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Seasonal variations of the fatty acid profiles in the edible portions of two freshwater fish species (*Pseudophoxinus fahrettini* and *Capoeta mauricii*)

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Abstract

In this study, seasonal and sexual variations of fatty acid profiles of *Pseudophoxinus fahrettini* and *Capoeta mauricii* which are freshwater fishes in Turkey inland waters were investigated. The monounsaturated fatty acids (MUFAs) values were higher than saturated fatty acids (SFAs) and polyunsaturated fatty acids (PUFAs) values for both species. Also the ratios of MUFAs in *P. fahrettini* were higher than those of *C. mauricii* in all seasons. The results indicated that palmitic acid was the most dominant SFA for both species in all seasons. Oleic acid in the muscle of *P. fahrettini* and palmitoleic acid in the muscle of *C. mauricii* were the highest MUFA respectively in both genders in all seasons. The highest ratios of ω3 PUFAs were 23.00% in the muscle of *C. mauricii* in summer and 18.44% in *P. fahrettini* in winter. The ratios of ω3 PUFAs in muscles of both species and genders were higher than the ratios of ω6 PUFAs during all the seasons. Eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) were significantly higher than the other PUFAs in both sexes of the species. The ratio of ω3 PUFAs to ω6 PUFAs was higher than 1.76 in both species and genders in all seasons.

Keywords: *Pseudophoxinus fahrettini*, *Capoeta mauricii*, Fatty acid, Season, Sex

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Introduction

Fish meat is a vital contributor to the survival and health of the world population (Dhanapal *et al*., 2012). This is due to their rich oil and essential fatty acid content. Essential fatty acids cannot be synthesized in the human body and they must be taken from dietary sources (Maylet *et al*., 2012; Usydus *et al*., 2012). Essential fatty acids are provided by ω3 and ω6 fatty acids which are found abundantly in seafood and vegetable oil (Mahaffey, 2004; Çelik, 2008). All fatty acids, especially essential fatty acids, constitute to a significant part of the human diet by providing a concentrated source of energy and they are substantial compounds in cell membrane structure in addition to their action as prostaglandins, hormones, and other molecules (Doyle, 2004). ω3 fatty acids, particularly eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), are mainly found in fish and fish oil. These fatty acids (EPA and DHA) are used to prevent and treat coronary heart disease, cardiovascular disease, hypertension, diabetes, obesity, prostate cancer, depression, thrombosis, lung disease, and some other diseases but also used for brain development and mental health (Augustsson *et al*., 2003; Holub and Holub, 2004; Vrablik *et al*., 2009; Dhanapal *et al*., 2012).

Due to its importance for human health, ω3 PUFAs capsules obtained from sea products are sold in pharmacies. The commercial fish oil capsules may lose their quality during their processing and conservation. That is why the consumption of fresh fish instead of fish oil capsules is more beneficial for human health. The consumption of fish twice a week is recommended. Thus, it is important to know the oil composition and the ω3 PUFAs quantity of eatable part of fishes as a human nutrient.

Pseudophoxinus fahrettini and *Capoeta mauricii* are freshwater fishes naturally living in central Anatolia, in Turkey. They live in the benthopelagic zone of subtropical waters. Both fishes are omnivorous and feed generally on phytoplankton, benthic algae, aquatic plants, zooplankton and invertebrates (Akın *et al*., 2010). Currently, no studies about the fatty acid composition of *P. fahrettini* and *C. mauricii* have been published. Therefore the aim of this study is to determine the sexual and seasonal variations of muscle fatty acid composition of *P. fahrettini* and *C. mauricii.*

Materials and methods

Fish sampling

The specimens of *P. fahrettini* used in this experiment were caught from Köprü River drainage and *C. mauricii* was caught from Sarıöz River in central Anatolia, in Turkey. Mean weights and lengths of the representative fishes were 27.56 g and 13.07 cm for *P. fahrettini*; 119.51 g and 21.94 cm for *C. mauricii* respectively. A total of 72 fishes (34 *C. mauricii*; 38 *P. fahrettini*) were used in experiments. The fishes were anaesthetized with clove oil and then dorsal muscle samples from each sex were taken and homogenized in a warring blender.

