PAPER

CHANGES IN THE FATTY ACIDS OF NEUTRAL AND POLAR LIPIDS OF *SILURUS GLANIS* AND *BARBUS CAPITO CAPITO* DURING AN ANNUAL CYCLE

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ABSTRACT

Seasonal changes in the fatty acid composition of neutral (NL) and polar lipid (PL) fractions of immature Wels (*Silurus glanis*) and Bulatmai barbel (*Barbus capito capito*) were investigated. The most abundant saturated, monounsaturated, and *n*-3 and *n*-6 polyunsaturated fatty acids (PUFA) for both fractions were palmitic, oleic, docosahexaenoic (DHA), and linoleic acid, respectively. The maximum *n*-3/*n*-6 PUFA ratio and eicosapentaenoic acid (EPA)+DHA content of the NL and PL fractions were found in the hot and cold seasons, respectively. The high *n*-3/*n*-6 ratio and the high EPA+DHA content of the NL fraction might be influenced by the composition of ingested food and that high amount in the PL fraction can be explained by the fact that fish acclimatize to the cold. Finally, these species are recommended for human consumption throughout the year, especially in winter due to the high *n*-3/*n*-6 ratio and the EPA+DHA content of the PL fraction during this season.

- Key words: seasonal changes, fatty acid, neutral lipid, polar lipid, *Silurus glanis*, *Barbus capito capito*, *n*-3/*n*-6 PUFA, EPA+DHA -

INTRODUCTION

Fish oil contains a high percentage of *n*-3 polyunsaturated fatty acids (*n*-3 PUFA), including eicosapentaenoic acid (EPA; 20:5 *n*-3) and docosahexaenoic acid (DHA, 22:6 *n*-3) (HENDERSON and TOCHER, 1987; SHIRAI *et al.*, 2002), that are useful for preventing and treating cardiovascular disease, inflammation, aggression, depression, hypertension, auto-immune disorders and cancer (PIKE *et al.*, 1999). Because EPA and DHA are effectively synthesised by aquatic organisms, humans can obtain these essential components by consuming marine and freshwater products (SUSHCHIK *et al.*, 2007). Fish production in Turkey in 2007 was 772,000 metric tonnes, with a per capita consumption of 8.6 kg per year (ANONYMOUS, 2009), meaning that Turkish people consume about 165 g of fish per week. The Fish Intercommittee Sub-group of the UK Scientific Advisory Committee on Nutrition (UK SACN) suggested that at least two portions of fish (one portion = about 145 g), of which one should be oily, should be eaten weekly to decrease cardiovascular disease risk (UK SACN, 2004). Therefore, the amount of fish consumed by Turkish people does not fulfil the daily requirements of *n*-3 PUFA.

Wels (*Silurus glanis* Linnaeus) is the largest freshwater fish species of Europe and is naturally distributed in large rivers and lakes from central and eastern Europe to the southern part of central Asia. This fish is also present in Turkey's lakes and rivers (GELDİAY and BALIK, 1996). Furthermore, *Silurus glanis* is one of the most popular freshwater fish for human consumption throughout the word and is a good source of PUFA (HWANG *et al.*, 2004). Although the primary distribution areas of Bulatmai barbel (*Barbus capito capito* Güldenstädt) are the Caspian Sea and the Aral Lake basins, this species is also found in the Çoruh and Aras basins in the East Anatolian Region of Turkey (GELDİAY and BALIK, 1996). *Barbus capito capito* is considered to be a potential species for fish farm owing to its tasty flesh and rapid growth rate (ARAS *et al.*, 2009a; YANIK *et al.*, 2009). In Turkey, both *S. glanis* and *B. capito capito* are endangered because of illegal catching methods, human pressure, degradation of spawning habitats and pollution (BAYIR *et al.*, 2011).

The fatty acid composition of fish lipids is extremely variable, even within a species, and depends upon different abiotic and biotic factors such as the season, the type and amount of feed available, the water temperature, the pH, the salinity and the reproduction cycle (SHIRAI *et al.*, 2002; HALİLOĞLU *et al.*, 2005; ARAS *et al.*, 2009a-b). Although seasonal changes of the fatty acid contents of freshwater fish species have been documented in the literature (RASOARAHONA *et al.*, 2005; BAYIR *et al.*, 2010), changes in the fatty acid compositions of *S. glanis* and *B. capito capito* throughout their yearly cycles have never been measured. Therefore, the goal of this study was to determine the fatty acid compositions of neutral and polar lipid fractions of the edible flesh of *Silurus glanis* and *Barbus capito capito* from the East Anatolian Region of Turkey during different seasons.

