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Fatty acids of neutral and phospholipids of three endangered trout: *Salmo trutta caspius* Kessler, *Salmo trutta labrax* Pallas and *Salmo trutta macrostigma* Dumeril

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ABSTRACT

Total (TL), neutral (NL) and phospholipid (PL) amounts and fatty acid (FA) composition of female *Salmo trutta caspius*, *Salmo trutta labrax* and *Salmo trutta macrostigma* were investigated during one year. Twenty-three FAs were identified in both NLs and PLs. The principal FAs of both fractions were palmitic acid in saturated fatty acid, oleic acid in monounsaturated fatty acid, docosahexaenoic acid (DHA) in *n*-3 polyunsaturated fatty acids (*n*-3 PUFAs) and linoleic acid in *n*-6 PUFAs. The highest values for TLs, NLs and PLs were found in winter. As a general trend, the highest *n*-3/*n*-6 ratios and eicosapentaenoic acid (EPA) + DHA amounts were found in the winter and this coincided with the lowest gonado-somatic index.

1. Introduction

Recently, there has been heightened interest in the lipid and fatty acid (FA) composition of fish. Fish naturally contain high levels of polyunsaturated fatty acids (PUFA) of the *n*-6 series, and especially of *n*-3 series such as eicosapentaenoic acid (EPA, 20:5 *n*-3) and docosahexaenoic acid (DHA, 22:6 *n*-3), that are recognised essential biochemical components of the human diet because of their beneficial effects of human health (Shirai, Terayama, & Takeda, 2002; Sushchik, Gladyshev, & Kalachova, 2007; Zenebe, Ahlgren, & Boberg, 1998). Recent studies have shown that *n*-3 PUFAs play a vital role in prevention and treatment of cardiovascular disease, inflammation, aggression, depression, hypertension, autoimmune disorders and cancer (Candela, Astiasarán, & Bello, 1997; Pike 1999).

FA composition of fish lipids is extremely variable, even within species, depending upon different abiotic and biotic factors such as season, the type and amount of feed available, water temperature, pH, salinity, and reproduction cycle (Kaushik, Corraze, Radunz-Neto, Larroquet, & Dumas, 2006; Shirai et al., 2002; Zenebe et al., 1998). Although seasonal changes of the FA content of freshwater fish species are documented in literature (Rasoarahona, Barnathan, Bianchini, & Gaydou, 2005; Sushchik et al., 2007), no study has been reported for Caspian Trout (*Salmo trutta caspius* Kessler), Black Sea Trout (*Salmo trutta labrax* Pallas) and Mountain Trout (*Salmo trutta macrostigma* Dumeril).

Brown trout (*S. trutta* Linnaeus) are native to North America, Europe, West and North Asia (Geldiay & Balık, 1996). In Turkey, streams of the Aras Basin (Aras, 1974), Çoruh Basin (Aras, 1974) and Karasu Basin (Kuru, 1975) contain naturally the sub-species of *S. trutta*: *S. t. caspius*, *S. t. labrax* and *S. t. macrostigma*, respectively. These species are endangered by illegal catching methods, human pressures and degradation of spawning habitats (Alp, Kara, & Büyükçapar, 2005). Consequently, there is need for good management practices for the conservation of stocks in Turkey. To fulfill this task, it is necessary to achieve a sound knowledge of the biology of these sub-species (Gortázar et al., 2007). Furthermore, determination of the changes in the FA composition of food fish is important for conclusions on their properties as a source of the essential components for humans (Sushchik et al., 2007). Therefore, the aim of the present study was to determine the seasonal fluctuations in the FA compositions of the neutral and phospholipids of muscle of the *S. t. caspius*, *S. t. labrax* and *S. t. macrostigma* throughout one year.

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2. Material and methods

2.1. Collection of samples

Female fish were collected quarterly between summer 2006 and autumn 2007 (14–16 July, October, February and April); *S. t. caspius* (96–117 g) were captured from Hamzalar Creek (Aras Basin), Tekman, Erzurum, Turkey (039° 27' 037"N; 041° 15' 39"E). *S. t. labrax* (86–124 g) were captured from Yağlı Creek (Çoruh Basin), İspir, Erzurum, Turkey (040° 21' 055"N; 041° 04' 22"E) and *S. t. macrostigma* specimens (111–123 g) were captured from Yeşildere Creek (Karasu Basin), Dumlu, Erzurum, Turkey (040° 07' 031"N; 041° 25' 18"E) by cast nets in spring and summer and by electrofishing in autumn and winter. Five fish of each of the three sub-species were killed with a sharp blow on the head; muscles were excised and frozen immediately in liquid nitrogen. Samples were transferred to laboratory and stored at −84 °C until analysis.

2.2. Lipid and fatty acid analysis

Muscle tissues of female trout (*c.* 1 g) were obtained from the point between linea lateral and dorsal fin. The Folch, Less, and Stanley (1957) method was used for lipid extraction. According to this method, samples were homogenised in chloroform/methanol (2:1 v/v) containing 0.01% (w/v) of butylated hydroxytoluene (Sigma, ⩾99.0% (GC), Product number: B1378) as antioxidant 20 vol (w/v) for 1 min. Homogenization was carried out in ice and others at room temperature (20–22 °C). The organic solvent was evaporated under a stream of nitrogen and the amount of lipid was determined gravimetrically. Muscle crude lipids were separated into the polar (phospholipids) and neutral lipids using Sep Pak silica cartridges (Waters, Milford, MA, USA). Physical and chemical characteristics of cartridges: Surface pH: 7; Activity grade: High; Pore size: 125 Å; Particle size: 80 μm; Flow rate: 2 ml min^{-1}. The residual lipid (*c.* 0.15 g) was applied on a silica filter and the neutral lipids were eluted with 30 ml chloroform (Sigma, ⩾99–99.4% (GC), product number: 24216). After elution of neutral lipids, the polar fraction was eluted with 30 ml methanol (Sigma, ⩾99.9%, Product number: 34885). Solvent from both fractions were then evaporated under nitrogen and the amounts of polar and neutral lipids were determined gravimetrically (Czesny & Dabrowski, 1998). Fatty acid methyl esters (FAME) were prepared from both fractions of lipids according to method of Metcalfe and Schmitz (1961). The crude lipid extract was saponified with NaOH in methanol and FAMEs were prepared by transmethylation with boron trifluoride (BF_3) in methanol. FAMEs were separated on a HP (Hewlet Packard) Agilent 6890N 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) ejector and detector temperature program was 190 °C for 35 min than increasing at 30 °C per min up to 220 °C where it was maintained for 5 min. Carrier gas was hydrogen (2 ml min^{-1} and split ratio was 30:1). The individual FAs were identified by comparing their retention times to that of a standard mix of FAs (Supelco 37 component FAME mix, Cat No.: 47885-U) and quantified by comparing their peak areas (David, Sandra, & Wylie, 2003).

2.3. Data analysis

The statistical analysis was performed with SPSS version 10.0 for Windows (SPSS, 1996). Data were presented as mean ± 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 ($n = 5$).