Total lipid extraction

Total lipid extraction was performed according to the Bligh and Dyer method (1959). Methyl esters were prepared by transmethylation using 2M KOH in methanol and hexane according to the method of Ichihara *et al.* (1996). Extracted lipids (10 mg) were dissolved in 2 ml hexane and methylated by 4 ml of 2 M methanolic KOH. After centrifugation at 4000 rpm for 10 min, the hexane layer was used for GC analyses application.

Gas chromatographic analyses

The fatty acid profiles were analyzed by GC using a flame ionization detector and a fused silica capillary column. The oven temperature was 140 ^oC, maintained for 5 min, then raised to 200 ^oC at a rate of 4 ^oC min^{-1} and to 220 ^oC at a rate of 1 ^oC min^{-1}, while the injector and the detector temperatures were set at 220 ^oC and 280 ^oC, respectively. The carrier gas was set at 16 psi. The split used was 1:40. Fatty acids were identified by comparing the retention times of fatty acid methyl ester mixtures.

Statistical analyses

The fatty acid results were given as % percentages±SE. Seasonal changes among the important fatty acids were statistically analyzed using One-way ANOVA test with SPSS Package Programme version 22. Variations between species and sexes were determined using the Student t-Test.

Results

Seasonal variations of fatty acid ratios in muscles of females and males are shown in Table 1 and 2 for *P. fahrettini* and *C. mauricii* respectively. In this study, the levels of total SFAs in muscle of *P. fahrettini* changed from 24.01% to 27.04% in female and from 24.27 % to 28.45% in male (Table 1). The ratios of SFAs in the muscle of *C. mauricii* reached the highest levels of 28.53% in female and 28.22% in males in summer and the lowest levels of 24.03% in females and 23.82% in males were observed in spring and in winter, respectively (Table 2).

Table 1: Seasonal and sexual variations of the fatty acid ratios in muscle of *Pseudophoxinus fahrettini* (% of total fatty acids).

