 2. Fatty acid composition (% of total fatty acids) and nutritional indices of total lipids of muscle of Chondrostoma regium collected in different seasons.

Fatty acid/Nutritional index	July	November	January	April
C14:0	2.96±0.20ª	2.11±0.16 <sup>b</sup>	1.75±0.10 <sup>b</sup>	2.16±0.13 <sup>b</sup>
C15:0	0.32±0.02 <sup>b</sup>	0.48±0.02 <sup>ab</sup>	0.41±0.03 <sup>ab</sup>	0.64±0.03ª
C16:0	18.93±0.85 <sup>b</sup>	20.94±1.02 <sup>b</sup>	24.25±1.25 <sup>a</sup>	18.16±0.74 <sup>b</sup>
C17:0	0.47±0.02 <sup>b</sup>	0.39±0.02 <sup>b</sup>	0.22±0.01 <sup>c</sup>	0.58±0.03ª
C18:0	6.53±0.35 <sup>a</sup>	5.90±0.25 <sup>ab</sup>	5.73±0.28 <sup>ab</sup>	4.49±0.25 <sup>b</sup>
∑SFA	29.21±1.42 <sup>b</sup>	29.82±1.40 <sup>b</sup>	32.36±1.67 <sup>a</sup>	26.03±1.18 <sup>c</sup>
C16:1n7	12.33±0.72 <sup>b</sup>	8.93±0.48°	8.9±0.34 <sup>c</sup>	14.19±0.60ª
C18:1 <i>n</i> 9	12.79±0.58ª	13.12±0.70ª	11.52±0.53ª	11.11±0.56ª
C20:1n9	0.41±0.03 <sup>a</sup>	0.28±0.01 <sup>b</sup>	0.49±0.02ª	0.35±0.01 <sup>ab</sup>
ΣMUFA	25.53±1.36 <sup>a</sup>	22.33±1.22 <sup>ab</sup>	20.91±1.05 <sup>b</sup>	25.65±1.19ª
C18:2n6	1.71±0.06 <sup>b</sup>	1.01±0.04 <sup>c</sup>	0.73±0.03 <sup>c</sup>	2.02±0.11 <sup>a</sup>
C18:3n3	1.02±0.04 <sup>a</sup>	1.03±0.06 <sup>a</sup>	0.61±0.02 <sup>b</sup>	0.80±0.04 <sup>ab</sup>
C20:2n6	0.38±0.02 <sup>b</sup>	0.20±0. <sup>01ab</sup>	0.27±0.01 <sup>ab</sup>	0.51±0.02 <sup>a</sup>
C20:3n6	0.51±0.03 <sup>b</sup>	0.92±0.04ª	0.39±0.02 <sup>b</sup>	0.53±0.02 <sup>b</sup>
C20:4n6	3.38±0.12 <sup>a</sup>	2.45±0.10 <sup>a</sup>	2.67±0.15 <sup>a</sup>	2.93±0.16 <sup>a</sup>
C20:5n3	20.33±1.02 <sup>b</sup>	23.63±1.28ª	20.1±0.92 <sup>b</sup>	20.22±0.87 <sup>b</sup>
C22:5n3	5.52±0.23 <sup>a</sup>	5.61±0.18 <sup>a</sup>	6.04±0.26 <sup>a</sup>	4.38±0.20 <sup>a</sup>
C22:6n3	12.33±0.62 <sup>b</sup>	12.93±0.55 <sup>b</sup>	15.84±0.70 <sup>a</sup>	16.88±0.88ª
∑PUFA	45.18±2.10 <sup>a</sup>	47.78±2.18 <sup>a</sup>	46.65±2.34 <sup>a</sup>	48.27±2.32 <sup>a</sup>
∑n3	39.2±1.76 <sup>b</sup>	43.2±2.16ª	42.59±2.18ª	42.28±1.89 <sup>a</sup>
∑n6	5.98±0.32 <sup>a</sup>	4.58±0.35 <sup>b</sup>	4.06±0.20 <sup>b</sup>	5.99±0.30 <sup>a</sup>
n3/n6	6.55±0.08 <sup>b</sup>	9.43±0.12ª	10.49±0.23ª	7.05±0.11 <sup>b</sup>
∑PUFA/∑SFA	1.55±0.45 <sup>b</sup>	1.60±0.88 <sup>b</sup>	1.44±0.39 <sup>b</sup>	1.85±0.56ª
Al	0.43±0.23 <sup>a</sup>	0.42±0.16 <sup>a</sup>	0.46±0.67 <sup>a</sup>	0.36±0.90 <sup>b</sup>
TI	0.20±0.09 <sup>a</sup>	0.19±0.08ª	0.21±0.01 <sup>a</sup>	0.17±0.22 <sup>b</sup>

Values reported are means  $\pm$  standard deviation (n=3). Means followed by different letters in the same line are significantly different (p<0.05). SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; Al; atherogenicity index; TI, thrombogenicity index.

The values of these indices determined for *C. regium* (**Table 2**) met the above criteria and, therefore, this species of fish can be considered as food eliciting health benefits.