3. Results and discussion

3.1. Total lipid, neutral lipid and phospholipid composition

The total lipid (TL), neutral lipid (NL) and phospholipid (PL) compositions of muscle of the *S. t. caspius*, *S. t. labrax* and *S. t. macrostigma* are presented in Table 1. Fat content ranged between 1.50% and 4.67%, showing that the three sub-species belong to low fat fish (2–4% fat) (Ackman, 1989). Our results are in agreement with previously given content by Blanchet et al. (2005) and Kaushik et al. (2006) since the range is comprised between 1.0% and 4.5% in wild salmonids. The NL and PL ratios were between 0.63 and 3.18 and 0.74 and 1.49, respectively. The maximal TL, NL and PL contents of the *S. t. caspius*, *S. t. labrax* and *S. t. macrostigma* were determined in the winter and the lowest fat content in the autumn for all fish. However, the lowest NL and PL values were found in the different seasons (Table 1). In an earlier study (Aras et al., 2009), we found that reproduction time was between the second half of autumn and first half of winter for same fish. Takashima, Hibiya, Watanabe, and Hara (1971) reported inverse relationships between lipid content of viscera and GSI in the cultured rainbow trout. Moreover, it is known that fish preferentially use lipids rather than carbohydrates as an energy source (Covey & Sargent, 1977) and need them during sexual maturation. Hence, the low muscle fat content in the autumn are probably due to ovary maturations of the female *S. t. caspius*, *S. t. labrax* and *S. t. macrostigma*.

3.2. Fatty acid composition of neutral and phospholipids

FA composition of NL and PL fractions of muscle tissues of the *S. t. caspius*, *S. t. labrax* and *S. t. macrostigma* are given in Tables 2–4. Twenty-three fatty acids were identified in both fractions.

The principal the FAs of NLs and PLs were palmitic acid (16:0) in saturated fatty acid (SFA), oleic acid (18:1 *n*-9) in monounsaturated fatty acid (MUFA), DHA (22:6 *n*-3) in *n*-3 PUFA and linoleic acid

Table 1
Total lipid, neutral lipid and phospholipid compositions of *Salmo trutta sp.* in different seasons.[A]

Sub-species	Component	Summer	Autumn	Winter	Spring
S. trutta caspius	Total lipid	2.33 ± 0.11^{b}	1.75 ± 0.06^{c}	3.10 ± 0.12^{a}	2.24 ± 0.07^{b}
	Neutral lipid	1.58 ± 0.17^{b}	0.63 ± 0.07^{c}	1.70 ± 0.15^{a}	1.29 ± 0.11^{b}
	Phospholipid	0.74 ± 0.30^{d}	1.12 ± 0.19^{b}	1.40 ± 0.09^{a}	0.95 ± 0.09^{c}
S. trutta labrax	Total lipid	1.87 ± 0.05^{c}	1.50 ± 0.06^{d}	4.67 ± 0.19^{a}	2.50 ± 0.08^{b}
	Neutral lipid	0.75 ± 0.05^{c}	1.32 ± 0.06^{b}	3.18 ± 0.66^{a}	1.36 ± 0.04^{b}
	Phospholipid	1.12 ± 0.02^{b}	1.18 ± 0.02^{b}	1.49 ± 0.74^{a}	1.14 ± 0.03^{b}
S. trutta macrostigma	Total lipid	2.97 ± 0.03^{b}	2.83 ± 0.05^{c}	3.22 ± 0.08^{a}	2.89 ± 0.08^{b}
	Neutral lipid	1.71 ± 0.05^{a}	1.71 ± 0.03^{a}	1.77 ± 0.06^{a}	1.59 ± 0.23^{b}
	Phospholipid	1.25 ± 0.03^{b}	1.12 ± 0.02^{c}	1.44 ± 0.08^{a}	1.29 ± 0.21^{b}

[A] Values were presented as mean sd. (a–b–c–d) means in a column with identical letters are not significantly different ($N = 5$) ($P < 0.05$).

Table 2
Fatty acid composition (% of total fatty acids[A]) of neutral and phospholipids of *Salmo trutta caspius* in different seasons.[B,C]