Fatty Acids	Spring		Summer		Autumn		Winter	
	male	female	male	female	male	female	male	female
C12:0	0.48±0.07	0.38±0.07	0.39±0.15	0.45±0.09	0.87±0.11	0.45±0.05	0.43±0.18	0.32±0.09
C14:0	2.11±0.32	1.89±0.18	2.48±0.06	2.42±0.07	2.88±0.12	2.21±0.09	2.43±0.16	2.09±0.45
C15:0	0.31±0.01	0.30±0.05	0.28±0.02	0.31±0.01	0.24±0.01	0.23±0.02	0.27±0.02	0.26±0.02
C16:0	17.28±0.71	16.71±0.47	18.79±0.82	17.41±0.36	17.72±0.29	16.61±0.28	15.03±0.53	16.41±0.21
C17:0	0.36±0.25	0.48±0.05	0.33±0.02	0.41±0.03	0.26±0.02	0.29±0.02	0.42±0.06	0.39±0.05
C18:0	5.01±1.27	6.33±0.68	3.53±0.17	3.73±0.17	3.36±0.08	3.45±0.17	3.5±0.12	3.99±0.25
C20:0	0.24±0.00	0.27±0.05	0.22±0.02	0.23±0.02	0.20±0.00	0.22±0.01	0.20±0.00	0.21±0.01
C21:0	0.58±0.06	0.69±0.03	0.52±0.05	0.55±0.03	0.6±0.03	0.55±0.03	0.51±0.04	0.60±0.04
C22:0	0.06±0.00	-	0.06±0.00	0.05±0.01	-	0.03±0.00	-	0.06±0.00
∑ SFA	**26.41±1.4**	**27.04±0.9**	**26.58±0.7**	**25.56±0.5**	**26.14±0.4**	**24.01±0.3**	**22.82 ±0.6**	**24.30 ±0.2**
C14:1	0.12±0.01	0.16±0.03	0.12±0.01	0.11±0.01	0.13±0.00	0.12±0.00	0.15±0.01	0.11±0.01
C15:1	0.10±0.00	0.10±0.03	0.11±0.03	0.08±0.03	-	-	0.07±0.00	0.17±0.03
C16:1	9.68±1.98	8.16±0.82	12.62±0.65	11.64±0.29	13.05±0.22	11.99±0.23	11.86±0.26	10.14±0.63
C17:1	0.37±0.14	0.32±0.04	0.31±0.05	0.33±0.01	0.37±0.03	0.28±0.02	0.37±0.02	0.32±0.04
C18:1ω9	22.11±2.75	20.92±1.27	22.01±0.79	21.70±0.78	19.68±0.67	20.29±1.12	18.57±0.26	18.51±1.05
C18:1ω7	5.38±0.39	6.35±0.13	5.5±0.41	5.94±0.28	5.29±0.16	6.28±0.30	5.97±0.23	5.92±0.14
C20:1	0.93±0.13	1.03±0.07	0.75±0.06	0.76±0.05	0.72±0.03	0.59±0.03	0.61±0.02	0.86±0.04
C22:1ω9	0.04±0.00	-	0.02±0.00	0.04±0.01	0.04±0.01	0.04±0.01	0.03±0.00	0.06±0.02
∑MUFA	**38.64 ±5.1**	**36.99 ±2.1**	**41.41 ±0.7**	**40.56 ±0.4**	**39.25 ±0.6**	**39.56 ±1.1**	**37.57 ±0.3**	**36.04 ±1.5**
C18:2ω6	4.99±0.61	3.59±0.25	3.70±0.66	5.16±0.60	3.00±0.32	3.43±0.28	3.54±0.16	3.27±0.15
C18:3ω3	2.76±0.30	3.27±0.47	4.05±0.15	4.22±0.19	4.41±0.32	4.20±0.29	3.41±0.45	3.26±0.55
C20:3ω6	0.85±0.26	0.62±0.16	0.39±0.15	0.39±0.10	0.13±0.08	0.33±0.08	0.49±0.10	0.81±0.12
C20:4ω6	0.30±0.00	0.30±0.02	0.25±0.02	0.27±0.02	0.30±0.02	0.28±0.03	0.42±0.04	0.38±0.03
C20:2ω6	2.33±1.13	2.58±0.46	1.27±0.18	1.32±0.07	1.58±0.99	1.74±0.10	1.80±0.10	2.82±0.42
C20:5ω3	6.04±1.02	6.21±0.46	6.83±0.66	6.67±0.30	8.09±0.27	8.68±0.31	8.34±0.18	7.65±0.34
C22:6ω3	6.13±3.50	8.31±1.60	3.21±0.49	3.38±0.19	4.04±0.16	4.58±0.23	4.27±0.30	7.53±0.94
∑ PUFA	**23.41 ±5.0**	**24.87 ±1.9**	**19.72±0.7**	**21.40±0.4**	**21.54 ±0.3**	**23.24 ±0.3**	**22.25 ±0.5**	**25.72 ±1.3**

Table 2: Seasonal and sexual variations of the fatty acid ratios in muscle of *Capoeta mauricii* (% of total fatty acids