# Fatty acid composition of the triacylglycerol fraction

The triacylglycerol (TAG) fraction separated from *C. regium* muscle was characterized by the share of the  $\Sigma$ SFA in the total fatty acids in the range of 22.75–27.21%, with the lowest level recorded in the fish caught in January (**Table 3**), which was the pre-breeding season, and when water temperature was low. The percentage of C16:0 was determined as 16.80% of total FAs (January) to 19.73% of total FAs (November). Other predominant SFAs were C14:0 and C18:0. The  $\Sigma$ MUFA ranged

from 22.86% to 36.85% of total FAs through the year. The highest contribution of MUFAs to FAs of the TAG fraction was found in the fish collected in April and November, and PUFAs were dominant in the fish from July and January. EPA and DHA among PUFAs, and C16:1*n*7 and C18:1*n*9 among MUFAs, were the most prevalent fatty acids in the TAG fraction of *C. regium*. Previous studies showed that the percentage of the main fatty acid of the fish muscle TAG fraction, C16:0, varied significantly within the species [Kaçar *et al.*, 2018; Kayhan *et al.*, 2015; Satar *et al.*, 2012]. In turn, Shirai *et al.* [2002] reported that C16:0, C16:1*n*7 and C18:1*n*9 were the major fatty acids of TAG of wild and cultured catfish, and concluded that these fatty acids were primarily used for energy production.

**Table 3.** Fatty acid composition (% of total fatty acids) and nutritional indices of the triacylglycerol fraction of muscle of *Chondrostoma regium* collected in different seasons.

Fatty acid/Nutritional index	July	November	January	April
C14:0	1.36±0.07 <sup>b</sup>	3.04±0.12 <sup>a</sup>	1.19±0.08 <sup>b</sup>	4.15±0.20 <sup>a</sup>
C15:0	0.37±0.02 <sup>b</sup>	0.53±0.03 <sup>ab</sup>	0.32±0.02 <sup>b</sup>	0.94±0.03ª
C16:0	18.16±0.62 <sup>a</sup>	19.73±0.85ª	16.8±0.64ª	17.82±0.70 <sup>a</sup>
C17:0	0.91±0.03 <sup>a</sup>	0.34±0.02 <sup>b</sup>	0.12±0.01°	0.38±0.02 <sup>b</sup>
C18:0	3.14±0.10 <sup>b</sup>	3.57±0.13 <sup>b</sup>	4.32±0.15 <sup>a</sup>	2.31±0.09 <sup>c</sup>
∑SFA	23.94±1.28 <sup>b</sup>	27.21±1.52ª	22.75±1.08 <sup>b</sup>	25.6±1.20 <sup>ab</sup>
C16:1n7	10.73±0.52 <sup>c</sup>	17.38±0.70 <sup>b</sup>	13.45±0.62°	20.64±1.01 <sup>a</sup>
C18:1 <i>n</i> 9	11.74±0.58 <sup>b</sup>	15.26±0.61ª	14.65±0.64ª	15.77±0.72 <sup>a</sup>
C20:1 <i>n</i> 9	0.39±0.02 <sup>b</sup>	0.54±0.03 <sup>b</sup>	1.34±0.10 <sup>a</sup>	0.44±0.02 <sup>b</sup>
ΣMUFA	22.86±1.02 <sup>b</sup>	33.18±1.82ª	29.44±1.33 <sup>ab</sup>	36.85±1.80 <sup>a</sup>
C18:2 <i>n</i> 6	2.05±0.06 <sup>b</sup>	2.57±0.09ª	1.98±0.06 <sup>b</sup>	2.33±0.11ª
C18:3n3	0.97±0.04 <sup>b</sup>	1.23±0.05ª	0.71±0.03°	1.17±0.04 <sup>ab</sup>
C20:2n6	0.50±0.02 <sup>a</sup>	0.16±0.01 <sup>b</sup>	0.43±0.02ª	0.29±0.01 <sup>b</sup>
C20:3n6	0.57±0.02 <sup>a</sup>	0.55±0.02°	0.65±0.03ª	0.49±0.02ª
C20:4n6	4.72±0.20 <sup>a</sup>	1.31±0.10 <sup>b</sup>	3.84±0.15ª	1.11±0.09 <sup>b</sup>
C20:5n3	24.04±1.11ª	20.64±0.97 <sup>b</sup>	22.75±1.13 <sup>ab</sup>	20.48±1.03 <sup>b</sup>
C22:5n3	7.34±0.32 <sup>a</sup>	4.70±0.20 <sup>ab</sup>	5.50±0.27 <sup>ab</sup>	3.31±0.19 <sup>b</sup>
C22:6n3	12.93±0.58 <sup>a</sup>	8.39±0.47 <sup>b</sup>	11.86±0.63ª	8.29±0.38 <sup>b</sup>
ΣPUFA	53.12±2.78 <sup>a</sup>	39.55±1.80 <sup>b</sup>	47.72±2.31 <sup>ab</sup>	37.47±1.89 <sup>b</sup>
Σn3	45.28±2.19 <sup>a</sup>	34.96±1.72 <sup>b</sup>	40.82±2.12 <sup>ab</sup>	33.25±1.82 <sup>b</sup>
Ση6	7.84±0.34 <sup>a</sup>	4.59±0.21 <sup>b</sup>	6.90±0.32 <sup>a</sup>	4.22±0.20 <sup>b</sup>
n3/n6	5.77±0.05 <sup>b</sup>	7.61±0.04 <sup>a</sup>	5.91±0.34 <sup>b</sup>	7.87±0.89ª
∑PUFA/∑SFA	2.22±0.32 <sup>a</sup>	1.45±0.48 <sup>b</sup>	2.10±0.09ª	1.46±0.98 <sup>b</sup>
Al	0.31±0.50 <sup>b</sup>	0.44±0.06 <sup>a</sup>	0.28±0.05°	0.46±0.33ª
TI	0.14±0.40 <sup>b</sup>	0.20±0.23 <sup>a</sup>	0.15±0.47 <sup>b</sup>	0.19±0.08 <sup>a</sup>

Values reported are means  $\pm$  standard deviation (n=3). Means followed by different letters in the same line are significantly different (p<0.05). SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; Al, atherogenicity index, TI, thrombogenicity index.