Fatty acids	Neutral lipid				Phospholipid			
	Summer	Autumn	Winter	Spring	Summer	Autumn	Winter	Spring
14:0	0.26 ± 0.003^{b}	0.89 ± 0.16^{a}	0.23 ± 0.005^{b}	0.39 ± 0.28^{b}	0.50 ± 0.07^{a}	0.23 ± 0.06^{b}	0.16 ± 0.05^{bc}	0.10 ± 0.01^{c}
15:0	1.83 ± 0.14^{a}	0.30 ± 0.09^{b}	0.08 ± 0.006^{c}	0.19 ± 0.009^{bc}	0.31 ± 0.08^{c}	0.20 ± 0.04^{d}	0.58 ± 0.09^{a}	0.43 ± 0.08^{b}
16:0	21.97 ± 0.11^{a}	15.57 ± 2.18^{b}	14.19 ± 0.18^{b}	15.24 ± 0.80^{b}	33.89 ± 0.98^{a}	33.18 ± 1.93^{a}	31.08 ± 0.35^{b}	31.23 ± 0.69^{b}
17:0	2.50 ± 0.03^{a}	0.52 ± 0.01^{c}	1.60 ± 0.13^{b}	1.72 ± 0.18^{b}	0.47 ± 0.02^{b}	0.55 ± 0.02^{b}	0.55 ± 0.04^{b}	0.81 ± 0.17^{a}
18:0	4.44 ± 0.02^{a}	3.95 ± 0.37^{b}	3.25 ± 0.09^{c}	3.27 ± 0.19^{c}	8.33 ± 0.76^{ab}	8.80 ± 0.61^{a}	7.67 ± 0.40^{b}	8.78 ± 0.88^{a}
20:0	0.75 ± 0.008^{c}	0.48 ± 0.11^{d}	1.13 ± 0.007^{a}	0.93 ± 0.05^{b}	0.64 ± 0.12^{a}	0.40 ± 0.22^{b}	0.57 ± 0.02^{ab}	0.64 ± 0.09^{a}
22:0	0.21 ± 0.003^{d}	0.78 ± 0.06^{b}	0.91 ± 0.03^{a}	0.72 ± 0.06^{c}	1.15 ± 0.80^{c}	2.77 ± 0.23^{a}	2.60 ± 0.50^{ab}	1.90 ± 0.54^{b}
24:0	0.19 ± 0.002^{b}	0.32 ± 0.01^{a}	0.18 ± 0.01^{b}	0.17 ± 0.05^{b}	0.12 ± 0.03^{b}	0.17 ± 0.05^{b}	0.27 ± 0.02^{a}	0.12 ± 0.08^{b}
Σ SFA	**32.15 ± 0.23^{a}**	**22.82 ± 2.64^{b}**	**21.57 ± 0.25^{b}**	**22.64 ± 0.83^{b}**	**45.40 ± 0.90^{ab}**	**46.30 ± 2.00^{a}**	**43.47 ± 0.76^{c}**	**44.00 ± 1.24^{bc}**
15:1	0.36 ± 0.004^{b}	0.42 ± 0.06^{b}	0.61 ± 0.07^{a}	0.14 ± 0.03^{c}	0.62 ± 0.05^{b}	0.92 ± 0.11^{a}	0.24 ± 0.003^{c}	0.05 ± 0.005^{d}
16:1 *n*-7	11.75 ± 0.05^{c}	14.26 ± 0.60^{a}	13.27 ± 0.09^{b}	13.86 ± 0.80^{a}	3.32 ± 0.10^{b}	3.16 ± 0.13^{b}	3.07 ± 0.08^{b}	4.44 ± 0.55^{a}
17:1	0.56 ± 0.006^{b}	0.21 ± 0.05^{c}	1.94 ± 0.03^{a}	1.98 ± 0.26^{a}	0.12 ± 0.02^{b}	0.06 ± 0.01^{c}	0.07 ± 0.005^{c}	0.32 ± 0.05^{a}
18:1 *n*-9	21.84 ± 0.14^{a}	15.84 ± 0.04^{c}	18.67 ± 0.06^{b}	18.83 ± 1.60^{b}	6.21 ± 0.15^{b}	8.31 ± 1.03^{a}	8.17 ± 0.93^{a}	8.26 ± 1.03^{a}
20:1 *n*-9	11.50 ± 0.13^{d}	19.52 ± 0.72^{c}	13.05 ± 0.10^{c}	14.98 ± 1.89^{b}	9.12 ± 0.91^{a}	7.50 ± 0.85^{b}	7.35 ± 0.73^{b}	6.09 ± 0.11^{c}
22:1 *n*-9	1.37 ± 0.02^{a}	0.53 ± 0.09^{c}	0.67 ± 0.01^{b}	0.68 ± 0.05^{b}	1.48 ± 0.37^{bc}	1.13 ± 0.16^{c}	3.02 ± 0.66^{a}	1.98 ± 0.62^{b}
Σ MUFA	**47.37 ± 0.25^{b}**	**50.47 ± 1.19^{a}**	**48.22 ± 0.27^{b}**	**50.77 ± 0.95^{a}**	**20.88 ± 0.53^{a}**	**21.09 ± 1.81^{a}**	**21.91 ± 2.21^{a}**	**20.79 ± 1.12^{a}**
18:3 *n*-3	0.27 ± 0.003^{b}	0.25 ± 0.01^{b}	0.82 ± 0.03^{a}	0.80 ± 0.08^{a}	0.39 ± 0.04^{a}	0.32 ± 0.03^{b}	0.26 ± 0.02^{c}	0.37 ± 0.02^{a}
20:5 *n*-3	0.34 ± 0.004^{c}	0.44 ± 0.04^{b}	0.50 ± 0.06^{a}	0.44 ± 0.03^{b}	0.32 ± 0.03^{c}	0.56 ± 0.08^{a}	0.48 ± 0.05^{b}	0.42 ± 0.07^{b}
22:5 *n*-3	0.005 ± 0.0004^{d}	0.14 ± 0.01^{c}	0.38 ± 0.06^{a}	0.20 ± 0.03^{b}	0.005 ± 0.0005^{b}	0.03 ± 0.008^{a}	0.005 ± 0.0009^{b}	0.006 ± 0.001^{b}
22:6 *n*-3	6.29 ± 0.08^{d}	8.18 ± 0.09^{c}	13.14 ± 0.22^{a}	11.22 ± 0.62^{b}	21.86 ± 1.71^{b}	19.77 ± 0.15^{c}	23.67 ± 1.10^{a}	23.83 ± 0.51^{a}
Σ *n*-3 PUFA	**6.91 ± 0.07^{d}**	**9.02 ± 0.12^{c}**	**14.84 ± 0.24^{a}**	**12.66 ± 0.65^{b}**	**22.57 ± 1.70^{b}**	**20.68 ± 0.13^{c}**	**24.41 ± 1.13^{a}**	**24.63 ± 0.58^{a}**
18:2 *n*-6	10.81 ± 0.02^{b}	14.56 ± 1.23^{a}	11.61 ± 0.13^{b}	10.94 ± 0.82^{b}	5.60 ± 0.32^{b}	6.76 ± 0.31^{a}	6.40 ± 0.35^{a}	6.55 ± 0.32^{a}
20:2 *n*-6	0.006 ± 0.00^{c}	0.22 ± 0.03^{c}	0.45 ± 0.09^{a}	0.30 ± 0.03^{b}	1.36 ± 0.03^{a}	0.97 ± 0.05^{c}	1.12 ± 0.05^{b}	1.42 ± 0.07^{a}
20:3 *n*-6	0.33 ± 0.004^{c}	0.80 ± 0.03^{a}	0.48 ± 0.05^{b}	0.49 ± 0.05^{b}	0.09 ± 0.01^{b}	0.40 ± 0.05^{a}	0.13 ± 0.05^{b}	0.09 ± 0.02^{b}
20:4 *n*-6	0.51 ± 0.006^{a}	0.22 ± 0.0007^{c}	0.32 ± 0.02^{b}	0.24 ± 0.04^{c}	0.18 ± 0.01^{a}	0.18 ± 0.006^{a}	0.19 ± 0.04^{a}	0.18 ± 0.01^{a}
22:2 *n*-6	1.91 ± 0.02^{b}	1.53 ± 0.12^{c}	2.27 ± 0.03^{a}	2.26 ± 0.20^{a}	0.15 ± 0.04^{c}	2.92 ± 0.67^{a}	2.31 ± 0.21^{b}	2.28 ± 0.92^{b}
Σ n–6 PUFA	**13.57 ± 0.05^{c}**	**17.33 ± 1.11^{a}**	**15.13 ± 0.13^{b}**	**14.22 ± 0.91^{bc}**	**7.38 ± 0.32^{c}**	**11.22 ± 0.75^{a}**	**10.15 ± 0.52^{b}**	**10.51 ± 0.37^{b}**
Σ n–3/n–6 PUFA	**0.51 ± 0.007^{c}**	**0.52 ± 0.03^{c}**	**0.98 ± 0.02^{a}**	**0.89 ± 0.04^{b}**	**3.07 ± 0.36^{a}**	**1.85 ± 0.12^{c}**	**2.41 ± 0.10^{b}**	**2.34 ± 0.13^{b}**
EPA + DHA	**6.63 ± 0.07^{d}**	**8.63 ± 0.14^{c}**	**13.64 ± 0.27^{a}**	**11.65 ± 0.64^{b}**	**22.18 ± 1.73^{b}**	**20.33 ± 0.12^{c}**	**24.15 ± 1.14^{a}**	**24.25 ± 0.58^{a}**

[A] Values are expressed as percentages of total fatty acids.
[B] (a–b–c–d) means in a row with identical letters are not significantly different. Values were presented as mean SD ($N = 5$) ($P < 0.05$).
[C] Neutral and phospholipids were not compared each other.

