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Original Article

Comparative Study of Lipid and Fatty Acid Profile in Liver Tissues of Male and Female *Silurus triostegus* During the Catching Seasons

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ABSTRACT

In this study, seasonal variations of total fatty acid (FA), phospholipid (PL) and triacylglycerol (TAG) compositions in liver tissues of catfish (*Silurus triostegus*) were investigated. Samples of *S. triostegus* were obtained from Atatürk Dam Lake, Turkey, in two month periods during one year as from May. The major components were palmitic acid (16:0), stearic acid (18:0), oleic acid (18:1n-9), palmitoleic acid (16:1n-7), docosahexaenoic acid (DHA, 22:6n-3) and arachidonic acid (AA, 20:4n-6) in total lipid, 16:0, 18:0, 18:1n-9, eicosapentaenoic acid (EPA, 20:5n-3), DHA and AA in PL, 16:0, 16:1n-7, 18:1n-9, linoleic acid (LA, 18:2n-6), AA, EPA, DHA and docosapentaenoic acid (22:5n-3) in TAG extracted from the liver of *S. triostegus* in all seasons. N-3/n-6 ratio was found 2.00-2.61 in females, 1.15-2.75 in males in total lipid. The highest lipid content was found in May (2.39%) in the females. In the males, the highest level was found in September (2.91%). In TAG fraction, the component with the lowest ratio in both sexes' TAG is PUFA. In PL fraction, SFA, MUFA, and PUFA percentages were found at similar rates in both sexes in all months.

Keywords: *Silurus triostegus*, fatty acid, phospholipid, triacylglycerol, comparison, male, female, season, total lipid

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INTRODUCTION

Mesopotamia catfish (*S. triostegus*) is a catfish species from the Siluridae family which is found in the Mesopotamia region namely Syria, Iraq, Iran, and Turkey. In *S. triostegus* living in the Atatürk Dam Lake, it was determined that egg laying started in May and continued until the end of June by utilizing parameters such as gonadosomatic index values and direct observation of gonads. Consequently, it can be said that the reproduction period is May, June, and July (Oymak *et al.*, 2001).

Catfish meat is delicious, and has high protein content. It is a carnivore and aggressive species. Their nutrients usually are water insects and their larvae, worms, frogs, and tadpoles (Geldiay & Balık 1996).

Today, there is a great interest in fish and fish oil due to the polyunsaturated fatty acids (PUFA) they contain. Analyzes usually constitute the fish muscle that forms the food. On the other hand, the fish liver, i.e., the main organ for long-chain PUFA (LC-PUFA), has not been analyzed much (Ackman *et al.*, 2002). Fish liver is the source of the essential oils for the prevention of problems related to vision and growth (Njinkoué *et al.*, 2002). Moreover, livers of some fish species are used for muscle pain and rheumatism in Pakistan (Saify *et al.*, 2003).

The liver is an important organ in terms of lipid metabolism. This organ also has an important role in the uptake, oxidation, and transformation of fatty acids (FA) and the supply of long-chain highly unsaturated fatty acids to other tissues (Rincon-Sanchez *et al.*, 1992).

Phospholipids (PL) and triacylglycerides (TAG) have different roles in fish metabolism. Phospholipids are the main components of cell membrane and structure, and serve as precursors of eicosanoids with the C20 PUFA they contain. Meanwhile, TAG function as energy reserves (Sargent *et al.*, 1995; Kiessling *et al.*, 2001).

Fish oil and fatty acid composition are the most changed biochemical compounds according to the ecological factors and the physiological state of the fish. The total fat content and fatty acid composition in fish vary depending on species, sex, season, nutritional environment, nutrient difference, water temperature, water pollution, and whether the species are in the culture or natural form. Fat and fatty acids differ structurally in different fish species. If the same species of fish live in different geographical regions, they may differ in terms of fatty acid diversity (Ackman *et al*, 2002; Akpınar *et al*., 2009; Kayhan *et al*., 2014). This difference is also seen in different organs of the fish (Crowford *et al.*, 1986; Suzuki *et al*., 1986).

Total, TAG, and PL fatty acids of *S. triostegus* liver have not studied before. Therefore, in this study, it was aimed to compare the seasonal changes of the total, TAG, and PL fatty acid contents of liver tissues of female and male individuals of *S. triostegus* living in Atatürk Dam Lake.

MATERIALS AND METHODS

Samples of *S. triostegus* fish species were gathered from the Atatürk Dam Lake in one year period by using a fishing net (between May, 2008 and March, 2009). On the same day, the caught fish samples were placed in heat-insulated protective containers filled with ice, and brought to the laboratory. The measurements of height and weight of the samples were made. Weight measurements were noted in grams, and height measurements were taken in cm using the fork length of the fish (Kaçar *et al.*, 2016). In this study, three male and three female fish were used. The sexes of fish samples were detected. After determining the wet weights of the liver samples taken out, they were put in tubes and stored in the chloroform-methanol mixture at -80 °C until analysis.