Among the nutritional indices of the TAG fraction, the  $\Sigma$ PUFA/ $\Sigma$ SFA ratio ranged from 1.45 (November) to 2.22 (July), Al was from 0.28 (January) to 0.46 (April) and TI varied between 0.14 (July) and 0.20 (November) (**Table 3**). In turn, the n3/n6 ratio was found to be in the range of 5.77–7.87. Previous research with freshwater fish (*Vimba vimba, Capoeta sieboldu, C. regium*) showed lower values [Görgün *et al.*, 2013, 2014; Kaçar *et al.*, 2018]. This was mainly because the two main n3 fatty acids, EPA and DHA, were highly abundant in the TAG

fraction of *C. regium* in our study, compared to the fish analyzed in other studies, whereas the share of *n*6 fatty acids, C18:2*n*6, C20:4*n*6 and C20:3*n*6, was low.

# Fatty acid composition of the phospholipid fraction and its subclasses

The fatty acid composition of the phospholipid (PL) fraction separated from of *C. regium* muscle is shown in **Table 4**. The share of C16:0 and  $\Sigma$ SFA was the highest in PL fatty acids of the fish

**Table 4.** Fatty acid composition (% of total fatty acids) and nutritional indices of the phospholipid fraction of muscle of *Chondrostoma regium* collected in different seasons.

Fatty acid/Nutritional index	July	November	January	April
C14:0	0.72±0.04 <sup>a</sup>	0.40±0.02 <sup>b</sup>	0.59±0.02 <sup>ab</sup>	0.51±0.03 <sup>ab</sup>
C15:0	0.26±0.01 <sup>b</sup>	0.30±0.02 <sup>a</sup>	0.21±0.02 <sup>b</sup>	0.36±0.02 <sup>a</sup>
C16:0	20.99±1.06 <sup>b</sup>	26.44±1.26 <sup>a</sup>	23.45±1.11 <sup>b</sup>	20.73±1.12 <sup>b</sup>
C17:0	0.04±0.01 <sup>b</sup>	0.55±0.03ª	0.55±0.02ª	0.68±0.05ª
C18:0	8.09±0.30ª	8.79±0.39 <sup>a</sup>	7.23±0.27ª	7.57±0.35ª
∑SFA	30.1±1.58 <sup>b</sup>	36.48±1.63ª	32.03±1.69 <sup>b</sup>	29.85±1.51 <sup>b</sup>
C16:1 <i>n</i> 7	4.90±0.22 <sup>a</sup>	3.37±0.20 <sup>a</sup>	4.66±0.21 <sup>a</sup>	3.93±0.17 <sup>a</sup>
C18:1 <i>n</i> 9	12.15±0.60 <sup>a</sup>	12.16±0.54 <sup>a</sup>	13.35±0.68 <sup>a</sup>	9.78±0.87 <sup>b</sup>
C20:1 <i>n</i> 9	0.30±0.02 <sup>b</sup>	0.59±0.03 <sup>a</sup>	0.64±0.03 <sup>a</sup>	0.31±0.02 <sup>b</sup>
∑MUFA	17.35±0.63ª	16.12±0.55ª	18.65±0.85ª	14.02±0.77ª
C18:2 <i>n</i> 6	1.82±0.11ª	0.65±0.04 <sup>b</sup>	1.47±0.05ª	1.57±0.07ª
C18:3n3	0.62±0.03 <sup>a</sup>	0.45±0.02 <sup>b</sup>	0.53±0.03 <sup>ab</sup>	0.27±0.01°
C20:2 <i>n</i> 6	0.47±0.02 <sup>b</sup>	0.21±0.01 <sup>c</sup>	0.44±0.02 <sup>b</sup>	0.69±0.04ª
C20:3 <i>n</i> 6	0.34±0.01 <sup>b</sup>	0.40±0.02 <sup>a</sup>	0.47±0.02 <sup>a</sup>	0.43±0.01 <sup>a</sup>
C20:4n6	3.15±0.17 <sup>a</sup>	3.62±0.30 <sup>a</sup>	2.92±0.10 <sup>a</sup>	3.92±0.34 <sup>a</sup>
C20:5n3	20.54±1.06 <sup>a</sup>	20.12±1.17ª	19.54±0.90°	20.19±1.03ª
C22:5n3	4.92±0.22 <sup>a</sup>	5.68±0.30 <sup>a</sup>	4.41±0.17 <sup>a</sup>	4.86±0.29ª
C22:6n3	20.62±1.12 <sup>b</sup>	16.19±0.82 <sup>c</sup>	19.46±0.98 <sup>b</sup>	24.12±1.68 <sup>a</sup>
∑PUFA	52.48±2.60 <sup>b</sup>	47.32±2.23 <sup>c</sup>	49.24±2.41°	56.05±2.76ª
∑n3	46.7±2.26 <sup>a</sup>	42.44±2.10 <sup>b</sup>	43.94±2.14 <sup>b</sup>	49.44±2.42ª
∑n6	5.78±0.28 <sup>a</sup>	4.88±0.23 <sup>a</sup>	5.30±0.24 <sup>a</sup>	6.61±0.33 <sup>a</sup>
n3/n6	8.07±0.67 <sup>a</sup>	8.69±0.45 <sup>a</sup>	8.29±0.30 <sup>a</sup>	7.47±0.40 <sup>a</sup>
∑PUFA/∑SFA	1.74±0.34 <sup>a</sup>	1.30±0.32 <sup>c</sup>	1.54±0.42 <sup>b</sup>	1.88±0.58ª
Al	0.34±0.30 <sup>b</sup>	0.44±0.09 <sup>a</sup>	0.38±0.06 <sup>b</sup>	0.32±0.38 <sup>b</sup>
П	0.19±0.40 <sup>b</sup>	0.24±0.20 <sup>a</sup>	0.21±0.11 <sup>a</sup>	0.17±0.07 <sup>b</sup>