Table 3
Fatty acid composition (% of total fatty acids[A]) of neutral and phospholipids of *Salmo trutta labrax* in different seasons.[B,C]

Fatty acids	Neutral lipid				Phospholipid			
	Summer	Autumn	Winter	Spring	Summer	Autumn	Winter	Spring
14:0	0.18 ± 0.004^{b}	0.30 ± 0.003^{a}	0.16 ± 0.02^{b}	0.17 ± 0.008^{b}	0.16 ± 0.008^{b}	0.17 ± 0.01^{b}	0.26 ± 0.04^{a}	0.15 ± 0.02^{b}
15:0	0.06 ± 0.0004^{c}	0.08 ± 0.005^{b}	0.07 ± 0.008^{b}	0.18 ± 0.01^{a}	0.66 ± 0.04^{b}	1.46 ± 0.10^{a}	0.41 ± 0.03^{c}	1.50 ± 0.08^{a}
16:0	18.74 ± 0.31^{a}	17.05 ± 0.83^{b}	16.19 ± 0.88^{b}	16.33 ± 0.04^{b}	30.57 ± 0.67^{a}	27.84 ± 1.83^{c}	29.65 ± 0.89^{ab}	28.70 ± 0.94^{bc}
17:0	1.85 ± 0.19^{a}	0.69 ± 0.02^{b}	0.53 ± 0.08^{c}	0.54 ± 0.04^{c}	0.26 ± 0.02^{b}	0.23 ± 0.03^{b}	0.39 ± 0.03^{a}	0.39 ± 0.01^{a}
18:0	3.49 ± 0.11^{a}	3.19 ± 0.10^{a}	3.25 ± 0.35^{a}	3.14 ± 0.40 ^{a}	9.68 ± 0.27^{a}	8.62 ± 0.20^{b}	8.74 ± 0.45^{b}	8.59 ± 0.37^{b}
20:0	0.36 ± 0.04^{c}	0.18 ± 0.02^{d}	0.62 ± 0.01^{a}	0.50 ± 0.01^{b}	0.38 ± 0.03^{a}	0.27 ± 0.01^{b}	0.13 ± 0.004^{d}	0.16 ± 0.007^{c}
22:0	0.69 ± 0.01^{c}	0.54 ± 0.04^{d}	1.49 ± 0.03^{a}	0.93 ± 0.05^{b}	3.03 ± 0.09^{d}	3.82 ± 0.12^{b}	4.49 ± 0.12^{a}	3.36 ± 0.16^{c}
24:0	0.28 ± 0.03^{b}	0.53 ± 0.04^{a}	0.24 ± 0.03^{b}	0.27 ± 0.009^{b}	0.28 ± 0.02^{d}	0.35 ± 0.009^{a}	0.32 ± 0.009^{c}	0.33 ± 0.01^{b}
Σ SFA	25.64 ± 0.31^{a}	22.56 ± 0.86^{b}	22.54 ± 0.75^{b}	22.06 ± 0.36^{b}	45.01 ± 0.63^{a}	42.76 ± 1.97^{b}	44.39 ± 1.19^{ab}	43.20 ± 1.23^{ab}
15:1	0.36 ± 0.02^{b}	0.28 ± 0.03^{c}	0.65 ± 0.05^{a}	0.19 ± 0.009^{d}	0.78 ± 0.05^{a}	0.09 ± 0.006^{d}	0.66 ± 0.01^{b}	0.32 ± 0.02^{c}
16:1 *n*-7	10.29 ± 0.27^{bc}	10.37 ± 0.45^{b}	9.72 ± 0.73^{c}	13.90 ± 0.11^{a}	3.57 ± 0.15^{a}	2.38 ± 0.09^{c}	2.50 ± 0.13^{c}	2.99 ± 0.24^{b}
17:1	0.58 ± 0.02^{a}	0.25 ± 0.03^{c}	0.29 ± 0.007^{b}	0.54 ± 0.04^{a}	0.28 ± 0.02^{c}	0.33 ± 0.02^{b}	0.93 ± 0.06^{a}	0.12 ± 0.004^{d}
18:1 *n*-9	16.96 ± 0.16^{d}	19.44 ± 0.65^{a}	16.46 ± 0.12^{c}	17.25 ± 0.10^{b}	8.54 ± 0.51^{c}	8.94 ± 0.38^{c}	10.53 ± 0.34^{a}	9.82 ± 0.20^{b}
20:1 *n*-9	17.67 ± 0.18^{a}	15.76 ± 0.42^{b}	15.22 ± 0.24^{c}	15.61 ± 0.23^{b}	7.78 ± 0.38^{a}	7.77 ± 0.19^{a}	7.47 ± 0.30^{a}	7.39 ± 0.19^{a}
22:1 *n*-9	0.78 ± 0.02^{a}	0.55 ± 0.02^{b}	0.57 ± 0.03^{b}	0.58 ± 0.02^{b}	0.90 ± 0.03^{b}	0.96 ± 0.02^{a}	0.96 ± 0.03^{a}	0.87 ± 0.02^{c}
Σ MUFA	46.63 ± 0.29^{b}	46.66 ± 0.72^{b}	42.91 ± 0.67^{c}	48.07 ± 0.16^{a}	21.85 ± 0.55^{b}	20.47 ± 0.43^{c}	23.05 ± 0.56^{a}	21.51 ± 0.46^{b}
18:3 *n*-3	0.24 ± 0.03^{c}	0.28 ± 0.01^{b}	0.35 ± 0.03^{a}	0.34 ± 0.03^{a}	0.42 ± 0.03^{b}	0.46 ± 0.02^{a}	0.28 ± 0.01^{c}	0.45 ± 0.008^{ab}
20:5 *n*-3	0.22 ± 0.02^{b}	0.52 ± 0.008^{a}	0.51 ± 0.07^{a}	0.51 ± 0.04^{a}	0.007 ± 0.001^{bc}	0.08 ± 0.008^{a}	0.004 ± 0.0008^{c}	0.009 ± 0.0005^{b}
22:5 *n*-3	0.05 ± 0.01^{d}	0.08 ± 0.005^{c}	0.36 ± 0.04^{a}	0.24 ± 0.03^{b}	18.99 ± 0.39^{b}	24.72 ± 0.20^{a}	19.74 ± 0.36^{b}	20.47 ± 0.36^{b}
22:6 *n*-3	13.51 ± 0.31^{d}	14.73 ± 0.51^{c}	17.27 ± 0.80^{a}	15.66 ± 0.38^{b}	19.62 ± 0.39^{d}	25.55 ± 0.21^{a}	20.09 ± 0.36^{c}	21.07 ± 0.37^{b}
Σ *n*-3 PUFA	14.02 ± 0.31^{d}	15.61 ± 0.50^{c}	18.49 ± 0.85^{a}	16.76 ± 0.37^{b}	6.56 ± 0.10^{a}	6.54 ± 0.08^{a}	5.41 ± 0.25^{b}	5.56 ± 0.34^{b}
18:2 *n*-6	9.65 ± 0.41^{b}	10.34 ± 0.64^{a}	10.19 ± 0.35^{ab}	8.37 ± 0.16^{c}	0.20 ± 0.01^{b}	0.29 ± 0.01^{a}	0.06 ± 0.005^{d}	0.14 ± 0.006^{c}
20:2 *n*-6	0.02 ± 0.003^{d}	0.45 ± 0.03^{b}	0.64 ± 0.07^{a}	0.32 ± 0.03^{c}	2.37 ± 0.11^{a}	0.39 ± 0.04^{d}	1.82 ± 0.08^{c}	2.17 ± 0.17^{b}
20:3 *n*-6	0.34 ± 0.04^{c}	0.85 ± 0.05^{a}	0.49 ± 0.03^{b}	0.46 ± 0.02^{b}	0.17 ± 0.006^{c}	0.23 ± 0.02^{b}	0.25 ± 0.007^{a}	0.22 ± 0.01^{b}
20:4 *n*-6	0.62 ± 0.06^{a}	0.31 ± 0.01^{c}	0.32 ± 0.01^{c}	0.40 ± 0.01^{b}	0.16 ± 0.005^{d}	0.26 ± 0.02^{c}	0.36 ± 0.02^{a}	0.33 ± 0.03^{b}
22:2 *n*-6	3.00 ± 0.07^{c}	3.12 ± 0.16^{c}	4.25 ± 0.17^{a}	3.60 ± 0.33^{b}	4.26 ± 0.23^{c}	2.68 ± 0.18^{d}	4.63 ± 0.21^{b}	5.50 ± 0.06^{a}
Σ n–6 PUFA	13.64 ± 0.44^{c}	15.06 ± 0.68^{b}	15.89 ± 0.17^{a}	13.20 ± 0.42^{c}	13.52 ± 0.22^{a}	10.11 ± 0.19^{c}	12.47 ± 0.40^{b}	13.79 ± 0.32^{a}
Σ n–3/n–6 PUFA	1.03 ± 0.05^{c}	1.04 ± 0.06^{c}	1.16 ± 0.05^{b}	1.28 ± 0.05^{a}	1.45 ± 0.02^{d}	2.53 ± 0.05^{a}	1.61 ± 0.05^{b}	1.53 ± 0.04^{c}
EPA + DHA	13.73 ± 0.31^{d}	15.25 ± 0.50^{c}	17.78 ± 0.84^{a}	16.18 ± 0.40^{b}	19.41 ± 0.39^{d}	25.18 ± 0.21^{a}	20.03 ± 0.36^{c}	20.92 ± 0.37^{b}

