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Seasonal variations in the fatty acid profiles of the liver and muscle of *Squalius cephalus* (Teleostei: Cyprinidae) living in Tödürge Lake (Sivas, Turkiye)

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Abstract

In this study, seasonal variations in the fatty acid profiles of the liver and muscle of *Squalius cephalus* from Tödürge Lake were determined by Gas Chromatography (GC). C22:6 n-3, C20:5 n-3, C20:4 n-6, C18:1 n-9, C18:1 n-7, C16:1 n-7, C18:0 and C16:0 were the principal fatty acids showing the highest levels in the muscle and liver of *Squalius cephalus* in all seasons. Although C22:6 n-3, which is regarded as the most important polyunsaturated fatty acid (PUFA), were found to be high in the spring (17.37%) and summer (20.45%) in the liver, the percentages of this acid changed between 15.10% (winter) and 17.77% (spring) in the muscle. In all seasons, total unsaturated fatty acids (n-3 and n-6 polyunsaturated plus monounsaturated fatty acids) of the muscle tissue were found to be between 60-70%. Based on these results, *S. cephalus* is a good food resource in terms of feeding with unsaturated fatty acids in all seasons, especially C22:6 n-3.

Keywords: *Squalius cephalus*, Season, Fatty acid variations, Tödürge Lake

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Introduction

The low rate of the cardiovascular diseases associated with the consumption of fish in Greenland Eskimos population led to the further investigation of the fatty acids of fish tissues (Varljen *et al*., 2003; Le Nechet *et al*., 2007). Since this starting point, nutritional investigations in terms of the health supporting effects of n-3 form of the PUFAs have been the primary focus (Watkins *et al*., 2003). Within this frame, the beneficial effects of C22:6 n-3 (docosahexaenoic acid; DHA) and C20:5 n-3 (eicosapentaenoic acid; EPA), which are two special n-3 PUFAs under investigation, on human health have been seen not only in heart problems but also cancer pathologies, neurodegenerative and chronic inflammatory diseases, plurimetabolic syndrome, and osteoblast functions (Lauritzen *et al*., 2000; Watkins *et al*., 2003; Lombardo *et al*., 2007).

Due to the lack of DHA and EPA in plant based oils, the best way to take these essential PUFAs is to consume fish and it has been recommended for healthy humans to take 650 mg day^{-1} of EPA/DHA (Holub and Holub, 2004). For these reasons, many studies have focused on the content of DHA and EPA, to a lesser degree ARA and C22:5 n-3 (docosapentaenoic acid; DPA) found in fish tissues. However, the fatty acid patterns of the fish have been showing substantial fluctuations depending on sex, age, food availability, reproduction period, location and season (Akpinar *et al*., 2009). Among these parameters, season affects diet compositions as one of the most important factors, depending on the temperature fluctuations (Szlınder-Rıchert *et al*., 2010). For this reason, many researchers from different countries have focused on the seasonal changes seen in the fatty acid patterns of fish tissues, especially in muscle tissues. Within this context, three tilapia species (*Tilapia rendalli, Oreochromis macrochir* and *O. niloticus*) from Madagascar (Rasoarahona *et al*., 2005), *Sander lucioperca* (Uysal *et al*., 2006), *Thymallus arcticus* (Sushchik *et al*., 2007), *Cyprinus carpio* (Guler *et al*., 2008), *Oncorhynchus mykiss* (Kalyoncu *et al*., 2010), *Engraulis encrasicolus* (Tufan *et al*., 2011) and *Alburnus chalcoides* (Görgün and Akpınar, 2012) are among the species investigated.

Fatty acid composition of lipid depots in fish body might be influenced seasonally in different manners. For example, liver consisting of mainly triacylglycerol is strongly affected by the fatty acid patterns of the diet taken (Lie *et al*., 1986; Dos Santos *et al*., 1993; Nanton *et al*., 2001). Muscle is an important fish part for humans to meet the EPA and DHA needs, by consuming fish (Gokce *et al*., 2004). For this reasons, Vasilyeva *et al*. (2016) investigated the liver and muscle of two fish species from Baikal Lake in terms of lipid and fatty acid composition. Nayak *et al*. (2017) showed the effects of dietary replacement of fish oil by linseed oil on the fatty acid composition and desaturase gene expression of the liver, muscle and intestine in *Puntius gonionotus*. The fatty acid compositions of the muscles of *Capoeta angorae* (Emre *et al*., 2015) and *Capoeta*

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caelestis (Emre *et al*., 2017) have been studied seasonally.