Lipid extraction and conversion of fatty acids to methyl esters

Liver samples homogenized in the chloroform-methanol mixture (Folch *et al.,* 1957). The thin layer chromatography technique was used to fractionate the total lipids in the samples. For this purpose, the mixture of 30 g of silica gel and 50 ml of pure water was applied as a thin layer to the plates of 20X20 size, and they were dried in an oven at 100 °C for one hour. Total lipid extracts of the samples were spotted onto the plates in a single row. The total lipids were run in a mixture of petroleum ether-diethyl ether-acetic acid (80:20:1). After drying the plates in the air, 2′7′ dichlorofluorescein was sprayed to make the lipid fractions visible under the UV lamp. The bands of phospholipids and triacylglycerol fractions that were determined by means of standards were scraped and transferred to the reaction tubes. To each fraction, 3 ml of methanol and 3-5 drops of sulfuric acid were added dropwise, and they were all heated at 85 °C under reflux for 2 hours (Stanley-Samuelson and Dadd,1983). The solution was extracted with methyl esters using hexane. Gas chromatography instrument with a FID detector was used for the analysis of fatty acid methyl esters.

Gas chromatography conditions

Fatty acid analyses of the oil samples converted to methyl esters were performed by using flame ionization detector and DB-23 capillary column in HP6890 model Gas Chromatography device. The features used were as follows. Detector temperature: 280 °C, injector temperature: 270 °C, and injection: split–model 1/20. Gas flow rates were; carrier gas: 2.8 ml/min (constant flow model), hydrogen: 30 ml/min, and air: 300 ml/min. Column (oven) temperature: at 130 °C, stand-by time 1 minute; to 170 °C with 6.5 °C/min, to 215 °C with 2.75 °C/min, standby time 12 minutes; to 230 °C with 40 °C/min, standby time 3 minutes; total analysis time: 38.8 minutes. For example, 1 microliter was injected into the device. A mixture of methyl esters of fatty acids was used as the standard in the detection of fatty acids. Chromatograms of fatty acids methyl esters and total fatty acids were obtained from the computer by the software HP 3365 Chem Station.

Evaluation of data

SPSS 16 computer program was used to compare fatty acid percent rates. All data obtained in our study were obtained from the average of three replicates. In the gas chromatographic analysis of fatty acid methyl esters, three samples of each period were injected separately, and the three values of the same fatty acid were averaged. Comparison of fatty acid percentages was made by one-way analysis of variance. Differences were determined by Tukey HSD test. As a result of the statistics, it was accepted that the differences were significant when the data were $p<0.05$.

RESULT AND DISCUSSION

Lipid content

The quantity of total lipids ranging from 1.29 to 2.39% (breeding period) in *S. triostegus* females decreased in July, September, and January. In male fish, this ratio was found between 0.50-2.91% (post-breeding period). In males, the amount of lipid decreased significantly after September, in November and January. It increased in March (pre-breeding period) and in May (breeding period) (Table 1).

In freshwater fish, the lipid content of the liver varies depending on the season, feeding cycle, and reproductive status (Ackman et al., 2002).

Table 1. Total lipid of liver tissue of female and male *S. triostegus*.

	Total lipid (%)	
	Female	Male
May	2.39±0.65a	2.14±0.45a
July	1.78±0.38b	1.04±0.12b
September	1.61±0.40b	2.91±0.13a
November	2.03±0.35a	0.50±0.07c
January	1.29±0.21b	0.60±0.14c
March	1.74±0.41b	2.09±0.52a

Superscript letters (a,b,c) denote significant differences ($p < 0.05$) in lipid content among months.

In the current study, it was found that liver lipid content decreased in the reproductive period of July. The decrease in total lipid and total fatty acid levels in the liver and muscles of the fish in the reproductive period shows that they obtain the energy they need from these lipids. During the reproductive period, the lipids in the liver and muscle are mobilized to the gonad for gonad development (Castell et al., 1972).

In previous studies, the liver lipid content of *Cyprinus carpio* increased in the spring (Kminkova et al., 2001; Akpınar, 1986a). Kandemir & Polat (2007) found the maximum total lipid amount in the liver of *Oncorhynchus mykiss* in the autumn season. In the present study, the highest lipid content of female fish was found in May in accordance with the previous studies.

The amount of lipid in the *S. triostegus* males decreased significantly in November and January, and increased in March and May. Jangaard et al., (1967) reported that seasonal changes in lipid level in liver and other organs are caused by irregular seasonal changes in fish feeding and water temperature.