[A] Values are expressed as percentages of total fatty acids.
[B] (a-b-c-d) means in a row with identical letters are not significantly different. Values were presented as mean SD ($N = 5$) ($P < 0.05$).
[C] Neutral and phospholipids were not compared each other.

(18:2 *n*-6) in *n*-6 PUFA. These four FAs represented 56.19–59.54% of NLs and 65.65–69.22% of PLs. All fish PLs were characterised by a high proportion of 16:0 (29.19–32.56%). These results are in agreement with Aras, Haliloğlu, Ayık, and Yetim (2003), Aras, Haliloğlu, Bayır, Atamanalp, and Sirkecioğlu (2003) and Akpınar, Görgün, and Akpınar (2009) who reported the most notable FA was 16:0 in the total lipids of the *S. t. labrax*, *S. t. macrostigma* and rainbow trout. The relative abundance of groups of FAs in the NLs was, in decreasing order, MUFA, SFA and PUFA. Wills and Hopkirk (1976) reported the same order in wild eels. Total PLs from wild fish tissues are characteristically rich in PUFA (Gunstone, Wijesundera, & Scrimgeour, 1978); however, in the present study, the most abundant fraction of PLs was SFA, not PUFA, due to high 16:0 ratio in SFA (18.45–33.89%). Little is known about the FA profiles of total lipids of the *S. t. labrax* and *S. t. macrostigma* (Aras, Haliloğlu, Ayık et al., 2003; Aras, Haliloğlu, Bayır et al., 2003; Akpınar et al., 2009) and nothing is known of those of the *S. t. caspius*. These researchers also reported higher PUFA than SFA in the total lipids. However, there is no record about FA compositions of NLs and PLs of the *S. t. caspius*, *S. t. labrax* and *S. t. macrostigma*. Therefore, high SFA ratio in PLs might be a special characteristic of them.

SFA contents ranged between 21.21% and 32.15% in the NLs. Similar results were reported for SFAs of total lipid of the *S. t. macrostigma*, *S. t. labrax* and *Oncorhynchus mykiss* (Aras, Haliloğlu, Ayık et al., 2003; Aras, Haliloğlu, Bayır et al., 2003; Akpınar et al., 2009). SFA contents of the PLs were quite higher than that of NLs (42.76–47.58%). SFA values in the NLs and PLs exhibited important seasonal variations in all fish (Tables 2–4) ($P < 0.05$). The maximal SFA contents of NLs in the *S. t. caspius*, *S. t. labrax* and *S. t. macrostigma* were found in the winter (post-spawning period), 3.10 ± 0.12%, 4.67 ± 0.19% and 3.22 ± 0.08%, respectively, and the lowest in the autumn (pre-spawning period) for the *S. t. caspius*, 1.49 ± 0.12% and *S. t. macrostigma*, 1.50 ± 0.06% and in the spring for the *S. t. labrax*, 2.83 ± 0.05% (Tables 2–4). The highest amounts SFAs of the PLs of the *S. t. labrax* and *S. t. macrostigma* were obtained in the summer; 45.01 ± 0.63% and 47.58 ± 0.85%, respectively, and autumn for the *S. t. caspius*, 46.30 ± 2.00%. The lowest amounts were found in the winter for the *S. t. caspius*, 43.47 ± 0.76% and in the autumn for the *S. t. labrax* and *S. t. macrostigma*, 42.76 ± 1.97% and 46.40 ± 0.62%, respectively (Tables 2–4).

Although MUFA contents of NLs (42.91–52.98%) were higher than the PLs, 16.00–23.05%, the inverse was seen for PUFA amounts (Tables 2–4). Gunstone et al. (1978) described that the PLs of fish contain much higher and lower amounts of the PUFA and MUFA than NLs, respectively. Therefore, our results were confirmed by previous studies. MUFA contents of NLs and PLs also showed important seasonal variations ($P < 0.05$). The highest amounts of MUFA of NLs in the *S. t. caspius*, *S. t. labrax* and *S. t. macrostigma* were found in the spring; 50.77 ± 0.95%, 48.07 ± 0.16% and 52.98 ± 0.67%, respectively (Tables 2–4). The lowest amounts were obtained in the winter for the *S. t. labrax*, 42.91 ± 0.67%, *S. t. macrostigma*, 47.40 ± 1.16% and *S. t. caspius*, 48.22 ± 0.27%. The highest MUFA amounts in the PLs were found in the winter for the *S. t. labrax*, 23.05 ± 0.56% and in the summer for the *S. t. macrostigma*, 20.71 ± 0.66%. The lowest activities were found in the autumn for the *S. t. labrax*, 20.47 ± 0.43% and *S. t. macrostigma*, 16.00 ± 0.85% and MUFA contents of PLs of the *S. t. caspius* were stable during the year (Tables 2–4).

n-3 PUFA values of NLs showed similar changes during the study, with significantly higher levels in the winter and decreases in the summer (Tables 2–4) ($P < 0.05$). The maximal *n*-3 PUFA of PLs were measured in the spring and winter for the *S. t. caspius*, 24.63 ± 0.58% and 24.41 ± 1.13%, respectively, and in the autumn for the *S. t. labrax*, 25.55 ± 0.21% and in the winter for *S. t. macrostigma*, 21.97 ± 0.47%. The lowest values were found in the autumn for the *S. t. caspius*, 20.68 ± 0.13%, and in the summer for the *S. t. labrax*, 19.62 ± 0.39% and *S. t. macrostigma*, 19.11 ± 0.75% (Tables 2–4) ($P < 0.05$).