Squalius cephalus is one of the fish species living in Tödürge Lake and this fish species has been consumed by the local folk as a meal. At the same time, as far as we can see, there is no comprehensive data on the fatty acid dynamics of *S. cephalus*, known as *Leuciscus cephalus* previously (Raikova-Petrova *et al*., 2012). For these reasons, the present study focused on the investigation of the fatty acid dynamics of the muscle and liver of *S. cephalus*, depending on the effect of season.

Materials and methods

Sample collection

Mature individuals of *Squalius cephalus* were selected for analyses. Sample collection and preparation studies were the same as a previously published article Görgün and Akpınar (2012) and carried out according to that study. Briefly, lipid and fatty acid analyses were performed with three fish specimens each caught in different seasons. Total lipids were extracted using one gram of the liver and one gram of the muscle taken from underneath the dorsal fin in chloroform/methanol (2/1, v/v). All of the experiments were carried out with three replicates.

Fatty acid analyses

Extraction procedure of the muscle and liver tissues was performed according to Folch *et al*. (1957). The method of Moss *et al*. (1974) was used to obtain saponifiable lipids from total lipids and their corresponding fatty acid methyl esters (FAMEs), using standard BF_3 method. Gas chromatographic analyses of the resultant FAME mixtures were carried out according to Görgün and Akpınar (2012) with a HP Agilent 6890N model gas chromatograph (GC) (Hewlett Packard, Palo Altu, CA, USA), equipped with a flame ionization detector (FID) and a HP-88 capillary column (0.25 µm, 100 m and 0.20 mm i.d) (Agilent Technologies Inc., USA). Each reported result is the average value of three GC analyses. The results are presented as mean±SE. Statistical analyses of the mean were carried out with SPSS 15.0 for windows. The differences between fatty acids data were evaluated by ANOVA using Tukey's comparison test at $p \leq 0.05$ levels.

Results

Seasonal variations in the fatty acid composition of the liver and muscle of *S. cephalus* have been exhibited in Table 1 and 2, respectively. 38 fatty acids were determined in both the muscle and liver tissue of *S. cephalus*, together with qualitative differences, depending on seasons.

It was found that the percentages of total saturated fatty acids (SFAs) were higher in the autumn in both liver (43.38%) and muscle (39.06%). In the SFA fraction, C16:0 (palmitic acid) was defined as the primary fatty acid in both tissues and all seasons. The highest amounts of C16:0 were found to be in autumn as 25.93% for the liver and as 22.56% for the muscle. At the same time, in the winter season, the amounts

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of this acid were in its lowest percentages in both tissues (Tables 1 and 2). C18:0 (stearic acid) was the second fatty acid with high percentages in SFA fraction. C18:0 amounts in the muscle tissue did not show any statistical differences in the summer (6.34%), autumn (5.98%) and winter (5.28%) ($p \geq 0.05$) and had its highest level in the spring (7.97%). However, C18:0 percentages of the liver were between 4.34% (winter) and 8.48% (spring).

Table 1: Seasonal variations of the liver fatty acids of *Squalius cephalus* (%)[A].