Values reported are means  $\pm$  standard deviation (n=3). Means followed by different letters in the same line are significantly different (p<0.05). SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; Al, atherogenicity index, TI, thrombogenicity index.

caught in November, but there were no significant ( $p \ge 0.05$ ) differences in C18:0 content throughout the year (7.23–8.79% of total FAs). The percentage of C18:1n9 was significantly (p < 0.05) lower in the sample from April, although the  $\Sigma$ MUFA did not differ significantly ( $p \ge 0.05$ ) depending on the month of fishing. The contribution of  $\Sigma$ PUFA to PL fatty acids was the higest in the fish caught in April. Throughout all seasons, the percentage of  $\Sigma$ PUFA was higher than  $\Sigma$ MUFA, and EPA and DHA have been determined as the major PUFAs of PLs. During the reproductive season in April, DHA was found at the highest level. In trun, C20:4n6 was detected in low amounts throughout the year.

The main fatty acids found in PLs of *C. regium* muscle were consistent with those found in other freshwater fish [Görgün *et al.*, 2014, Kayhan *et al.*, 2015; Shirai *et al.*, 2002].

The C18:0 was detected at a higher share in PSs than in total lipids (**Table 2** and **4**). Although C18:0 is a saturated fatty acid, it tended to accumulate in the PL fraction. There were no significant ( $p \ge 0.05$ ) differences in EPA content throughout the year (**Table 4**). It is expected that phospholipids, which are structural lipids, are rich in PUFAs.

Henderson & Tocher [1987] reported that the *n*3/*n*6 ratio of the PLs of freshwater fish was between 1.6 and 2.0, whereas

Table 5. Fatty acid composition (% of total fatty acids) and nutritional indices of phosphatidylcholine of muscle of Chondrostoma regium collected in different seasons.

Fatty acid/Nutritional index	July	November	January	April
C14:0	0.40±0.02 <sup>b</sup>	0.42±0.02 <sup>b</sup>	0.72±0.03ª	0.68±0.04ª
C15:0	0.23±0.01 <sup>b</sup>	0.31±0.02 <sup>ab</sup>	0.39±0.02ª	0.40±0.02ª
C16:0	21.95±1.04 <sup>b</sup>	30.53±1.55ª	28.83±1.32ª	25.8±1.34 <sup>ab</sup>
C17:0	0.83±0.04 <sup>a</sup>	0.51±0.02 <sup>b</sup>	0.51±0.02 <sup>b</sup>	0.46±0.03 <sup>b</sup>
C18:0	6.90±0.31 <sup>a</sup>	2.79±0.12 <sup>b</sup>	2.99±0.27 <sup>b</sup>	5.29±0.55ª
∑SFA	30.31±1.58 <sup>a</sup>	34.56±1.60ª	33.44±1.55ª	32.63±1.74 <sup>a</sup>
C16:1 <i>n</i> 7	4.73±0.20 <sup>b</sup>	4.71±0.28 <sup>b</sup>	7.40±0.44 <sup>a</sup>	4.16±0.18 <sup>b</sup>
C18:1 <i>n</i> 9	12.68±0.64ª	13.19±0.55ª	14.1±0.63 <sup>a</sup>	12.81±0.57ª
C20:1 <i>n</i> 9	0.34±0.02 <sup>b</sup>	0.25±0.01 <sup>b</sup>	0.27±0.02 <sup>b</sup>	0.88±0.05ª
∑MUFA	17.75±0.70 <sup>b</sup>	18.15±0.78 <sup>b</sup>	21.77±1.12 <sup>a</sup>	17.85±0.83 <sup>b</sup>
C18:2n6	1.15±0.07 <sup>b</sup>	1.81±0.06 <sup>ab</sup>	1.72±0.10 <sup>ab</sup>	2.57±0.10 <sup>a</sup>
C18:3n3	0.34±0.03 <sup>b</sup>	0.55±0.03 <sup>a</sup>	0.43±0.02 <sup>ab</sup>	0.36±0.02 <sup>b</sup>
C20:2n6	0.54±0.02 <sup>a</sup>	0.14±0.01 <sup>b</sup>	0.13±0.01 <sup>b</sup>	0.31±0.03 <sup>a</sup>
C20:3n6	0.40±0.04ª	0.26±0.02 <sup>b</sup>	0.46±0.02°	0.44±0.03ª
C20:4n6	4.88±0.23ª	2.20±0.16 <sup>b</sup>	2.79±0.14 <sup>b</sup>	2.74±0.17 <sup>b</sup>
C20:5n3	17.63±0.69 <sup>b</sup>	22.32±1.17ª	20.54±1.03 <sup>a</sup>	17.15±0.77 <sup>b</sup>
C22:5n3	6.05±0.32 <sup>a</sup>	4.35±0.24 <sup>b</sup>	3.15±0.30 <sup>b</sup>	4.21±0.21 <sup>b</sup>
C22:6n3	20.88±1.13 <sup>a</sup>	15.58±0.81 <sup>b</sup>	15.48±0.68 <sup>b</sup>	21.66±1.10 <sup>a</sup>
∑PUFA	51.87±2.81 <sup>a</sup>	47.21±2.30 <sup>ab</sup>	44.70±2.42 <sup>b</sup>	49.44±2.49 <sup>ab</sup>
∑n3	44.9±2.21ª	42.8±2.15ª	39.6±2.10 <sup>b</sup>	43.38±2.13ª
∑n6	6.97±0.28 <sup>a</sup>	4.41±0.23b	5.10±0.34 <sup>b</sup>	6.06±0.28 <sup>a</sup>
n3/n6	6.44±0.39°	9.70±0.56ª	7.76±0.35 <sup>b</sup>	7.15±0.22 <sup>b</sup>
∑PUFA/∑SFA	1.71±0.11 <sup>a</sup>	1.37±0.15 <sup>c</sup>	1.34±0.36 <sup>c</sup>	1.52±0.78 <sup>b</sup>
Al	0.34±1.22 <sup>b</sup>	0.49±1.09ª	0.48±1.05 <sup>a</sup>	0.42±0.35ª
П	0.19±0.05 <sup>a</sup>	0.23±0.34ª	0.23±0.25ª	0.21±0.22 <sup>a</sup>