The minimum *n*-6 PUFA amounts of NLs were recorded in the summer for the *S. t. caspius*, 13.57 ± 0.05%, and in the spring for the *S. t. labrax*, 13.64 ± 0.44% and *S. t. macrostigma*, 15.63 ± 0.43%, with the maximum values found in the autumn for the *S. t. caspius*, 17.33 ± 1.11% and *S. t. macrostigma*, 18.43 ± 0.36% and in the winter for the *S. t. labrax*, 15.89 ± 0.17% (Tables 2–4) ($P < 0.05$). The *n*-6 PUFA levels of PLs of the *S. t. caspius* and *S. t. labrax* exhibited the highest values in the spring, 11.15 ± 0.73% and 13.79 ± 0.32%, respectively and lowest in the summer for the *S. t. caspius*, 9.37 ± 0.32% and autumn, 10.11 ± 0.19% for the *S. t. labrax*. *n*-6 PUFA levels of PLs in the *S. t. macrostigma* displayed significant increases during the autumn, 16.25 ± 0.27% and decreases during the winter, 11.46 ± 0.31% (Tables 2–4) ($P < 0.05$).

The *n*-3/*n*-6 ratios of PLs (1.29–2.42) were quite higher compared to those of NLs (0.46–1.28). Akpınar et al. (2009) described that the *n*-3/*n*-6 ratio was 2.26 in the female *S. t. caspius* and Ackman (1967) and Standsby (1967) found that this ratio ranged from 1.7 to 3.5 in the freshwater species. Hence, our findings are in accordance with earlier studies. The highest *n*-3/*n*-6 ratios of NLs were recorded in the winter for the *S. t. caspius*, 0.98 ± 0.002 and *S. t. macrostigma*, 0.70 ± 0.01 and in the spring for the *S. t. labrax*, 1.28 ± 0.05. The lowest values were found in the autumn and summer for all fish (Tables 2–4) ($P < 0.05$). The highest *n*-3/*n*-6 ratios of PLs were measured in the different seasons: in the summer for the *S. t. caspius*, 3.07 ± 0.36, in the autumn for *S. t. labrax*, 2.53 ± 0.05, and in the winter for the *S. t. macrostigma*, 1.92 ± 0.09. The lowest levels where measured in the autumn for the *S. t. caspius* and *S. t. macrostigma*, 1.85 ± 0.12 and 1.29 ± 0.08 and in summer for the *S. t. labrax*, 1.45 ± 0.02 (Tables 2–4) ($P < 0.05$).

EPA + DHA amounts of PLs (18.90–25.18%) were higher than those of NLs (6.63–17.78%). It has been reported that the major PUFA in PLs is DHA (Gunstone et al., 1978). Hence, the high EPA + DHA amounts in PLs are due to the high DHA ratio in this fraction. EPA + DHA values of NLs showed similar behaviour, i.e., the highest amounts were recorded during winter ($P < 0.05$) and lowest in the summer (Tables 2–4). However, the highest and lowest amounts of them were found in the different seasons in PLs (Tables 2–4).

It is known that species, geographical origin and season change the lipid content of fish (Bayır, Haliloğlu, Sirkecioğlu, & Aras, 2006; Rasoarahona et al., 2005). The FA compositions of tissue lipids in wild fish are markedly influenced by the patterns of FAs in their dietary lipids and reflect the availability of FAs in the aquatic food chain (Henderson & Tocher, 1987).

Theoretically, the FA compositions of NLs and PLs in fish change during an annual cycle since they are fractions of total FAs, but they have not been well studied in this respect. In the present investigation, the highest PUFA values of NLs were determined in the cold seasons which are also include spawning period for three sub-species. The major PUFAs in NLs were 18:2 *n*-6 and docosadienoic acid (22:2 *n*-6). It is known that 18:2 *n*-6 is dominant FAs in total and NLs of fish (Aras, Haliloğlu, Ayık et al., 2003; Aras, Haliloğlu, Bayır et al., 2003; Almeida & Franco, 2007). But, this is the first record showing high 22:2 *n*-6 in NLs in fish, especially in the *S. trutta*. Hazel (1979) reported that *n*-3 and *n*-6 PUFA amounts of triacylglycerols, which are major classes of NLs, of liver of rainbow trout increased during acclimatisation to low temperature. At the same time; Shirai, Suzuki, Toukairin, and Wada (2001) investigated effects of season and spawning on the lipid content of ovary and liver of Japanese catfish. They reported that triacylglycerols were higher in the spawning than post-spawning season and that the inverse was seen for free fatty acids. Moreover, the highest and lowest FA composi-

Table 4
Fatty acid composition (% of total fatty acids[A]) of neutral and phospholipids of *Salmo trutta macrostigma* in different seasons.[B,C]