MATERIAL AND METHODS

Collection of samples

Immature Wels and Bulatmai barbel were caught quarterly between autumn 2006 and summer 2007 from the Aras River, Karakurt, Kars, Turkey (altitude: 1,479 m; latitude: 040°09'36"N; longitude: 042°35'32.4"E). The mean weights of Wels and Bulatmai barbel were 807.0±41.2 g and 363.8±29.2 g in the autumn, 738.8±38.3 g and 373.6±50.8 g in the winter, 778.6±54.5 g and 309.0±40.3 g in the spring and 755.2±47.4 g and 334.4±39.4 g in the summer, respectively. The fish were caught using monofilament gill nets. Five fish of each species in each season were killed with a sharp blow to the head. Muscles excised from the point between the Linea lateral and the dorsal fin, were frozen immediately in liquid nitrogen. Samples were transferred to the laboratory and stored at -84°C until analysis.

Lipid extraction and quantification

The method described by FOLCH *et al.* (1957) was used for lipid extraction. According to this method, samples (*c.* 1 g) were homogenised in chloroform/methanol (2:1 v/v) containing 0.01% (w/v) butylated hydroxytoluene [Sigma, ≥ 99.0% (GC), product number: B1378] as an antioxidant (20 vol. (w/v)) for 1 min. The organic solvent was evaporated under a stream of nitrogen, and the amount of lipid was determined gravimetrically.

Lipid fraction separation

Crude muscle lipids were separated into the polar (phospholipids) and neutral lipid fractions using Sep Pak silica cartridges (Waters, Milford, MA, USA). The residual lipid (*c.* 0.15 g) was applied to a silica filter, and the neutral lipids were eluted with 30 mL chloroform [Sigma, ≥ 99-99.4% (GC), product number: 24216]. After the elution of the neutral lipids, the polar fractions were eluted with 30 mL methanol (Sigma, ≥ 99.9%, product number: 34885). Solvent from both fractions was then evaporated under nitrogen, and the amounts of neutral and polar lipids were determined gravimetrically (CZESNY and DABROWSKI, 1998).

Fatty acid analysis

Fatty acid methyl esters (FAMEs) were prepared from both fractions of lipids according to the method of METCALFE and SCHMITZ (1961). The crude lipid extract was saponified with sodium hydroxide in methanol, and FAMEs were prepared by transmethylation with boron trifluoride in methanol. FAMEs were obtained using an HP (Hewlet Packard) Agilent 6890 N model gas chromatography (GC) equipped with a flame ionisation detector and fitted with a DB 23 capillary column (60 m, 0.25 mm i.d. and 0.25 μm). The ejector and detector temperature program was 190°C for 35 min followed by an increase of 30°C per min up to 220°C; this temperature was maintained for 5 min. The carrier gas was hydrogen (2 mL min^{-1}) and split ratio was 30:1. The individual fatty acids (FAs) were identified by comparing their retention times to those of a standard mix of FAs (Supelco 37 component FAME mix, Cat No: 47885-U), and the FAs were quantified by comparing their peaks to those of the standards (DAVID *et al.*, 2003).

Data analysis

The statistical analyses were performed with SPSS version 10.0 for Windows (SPSS, 1996). Data are presented as the mean ± the standard deviation (SD) of the mean. Data were analysed by one-way analysis of variance (ANOVA). The significant means were compared by Duncan's multiple range tests at $\alpha = 0.05$ level (n=5).

RESULTS AND DISCUSSION

Total lipid, neutral lipid and polar lipid levels

The amounts of total, neutral and polar lipids of wet muscle of immature Wels and Bulatmai barbel for different seasons are presented in Table 1.

We found that the lipid contents (% wet weight of muscle tissues) of the two species were greatest in the winter (Table 1). Total lipids (% w/w) from the muscles of *S. glanis* and *B. capito capito* during the winter were 2.34 and 4.57%, respectively. Additionally, the highest polar lipid amounts were found in the winter and were 1.77 and 1.26%, respectively. However, the highest amounts of neutral lipid were obtained in the summer for *S. glanis* (0.84%) and in the winter for *B. capito capito* (3.31%). It has been reported that the amount of lipids in *Silurus asotus* during the winter was slightly higher than that during the summer, although the difference was not statistically significant (HWANG *et al.*, 2004). Lipids play a major role as regulators of body density when organisms adapt to specific habitat conditions (KOZLOVA and KHOTIMCHENKO, 2000). Phospholipids are a major component of biomembranes. As for other poikilothermic organisms, freshwater fish undergo alterations of the composition and amount of their biomembrane lipids in response to changes in the environmental temperature (HENDERSON and TOCHER, 1987; BAYIR *et al.*, 2010). Thus, the reason behind the high total lipid and polar lipid amounts of *S. glanis* and *B. capito capito* during the cold seasons is likely the response to the low water temperature (winter 4.1°C and autumn 6.2°C).