In our study, the amount of lipid increased in both sexes in the autumn months, which was the post-reproductive period. Stored lipids vary during the breeding and feeding period. Especially when fish find enough food, they can control their reproduction and lipid storage period. The lipid storage cycle is directly related to the abundance of food. Lipid variation is high throughout the year if nutrients are abundant, whereas lipid variation is low if nutrients are scarce (Kluytmans & Zandee, 1973, Ackman & Eaton, 1976, Kinsella et al., 1977, Mute et al., 1989).

The FA composition of total lipid

The total fatty acid content of female and male *S. triostegus* liver tissue is given in Table 2. The highest saturated fatty acids (SFA) in females were found in the summer whereasin males, they were found in spring which is the reproductive period. The lowest SFA was found in January in both sexes. The SFA was found to have 16:0 and 18:0 most commonly. The 16:0 ratio with saturated fatty acids decreased in males and females in January and in the pre-breeding period in March In males, both components were found to be the highest in May, which is in the breeding period. The 18:0 were highest in females during November, yet no statistically significant difference was found in other seasons. This fatty acid in males showed fluctuations throughout the year. In males and females, monounsaturated fatty acids (MUFA) in liver tissue increased in January.

The MUFA and 18:1n-9 ratio was high in January and March, and decreased in September in both males and females. In September, the amount of 16:1n-7 in females reached the lowest value. This fatty acid did not show a significant change except June and September. The highest value for males was found in January. Also, 18:1n-9 did not differ in females in all seasons. In males, it is low in May and September and close to each other. The PUFA in both sexes was found to be high in September. It fell in females in May and in July in males. The 20:5n-3, 22:5n-3, and 22:6n-3 are major n-3PUFA while 18:2n-6 and 20:4n-6 are dominant n-6PUFA. Moreover, the 20: 5n-3 was found to decrease in September after the breeding period, and has the highest value in winter in female *S. triostegus* liver tissue. It was also observed that it decreased in July and increased in May in males. In May, when the arachidonic acid was the lowest in females, it was the highest in males. Docosahexaenoic acid (DHA) increased in both sexes in July and September. In females, in May, July, and November the highest SFA, in January the highest PUFA and the lowest MUFA was found while in males, in September the highest PUFA and in July, November, January, and March the highest MUFA was determined. The dominant fatty acids throughout the year are 16:0 from SFA, 18:1n-9 from MUFA, and DHA from PUFA. N-3/n-6 ratio was found 2.00-2.61 in females and 1.15-2.75 in males. In both sexes, the highest value was determined in the same month, and it was close to each other.

Fish are not only important protein sources but also contain nutritionally valuable lipids. Analyzes usually focus on the fish muscle that forms the food. But the fish liver is the main organ of long-chain PUFA and has not been analyzed much (Ackman et al., 2002).

In fish liver, lipids major fatty acids are similar to the ones in fish muscle. Most of the fish that have been studied have 16:0 most among the saturated fatty acids, 18:1n-9 among the monounsaturated, and 22:6n-3 among the polyunsaturated (Kminkova et al., 2001; Uysal et al., 2006; Akpınar et al., 2009; Njinkoué et al., 2002; Aras et al., 2003b). Similar results were found in *S. triostegus* liver. However, quantitative fatty acid content in the liver varies. In accordance with the present study, in the previous studies, 14:0 and 18:0 found least among the saturated fatty acids, 16:1n-7 among the monounsaturated fatty acids, 18:2n-6 and 18:3n-3 among the highly unsaturated fatty acids, 20:3n-6 and 20:4n-6 acids which are the precursors of eicosanoids (Tufan et al., 2013; Misir et al., 2016; Kaçar & Başhan, 2017).

Sander lucioperca's n-3 fatty acids increased most in November when the temperature fell (Uysal et al., 2006). In both sexes of *S. triostegus,* PUFA increased in the autumn and winter when the temperature fell (Kaçar *et al.*, 2016).

In the previous studies, in line with the current research, it was observed that long-chain unsaturated fatty acids have changed more than saturated fatty acids. It was concluded that gonad development and breeding periods had directly an effect on these changes (Akpınar, 1986b).

The data indicate that the ratio of n-3/n-6 in total lipids of the liver may be different in both sexes. This depends on the ratios of 18:3n-3, 20:5n-3, 22:5n-3, and 22:6n-3, forming n-3PUFA and 18:2n-6, 20:3n- 6, and 20:4n-6, forming n-6PUFA.

In the current investigation, it was determined that the fatty acid content of fish liver lipids is affected by the sex and the season, thus, the reproductive period.

The FA composition of TAG fraction

Table 3 shows the TAG fatty acid content of male and female *S. triostegus* liver. Palmitic acid and therefore the SFA ratio in males increased in September, which is the post-breeding period, and decreased in March, just before the breeding season. There was no significant difference in females for 16:0 throughout the year.