Fatty Acids	Spring 12 °C Mean±S.E	Summer 24 °C Mean±S.E	Autumn 19 °C Mean±S.E	Winter 6 °C Mean±S.E
C 8:0[c]	0.02±0.01a[B]	0.04±0.02a	0.02±0.00a	0.02±0.01a
C 10:0	0.06±0.04a	0.05±0.03ab	0.03±0.01ab	0.01±0.00b
C 11:0	0.05±0.04a	0.14±0.01b	0.44±0.06c	0.11±0.01ab
C 12:0	0.24±0.06a	0.13±0.04ab	0.16±0.03ab	0.09±0.01b
C 13:0	0.02±0.01a	0.06±0.02b	0.06±0.00b	0.07±0.04b
C 14:0	1.36±0.10a	1.44±0.03a	2.44±0.30b	3.23±0.44b
C 15:0	0.57±0.24a	0.37±0.02a	0.58±0.10a	0.92±0.10b
C 16:0	19.88±0.32a	19.05±0.54a	25.93±0.53b	17.46±0.27c
C 17:0	1.44±0.14ac	1.65±0.37ab	2.36±0.21b	0.72±0.16c
C 18:0	8.48±0.27a	6.61±0.11b	7.14±0.27b	4.34±0.08b
C 19:0	0.26±0.09a	0.28±0.02a	0.28±0.00a	0.14±0.07b
C 20:0	0.21±0.03a	0.62±0.18b	1.16±0.26c	0.38±0.11ab
C 21:0	0.18±0.15a	0.73±0.11b	0.69±0.12bc	0.35±0.03ac
C 22:0	0.97±0.09a	0.81±0.11a	1.26±0.15b	0.84±0.09a
C 24:0	0.10±0.02a	0.21±0.08a	0.86±0.12b	0.15±0.04a
Σ SFA	**33.83±0.26a**	**32.16±0.31a**	**43.38±0.29b**	**28.80±0.24c**
C 14:1 n-5	0.19±0.06a	0.27±0.02a	0.18±0.11a	0.73±0.09b
C 15:1 n-5	0.10±0.02a	0.20±0.06a	0.28±0.16a	0.55±0.13b
C 16:1 n-7	7.78±0.19a	4.44±0.33b	7.60±0.22a	18.03±0.34c
C 17:1 n-8	1.31±0.31a	0.81±0.16a	1.16±0.42a	1.82±0.20a
C 18:1 n-9	11.18±0.64a	11.71±0.40a	16.61±0.35b	18.79±0.74c
C 18:1 n-7	9.74±0.27a	4.83±0.11b	6.84±0.24c	13.61±0.48d
C 20:1 n-9	0.26±0.02ab	0.74±0.17ac	0.85±0.25c	1.09±0.03c
C 22:1 n-9	0.02±0.01a	0.36±0.11b	0.05±0.02a	0.02±0.00a
C 24:1 n-9	0.02±0.01a	0.05±0.02ab	0.09±0.04b	0.01±0.00a
Σ MUFA	**30.57±0.32a**	**23.39±0.21b**	**33.64±0.24c**	**54.64±0.37d**
C 18:2 n-6	2.71±0.17a	2.04±0.08b	2.48±0.19ac	1.72±0.12b
C 18:3 n-6	0.15±0.02ac	0.24±0.02ab	0.21±0.05ab	0.09±0.02c
C 20:2 n-6	0.07±0.02a	0.15±0.02a	0.60±0.11b	0.25±0.06a
C 20:3 n-6	0.06±0.02a	0.08±0.02a	0.05±0.00a	0.03±0.01a
C 20:4 n-6	5.03±0.01a	6.27±0.15b	3.33±0.21c	2.66±0.13d
C 22:2 n-6	0.02±0.00a	0.02±0.01a	0.01±0.00a	0.02±0.00a
C 22:4 n-6	0.30±0.06a	0.47±0.07ab	0.69±0.11b	0.25±0.08a
C 22:5 n-6	0.16±0.08a	0.27±0.04a	0.17±0.00a	0.13±0.05a
Σ n-6 PUFA	**8.50±0.21a**	**9.50±0.27b**	**7.51±0.33c**	**5.11±0.18d**
C 18:3 n-3	0.84±0.09a	1.54±0.12b	0.73±0.10a	0.64±0.06a
C 20:3 n-3	0.04±0.01a	0.10±0.03b	0.09±0.00b	0.08±0.00ab
C 20:5 n-3	6.00±0.21a	9.32±0.57b	6.13±0.33a	3.39±0.41c

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Table 1 continued:				
C 22:3 n-3	0.04±0.01a	0.11±0.04a	0.07±0.00a	0.66±0.18b
C 22:5 n-3	2.84±0.25a	3.45±0.10b	2.35±0.20a	1.31±0.06c
C 22:6 n-3	17.37±0.56a	20.45±0.31b	6.13±0.18c	5.39±0.23c
Σ n-3 PUFA	**27.10±0.25a**	**34.97±0.23b**	**15.50±0.18c**	**11.47±0.16d**
Σ PUFA	**35.60±0.28a**	**44.47±0.26b**	**23.01±0.22c**	**16.58±0.15d**