Values reported are means  $\pm$  standard deviation (n=3). Means followed by different letters in the same line are significantly different (p<0.05). SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; Al, atherogenicity index, Tl, thrombogenicity index.

in in our study it was in the range of 7.47–8.69 (**Table 4**). The main cause for the high n3/n6 ratio of the PLs from C. regium muscle was the relatively high levels of EPA, one of the essential n3 fatty acids, and the very low levels of n6 fatty acids. Other nutritional indices of PLs from C. regium muscle ranged from 1.30 to 1.88 for the  $\Sigma PUFA/\Sigma SFA$  ratio, from 0.32 to 0.44 for Al and from 0.17 to 0.24 for Tl.

Considering the fatty acid composition of the phospholipid subclasses, it was observed that the seasonal fatty acid distribution in the phosphatidylcholine (PC) of *C. regium* muscle (**Table 5**) was similar to the fatty acid distribution in the PL fraction (**Table 4**). C16:0, C18:1*n9*, EPA and DHA were found to be

characteristic of the PC (**Table 5**). The fatty acid composition allowed to determine quality indices of PC at the levels of 1.34–1.71 for the  $\Sigma PUFA/\Sigma SFA$  ratio, 0.34–0.49 for Al and 0.19–0.23 for Tl.

The predominant contribution of C16:0, C18:1*n*9, EPA, and DHA in the fatty acids of PC found in our study was consistent with literature data; these fatty acids have been determined to be prevalent in *Alburnus mossulensis* [Kızmaz, 2021], *Myoxocephalus jaok* [Kostetsky *et al.*, 2018], and *Cololabis saira* [Tao *et al.*, 2024]. It has been shown that season, temperature and environment of fish growth, and dietary fatty acids have an effect on the composition of PC fatty acid in fish muscle [Lie *et al.*, 1992a]. For example, it was emphasized that the share of C16:0

**Table 6.** Fatty acid composition (% of total fatty acids) and nutritional indices of phosphatidylethanolamine of muscle of *Chondrostoma regium* collected in different seasons.

Fatty acid/Nutritional index	July	November	January	April
C14:0	0.93±0.03ª	0.13±0.01 <sup>b</sup>	0.48±0.02 <sup>ab</sup>	0.70±0.03 <sup>b</sup>
C15:0	0.09±0.01 <sup>a</sup>	0.05±0.01 <sup>b</sup>	0.10±0.02 <sup>a</sup>	0.09±0.01 <sup>a</sup>
C16:0	8.92±0.37 <sup>b</sup>	13.08±0.60 <sup>a</sup>	14.51±0.63 <sup>a</sup>	12.71±0.57 <sup>a</sup>
C17:0	1.16±0.05 <sup>a</sup>	0.79±0.03 <sup>b</sup>	0.91±0.03 <sup>b</sup>	0.77±0.02 <sup>b</sup>
C18:0	11.09±0.51ª	11.61±0.42 <sup>a</sup>	7.63±0.37 <sup>b</sup>	10.58±0.58 <sup>a</sup>
∑SFA	22.19±1.06 <sup>a</sup>	25.66±1.13 <sup>a</sup>	23.63±1.09ª	24.85±1.15ª
C16:1 <i>n</i> 7	3.43±0.18 <sup>a</sup>	1.33±0.06 <sup>b</sup>	2.90±0.14 <sup>ab</sup>	2.40±0.11 <sup>ab</sup>
C18:1 <i>n</i> 9	8.53±0.33ª	7.82±0.36 <sup>a</sup>	9.11±0.44ª	10.47±0.55 <sup>a</sup>
C20:1 <i>n</i> 9	0.41±0.02 <sup>a</sup>	0.35±0.02 <sup>a</sup>	0.58±0.03 <sup>a</sup>	0.34±0.02 <sup>a</sup>
∑MUFA	12.37±0.58 <sup>a</sup>	9.50±0.49 <sup>b</sup>	12.59±0.63ª	13.21±0.54 <sup>a</sup>
C18:2 <i>n</i> 6	1.04±0.04 <sup>a</sup>	0.54±0.02 <sup>b</sup>	0.80±0.03 <sup>b</sup>	1.16±0.07 <sup>a</sup>
C18:3n3	0.35±0.02ª	0.26±0.01ª	0.35±0.03ª	0.35±0.03 <sup>a</sup>
C20:2 <i>n</i> 6	0.52±0.03 <sup>a</sup>	0.20±0.01 <sup>b</sup>	0.34±0.01 <sup>ab</sup>	0.57±0.03 <sup>a</sup>
C20:3n6	0.56±0.03 <sup>b</sup>	0.53±0.02 <sup>b</sup>	1.13±0.05ª	0.76±0.03 <sup>ab</sup>
C20:4n6	6.02±0.27 <sup>a</sup>	3.68±0.15 <sup>b</sup>	4.64±0.17 <sup>b</sup>	5.58±0.25 <sup>a</sup>
C20:5n3	17.85±0.88 <sup>b</sup>	22.71±1.10 <sup>a</sup>	19.12±0.92 <sup>b</sup>	17.32±0.80 <sup>b</sup>
C22:5n3	9.18±0.43 <sup>b</sup>	11.27±0.58 <sup>a</sup>	7.01±0.38 <sup>b</sup>	7.65±0.33 <sup>b</sup>
C22:6n3	29.84±1.39ª	25.59±1.23 <sup>b</sup>	30.31±1.53ª	28.47±1.37 <sup>a</sup>
∑PUFA	65.36±3.24ª	64.78±3.19ª	63.7±3.22ª	61.86±3.02°
∑n3	57.22±2.21ª	59.83±2.90ª	56.79±2.73ª	53.79±2.55 <sup>b</sup>
∑n6	7.45±0.45°	4.95±0.20 <sup>b</sup>	6.91±0.29 <sup>ab</sup>	8.07±0.37 <sup>a</sup>
n3/n6	7.68±0.56 <sup>c</sup>	12.08±0.30 <sup>a</sup>	8.21±0.40 <sup>b</sup>	6.66±0.32 <sup>d</sup>
∑PUFA/∑SFA	2.95±0.11ª	2.52±0.13 <sup>a</sup>	2.70±0.09ª	2.49±0.65 <sup>a</sup>
Al	0.16±0.08 <sup>b</sup>	0.18±0.04 <sup>b</sup>	0.22±0.02 <sup>a</sup>	0.21±0.18 <sup>a</sup>
П	0.11±0.22 <sup>a</sup>	0.12±0.34 <sup>a</sup>	0.12±0.45 <sup>a</sup>	0.13±0.29 <sup>a</sup>