Table 2. Seasonal variations of fatty acid composition of total lipid of liver tissue from female and male *S. triostegus* (% of total FA)*.

	May		July		September		November		January		March	
Fatty Acids	Female	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female	Male
10:00	-	-	-	-	-	0.03±0.01	-	-	-	-	-	-
12:00	0.05±0.02a	0.06±0.02a	-	-	0.01±0.01b	-	-	0.01±0.01b	0.12±0.05c	0.12±0.04c	0.07±0.04d	0.11±0.01c
13:00	0.32±0.03a	0.13±0.05b	0.21±0.02c	0.15±0.03b	-	0.07±0.04d	-	-	-	-	-	-
14:00	1.59±0.16a	2.47±0.21b	1.75±0.11c	1.19±0.11d	1.47±0.17a	2.07±0.25ab	1.10±0.10d	1.45±0.17a	1.14±0.16d	1.26±0.16d	1.98±0.67ab	1.96±0.17ab
15:00	0.72±0.07a	0.54±0.04b	0.26±0.02c	0.17±0.03d	0.47±0.03e	0.54±0.04b	0.01±0.01f	0.11±0.03d	0.35±0.02c	0.25±0.02c	0.47±0.05e	0.60±0.05b
16:00	25.53±1.26a	23.46±1.26a	26.85±1.20a	20.48±1.28b	24.60±1.22a	20.89±1.09b	22.49±1.25b	21.00±1.30b	19.49±1.09b	17.28±1.17c	19.91±1.14b	18.43±1.08b
17:00	0.42±0.03a	0.42±0.04a	0.18±0.01b	0.23±0.01b	0.28±0.02b	0.51±0.05c	0.31±0.03d	0.26±0.01b	0.36±0.02d	0.27±0.03b	0.56±0.05e	0.64±0.05e
18:00	9.95±0.99a	9.94±0.99a	10.47±1.10a	11.55±1.01a	10.66±1.01a	8.58±0.87a	14.15±1.14b	7.78±0.77c	8.95±0.88a	4.84±0.45d	9.21±0.91a	5.97±0.57d
∑SFA***	38.58±1.37a	36.96±1.33a	39.72±1.40a	33.77±1.44b	37.49±1.33a	32.69±1.29b	38.06±1.36a	30.61±1.38b	30.41±1.30b	24.02±1.28c	32.20±1.44b	27.71±1.29c
16:1n-7	7.97±0.77a	5.77±0.55b	4.11±0.41b	4.25±0.43b	3.26±0.30c	5.68±0.45b	7.41±0.66a	5.55±0.56b	7.40±0.59a	8.60±0.83a	7.72±0.69a	6.64±0.63b
18:1n-9	22.24±1.28a	21.80±1.26a	20.93±1.20a	30.91±1.30b	20.57±1.19a	21.59±1.27a	20.13±1.10a	30.01±1.38b	21.36±1.17a	30.79±1.36b	23.52±1.22a	29.10±1.28b
20:1n-9	0.71±0.07a	0.86±0.05a	2.17±0.29b	3.16±0.32b	1.64±0.11c	1.31±0.10c	2.07±0.24b	0.99±0.08a	3.21±0.33b	4.78±0.43d	1.93±0.17c	2.70±0.29bc
∑MUFA	30.92±1.33a	28.43±1.27a	27.21±1.28a	38.32±1.43b	25.47±1.26c	28.58±1.26a	29.61±1.30a	36.55±1.33b	31.97±1.38a	44.17±1.54d	33.17±1.42e	38.44±1.45b
18:2n-6	2.52±0.24a	3.51±0.32b	1.40±0.11c	0.88±0.07d	1.69±0.16c	2.96±0.27a	2.55±0.22a	2.79±0.22a	1.94±0.18c	1.55±0.22c	2.14±0.25a	3.80±0.36b
18:3n-3	1.22±0.17a	1.22±0.13a	0.57±0.05b	0.49±0.05b	0.93±0.08a	1.93±0.16c	1.20±0.12a	3.29±0.37c	1.34±0.16a	0.79±0.07ab	1.14±0.18a	2.32±0.25c
20:2n-6	0.45±0.04a	0.49±0.03a	0.61±0.06b	0.34±0.02c	0.76±0.07b	0.80±0.07b	0.90±0.09b	0.38±0.04c	0.54±0.05a	0.70±0.05b	0.72±0.07b	1.26±0.17d
20:3n-6	0.48±0.04a	0.54±0.04a	0.46±0.03a	0.51±0.03a	0.58±0.05a	0.70±0.07b	1.25±0.13c	0.33±0.02d	0.57±0.05a	0.68±0.06b	0.46±0.04a	0.72±0.07b
20:4n-6	4.37±0.39a	11.42±1.01b	7.78±0.76c	5.75±0.56a	9.29±0.91c	8.93±0.88c	4.64±0.44a	6.26±0.65ac	7.09±0.78c	5.28±0.58a	7.05±0.71c	4.96±0.44a
20:5n-3	3.97±0.36a	5.38±0.59b	2.08±0.23c	1.97±0.17c	1.74±0.14c	4.68±0.42a	4.56±0.48a	2.84±0.21c	5.10±0.56b	3.86±0.36a	4.68±0.41a	5.42±0.55b
22:5n-3	1.05±0.10a	3.84±0.37b	2.28±0.22c	2.21±0.20c	3.03±0.38c	4.47±0.45b	3.13±0.33c	2.05±0.36c	5.80±0.59b	3.30±0.31c	4.62±0.48b	3.51±0.33b
22:6n-3	15.42±1.05a	8.05±0.86b	17.80±1.17a	15.67±1.15a	18.96±1.15a	14.21±1.07c	14.02±1.04c	13.91±1.19c	14.27±1.14c	14.69±1.04c	13.80±1.13c	10.86±0.98b
∑PUFA	30.48±1.30a	34.45±1.39a	32.98±1.22a	27.82±1.22b	36.98±1.33c	38.68±1.44c	32.25±1.38a	31.85±1.30c	36.65±1.34c	30.85±1.27a	34.61±1.42aa	32.85±1.43a
n-3	21.66±1.27a	18.49±1.08b	22.73±1.20a	20.34±1.29a	24.66±1.30a	25.29±1.33a	22.91±1.25a	22.09±1.32a	26.51±1.29c	22.64±1.20a	24.24±1.09a	22.11±1.38a
n-6	8.82±0.89a	15.96±1.05b	10.25±0.99c	7.48±0.77a	12.32±1.02c	13.39±1.04c	9.34±0.87a	9.76±0.98a	10.14±1.10c	8.21±0.87a	10.37±1.00c	10.74±1.10c
n-3/n-6	2.45	1.15	2.21	2.71	2.00	1.88	2.45	2.26	2.61	2.75	2.33	2.05