Fatty acid composition of neutral and polar lipid fractions

Tables 2-5 show the overall average fatty acid compositions of the neutral and polar lipid fractions of wet muscle tissues of *S. glanis* and *B. capito capito* together with the range of individual fatty acids observed.

Twenty-three fatty acids were identified in the neutral and polar lipid fractions of both species. The most abundant saturated (SFA), monounsaturated (MUFA) and *n*-3 and *n*-6 polyunsaturated (*n*-3 and *n*-6 PUFA) fatty acids were palmitic acid (16:0), oleic acid (18:1 *n*-9), DHA (22:6 *n*-3) and linoleic acid (18:2 *n*-6), respectively. These four fatty acids represented 56-63% of neutral lipids and 59-69% of polar lipids for *S. glanis* and 65-71% and 62-66% for *B. capito capito*, respectively. The most abundant polar SFA was palmitic acid; 23-26% for *S. glanis*

Table 1 - Total lipid, neutral lipid and polar lipid contents of *Silurus glanis* and *Barbus capito capito* in different seasons[1].

Species	Component	Autumn	Winter	Spring	Summer
Silurus glanis	Total lipid	2.33±0.25^{a}	2.34±0.06^{a}	1.11±0.19^{c}	1.31±0.41^{b}
	Neutral lipid	0.76±0.09^{a}	0.57±0.04^{b}	0.67±0.11^{ab}	0.84±0.22^{a}
	Phospholipid	1.57±0.19^{a}	1.77±0.04^{a}	0.43±0.15^{b}	0.46±0.27^{b}
Barbus capito capito	Total lipid	2.06±0.28^{b}	4.57±1.09^{a}	1.77±0.16^{c}	1.34±0.74^{d}
	Neutral lipid	0.95±0.12^{c}	3.31±1.19^{a}	1.29±0.20^{b}	0.74±0.05^{c}
	Phospholipid	1.11±0.16^{a}	1.26±0.14^{a}	0.48±0.09^{b}	0.60±0.04^{b}

[1]Values are means of five replicates (n=5) ± standard deviation (SD). Values in the rows with different superscript (a-d) are significantly different ($P < 0.05$).

Table 2 - Fatty acid content of neutral lipids of *Silurus glanis* along the year seasons[1,2].

Fatty acids	Neutral lipids			
	Autumn	Winter	Spring	Summer
14:0	0.35±0.0^{c}	0.44±0.0^{b}	0.57±0.0^{a}	0.35±0.0^{c}
15:0	0.16±0.0^{c}	0.38±0.0^{a}	0.34±0.0^{b}	0.19±0.0^{c}
16:0	17.3±0.2^{c}	20.3±0.2^{a}	19.6±0.3^{b}	17.4±0.0^{c}
17:0	0.65±0.1^{bc}	0.73±0.1^{b}	0.59±0.0^{c}	1.26±0.0^{a}
18:0	3.88±0.1^{d}	4.17±0.0^{c}	6.47±0.3^{a}	4.78±0.0^{b}
20:0	0.36±0.0^{b}	0.39±0.0^{b}	0.35±0.0^{b}	0.57±0.0^{a}
22:0	1.43±0.0^{b}	1.24±0.0^{c}	2.97±0.2^{a}	1.37±0.0^{bc}
24:0	0.23±0.0^{c}	0.50±0.0^{b}	0.77±0.0^{a}	0.21±0.0^{c}
Σ SFA	**22.9±0.2^{d}**	**27.0±0.2^{b}**	**28.7±0.3^{a}**	**24.8±0.1^{c}**
15:1	0.24±0.0^{d}	1.15±0.0^{a}	0.37±0.1^{c}	0.54±0.0^{b}
16:1 n-7	14.1±0.6^{d}	19.5±0.6^{a}	18.5±0.1^{b}	16.1±0.2^{c}
17:1	0.25±0.0^{c}	0.47±0.0^{b}	0.25±0.0^{c}	0.54±0.0^{a}
18:1 n-9	29.5±0.7^{a}	20.7±0.4^{c}	19.5±0.3^{d}	24.0±0.3^{b}
20:1 n-9	6.01±0.3^{a}	3.60±0.2^{d}	4.12±0.4^{c}	5.46±0.2^{b}
22:1 n-9	0.56±0.0^{c}	0.72±0.0^{b}	0.79±0.0^{a}	0.58±0.0^{c}
Σ MUFA	**50.1±0.7^{a}**	**45.5±0.9^{c}**	**42.7±0.4^{d}**	**46.7±0.3^{b}**
18:3 n-3	0.22±0.0^{b}	0.37±0.0^{a}	0.34±0.0^{a}	0.35±0.0^{a}
20:5 n-3	0.54±0.0^{c}	0.74±0.0^{a}	0.60±0.0^{b}	0.56±0.0^{c}
22:5 n-3	0.08±0.0^{c}	0.15±0.0^{c}	1.00±0.1^{a}	0.39±0.0^{b}
22:6 n-3	6.23±0.3^{b}	6.08±0.2^{b}	5.55±0.3^{c}	7.28±0.1^{a}
Σ n-3 PUFA	**7.08±0.4^{b}**	**7.34±0.2^{b}**	**7.50±0.4^{b}**	**8.58±0.1^{a}**
18:2 n-6	16.0±0.5^{a}	13.5±0.3^{c}	14.5±0.2^{b}	13.4±0.3^{c}
20:2 n-6	0.27±0.0^{c}	0.64±0.0^{a}	0.25±0.0^{c}	0.47±0.0^{b}
20:3 n-6	2.05±0.0^{b}	1.69±0.0^{c}	2.45±0.1^{a}	2.60±0.2^{a}
20:4 n-6	0.11±0.0^{d}	0.58±0.0^{c}	0.82±0.0^{a}	0.68±0.0^{b}
22:2 n-6	0.78±0.0^{b}	0.77±0.02	1.06±0.0^{a}	0.81±0.0^{b}
Σ n-6 PUFA	**19.2±0.5^{a}**	**17.2±0.4^{c}**	**19.1±0.2^{a}**	**17.9±0.3^{b}**
Unknown	**-**	**~2.07**	**~4.04**	**~2.25**
Σ PUFA	**26.3±0.62^{a}**	**26.2±0.5^{ab}**	**24.1±0.2^{c}**	**25.5±0.7^{b}**
Σ n-3/n-6 PUFA	**0.37±0.0^{d}**	**0.43±0.0^{b}**	**0.39±0.0^{c}**	**0.48±0.0^{a}**
EPA+DHA	**6.77±0.3^{b}**	**6.82±0.2^{b}**	**6.16±0.3^{c}**	**7.84±0.1^{a}**