 $Values \ reported \ are \ means \pm standard \ deviation \ (n=3). \ Means \ followed \ by \ different \ letters in the same line \ are significantly \ different \ (p<0.05). \ SFA, saturated \ fatty \ acids; \ MUFA, monouns \ attracted \ fatty \ acids; \ Al, \ atherogenicity \ index.$ 

and C18:1*n*9 increased in PC fatty acids of *A. mossulensis* in November, while the share of EPA and DHA decreased in the same period [Kızmaz, 2021]. In the present study, it was determined that the percentage of EPA in PC fatty acids was significantly higher in the fish caught in November and January than in those from April and July.

Similar to the PC, C16:0, C18:1n9, EPA and DHA were shown to be predominant in the fatty acid composition of phosphatidyle-thanolamine (PE) of *C. regium* muscle (**Table 6**). However, the nutritional indices of PE differed from those of PC; the  $\Sigma$ PUFA/ $\Sigma$ SFA ratio of PE was found in the range of 2.49 (April) to 2.95 (July); Al was between 0.16 (July) and 0.22 (January) and TI varied from

0.11 (July) to 0.13 (April). The contribution of ∑MUFA to fatty acids of PE of *C. regium* decreased slightly in November. In addition, the *n*3/*n*6 ratio of PE was found to be higher in lipids of the fish caught in November compared to those caught in other periods. Compared to the results obtained for the PL fraction, the fatty acid composition of PE was richer in C18:0, C20:4*n*6, EPA, C22:5*n*3 and DHA. However, the contribution of C16:0 and C18:1*n*9 to PE was found to be lower than to the PL fraction. Compared to *A. mossulensis* [Kızmaz, 2021], *C. regium*, in our analysis, had a higher EPA and a lower C20:4*n*6 contribution to fatty acids of PE. The high proportions of DHA and EPA in the PE of *C. regium* lipids were consistent with results of studies conducted with halibut

Table 7. Fatty acid composition (% of total fatty acids) and nutritional indices of phosphatidylinositol of muscle of Chondrostoma regium collected in different seasons.