* Means are the averages of 3 replicates ** Values reported are means ±standard deviation; means followed by different letters in same line are significantly different (p<0.05) by Tukey's test *** SFA: saturated fatty acids, MUFA: monounsaturated fatty acids, PUFA: polyunsaturated fatty acids

Table 3. Seasonal variations of fatty acid composition in triacylglycerol fraction of liver tissue from female and male *S. triostegus* (% of total FA)*.

	May		July		September		November		January		March	
Fatty Acids	Female	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female	Male
10:00	0.18±0.01a	0.30±0.03b	0.48±0.03b	0.11±0.01c	-	-	-	-	-	-	-	-
12:00	0.49±0.03a	0.14±0.02b	0.19±0.01c	0.16±0.05b	-	-	-	-	-	-	-	-
13:00	0.94±0.08a	0.34±0.05b	0.50±0.04c	0.33±0.06b	0.66±0.05c	0.77±0.06c	0.92±0.08a	-	-	-	-	-
14:00	4.00±0.40a	2.61±0.22b	2.51±0.25b	2.69±0.26b	2.84±0.24b	4.56±0.44a	2.70±0.22b	1.95±0.19c	2.01±0.20c	2.35±0.26b	3.73±0.33a	2.96±0.30a
15:00	0.92±0.07a	0.52±0.04b	0.47±0.04b	0.35±0.03b	0.88±0.07a	1.16±0.16c	0.40±0.03b	0.31±0.03b	0.74±0.07a	0.40±0.02b	1.09±0.10a	0.70±0.06a
16:00	30.30±1.33a	21.88±1.20b	30.78±1.30a	21.30±1.19b	28.02±1.27a	28.52±1.27a	30.27±1.29a	27.42±1.30a	27.16±1.28a	26.48±1.27a	29.07±1.28a	20.22±1.27b
17:00	0.94±0.09a	0.40±0.04b	0.44±0.04b	0.27±0.01c	0.30±0.03c	0.21±0.08d	1.03±0.10a	0.16±0.01e	0.55±0.04b	0.25±0.01c	0.59±0.05b	0.61±0.05b
18:00	4.09±0.43a	6.69±0.65b	3.62±0.34c	5.23±0.52ab	5.41±0.54ab	7.24±0.77b	4.72±0.44a	6.55±0.56b	4.82±0.40ab	3.75±0.31c	5.19±0.51ab	4.06±0.40a
∑SFA***	41.86±1.40a	32.88±1.33b	38.99±1.33a	30.44±1.30b	38.11±1.38a	42.46±1.40a	40.04±1.44a	36.39±1.44d	35.28±1.33d	33.23±1.36b	39.67±1.42a	28.55±1.29b
16:1n-7	9.01±0.07a	7.32±0.67b	10.54±1.08a	16.74±1.06c	11.62±1.10a	13.99±1.03d	15.17±1.05c	14.00±1.14d	14.05±1.14d	15.99±1.05c	12.59±1.02d	11.09±0.99a
18:1n-9	27.56±1.28a	28.74±1.27a	26.91±1.22a	33.69±1.33b	26.81±1.25a	26.63±1.25a	26.58±1.39a	32.80±1.29b	29.60±1.34a	32.76±1.39b	29.44±1.30a	30.78±1.30ab
20:1n-9	0.92±0.08a	1.23±0.10a	1.88±0.16b	1.07±0.09a	1.47±0.11c	0.83±0.07a	1.35±0.19a	0.42±0.04d	1.74±0.20b	2.09±0.02b	1.07±0.17a	1.69±0.11b