[1]Values are expressed as percentages of total fatty acids.
[2]Values are means of five replicates (n=5) ± standard deviation (SD). Values in the rows with different superscript (a-d) are significantly different ($P < 0.05$).

Table 3 - Fatty acid content of polar lipids in the edible portions of immature *Silurus glanis* along the year seasons[1,2].

Fatty acids	Polar lipids			
	Autumn	Winter	Spring	Summer
14:0	0.24±0.0^{ab}	0.22±0.02^{b}	0.25±0.0^{a}	0.25±0.0^{a}
15:0	1.96±0.1^{c}	2.44±0.20^{a}	2.23±0.1^{b}	1.57±0.0^{d}
16:0	23.2±0.2^{b}	26.1±0.70^{a}	23.9±0.8^{b}	25.7±0.6^{a}
17:0	0.21±0.0^{b}	0.18±0.01^{b}	0.30±0.0^{a}	0.29±0.0^{a}
18:0	9.79±0.6^{a}	9.62±0.33^{a}	10.1±0.3^{a}	9.86±0.2^{a}
20:0	0.24±0.0^{b}	0.18±0.01^{c}	0.16±0.0^{c}	0.28±0.0^{a}
22:0	7.33±0.3^{c}	8.16±0.36^{b}	11.1±0.3^{a}	7.20±0.2^{c}
24:0	0.73±0.1^{b}	0.57±0.05^{b}	1.55±0.1^{a}	0.58±0.0^{b}
Σ SFA	**43.7±0.7^{d}**	**47.5±0.28^{b}**	**49.7±0.9^{a}**	**45.7±0.4^{c}**
15:1	0.05±0.0^{c}	0.07±0.01^{b}	0.25±0.0^{a}	0.06±0.0^{bc}
16:1 n-7	5.59±0.3^{b}	5.51±0.22^{b}	7.06±0.1^{a}	5.05±0.1^{c}
17:1	2.48±0.2^{a}	2.38±0.13^{a}	1.50±0.1^{c}	1.88±0.1^{b}
18:1 n-9	13.8±0.4^{a}	10.1±0.25^{b}	13.4±0.9^{a}	13.6±0.6^{a}
20:1 n-9	2.38±0.1^{b}	1.91±0.06^{a}	1.44±0.2^{d}	2.41±0.1^{c}
22:1 n-9	0.96±0.0^{b}	0.83±0.06^{c}	0.80±0.0^{c}	1.11±0.0^{a}
Σ MUFA	**25.2±1.0^{a}**	**20.8±0.28^{c}**	**24.4±0.8^{ab}**	**24.1±0.5^{b}**
18:3 n-3	0.30±0.0^{a}	0.17±0.03^{c}	0.18±0.0^{c}	0.23±0.0^{b}
20:5 n-3	1.33±0.0^{b}	1.50±0.08^{a}	1.03±0.0^{c}	1.12±0.0^{c}
22:5 n-3	0.04±0.0^{b}	0.04±0.00^{b}	0.11±0.0^{a}	0.02±0.0^{c}
22:6 n-3	11.9±0.5^{b}	15.2±0.34^{a}	7.94±0.1^{d}	10.7±0.2^{c}
Σ n-3 PUFA	**13.6±0.4^{b}**	**16.9±0.40^{a}**	**9.26±0.1^{d}**	**12.0±0.2^{c}**
18:2 n-6	13.4±1.0^{a}	11.4±0.59^{b}	11.2±0.2^{b}	13.8±0.4^{a}
20:2 n-6	0.21±0.1^{a}	0.30±0.03^{a}	0.23±0.0^{a}	0.24±0.0^{a}
20:3 n-6	1.45±0.1^{b}	1.09±0.05^{c}	1.89±0.0^{a}	1.81±0.1^{a}
20:4 n-6	1.43±0.2^{ab}	1.01±0.09^{c}	1.32±0.0^{b}	1.57±0.0^{a}
22:2 n-6	0.58±0.0^{a}	0.55±0.03^{a}	0.60±0.0^{a}	0.56±0.0^{a}
Σ n-6 PUFA	**17.0±1.1^{a}**	**14.2±0.72^{c}**	**15.3±0.3^{b}**	**18.0±0.3^{a}**
Σ PUFA	**30.0±0.2^{b}**	**30.6±1.11^{ab}**	**31.5±0.4^{a}**	**24.5±0.4^{c}**
Σ n-3/n-6 PUFA	**0.80±0.0^{b}**	**1.19±0.08^{a}**	**0.60±0.0^{c}**	**0.67±0.0^{c}**
EPA+DHA	**13.2±0.5^{b}**	**16.7±0.38^{a}**	**8.97±0.1^{d}**	**11.8±0.2^{c}**