Fatty acids	Neutral lipid				Phospholipid			
	Summer	Autumn	Winter	Spring	Summer	Autumn	Winter	Spring
14:0	0.27 ± 0.03^{b}	0.54 ± 0.02^{a}	0.28 ± 0.02^{b}	0.27 ± 0.01^{b}	0.13 ± 0.006^{c}	0.34 ± 0.03^{b}	0.63 ± 0.03^{a}	0.15 ± 0.01^{c}
15:0	0.27 ± 0.009^{b}	0.35 ± 0.03^{a}	0.09 ± 0.004^{d}	0.11 ± 0.004^{c}	0.49 ± 0.02^{b}	0.62 ± 0.02^{a}	0.06 ± 0.003^{d}	0.13 ± 0.004^{c}
16:0	17.84 ± 0.48^{a}	17.46 ± 0.31^{a}	16.19 ± 0.47^{b}	14.92 ± 0.26^{c}	33.57 ± 0.49^{a}	31.36 ± 0.87^{c}	33.24 ± 0.93^{a}	32.08 ± 0.32^{b}
17:0	0.94 ± 0.04^{a}	0.50 ± 0.04^{d}	0.87 ± 0.05^{b}	0.56 ± 0.02^{c}	0.59 ± 0.04^{a}	0.41 ± 0.04^{c}	0.52 ± 0.06^{a}	0.39 ± 0.01^{c}
18:0	4.79 ± 0.35^{a}	3.96 ± 0.13^{b}	3.08 ± 0.19^{c}	3.29 ± 0.33^{c}	8.79 ± 0.42^{a}	8.50 ± 0.21^{b}	7.51 ± 0.23^{b}	10.57 ± 0.55^{a}
20:0	0.77 ± 0.05^{c}	0.59 ± 0.06^{d}	1.16 ± 0.08^{a}	0.89 ± 0.08^{b}	0.27 ± 0.03^{b}	0.32 ± 0.02^{a}	0.14 ± 0.02^{c}	0.26 ± 0.01^{b}
22:0	1.07 ± 0.13^{c}	1.29 ± 0.11^{b}	1.45 ± 0.06^{a}	1.04 ± 0.04^{c}	3.57 ± 0.19^{b}	4.54 ± 0.30^{a}	4.31 ± 0.33^{a}	3.33 ± 0.14^{b}
24:0	0.16 ± 0.006^{b}	0.20 ± 0.01^{a}	0.21 ± 0.03^{a}	0.13 ± 0.008^{c}	0.16 ± 0.008^{c}	0.32 ± 0.01^{a}	0.25 ± 0.02^{b}	0.26 ± 0.02^{b}
Σ SFA	26.12 ± 0.25^{a}	24.89 ± 0.50^{b}	23.33 ± 0.36^{c}	21.21 ± 0.60^{d}	47.58 ± 0.85^{a}	46.40 ± 0.62^{b}	46.66 ± 0.62^{b}	47.18 ± 0.53^{ab}
15:1	0.28 ± 0.02^{c}	0.37 ± 0.04^{b}	0.65 ± 0.04^{a}	0.29 ± 0.008^{c}	0.47 ± 0.03^{b}	0.15 ± 0.009^{c}	1.16 ± 0.15^{a}	1.05 ± 0.11^{a}
16:1 *n*-7	11.36 ± 0.33^{b}	11.29 ± 0.48^{b}	11.45 ± 0.56^{b}	12.95 ± 0.41^{a}	3.59 ± 0.25^{a}	3.74 ± 0.41^{a}	3.15 ± 0.37^{b}	3.82 ± 0.11^{a}
17:1	0.54 ± 0.04^{b}	0.35 ± 0.03^{d}	0.73 ± 0.06^{a}	0.48 ± 0.03^{c}	0.06 ± 0.003^{c}	0.25 ± 0.03^{a}	0.08 ± 0.002^{c}	0.16 ± 0.01^{b}
18:1 *n*-9	17.30 ± 0.27^{d}	22.32 ± 0.77^{a}	18.33 ± 0.75^{c}	19.46 ± 0.86^{b}	10.33 ± 0.10^{a}	8.89 ± 0.70^{d}	8.78 ± 0.33^{b}	9.86 ± 0.21^{a}
20:1 *n*-9	19.10 ± 0.51^{a}	13.37 ± 0.44^{c}	15.58 ± 0.45^{b}	19.36 ± 0.85^{a}	5.47 ± 0.14^{b}	2.62 ± 0.22^{d}	5.76 ± 0.16^{a}	4.48 ± 0.06^{c}
22:1 *n*-9	0.74 ± 0.06^{a}	0.67 ± 0.06^{b}	0.66 ± 0.03^{b}	0.44 ± 0.03^{c}	0.79 ± 0.06^{b}	0.35 ± 0.04^{d}	0.97 ± 0.05^{a}	0.55 ± 0.06^{c}
Σ MUFA	49.32 ± 0.36^{b}	48.36 ± 0.39^{b}	47.40 ± 1.16^{c}	52.98 ± 0.67^{a}	20.71 ± 0.66^{a}	16.00 ± 0.85^{c}	19.90 ± 0.71^{b}	19.93 ± 0.08^{b}
18:3 *n*-3	0.48 ± 0.07^{ab}	0.52 ± 0.05^{a}	0.53 ± 0.01^{a}	0.44 ± 0.04^{b}	0.46 ± 0.03^{a}	0.35 ± 0.04^{b}	0.33 ± 0.01^{b}	0.35 ± 0.02^{b}
20:5 *n*-3	0.42 ± 0.06^{c}	0.58 ± 0.03^{b}	0.66 ± 0.05^{a}	0.63 ± 0.02^{ab}	0.002 ± 0.0004^{c}	0.01 ± 0.001^{a}	0.001 ± 0.0005^{c}	0.007 ± 0.0008^{b}
22:5 *n*-3	0.18 ± 0.01^{c}	0.15 ± 0.01^{d}	0.36 ± 0.03^{a}	0.28 ± 0.01^{b}	18.46 ± 0.74^{c}	20.24 ± 0.96^{b}	21.56 ± 0.49^{a}	18.55 ± 0.43^{c}
22:6 *n*-3	6.67 ± 0.40^{c}	7.13 ± 0.50^{c}	10.46 ± 0.38^{a}	8.29 ± 0.35^{b}	19.11 ± 0.75^{c}	20.96 ± 0.98^{b}	21.97 ± 0.47^{a}	19.05 ± 0.44^{c}
Σ *n*-3 PUFA	7.76 ± 0.46^{d}	8.38 ± 0.48^{c}	12.01 ± 0.42^{a}	9.64 ± 0.35^{b}	6.52 ± 0.25^{b}	10.52 ± 0.31^{a}	6.46 ± 0.30^{b}	6.46 ± 0.38^{b}
18:2 *n*-6	12.27 ± 0.33^{b}	14.57 ± 0.35^{a}	12.16 ± 0.38^{b}	11.38 ± 0.41^{c}	0.18 ± 0.02^{b}	0.35 ± 0.02^{a}	0.09 ± 0.006^{d}	0.14 ± 0.01^{c}
20:2 *n*-6	0.008 ± 0.001^{d}	0.32 ± 0.01^{b}	0.68 ± 0.04^{a}	0.25 ± 0.02^{c}	1.97 ± 0.06^{b}	1.70 ± 0.14^{c}	1.53 ± 0.07^{d}	2.51 ± 0.16^{a}
20:3 *n*-6	0.23 ± 0.008^{c}	0.77 ± 0.07^{a}	0.66 ± 0.04^{b}	0.69 ± 0.01^{b}	0.10 ± 0.008^{c}	0.23 ± 0.02^{a}	0.14 ± 0.003^{b}	0.13 ± 0.03^{b}
20:4 *n*-6	1.28 ± 0.13^{a}	0.15 ± 0.02^{b}	0.15 ± 0.01^{b}	0.12 ± 0.004^{b}	0.17 ± 0.02^{d}	0.19 ± 0.008^{c}	0.29 ± 0.01^{a}	0.23 ± 0.02^{b}
22:2 *n*-6	2.87 ± 0.11^{c}	2.62 ± 0.14^{d}	3.50 ± 0.30^{a}	3.20 ± 0.10^{b}	3.53 ± 0.14^{b}	3.61 ± 0.11^{ab}	3.05 ± 0.16^{c}	3.76 ± 0.10^{a}
Σ n–6 PUFA	16.66 ± 0.19^{b}	18.43 ± 0.36^{a}	17.14 ± 0.53^{b}	15.63 ± 0.43^{c}	12.28 ± 0.37^{c}	16.25 ± 0.27^{a}	11.46 ± 0.31^{d}	13.09 ± 0.30^{b}
Σ n–3/n–6 PUFA	0.47 ± 0.03^{c}	0.46 ± 0.03^{c}	0.70 ± 0.01^{a}	0.62 ± 0.04^{b}	1.56 ± 0.07^{b}	1.29 ± 0.08^{d}	1.92 ± 0.09^{a}	1.46 ± 0.05^{c}
EPA + DHA	7.09 ± 0.42^{d}	7.71 ± 0.48^{c}	11.12 ± 0.40^{a}	8.92 ± 0.36^{b}	18.92 ± 0.73^{c}	20.60 ± 0.97^{b}	21.89 ± 0.48^{a}	18.90 ± 0.43^{c}