Fatty acid/Nutritional index	July	November	January	April
C14:0	1.90±0.06ª	0.15±0.01 <sup>c</sup>	1.05±0.04 <sup>b</sup>	1.90±0.05ª
C15:0	0.17±0.01 <sup>b</sup>	0.06±0.01°	0.19±0.02 <sup>b</sup>	0.24±0.01 <sup>a</sup>
C16:0	22.54±1.08 <sup>a</sup>	8.03±0.32 <sup>c</sup>	15.37±0.68 <sup>b</sup>	15.11±0.73 <sup>b</sup>
C17:0	1.34±0.07 <sup>a</sup>	0.65±0.03 <sup>b</sup>	0.79±0.04 <sup>b</sup>	1.08±0.04 <sup>a</sup>
C18:0	31.62±1.31 <sup>b</sup>	42.53±2.12 <sup>a</sup>	27.07±1.27 <sup>c</sup>	39.65±1.94ª
∑SFA	57.57±2.77ª	51.41±2.25 <sup>ab</sup>	44.47±2.19 <sup>b</sup>	57.98±2.80°
C16:1 <i>n</i> 7	4.40±0.22 <sup>a</sup>	1.0±0.05 <sup>c</sup>	2.30±0.09 <sup>b</sup>	4.48±0.15 <sup>a</sup>
C18:1 <i>n</i> 9	6.85±0.31 <sup>b</sup>	8.51±0.45 <sup>a</sup>	6.83±0.28 <sup>b</sup>	9.97±0.40 <sup>a</sup>
C20:1 <i>n</i> 9	0.45±0.02 <sup>a</sup>	0.30±0.02 <sup>a</sup>	0.15±0.01 <sup>b</sup>	0.38±0.02 <sup>a</sup>
MUFA	11.7±0.54 <sup>b</sup>	9.81±0.37 <sup>b</sup>	9.28±0.45 <sup>b</sup>	14.83±0.67ª
C18:2n6	0.89±0.04 <sup>b</sup>	0.26±0.01°	0.76±0.03 <sup>b</sup>	1.34±0.04ª
C18:3n3	1.32±0.03 <sup>a</sup>	1.65±0.07ª	0.37±0.02 <sup>b</sup>	0.50±0.03b
C20:2n6	0.12±0.01 <sup>b</sup>	0.10±0.01 <sup>b</sup>	0.25±0.02 <sup>a</sup>	0.12±0.01 <sup>b</sup>
C20:3n6	0.41±0.03 <sup>c</sup>	2.15±0.10 <sup>a</sup>	0.38±0.02 <sup>c</sup>	0.63±0.04 <sup>b</sup>
C20:4n6	5.16±0.18 <sup>b</sup>	9.03±0.46 <sup>a</sup>	9.61±0.39 <sup>a</sup>	6.03±0.27 <sup>b</sup>
C20:5n3	11.39±0.59 <sup>b</sup>	15.87±0.68ª	17.21±0.83ª	9.26±0.48 <sup>b</sup>
C22:5n3	3.36±0.11ª	3.0±0.12 <sup>a</sup>	3.72±0.17 <sup>a</sup>	2.32±0.08 <sup>b</sup>
C22:6n3	8.0±0.31 <sup>b</sup>	6.61±0.30 <sup>b</sup>	13.86±0.71ª	6.93±0.26 <sup>b</sup>
<u>P</u> UFA	30.65±1.45 <sup>b</sup>	38.67±1.67 <sup>ab</sup>	46.16±2.24 <sup>a</sup>	27.13±1.23 <sup>b</sup>
<u>7</u> n3	24.07±1.14 <sup>b</sup>	27.13±1.25 <sup>b</sup>	35.16±1.60 <sup>a</sup>	19.01±1.08 <sup>c</sup>
<u>-</u> n6	6.58±0.32 <sup>b</sup>	11.54±0.55ª	11.0±0.56ª	8.12±0.48 <sup>b</sup>
n3/n6	3.65±0.34 <sup>a</sup>	2.35±1.20 <sup>b</sup>	1.05±1.00 <sup>c</sup>	2.34±0.49 <sup>b</sup>
_PUFA/∑SFA	0.53±0.04 <sup>c</sup>	0.75±0.03 <sup>b</sup>	1.04±0.04ª	0.47±0.21°
Al	0.71±0.33 <sup>a</sup>	0.18±0.26 <sup>d</sup>	0.35±0.03 <sup>c</sup>	0.54±0.01 <sup>b</sup>
7	0.66±0.03°	0.54±0.45 <sup>b</sup>	0.37±0.32 <sup>d</sup>	0.80±0.08ª

Values reported are means  $\pm$  standard deviation (n=3). Means followed by different letters in the same line are significantly different (p<0.05). SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; Al, atherogenicity index, TI, thrombogenicity index.

(*Hippoglossus hippoglossus*) [Lie *et al.*, 1992b] and horse mackerel (*Trachurus trachurus*) [Bandarra *et al.*, 2001].

Phosphatidylinositol (PI) isolated from *C. regium* lipids contained mainly SFAs, which accounted for 44.47% to 57.98% of total FAs. The share of PUFAs was 27.13–46.16% of total FAs, and that of MUFAs ranged from 9.28% to 14.83% of total FAs (**Table 7**). The percentages of C16:0 and C18:0 were the lowest in PI of fish from November and January, respectively. Compared to the fatty acid composition of the phospholipid fraction (**Table 4**), contents of C18:0 and C20:4*n*6 were higher, but the share of C18:1*n*9, EPA (except sample from April), and DHA was lower in the PI (**Table 7**). The  $\Sigma$ PUFA/ $\Sigma$ SFA ratio of PI was from 0.47 (April) to 1.04 (January) and AI ranged from 0.18

(November) to 0.71 (July). In turn, TI was found in the range of 0.37 (January) to 0.80 (April).

In general, when the water temperature was low in January, the share of PUFAs in PI was high compared to the other months, while the percentage of the predominant saturated fatty acids, C18:0 and C16:0, was low. The reason for this could be that membrane lipids have adapted to the cold environment. C18:0 and C20:4n6 were characteristics of the PI fraction. The same dominant fatty acids in the PI fraction have been found in previous studies [Lie *et al.*, 1992b; Kızmaz, 2021].

Analysis of the fatty acid composition of phosphatidylserine (PS) of *C. regium* muscle showed that the main FAs were C16:0, C18:0, C16:1n7, C18:1n9, EPA, and DHA (**Table 8**).

Table 8. Fatty acid composition (% of total fatty acids) and nutritional indices of phosphatidylserine of muscle of Chondrostoma regium collected in different seasons.