[1]Values are expressed as percentages of total fatty acids.
[2]Values are means of five replicates (n=5) ± standard deviation (SD). Values in the rows with different superscript (a-d) are significantly different ($P < 0.05$).

(Table 3) and 22-28% for *B. capito capito* (Table 5). Numerous researchers have reported similar results for tilapia species, Bulatmai barbel (total lipids) and brown trout subspecies (RASOARAHONA *et al.*, 2005; ARAS *et al.*, 2009a; BAYIR *et al.*, 2010). It has been suggested that decreasing the water temperature results in a decrease in the 16:0 content (HENDERSON and TOCHER, 1987). However, an inverse result was found in this study except for the neutral lipids of *B. capito capito*; this discrepancy could be explained as a metabolic response of fish to extreme environmental conditions.

The highest and lowest total SFA contents of both fractions in *S. glanis* and *B. capito capito* were found during the hot (spring and summer) and cold seasons (autumn and winter), respectively (Tables 2-5) ($P < 0.05$). The SFA content of the polar lipid fraction was higher than that of the neutral lipid fraction (Tables 2-5). It has been suggested that in most freshwater fish species, the polar lipid fraction contains more PUFA than and similar amounts of SFA as the neutral lipid fraction (HENDERSON and TOCHER, 1987). However, it is also known that water temperature can have a substantial impact on the fatty acid profiles of fish (JANKOWSKA *et al.*, 2008). In this study, because a substantial temperature difference was observed between winter (4.1°C) and summer (20°C), it was assumed that the adaptation of fish to this extreme environmental condition was a possible reason for the higher SFA amounts in the polar lipid fraction than in the neutral lipid fraction. Similarly, in a previous study, we reported that the polar lipid fraction of *Salmo trutta* contained higher SFA levels than the neutral lipid fraction (BAYIR *et al.*, 2010).

As seen in Tables 3 and 5, the highest MUFA levels in the polar lipid fraction were recorded in the autumn (25% for Wels and 23% for Bulatmai barbel). Similarly, the maximum MUFA level in the neutral lipid fraction was also ob-

tained during the cold seasons (in the autumn for Wels (50%) and in the winter for Bulatmai barbel (49%); Tables 2 and 4). Oleic acid was the most abundant fatty acid among MUFAs of both fractions, which is a characteristic property of freshwater fish oils (GÜLER *et al.*, 2008), and the highest level of oleic acid was found during the cold seasons (Tables 2-5) ($P < 0.05$). However, CSENGERI *et al.* (1978) suggested that the low level oleic acid in carp was related to the low level oleic acid in their natural diets, which is a possible explanation for our results. Although the MUFA content in the neutral lipid fraction was higher than that of in the polar lipid fraction, PUFA contents of both fractions were similar for the two species. GUNSTONE *et al.* (1978) reported that the polar lipid fractions of fish contain much lower contents of MUFA than the neutral lipid fraction does. Thus, the findings of our study are in accordance with those published in literature.