[A] Values are expressed as percentages of total fatty acids.
[B] (a–b–c–d) means in a row with identical letters are not significantly different. Values were presented as mean ± SD ($N = 5$) ($P < 0.05$).
[C] Neutral and phospholipids were not compared each other.

tion of muscle in Siberian grayling (*Thymallus arcticus*) was found in the pre-spawning and post-spawning period, respectively (Sushchik et al., 2007). As mentioned above, autumn and winter are the pre and post-spawning periods for all sub-species, respectively. Hence, our results agree with previous studies and the high PUFA contents in NLs suggest that there is an important spawning influence on the FA metabolism in the *S. trutta*. The highest SFA and MUFA contents of NLs were obtained in the summer and spring, respectively. Palmitic acid was the predominant FA in SFAs of NLs (Tables 2–4). Ackman, Eaton, and Linne (1975) described that the levels of palmitic acid of fish was not affected by diet. However, many other factors could influence metabolism and FA metabolism in fish, e.g. water temperature and life mode. Therefore, these factors may be critical for high SFA level in the summer. The major MUFAs in NLs were palimitoleic acid (16: *n*-7), oleic acid (18:1 *n*-9) and eicosenoic acid (20:1 *n*-9). Henderson and Tocher (1987) described that fish NLs contain high level of 18:1 *n*-9 and 16:1 *n*-7. Since diet is main factor affecting tissue FA composition in fish, high MUFA ratios in the spring probably related to increasing feeding activity in the *S. trutta*.

The highest total PUFA amounts of PLs were found in the pre-spawning period in the *S. t. labrax* and *S. t. macrostigma*. On the contrary, the lowest total PUFA levels were found in this period for the *S. t. caspius* (Tables 2–4). FAs are not only the major source of metabolic energy in fish for growth, they are also the major source of metabolic energy for reproduction (Tocher, 2003). Sushchik et al. (2007) reported that spawning was the main cause of the seasonal variations of contents of many FA species in muscles of Sberian grayling. Similarly, Marshall, Yaragina, and Lambert (1999) determined clear correlation between oil levels and fecundity in wild cod. Hence, the high PUFA rations of PLs during the pre-spawning period related to energy requirements of the *S. t. labrax* and *S. t. macrostigma* for reproduction. 22:6 *n*-3, 18:2 *n*-6 and 22:2 *n*-6 were the abundant PUFAs in PLs. It has been known that DHA and 18:2 *n*-6 are abundant FAs in the total lipids of the *S. t. labrax* and *S. t. macrostigma* (Aras, Haliloğlu, Ayık et al., 2003; Aras, Haliloğlu, Bayır et al., 2003) and PLs of *Brycon cephalus* (Almeida & Franco, 2007). However, there is not any record showing high 22:2 *n*-6 amount in NLs and PLs of the *S. trutta*. This can be explained by GC column differences between our study and earlier studies.

While the highest SFA contents of PLs were obtained in the summer for the *S. t. labrax* and *S. t. macrostigma*, it was found in the pre-spawning period for the *S. t. caspius*. Also in this fraction palmitic acid was the predominant FA in SFA (Tables 2–4). Since amount of 16:0 is not influenced by diet (Ackman et al., 1975), the reasons for high 16:0 ratios of PLs in the summer and autumn may be related to water temperature and reproduction activity, respectively.

There was no harmony in the seasonality of MUFAs of PLs in the three sub-species. They were stable during the year in the *S. t. caspius*, the highest values were in the winter for the *S. t. labrax* and in the summer for the *S. t. macrostigma* (Tables 2–4). Developmental and seasonal metabolic cycles reflect the complex interactions between exogenous factors such as food availability, temperature and growth and endogenous factors such as reproductive activities (Gabbott, 1983), that may influence the results. Similarly NLs, the major MUFAs in PLs were also 16: *n*-7, 18:1 *n*-9 and 20:1 *n*-9. Almeida and Franco (2007) reported that 18:1 *n*-9 and 16:1 *n*-7 were the major FAs in PLs of *Brycon cephalus*.

The *n*-3/*n*-6 ratio has been suggested to be the best index when comparing relative nutritional value of fish oils (Piggott & Tucker, 1990). Our results show that *n*-3/*n*-6 ratio vary according to season, being the highest in the winter for both fractions of the *S. t. caspius* and *S. t. macrostigma* (Tables 2–4). Similar results were also

observed by Rasoarahona et al. (2005) who reported the highest *n*-3/*n*-6 ratios were in the winter for three tilapia species. However, the highest *n*-3/*n*-6 ratio was found in the spring and autumn for NLs and PLs of *S. t. labrax*, respectively (Table 3). FA composition of fish lipids is extremely variable, even within species (Kaushik et al., 2006; Shirai et al., 2002; Zenebe et al., 1998), and this is probably the reason for the differences between the two groups (*S. t. caspius*, *S. t. macrostigma* and *S. t. labrax*). In contrast to the PLs of the *S. t. labrax*, the contents of EPA + DHA were the highest in the winter for the sub-species. This can be explained by the fact that in the post-spawning season the fish feed mainly on insects, smaller fish, mollusks and crustaceans, which, according to Henderson and Tocher (1987), are known to be rich sources of PUFA.

In conclusion, we found that fatty compositions of neutral and phospholipids of the *S. t. caspius*, *S. t. labrax* and *S. t. macrostigma* were affected by some endogenous factors, especially reproduction, and exogenous factors, especially diet, and provide reference values throughout the seasons of FA contents for these three sub-species. However, *n*-3/*n*-6 ratios and EPA + DHA amounts, generally, were higher in the winter than other seasons, and it is of interest to note that the increase in *n*-3/*n*-6 ratios and EPA + DHA amounts occurred during the season where the fish exhibit the lowest GSI and is the period immediately after spawning. Sargent (1996) reported that *n*-3 PUFA, especially DHA, has a role in maintaining the structure and functional integrity of fish cells. In addition DHA has a specific and important role in neural cell membranes, i.e. the brain and eyes. For these reasons, according to *n*-3/*n*6 ratios and EPA + DHA amounts edible flesh, the best period for consumption of the *S. trutta* would be winter. This is the first study to report seasonal changes of FAs of NLs and PLs of *S. trutta sp.* Therefore, more detailed studies showing relationships between season and fatty acid metabolism of these economically valuable and endangered sub-species should be taken into consideration. Furthermore, controlled aquarium experiments could be conducted to determine the specific effect of a certain factor or a combination of factors on fatty acid metabolism of the *S. t. caspius*, *S. t. labrax* and *S. t. macrostigma*.

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