Fatty acid/Nutritional index	July	November	January	April
C14:0	3.35±0.12 <sup>b</sup>	2.12±0.08 <sup>b</sup>	2.63±0.11 <sup>b</sup>	4.60±0.25 <sup>a</sup>
C15:0	1.22±0.05 <sup>a</sup>	0.40±0.02 <sup>b</sup>	0.23±0.01 <sup>c</sup>	0.38±0.02 <sup>c</sup>
C16:0	18.91±0.74 <sup>b</sup>	20.74±0.92 <sup>b</sup>	17.47±0.60 <sup>b</sup>	24.08±1.22 <sup>a</sup>
C17:0	2.53±0.10 <sup>a</sup>	0.99±0.04 <sup>b</sup>	1.80±0.05 <sup>ab</sup>	1.13±0.05 <sup>ab</sup>
C18:0	20.49±1.07 <sup>a</sup>	7.5±0.35 <sup>b</sup>	6.29±0.28 <sup>b</sup>	9.19±0.42 <sup>b</sup>
∑SFA	46.5±2.14ª	31.75±1.72 <sup>b</sup>	28.42±1.30 <sup>b</sup>	39.38±2.02ab
C16:1n7	13.96±0.54ª	7.70±0.37 <sup>b</sup>	10.89±0.56 <sup>ab</sup>	14.11±0.58ª
C18:1 <i>n</i> 9	16.5±0.70 <sup>b</sup>	15.62±0.63 <sup>b</sup>	23.44±1.10ª	24.5±1.05 <sup>a</sup>
C20:1 <i>n</i> 9	0.43±0.02 <sup>c</sup>	0.30±0.02°	0.64±0.04 <sup>b</sup>	3.62±0.12 <sup>a</sup>
∑MUFA	30.89±1.13 <sup>b</sup>	23.62±1.18°	34.97±1.56 <sup>b</sup>	42.23±2.14 <sup>a</sup>
C18:2n6	1.96±0.04 <sup>b</sup>	4.25±0.19 <sup>a</sup>	3.40±0.15 <sup>ab</sup>	2.58±0.10 <sup>ab</sup>
C18:3n3	0.76±0.03 <sup>b</sup>	1.30±0.05 <sup>ab</sup>	1.19±0.05 <sup>ab</sup>	2.36±0.10 <sup>a</sup>
C20:2n6	0.67±0.03°	0.17±0.01 <sup>b</sup>	0.55±0.03 <sup>a</sup>	0.23±0.01 <sup>b</sup>
C20:3n6	0.24±0.02 <sup>b</sup>	0.70±0.03 <sup>a</sup>	0.64±0.03 <sup>a</sup>	0.21±0.01 <sup>b</sup>
C20:4n6	1.77±0.06 <sup>b</sup>	4.54±0.14 <sup>a</sup>	4.04±0.20 <sup>a</sup>	0.88±0.05 <sup>b</sup>
C20:5n3	7.16±0.35 <sup>b</sup>	10.66±0.54 <sup>a</sup>	9.47±0.47 <sup>a</sup>	5.23±0.30 <sup>b</sup>
C22:5n3	2.96±0.17 <sup>b</sup>	7.53±0.31a	5.37±0.28 <sup>a</sup>	2.01±0.10 <sup>b</sup>
C22:6n3	7.01±0.36 <sup>b</sup>	15.4±0.73ª	11.86±0.52 <sup>ab</sup>	4.82±0.28 <sup>c</sup>
ΣPUFA	22.53±1.13 <sup>b</sup>	44.55±2.10 <sup>a</sup>	36.52±1.72 <sup>ab</sup>	18.32±0.70 <sup>b</sup>
Σn3	17.89±0.74 <sup>b</sup>	34.89±1.55ª	27.89±1.58 <sup>ab</sup>	14.42±0.68 <sup>b</sup>
Σn6	4.64±0.15 <sup>b</sup>	9.66±0.55ª	8.63±0.41 <sup>a</sup>	3.90±0.13 <sup>b</sup>
n3/n6	3.85±0.05 <sup>a</sup>	3.61±0.08 <sup>a</sup>	3.23±0.11 <sup>a</sup>	3.69±0.67 <sup>a</sup>
∑PUFA/∑SFA	0.48±0.04 <sup>b</sup>	1.40±0.55ª	1.29±0.40 <sup>c</sup>	0.47±0.06 <sup>b</sup>
Al	0.60±0.34 <sup>b</sup>	0.43±0.05 <sup>b</sup>	0.39±0.10 <sup>c</sup>	0.70±0.07 <sup>a</sup>
TI	0.57±0.05ª	0.24±0.02 <sup>b</sup>	0.24±0.01 <sup>b</sup>	0.54±0.08ª

Values reported are means  $\pm$  standard deviation (n=3). Means followed by different letters in the same line are significantly different (p<0.05). SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; Al, atherogenicity index.

The contribution of C16:1n7 to total FAs of PS was the lowest in the sample from November (7.70% of total FAs) and the highest in that from April (14.11% of total FAs). Depending on the month of fishing, the  $\Sigma$ PUFAV $\Sigma$ SFA ratio ranged from 0.47 to 1.40, Al varied from 0.39 to 0.70, and TI was found in the range of 0.24 to 0.57. Interestingly, levels of EPA and DHA were lower, while these of C16:1n7 and C18:1n9 were higher in the PS than in the PL (**Table 4** and **8**). In the study conducted with tuna (*Thunnus obesus*), bluefin (*Thunnus thynnus*), bonito (*Sarda sarda*), frigate (*Auxis thazard*), skipjack (*Katsuwonus pelamis*), and yellowfin (*Thunnus albacares*) fishes, PS was the subclass with the highest contribution of SFAs among PC, PE and PI subclasses [Medina *et al.*, 1995].

#### **CONCLUSIONS**

The findings demonstrated the high quality of lipids of *C. regium* because atherogenicity and thrombogenicity indices had low values, indicating no risk to human health. The *C. regium* muscle total lipid fraction was found to have a high *n3/n6* ratio and contain high levels of DHA and EPAs. The nutritional value of the fish species found in the Munzur River was high, as evidenced by the fact that this value was significantly greater than those of any other freshwater fish previously researched in Turkey.

The study results also showed that, in the reproductive period (January), the share of SFAs in total lipids increased and the share of MUFAs decreased compared to the other months. Season had an impact on the TAG, PL and PL subclass fatty acid (SFA, MUFA,

PUFA) levels as well. In summary, the fish of *C. regium* species are excellent sources of high quality, PUFA-rich lipids. As a result, it is stated that the consumption of *C. regium* is recommended for humans.

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#### **CONFLICT OF INTERESTS**

The authors declare no conflict of interest.

# **ADDITIONAL INFORMATION**

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

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