Fatty Acids	Spring		Summer		Autumn		Winter	
	male	female	male	female	male	female	male	female
C12:0	0.12±0.03	0.26±0.12	0.07±0.01	0.27±0.01	0.12±0.04	0.37±0.14	0.12±0.02	0.16±0.01
C14:0	3.21±0.18	3.16±0.12	3.35±0.22	2.70±0.19	3.74±0.34	3.19±0.26	2.72±0.45	3.41±0.03
C15:0	0.45±0.04	0.39±0.05	0.79±0.16	0.64±0.20	1.05±0.35	0.68±0.25	0.33±0.02	1.67±0.02
C16:0	16.74±0.67	16.62±0.34	18.80±0.99	18.56±0.62	15.98±0.23	15.54±0.57	16.89±0.61	16.20±0.13
C17:0	0.24±0.02	0.24±0.02	0.27±0.02	0.41±0.02	0.28±0.04	0.30±0.03	0.29±0.03	0.39±0.00
C18:0	2.71±0.13	2.75±0.11	4.36±0.65	5.09±0.5	3.07±0.24	3.51±0.37	2.42±0.32	4.22±0.16
C20:0	0.14±0.00	0.18±0.02	0.16±0.00	0.19±0.02	0.17±0.02	0.18±0.02	0.17±0.01	0.14±0.00
C21:0	0.48±0.04	0.35±0.06	0.33±0.06	0.58±0.12	0.30±0.03	0.42±0.03	0.79±0.18	0.16±0.00
C22:0	0.07±0.05	0.08±0.00	0.09±0.00	0.09±0.01	0.09±0.01	0.11±0.02	0.09±0.01	-
∑ SFA	**24.25±1.80**	**24.03±1.79**	**28.22±2.02**	**28.53±2.00**	**24.8±1.71**	**24.3±1.66**	**23.82±1.81**	**26.35±1.93**
C14:1	0.17±0.02	0.17±0.00	0.11±0.00	0.09±0.00	0.13±0.01	0.14±0.00	0.12±0.03	0.23±0.07
C15:1	0.07±0.01	0.05±0.00	0.14±0.01	0.10±0.01	0.15±0.07	0.12±0.04	0.12±0.08	0.23±0.03
C16:1	15.63±0.73	16.68±0.77	13.93±1.00	11.60±1.06	14.74±0.73	13.31±0.82	14.74±1.97	10.78±0.10
C17:1	0.50±0.07	0.45±0.08	0.52±0.12	0.82±0.24	0.45±0.04	0.65±0.10	1.19±0.52	0.41±0.00
C18:1ω9	11.18±1.00	10.77±0.42	9.05±1.08	8.18±0.88	8.38±0.86	9.30±1.17	9.01±0.34	12.86±0.14
C18:1ω7	5.46±0.44	5.60±0.10	4.95±0.11	5.08±0.16	5.01±0.32	5.37±0.31	5.84±0.25	5.25±0.05
C20:1	1.37±0.10	1.13±0.14	1.41±0.17	1.13±0.08	1.16±0.18	0.93±0.22	0.57±0.09	1.93±0.00
C22:1ω9	0.14±0.01	0.09±0.01	0.14±0.03	0.15±0.03	0.23±0.01	0.18±0.05	0.11±0.05	-
∑MUFA	**34.52±2.12**	**34.94±2.21**	**30.7±1.83**	**27.15±1.56**	**30.25±1.89**	**30±1.80**	**31.7±1.93**	**31.69±2.01**
C18:2ω6	2.91±0.47	3.04±0.18	1.93±0.33	2.41±0.42	2.00±0.23	2.91±0.54	3.05±0.44	3.38±0.02
C18:3ω3	3.84±0.28	3.25±0.58	2.67±0.54	4.93±1.27	2.66±0.33	3.81±0.65	7.06±1.48	1.63±0.05
C20:3ω6	0.20±0.08	0.17±0.02	0.15±0.00	0.18±0.02	0.12±0.01	0.19±0.03	0.18±0.01	0.14±0.00
C20:4ω6	0.17±0.02	0.13±0.02	0.14±0.01	0.17±0.02	0.14±0.01	0.14±0.01	0.21±0.03	0.17±0.00
C20:2ω6	1.88±0.22	1.38±0.14	2.08±0.26	2.53±0.37	1.67±0.09	2.11±0.23	1.77±0.32	2.47±0.04
C20:5ω3	8.26±0.68	9.88±0.81	9.96±0.67	9.69±0.91	12.15±0.31	11.55±0.69	7.94±0.27	9.98±0.07
C22:6ω3	6.25±0.68	4.24±0.21	8.41±1.00	8.38±0.64	6.20±0.35	6.54±0.51	5.28±1.46	8.36±0.04
∑ PUFA	**23.51±1.14**	**22.09±1.27**	**25.34±1.49**	**28.29±1.43**	**24.94±1.63**	**27.25±1.52**	**25.49±1.20**	**26.13±1.48**

According to our results, palmitic acid was the major SFA. The highest ratios of palmitic acid in males and females in summer were found to be 18.79% and 17.41% for *P. fahrettini* and 18.80% and 18.56% for *C. mauricii*, respectively.