[A]: Average of three lots analyzed. [B]: Values reported are means± S.E. [C](a-b-c-d): Values for each sample with different superscript letters in the same fraction are significantly different at $p \leq 0.05$. Σ SFA-total saturated fatty acid; Σ MUFA-total monounsaturated fatty acid; Σ n-6 PUFA-total n-6 polyunsaturated fatty acid; Σ n-3 PUFA-total n-3 polyunsaturated fatty acid, Σ PUFA- total polyunsaturated fatty acid.

Table 2: Seasonal variations of the muscle fatty acids of *Squalius cephalus* (%)[A].

Fatty Acids	Spring Mean±S.E	Summer Mean±S.E	Autumn Mean±S.E	Winter Mean±S.E
C 8:0[C]	0.12±0.02a	0.02±0.00b[B]	0.02±0.00b	0.01±0.00b
C 10:0	0.18±0.04a	0.02±0.00b	0.03±0.01b	0.02±0.00b
C 11:0	0.25±0.02a	0.02±0.00b	0.38±0.05c	0.24±0.04a
C 12:0	0.60±0.12a	0.17±0.04b	0.33±0.07b	0.11±0.02b
C 13:0	0.11±0.02ac	0.03±0.00b	0.06±0.02b	0.07±0.00bc
C 14:0	1.76±0.02a	2.00±0.17a	2.97±0.28b	2.12±0.06a
C 15:0	0.47±0.03	0.57±0.13	0.85±0.25	1.00±0.20
C 16:0	18.22±0.13a	20.33±0.21b	22.56±0.28c	17.09±0.11d
C 17:0	0.61±0.00a	1.05±0.21ab	1.36±0.21b	1.04±0.03ab
C 18:0	7.97±0.56a	6.34±0.32b	5.98±0.27b	5.28±0.17b
C 19:0	0.26±0.02a	0.29±0.01a	0.23±0.03a	0.23±0.01a
C 20:0	0.65±0.08a	0.74±0.01a	1.57±0.32b	0.80±0.09a
C 21:0	0.61±0.06a	1.12±0.16b	1.53±0.07c	0.60±0.06a
C 22:0	1.00±0.12a	0.88±0.22a	0.81±0.11a	1.10±0.12a
C 24:0	0.17±0.01a	0.01±0.00b	0.42±0.17c	0.07±0.02b
Σ SFA	**32.96±0.19a**	**33.58±0.25b**	**39.06±0.22c**	**29.75±0.13d**
C 14:1 n-5	0.23±0.04a	0.16±0.02a	0.14±0.01a	0.69±0.09b
C 15:1 n-5	0.18±0.02ab	0.15±0.07a	0.12±0.00a	0.36±0.05b
C 16:1 n-7	7.41±0.35ac	6.66±0.48a	4.87±0.25b	8.71±0.51c
C 17:1 n-8	0.74±0.02a	0.68±0.11a	0.71±0.01a	1.19±0.21b
C 18:1 n-9	10.71±0.40a	11.32±0.24a	10.96±0.38a	13.33±0.12b
C 18:1 n-7	6.40±0.11a	6.00±0.20a	4.09±0.17b	8.83±0.28c
C 20:1 n-9	0.44±0.02a	0.37±0.02a	0.35±0.09a	0.56±0.10a
C 22:1 n-9	0.07±0.02a	0.09±0.02a	0.04±0.01a	0.04±0.01a
C 24:1 n-9	0.07±0.02a	0.02±0.00a	0.07±0.00a	0.04±0.01a
Σ MUFA	**26.23±0.37a**	**25.42±0.11b**	**21.32±0.10c**	**33.74±0.12d**
C 18:2 n-6	3.60±0.11a	3.44±0.25a	2.85±0.09b	2.44±0.13b
C 18:3 n-6	0.32±0.07a	0.15±0.01b	0.17±0.04b	0.14±0.03b
C 20:2 n-6	0.11±0.02a	0.07±0.01a	0.29±0.08b	0.27±0.04b
C 20:3 n-6	0.11±0.01a	0.05±0.00b	0.08±0.02ab	0.05±0.02b
C 20:4 n-6	3.52±0.05ab	3.78±0.10b	3.30±0.08a	4.45±0.14c
C 22:2 n-6	0.09±0.01a	0.01±0.00b	0.02±0.00b	0.07±0.02c
C 22:4 n-6	0.22±0.03a	0.03±0.01b	0.57±0.04c	0.50±0.06c
C 22:5 n-6	0.39±0.03a	0.18±0.05b	0.19±0.01b	0.24±0.02b
Σ n-6 PUFA	**8.32±0.05a**	**7.68±0.10b**	**7.43±0.07b**	**8.13±0.13a**
C 18:3 n-3	1.25±0.03a	1.21±0.04a	1.28±0.01a	1.04±0.02b
C 20:3 n-3	0.18±0.01ac	0.05±0.00b	0.20±0.05a	0.11±0.01bc
C 20:5 n-3	9.48±0.10a	11.72±0.17b	12.65±0.12c	8.14±0.21d
C 22:3 n-3	0.28±0.04a	0.20±0.06a	0.05±0.02b	0.24±0.03a