There was no harmony in the seasonal fluctuations of the highest total PUFA value of polar lipids. While the maximum total PUFA value was measured in the spring for Wels (31%), the maximum value was observed in the winter for Bulatmai barbel (32%). In contrast, the total PUFA contents of neutral lipids in both species were higher in the autumn than during the other seasons (Tables 2-5) ($P < 0.05$). Additionally, we found that fish have more total PUFA during cold seasons than during hot seasons except for polar lipids in Bulatmai barbel. Similar to our results, FARKAS *et al.* (1980) reported that exposure to cold temperatures for only a few hours was sufficient to increase the PUFA content of the polar lipid fraction of the liver of *Cyprinus carpio*. It is also known that as the temperature decreases, the level of unsaturation tends to increase to assist in maintaining the freezing point below that of the surrounding water to ensure

Table 4 - Fatty acid content of neutral lipids in the edible portions of immature *Barbus capito capito* along the year seasons[1,2].

Fatty acid	Neutral lipids			
	Autumn	Winter	Spring	Summer
14:0	0.30±0.0^{c}	0.45±0.0^{b}	0.90±0.0^{a}	0.30±0.0^{c}
15:0	0.14±0.0^{b}	0.47±0.0^{a}	0.48±0.0^{a}	0.13±0.0^{b}
16:0	11.5±0.6^{b}	12.6±1.4^{b}	17.8±0.8^{a}	11.9±0.6^{b}
17:0	0.24±0.0^{b}	1.58±0.1^{c}	0.77±0.0^{c}	0.24±0.0^{a}
18:0	2.16±0.1^{b}	2.62±0.2^{a}	2.77±0.0^{a}	2.32±0.2^{b}
20:0	0.18±0.0^{d}	0.62±0.0^{a}	0.50±0.0^{b}	0.36±0.0^{c}
22:0	0.95±0.0^{b}	0.83±0.1^{b}	1.32±0.1^{a}	0.85±0.1^{b}
24:0	0.22±0.0^{c}	0.31±0.0^{b}	0.45±0.0^{a}	0.28±0.0^{bc}
Σ SFA	**14.7±0.6^{c}**	**18.4±1.3^{b}**	**23.6±0.9^{a}**	**15.4±0.8^{c}**
15:1	0.24±0.0^{c}	0.43±0.0^{b}	1.06±0.0^{a}	0.16±0.0^{d}
16:1 n-7	7.05±0.4^{c}	12.6±0.9^{b}	15.7±1.0^{a}	5.92±0.4^{d}
17:1	0.16±0.0^{c}	1.13±0.1^{a}	0.29±0.0^{b}	0.16±0.0^{c}
18:1 n-9	25.9±0.5^{ab}	26.4±1.0^{a}	24.9±1.1^{b}	26.5±0.4^{a}
20:1 n-9	12.3±0.4^{b}	8.83±0.9^{c}	4.92±0.3^{d}	14.8±0.4^{a}
22:1 n-9	0.27±0.0^{a}	0.26±0.0^{a}	0.28±0.0^{a}	0.27±0.0^{a}
Σ MUFA	**45.7±0.7^{c}**	**49.4±0.6^{a}**	**7.00±1.6^{bc}**	**47.7±0.7^{b}**
18:3 n-3	1.21±0.0^{a}	0.73±0.0^{c}	0.40±0.0^{d}	0.87±0.0^{b}
20:5 n-3	0.70±0.0^{a}	0.63±0.1^{a}	0.59±0.0^{a}	0.59±0.0^{a}
22:5 n-3	0.05±0.0^{c}	0.26±0.0^{a}	0.27±0.0^{a}	0.09±0.0^{b}
22:6 n-3	2.24±0.1^{b}	5.96±0.3^{a}	6.07±0.4^{a}	1.51±0.0^{c}
Σ n-3 PUFA	**4.19±0.1^{b}**	**7.57±0.2^{a}**	**7.32±0.4^{a}**	**3.05±0.1^{c}**
18:2 n-6	32.1±0.6^{a}	21.2±1.1^{c}	16.2±0.3^{d}	30.4±1.0^{b}
20:2 n-6	0.25±0.0^{b}	0.31±0.0^{a}	0.24±0.0^{b}	0.26±0.0^{b}
20:3 n-6	1.67±0.1^{b}	1.64±0.1^{b}	2.81±0.1^{a}	1.70±0.1^{b}
20:4 n-6	0.52±0.0^{c}	0.65±0.1^{b}	0.78±0.0^{a}	0.51±0.0^{c}
22:2 n-6	0.34±0.0^{a}	0.35±0.0^{a}	0.39±0.0^{a}	0.35±0.0^{a}
Σ n-6 PUFA	**34.8±0.6^{a}**	**24.1±1.2^{c}**	**20.4±0.4^{d}**	**33.2±1.1^{b}**
Σ PUFA	**39.0±0.4^{a}**	**31.7±1.3^{c}**	**27.8±0.8^{d}**	**36.2±1.0^{b}**
Σ n-3/n-6 PUFA	**0.12±0.0^{c}**	**0.31±0.0^{b}**	**0.36±0.0^{a}**	**0.09±0.0^{d}**
EPA+DHA	**2.94±0.1^{b}**	**6.59±0.2^{a}**	**6.66±0.4^{a}**	**2.09±0.1^{c}**