The levels of monounsaturated fatty acids (MUFA) in the present study were found to be in the range of 27.10% to 34.92% in *C. mauricii* and 36.04% to 41.41% in *P. fahrettini*. The ratios of MUFAs in the muscle of both species were generally higher than other fatty acid groups (Tables 1 and 2). MUFAs values in *P. fahrettini* were higher than those of *C. mauricii* in all seasons. Particularly, these differences between species were statistically significant in autumn and summer (p<0.05). Oleic acid in *P. fahrettini* and palmitoleic acid in *C. mauricii* were the major MUFAs in all seasons (Tables 1 and 2). Oleic acid content of female *P. fahrettini* was at minimum in the winter (18.51%) and at maximum in summer (21.70%). However the ratio of oleic acid content of male *P. fahrettini* significantly decreased to minimum level in summer (22.18%) and increased to maximum in winter (18.58%) (Table 1). Palmitoleic acid of female *C. mauricii* reached the highest level in spring and decreased to the lowest level in winter, but there were no significant changes in both sexes (p>0.05). This situation may be associated to the differences in nutrition and reproduction physiologies of the sexes.

The levels of total PUFAs in muscle of *P. fahrettini* changed from 21.40% to 25.72% in females and from 19.72% to 23.41% in males. The levels of total PUFAs in muscle of *C. mauricii* also ranged from 22.09% to 28.29% in females and from 23.51% to 25.49% in males.

The ratios of ω3 PUFAs in the muscle of *P. fahrettini* ranged from 14.09% to 16.54% in males and from 14.26% to 18.44% in females (Fig. 1); the levels of ω3 PUFAs of *C. mauricii* ranged from 17.37% to 23.00% in females and from 18.35% to 21.04% in males (Fig. 2). EPA and DHA were the two major ω3 PUFAs in the muscle of the species. The ratio of EPA in the muscle of female *P. fahrettini* significantly increased to a maximum level in autumn (8.68%) and decreased to a minimum in spring (6.21%), and EPA level of males was at the highest point in winter (8.34%) and at the lowest point in spring (6.04%) (Table 1). On the other hand, the EPA levels of *C. mauricii* reached the highest level in males (12.15%) and in females (11.55%) in autumn. The EPA ratios in the muscle of *C. mauricii* were significantly higher than those of *P. fahrettini* in both sexes for all seasons (p<0.05), except in males in winter.