∑MUFA	37.49±1.38a	37.29±1.33a	39.33±1.33a	51.50±1.59b	39.90±1.40a	41.45±1.44c	43.10±1.41c	47.22±1.52d	45.39±1.52d	50.84±1.56b	43.10±1.55c	43.56±1.43c
18:2n-6	3.05±0.34a	4.31±0.46b	2.83±0.22a	3.89±0.32a	2.54±0.35a	3.88±0.33a	2.57±0.29a	2.50±0.22a	2.40±0.28a	1.93±0.19b	2.86±0.27a	5.02±0.56b
18:3n-3	1.77±0.11a	2.14±0.21b	1.07±0.16c	2.50±0.28b	1.45±0.19a	3.38±0.33d	1.07±0.20c	2.27±0.28b	1.39±0.16a	3.95±0.30d	1.27±0.18ac	2.62±0.20b
20:2n-6	0.25±0.02a	0.33±0.03a	0.50±0.04b	0.36±0.02a	1.07±0.19c	0.40±0.03b	0.42±0.04b	0.15±0.01d	0.67±0.05b	0.33±0.02a	0.83±0.07bc	1.81±0.11e
20:3n-6	0.30±0.02a	0.63±0.05b	0.46±0.03a	0.43±0.04a	0.29±0.05a	0.14±0.01c	0.24±0.03a	0.18±0.01d	0.27±0.02a	0.50±0.04b	0.61±0.05b	0.86±0.07e
20:4n-6	4.53±0.44a	5.97±0.55a	5.38±0.50a	2.45±0.22b	3.68±0.36c	2.47±0.28b	2.50±0.20b	2.31±0.30b	3.71±0.32c	2.80±0.31b	3.03±0.33b	3.92±0.38c
20:5n-3	2.54±0.29a	5.73±0.55b	2.64±0.23a	1.28±0.11c	3.49±0.44d	1.75±0.10e	1.86±0.16e	1.31±0.17c	2.35±0.22a	1.48±0.15c	1.74±0.19e	2.59±0.27a
22:5n-3	2.23±0.20a	2.81±0.29a	2.32±0.22a	1.12±0.10b	1.85±0.16c	1.01±0.13b	1.30±0.15c	0.26±0.01d	2.38±0.27a	0.94±0.07b	2.08±0.31a	4.76±0.04e
22:6n-3	5.89±0.55a	7.83±0.71b	6.38±0.34a	5.93±0.56a	7.52±0.77b	2.98±0.23c	6.83±0.66a	6.50±0.68a	6.07±0.60a	3.90±0.33d	4.76±0.45a	6.21±0.56a
∑PUFA	20.56±1.22a	29.75±1.28b	21.58±1.39a	17.96±1.07c	21.89±1.29a	16.01±1.16c	16.79±1.06c	15.48±1.05c	19.24±1.09a	15.83±1.03c	17.18±1.17c	27.79±1.22d
n-3	12.43±1.02a	18.51±1.08b	12.41±1.12a	10.83±1.10a	14.31±1.04c	9.12±0.99a	11.06±1.10a	10.34±1.00a	12.19±1.03a	10.27±1.07a	9.85±0.99a	16.18±1.10b
n-6	8.13±0.78a	11.24±1.12b	9.17±0.88a	7.13±0.88a	7.58±0.70a	6.89±0.65c	5.73±0.51c	5.14±0.51c	7.05±0.69a	5.56±0.45c	7.33±0.77a	11.61±1.03b
n-3/n-6	1.52	1.64	1.35	1.51	1.88	1.32	1.93	2.01	1.72	1.84	1.34	1.39

* Means are the averages of 3 replicates ** Values reported are means ±standard deviation; means followed by different letters in same line are significantly different ($p<0.05$) by Tukey's test *** SFA: saturated fatty acids, MUFA: monounsaturated fatty acids, PUFA: polyunsaturated fatty acids

Table 4. Seasonal variations of fatty acid composition in phospholipid fraction of liver tissue from female and male *S. triostegus* (% of total FA)*.