[1]Values are expressed as percentages of total fatty acids.
[2]Values are means of five replicates (n=5) ± standard deviation (SD). Values in the rows with different superscript (a-d) are significantly different ($P < 0.05$).

Table 5 - Fatty acid content of polar lipids in the edible portions of immature *Barbus capito capito* along the year seasons[1,2].

Fatty acid	Polar lipids			
	Autumn	Winter	Spring	Summer
14:0	0.18±0.0^{bc}	0.14±0.0^{c}	0.24±0.0^{b}	0.30±0.0^{a}
15:0	1.73±0.0^{b}	0.88±0.1^{c}	2.55±0.0^{a}	2.50±0.0^{a}
16:0	28.2±0.2^{a}	27.7±1.4^{a}	24.2±0.4^{b}	22.8±0.1^{c}
17:0	0.29±0.0^{b}	0.43±0.0^{a}	0.26±0.0^{b}	0.41±0.0^{a}
18:0	8.46±0.2^{c}	9.42±0.3^{b}	9.54±0.5^{b}	12.3±0.4^{a}
20:0	0.06±0.0^{c}	0.13±0.0^{b}	0.24±0.0^{a}	0.11±0.0^{b}
22:0	9.38±0.0^{c}	7.51±0.2^{d}	10.1±0.2^{b}	11.5±0.1^{a}
24:0	1.66±0.1^{a}	0.21±0.0^{c}	0.16±0.0^{c}	0.54±0.0^{b}
Σ SFA	**50.0±0.1^{a}**	**46.4±1.6^{b}**	**47.4±0.7^{b}**	**50.6±0.2^{a}**
15:1	0.50±0.0^{b}	0.54±0.0^{a}	0.55±0.0^{a}	0.38±0.0^{c}
16:1 n-7	2.77±0.1^{b}	3.66±0.1^{a}	3.68±0.2^{a}	2.21±0.0^{c}
17:1	0.12±0.0^{b}	0.15±0.0^{b}	0.24±0.0^{a}	0.25±0.0^{a}
18:1 n-9	14.1±0.4^{ab}	10.2±0.6^{c}	14.9±1.2^{a}	13.6±0.1^{b}
20:1 n-9	5.46±0.1^{b}	6.00±0.5^{a}	1.56±0.3^{d}	2.58±0.0^{c}
22:1 n-9	0.42±0.0^{c}	0.69±0.0^{a}	0.48±0.0^{b}	0.23±0.0^{d}
Σ MUFA	**23.4±0.6^{a}**	**21.3±0.9^{b}**	**21.4±0.9^{b}**	**19.3±0.1^{c}**
18:3 n-3	0.49±0.0^{a}	0.13±0.0^{c}	0.31±0.0^{b}	0.32±0.0^{b}
20:5 n-3	0.92±0.0^{a}	0.53±0.0^{c}	0.89±0.1^{a}	0.70±0.0^{b}
22:5 n-3	0.02±0.0^{a}	0.007±0.0^{c}	0.03±0.0^{b}	0.006±0.0^{bc}
22:6 n-3	6.55±0.4^{d}	16.9±0.5^{a}	16.0±0.9^{b}	11.2±0.1^{c}
Σ n-3 PUFA	**7.98±0.4^{c}**	**17.6±0.5^{a}**	**17.3±1.0^{a}**	**12.2±0.2^{b}**
18:2 n-6	14.7±0.2^{a}	10.3±0.5^{b}	11.1±0.9^{b}	14.7±0.2^{a}
20:2 n-6	0.75±0.0^{d}	2.30±0.1^{c}	0.30±0.0^{b}	1.01±0.1^{a}
20:3 n-6	1.97±0.0^{b}	0.29±0.0^{a}	0.24±0.0^{b}	0.24±0.0^{c}
20:4 n-6	0.12±0.0^{c}	0.26±0.0^{b}	1.61±0.1^{a}	1.55±0.0^{a}
22:2 n-6	0.56±0.0^{d}	1.39±0.0^{b}	0.35±0.0^{a}	0.16±0.0^{c}
Σ n-6 PUFA	**17.9±0.4^{a}**	**14.6±0.5^{b}**	**13.6±0.9^{c}**	**17.7±0.2^{a}**
Σ PUFA	**25.9±0.6^{c}**	**32.2±0.8^{a}**	**30.9±1.6^{ab}**	**29.9±0.2^{b}**
Σ n-3/n-6 PUFA	**0.44±0.0^{c}**	**1.21±0.0^{a}**	**1.27±0.0^{a}**	**0.69±0.0^{b}**
EPA+DHA	**7.47±0.4^{d}**	**17.4±0.5^{a}**	**16.9±1.0^{b}**	**11.9±0.2^{c}**