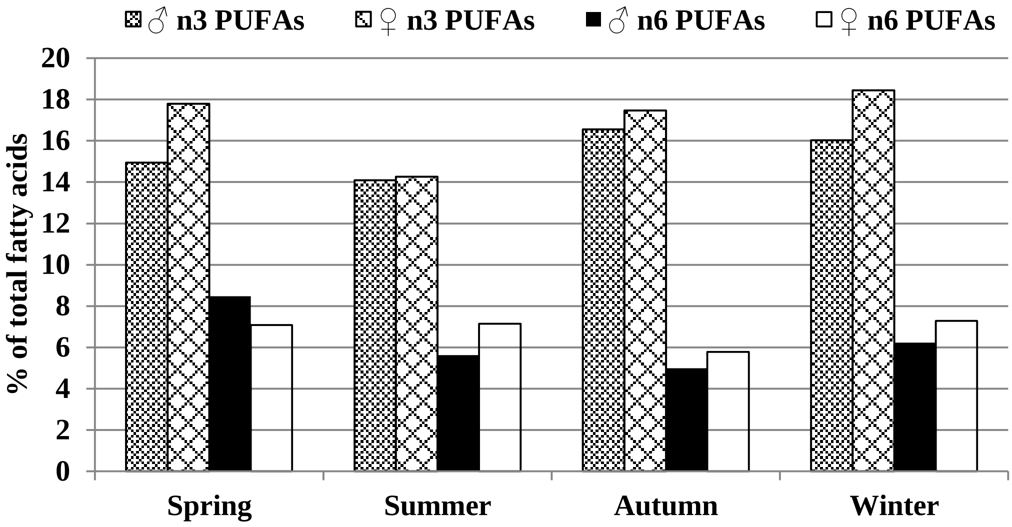

Figure 1: Seasonal and sexual variations of total ω3 PUFAs and ω6 PUFAs ratios in muscle of *Pseudophoxinus fahrettini* (% of total fatty acids).

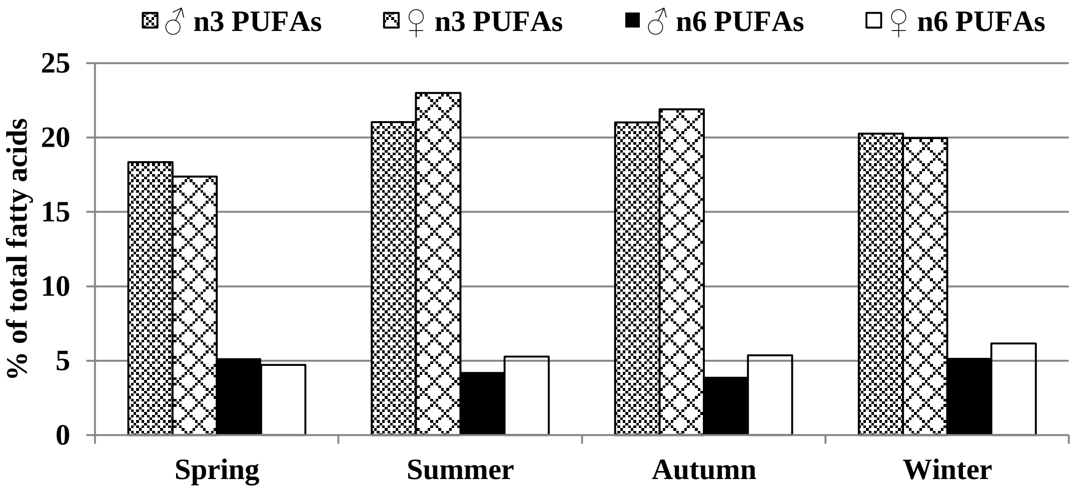

Figure 2: Seasonal and sexual variations of total n3 PUFAs and n6 PUFAs ratios in muscle of *Capoeta mauricii* (% of total fatty acids).

DHA levels of *P. fahrettini* were the highest in females (8.31%) and males (6.13%) in spring, while DHA levels of *C. mauricii* were the highest in summer with a rate of 8.41% in males and 8.38% in females. DHA values were higher in *C. mauricii* than in *P. fahrettini*, except in spring in females.

The ω6 PUFAs ratios of *P. fahrettini* ranged from 5.00% to 8.47% in males and from 5.79% to 7.28% in females (Fig. 1); the levels of ω6 PUFAs ratios of *C. mauricii* ranged from 4.71% to 6.15% in females and from 3.93% to 5.20% in males (Fig. 2). The ratios of total ω6 PUFAs in both sexes of the species were remarkably lower than the ω3 PUFAs ratios.

Discussion

Fish adaptation to cold may be the reason for high level of SFA in temperate conditions and low level in winter (Logue *et al.,* 2000). For instance, it was also reported that the highest concentrations of SFAs, mainly in the form of palmitic acid, were found in summer in *Capoeta erhani* (Emre *et al.,* 2014). In this study, there were no statistical differences between the SFAs ratios in the muscle of the species. This situation may be due to the fact that the studied species belong to the same family and live in neighboring habitats.