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Table 2 continued:				
C 22:5 n-3	3.54±0.08a	3.97±0.21a	2.88±0.11b	3.71±0.14a
C 22:6 n-3	17.77±0.24a	16.14±0.14b	15.16±0.19c	15.10±0.12c
Σ n-3 PUFA	**32.50±0.18a**	**33.31±0.11b**	**32.22±0.10c**	**28.38±0.09d**
Σ PUFA	**40.82±0.16a**	**40.99±0.10a**	**39.65±0.11b**	**36.51±0.13c**

[A]: Average of three lots analyzed. [B]: Values reported are means± S.E. [C](a-b-c-d): Values for each sample with different superscript letters in the same fraction are significantly different at $p \leq 0.05$. Σ SFA-total saturated fatty acid; Σ MUFA-total monounsaturated fatty acid; Σ n-6 PUFA-total n-6 polyunsaturated fatty acid; Σ n-3 PUFA-total n-3 polyunsaturated fatty acid, Σ PUFA- total polyunsaturated fatty acid.

C18:1 n-9 (oleic acid) was identified as the main monounsaturated fatty acid (MUFA) in both tissues investigated of *S. cephalus*. In the liver, C18:1 n-9 amounts showed increases in the autumn (16.61%) and winter (18.79%) ($p \leq 0.05$). Similarly, the highest percentage of this acid in the muscle tissue was found to be in the winter season as 13.33%. The present study showed that other notable compounds of the MUFA fraction were C18:1 n-7 and C16:1 n-7 in both liver and muscle tissues. In general, there were clear increases in the percentages of these acids both in the liver and muscle tissues, together with the winter season. For example, in the liver, the level of C16:1 n-7 in the winter (18.03%) period was almost two times higher than the level determined for the autumn (7.60%) season. The highest total MUFA percentages were determined in the winter for both liver and muscle as 54.64% and 33.74%, respectively. The lowest percentages of total MUFA were found to be in the summer (23.39%) for liver and in the autumn (21.32%) for muscle.

C20:4 n-6 (ARA) was identified as the principal n-6 polyunsaturated fatty acid (PUFA) in both tissues investigated of *S. cephalus*. In the liver, there were significant differences in terms of ARA percentages ($p \leq 0.05$) in all seasons and the highest ARA amount was determined as 6.27% in the summer period. However, the amount of this acid in the muscle tissue changed between 3.30% (autumn) and 4.45% (winter). Other fatty acid of n-6 PUFAs exceeding 1% in the liver and muscle was C18:2 n-6 (oleic acid).