	May		July		September		November		January		March	
Fatty Acids	Female	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female	Male
14:00	0.45±0.03a	1.79±0.10b	1.44±0.12b	1.15±0.11c	1.61±0.16b	1.89±0.13b	1.00±0.10c	1.47±0.16b	1.01±0.34c	1.18±0.18c	1.25±0.33c	1.41±0.19b
15:00	0.40±0.03a	0.49±0.04a	0.24±0.02b	0.26±0.02b	0.58±0.05a	0.57±0.03a	0.25±0.02b	0.11±0.01c	0.44±0.03a	0.36±0.03ab	0.43±0.02a	0.74±0.06d
16:00	22.75±1.20a	20.98±1.20a	20.34±1.19a	23.47±1.19a	24.79±1.22a	23.99±1.29a	23.79±1.28a	22.66±1.22a	20.63±1.18a	26.56±1.32b	20.31±1.10a	23.51±1.22a
17:00	0.18±0.01a	0.48±0.04b	0.15±0.02a	0.17±0.01a	0.28±0.03c	0.34±0.03b	0.40±0.04b	0.27±0.02c	0.62±0.05b	0.40±0.03b	0.85±0.07d	0.99±0.01d
18:00	11.71±1.09a	12.56±1.02a	14.74±1.14b	11.38±1.01a	11.86±1.01a	12.78±1.13a	14.43±1.09b	10.05±0.98a	13.81±1.12b	9.00±1.01a	14.68±1.17b	9.13±0.97a
∑SFA***	35.49±1.36a	36.30±1.33a	36.91±1.39a	36.43±1.35a	39.12±1.40a	39.57±1.40a	39.87±1.33a	34.56±1.29a	36.50±1.45a	37.50±1.30a	37.52±1.37a	35.78±1.34a
16:1n-7	2.40±0.29a	3.36±0.34a	3.21±0.31a	3.07±0.32a	3.01±0.33a	3.76±0.31a	3.26±0.27a	5.58±0.54b	2.02±0.20a	4.04±0.40a	3.88±0.41a	3.32±0.32a
18:1n-9	16.01±1.17a	15.74±1.05a	17.24±1.15a	19.83±1.09b	17.05±1.08a	15.66±1.15a	14.48±1.17a	16.72±1.06a	19.16±1.09b	17.00±1.07a	15.66±1.63a	15.09±1.15a
20:1n-9	0.62±0.05a	0.90±0.09b	2.56±0.21c	1.08±0.10b	0.98±0.07b	1.31±0.11b	1.40±0.10b	0.52±0.04a	2.05±0.23c	1.03±0.15b	1.13±0.18b	1.68±0.13d
∑MUFA	19.03±1.09a	20.00±1.20a	23.01±1.22a	23.98±1.26a	21.04±1.20a	20.73±1.19a	19.14±1.09a	22.82±1.29a	23.23±1.29a	22.07±1.21a	20.67±1.30a	20.09±1.25a
18:2n-6	1.58±0.16a	1.24±0.11a	0.79±0.07b	1.72±0.16a	1.42±0.11a	2.18±0.20c	2.58±0.28c	1.24±0.13a	1.66±0.27a	1.22±0.16a	1.39±0.16a	2.05±0.20c
18:3n-3	0.47±0.05a	0.53±0.04a	0.44±0.03a	0.47±0.03a	0.75±0.06b	1.34±0.12c	0.68±0.05b	0.22±0.02d	0.72±0.07b	0.59±0.04b	0.51±0.03b	0.78±0.06b
20:2n-6	0.42±0.04a	0.69±0.05b	0.88±0.07c	0.25±0.02d	0.73±0.05c	0.84±0.07c	0.49±0.04a	0.26±0.02d	0.53±0.04a	0.50±0.03a	0.47±0.03a	0.73±0.07c
20:3n-6	0.76±0.07a	0.83±0.05a	0.59±0.03b	0.55±0.04b	0.57±0.04b	0.71±0.06a	1.25±0.12c	0.31±0.02d	0.46±0.03b	0.75±0.06a	0.28±0.01d	0.50±0.04b
20:4n-6	12.82±1.02a	12.54±1.02a	9.03±0.99b	9.33±0.98b	9.34±0.97b	10.02±1.00b	10.53±1.13b	12.46±1.03a	7.46±0.77c	8.25±0.83bc	10.80±1.01b	8.83±0.78c
20:5n-3	5.23±0.55a	5.35±0.54a	2.08±0.22b	4.69±0.43a	4.02±0.40a	4.46±0.34a	4.55±0.34a	4.86±0.44a	5.11±0.51a	5.31±0.54a	5.28±0.50a	8.89±0.87c
22:5n-3	4.23±0.33a	4.84±0.47a	4.70±0.45a	3.00±0.31a	3.35±0.31a	4.54±0.42a	3.04±0.39a	3.39±0.33a	3.59±0.54a	2.00±0.21b	3.54±0.33a	3.64±0.35a
22:6n-3	19.89±1.09a	17.60±1.07b	21.40±1.20a	19.49±1.09a	19.60±1.20a	15.53±1.15b	17.79±1.07b	19.79±1.20a	20.69±1.27a	21.78±1.21c	19.45±1.08a	18.62±1.08a
∑PUFA	45.40±1.46a	43.62±1.43a	39.91±1.38b	39.50±1.40b	39.78±1.35b	39.62±1.38b	40.91±1.44b	42.53±1.44a	40.22±1.39b	40.40±1.40b	41.72±1.50b	44.04±1.41a
n-3	29.82±1.22a	28.32±1.22a	28.62±1.34a	27.65±1.26a	27.72±1.28a	25.87±1.34a	26.06±1.27a	28.26±1.21a	30.11±1.31a	29.68±1.30a	28.78±1.32a	31.93±1.34b
n-6	15.58±1.05a	15.30±1.54a	11.29±1.01b	11.85±1.01b	12.06±1.12b	13.75±1.03a	14.85±1.04a	14.27±1.14a	10.11±1.10b	10.72±1.01b	12.94±1.02b	12.11±1.12b
n-3/n-6	1.91	1.85	2.53	2.33	2.29	1.88	1.75	1.98	2.97	2.76	2.22	2.63