It was reported that palmitic acid was the primary SFA for both sea water and freshwater fish species such as *Dicentrarchus labrax* (Alasalvar *et al*., 2002), *Acipenser oxyrinchus desotoi, Pomoxis* spp. (Chen *et al*., 1995; Grün *et al*., 1999), *Cyprinus carpio, Labeo rohita* and *Oreochromis mossambicus* (Jabeen and Chaudhry, 2011). The ratios of palmitic acid in many freshwater and seawater fish species ranged from 17.83% to 46.00% (Uysal and Aksoylar 2005; Jabeen and Chaudhry, 2011; Marichamy *et al*., 2012; Murillo *et al*., 2014). In consideration of these values, it can be said that the palmitic acid is the key metabolite for fishes.

It was proposed that oleic acid was the dominant MUFA in Baltic sprat (*Sprattus sprattus balticus*) with a mean ratio of 25.18% (Usydus *et al*., 2012), in *Epinephelus aeneus*, *Cephalopholis taeniops and Serranus scriba* with the range of 11 - 16% seasonally (Louly *et al*., 2011). It was also known that oleic and palmitoleic acids were the most abundant MUFAs in fish tissues.

As shown in Tables 1 and 2, the PUFA rates of both species have not been significantly affected by seasons. The PUFA quantities in fishes are quite important for adaptation to cold conditions. As the total PUFA quantities studied in both species are not significantly affected by season variations, it is supposed that the fatty acid compositions of the studied species are not very affected by the temperature changes between seasons. The species was studied live near to the source sections of the streams and these parts of streams are slightly affected by seasonal temperature changes. No important differences have been detected between the PUFA rates of the species except from summer. But the ratio of PUFAs in the muscle of *C. mauricii* was higher than in *P. fahrettini* in summer ($p<0.05$).

It was reported that DHAs were the most abundant PUFAs in *Chondrostoma regium*, *Barbus rajonorum*, *Carasobarbus luteus*, *Leuciscus lepidus*, *Acanthobrama marmid*, *Cyprinion macrostomus*, and *Silurus triostegus* as freshwater fishes (Cengiz *et al*., 2010). Louly *et al.* (2011) reported that DHA ratios ranged from 5% to 9% for *C. taeniops*, from 13% to 17% for *S. scriba*, and from 10% to 16% for *E. aeneus*. The DHA levels of the species studied were similar to the DHA levels of many fish species mentioned in the literature.

The ratio of ω3 PUFAs to ω6 PUFAs is mostly used for the determination of nutritional value of fishes. Diet including food with appropriate ratio of ω3 PUFAs / ω6 PUFAs reduces heart diseases and risk of cancer (Kinsella *et al*., 1990). Ackman *et al*. (1975) have shown that the ω3 PUFAs sources like linoleic acids were lower in freshwater fish oils in comparison with marine oils. However, our results have shown that the ω3 PUFAs /ω6 PUFAs ratios reached the levels of 3.31 in *P. fahrettini* and 5.35 in *C. mauricii* in autumn and these results were quite high compared to the literature, in particular for *C. mauricii*. The ω3 PUFAs /ω6 PUFAs ratios in *Salmo trutta macrostigma* tissues were found to be 2.59 in male and 2.26 in female muscle (Akpinar *et al*., 2009). Güler *et al*. 2007 reported that ω3 PUFAs /ω6 PUFAs ratios were 1.49, 1.45, 1.22, 0.72 in spring, autumn, winter, and summer, respectively. Rasoarahona *et al*. (2005) proposed that ω3 PUFAs /ω6 PUFAs ratios vary between 0.5 and 1.6 for three tilapia species (*Oreochromis niloticus, O. macrochir and Tilapia rendalli*). Zenebe *et al.* (1998) observed that the ω3 PUFAs /ω6 PUFAs ratios varied considerably (1.1–7.6) for freshwater fish of commercial importance.

The results obtained in this study have shown that ω3 PUFAs /ω6 PUFAs ratios (an important indicator of the dietary quality in human nutrition) of the species studied were quite high. Thus, it is possible to say that these species are an important source of ω3 PUFAs for local population. Besides, as the ω3 PUFAs rates of the species were not affected too much by seasons.

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