In this study, it was found that the most dominant n-3 form of PUFA was C22:6 n-3 (DHA) and the most radical changes, especially in the liver, were observed in the level of this acid, depending on the season. In the liver, C22:6 n-3 values determined for the spring (17.37%) and summer (20.45%) seasons were higher than those found for the autumn (6.13%) and winter (5.39%) seasons. In the muscle, the percentages of C22:6 n-3 were determined to be 17.77% in spring, 16.14% in summer, 15.16% in autumn, and 15.10% in winter. The second fatty acid with high percentages in n-3 PUFAs fraction was C20:5 n-3 (EPA). The highest percentages of C20:5 n-3 in the liver and muscle were determined in the summer (9.32%) and autumn (12.65%), respectively. In both tissues investigated, during summer and autumn seasons, there were similarities for total n-3 PUFA, EPA and DHA percentages. At the same time, total n-3

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PUFA percentages showed clear decreases in the liver and muscle tissue of *S. cephalus* during the winter season. We also found that total PUFA and C22:6 n-3 levels were high in all seasons investigated in the muscle tissue.

Discussion

Steffens (1997) indicated that C16:0 and C18:0 are the principal compounds of SFA fraction, emphasizing that C16:0 is much more stable than C18:0. Two previous studies conducted on *Oncorhynchus mykiss* (Görgün and Akpinar, 2007) and *Salmo trutta labrax* (Aras *et al*., 2003) showed that C16:0 and C18:0 were the principal SFA elements. These kinds of findings were also determined in marine fish species in Turkish waters (Bayır *et al*., 2006). The present study also reported similar data.

The most dominant fatty acid of MUFAs fraction of both liver and muscle was C18:1 n-9. Studies carried out on the muscles of the carp from different regions exhibited similar findings. For example, Guler *et al*. (2008) determined the seasonal fatty acid composition of the muscle of carp from Beyşehir Lake and found that C18:1 n-9 was the principal MUFA. Kalyoncu *et al*. (2010) investigated the seasonal variations in the muscle fatty acid composition of the carp living in Ivriz Dam Lake and repeated the same data with quantitative differences. Similar findings were found by Fajmonova *et al*. (2003) and Buchotova *et al.* (2010).

Sargent *et al*. (1999) indicated that ARA is an important fatty acid for the growth and reproduction of fish and is the precursor of eicosanoids. Due to these physiological and metabolic statuses of this acid, high levels of ARA might not be stored in the livers and muscles of fish. However, the percentages of EPA, which is a competitive molecule for ARA in the metabolic pathways (Watkins *et al*., 2003; Le *et al*., 2009), were found to be strongly higher than ARA in the spring, summer and autumn in both liver and muscles of *S. cephalus*. At the same time, the percentages of EPA in the muscle were a little bit higher than that of found in the liver. It has been indicated that EPA found in fish tissues is much more beneficial for human health than the effects of ARA. Because when humans ingest EPA and DHA from fish oil, these fatty acids will replace ARA, especially in the cell membranes such as platelets, liver cells, monocytes and neutrophils. This event will reduce the effect of molecules such as prostaglandin E_2, thromboxane A_2, leukotriene B_4 which will arise from ARA (Simopoulos, 1999).

The most radical changes seen in the fatty acid composition of fish include reproduction period, season, and feeding (Görgün and Akpınar, 2012; Szlınder-Rıchert *et al*., 2010). However, it is well documented that fish have the ability to change their fatty acid composition depending on the temperature and it is believed that PUFA levels in fish tissue have increased with the decrease in temperature (Uysal *et al*., 2008). When

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compared with this data, in our study, high percentages of C22:6 n-3 and total PUFA in the spring and summer in the liver might explain the intensive feeding period of *S. cephalus*. From this point of view, the decreases and increases in the total PUFA and C22:6 n-3 amounts seen in different seasons in the present study might be a possible result of the changing food composition, depending on the water temperature changes. The reproduction period of *S. cephalus* from Turkish and Bulgarian Rivers was compared in detail by Raikova-Petrova *et al*. (2012). According to the article of Raikova-Petrova *et al*. (2012), the reproduction period of *S. cephalus* covers the months between May and July from different Turkish water bodies. However, we could not determine clear decreases in the levels of important PUFAs such as ARA, EPA and DHA in the determined reproduction period for the species. This means that the species under investigation also might have intensive n-3 PUFA intake from food in this period. Taking all of these data into account, our study shows that *S. cephalus* from Tödürge Lake is a good food source in terms of feeding with unsaturated fatty acids in all seasons.

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