[1]Values are expressed as percentages of total fatty acids.
[2]Values are means of five replicates (n=5) ± standard deviation (SD). Values in the rows with different superscript (a-d) are significantly different ($P < 0.05$).

membrane fluidity and general body flexibility (MARTINO and CRUZ, 2004).

The *n*-3/*n*-6 ratio of the polar lipid fraction was higher than that of the neutral lipid fraction (Tables 2-5) ($P < 0.05$). Similarly, BAYIR *et al.* (2010) found that phospholipids have a higher *n*-3/*n*-6 ratio than neutral lipids in female *Salmo trutta.* Whereas the highest *n*-3/*n*-6 ratio of the neutral lipid fraction was observed during the summer for Wels (0.48) and during the spring for Bulatmai barbel (0.36), it was obtained in the winter for the polar lipid fraction of Wels (1.19) and in the spring and winter for Bulatmai barbel (1.27 and 1.21, respectively). Previously, RASOARAHONA *et al.* (2005) and GÜLER *et al.* (2008) suggested that the highest *n*-3/*n*-6 ratio of muscle lipids of tilapia and carp were present in the winter. On the other hand, SHIRAI *et al.* (2001) reported that there was no seasonal variation in the *n*-3/*n*-6 ratio of liver phosphatidylcholine in *Silurus asotus*. Because many factors may be responsible for the variations in the levels of lipids and fatty acids of fish, e.g., internal factors such as genetic variation, age, sex, condition, starvation and reproductive cycle and external factors such as diet, season and temperature (HALİLOĞLU *et al.*, 2004-2005; BAYIR *et al.*, 2006; KWETEGYEKA *et al.*, 2008), the fatty acid composition of fish lipids is extremely variable (SHIRAI *et al.*, 2002; ARAS *et al.*, 2009a-b). This variability may have influenced the results of this study.

The EPA+DHA content of the polar lipid fraction was higher than that of neutral lipid fraction for both species. This result was in accordance with that of PETURSDOTTIR *et al.* (2008), who found that dietary EPA and DHA were present in much higher proportions in the polar lipid fraction than in the neutral lipid fraction of the deep-sea redfish *Sebastes mentella.* In our present study, the highest amounts of EPA+DHA of both species were recorded during the summer for neutral lipids and during the winter for polar lipids ($P < 0.05$). The general assumption is that the fatty acid composition of the neutral storage lipids reflects trophic influences much better than the physiologically more important polar lipids (KWETEGYEKA *et al.*, 2008). The lipids of predatory fish such as *S. glanis* that prey on small fish are expected to be influenced by the fatty acid composition of their prey, which are rich sources of PUFA (HENDERSON and TOCHER, 1987). *B. capito capito* is an omnivorous fish, and its natural food items are invertebrates, algae, detritus, plant material, and small fishes (KOTTELAT and FREYHOF, 2007). The fatty acid composition of *B. capito capito* lipids is also influenced by its nutritional habits. These results indicate that the high EPA+DHA content of neutral lipids in hot seasons is considerably influenced by the composition of the ingested food and that low water temperature is the main reason for the high EPA+DHA content of the polar lipid fraction during the winter.

CONCLUSIONS

We found that the season significantly influences the fatty acid contents of the studied species. However, the *n*-3/*n*-6 ratio and the EPA+DHA content of the polar lipid fraction of edible flesh, which contained a higher amount of PUFA than did the neutral lipid fraction (ACKMAN, 1989), were higher during the winter than during other seasons. In recent years, *n*-3 PUFA has been determined to have greater potency in the amelioration of heart disorders than *n*-6 PUFA. Therefore, the *n*-3/*n*-6 PUFA ratio is a marker of the biomedical relevance of fish oils (HAZRA *et al.*, 1998). The International Society for the Study of Fatty Acids and Lipids advocates 500 mg per day EPA+DHA for cardiovascular health (ISSFAL, 2004). Therefore, we recommend eating about 2.5 portions of *S. glanis* or *B. capito capito* per week throughout the year. Moreover, to investigate in detail the fatty acid metabolism of these economically important and endangered species, controlled aquarium experiments should be conducted.

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