* Means are the averages of 3 replicates ** Values reported are means ±standard deviation; means followed by different letters in same line are significantly different (p<0.05) by Tukey's test *** SFA: saturated fatty acids, MUFA: monounsaturated fatty acids, PUFA: polyunsaturated fatty acids

In males, it was low in May, July, and March, and it was close to each other,while in September, November, and January it was high and close to each other. Palmitoleic acid and MUFA ratio were found to be highest in females in November and January and in males in July and January. These components decreased in both sexes in May, which is the reproductive period. The 18:1n-9 and 16:1n-7 are the most frequently detected MUFA in both sexes. In addition, 20:1n-9 were detected in both sexes, albeit in small amounts.

In the female TAG fraction, SFA was found most in May, and MUFA was found in November, January, and March. The ratio of these components in the other two months is similar. In males, MUFA was found in all months most commonly in males except for September. The component with the lowest ratio in both sexes' TAG is PUFA. It is noteworthy that MUFA ratios are higher in this fraction. In females, the PUFA ranged 16.79 to 21.89% and in males ranged from 15.48 to 29.75% through the year. The 20:4n-6 and DHA were found as major PUFA. The ratio of n-3/n-6 in the liver TAG fraction of *S. triostegus* was 1.34-1.93 in females and 1.32-2.01 in males. The highest n-3/n-6 ratio was determined in November in both sexes.

Fat-free fish store 50-80% of their fat in their livers in the form of TAG, which are good sources for fat-soluble vitamins, especially A and D vitamins (Jacquot, 1961).

In fish caught in nature, in TAG mostly monoenes, then saturated followed by PUFA are found (Henderson & Tocher, 1987). Based on this, it has been found that fish species accumulate mainly SFA and MUFA as stored lipids (Pinela et al., 2009). The reason for the high rate of monoenes in triacylglycerol is that among the components in this group, the ratio of 18:1-9 is higher. In addition, n-3HUFAs were found to be low in this fraction since the amount of n-3 components such as 20:5n-3 and 22:6n-3 was low (Cejas et al., 2003).

Fatty acids such as palmitic acid, 16:1n-7, and 18:1n-9 are found to be excessively present in storage lipids (Ackman 1967). In the present research, MUFA were found in the liver TAG of both sexes most, then SFA followed by PUFA were found. Similar results were found by Kozlova & Khotimchenko (2000) as well. In May (reproduction period) the percentage of MUFA decreased. Their amounts may be reduced because these components are mobilized to the gonad for reproduction.

In *Comephorus baicalensis*, MUFA were found to be the most, SFA next, and then PUFA the least. Among the monounsaturated fatty acids 18:1-9 were found most. The 16:0 was determined most within the SFA group. Other major fatty acids are 16:1n-7, 18:1n-7, and 20:5n-3(Kozlova & Khotimchenko, 2000). Ackman et al., (2002) identified the 16:0, 18:1n-9, 16:1n-7, 18:2n-2, 18:3n-3, 20:4n-6, 20:5n-3, and 22: 6n-3 as the major fatty acids. This finding is consistent with the findings of the current study as well.

The FA composition of PL fraction

In the liver PL fraction of *S. triostegus*, the seasonal rates of SFA was 35.49-39.87% in females and 34.56-39.57% in males, MUFA was 19.03-23.23% in females and 20.0-23.98% in males, and PUFA was 39.78-45.40% in females and 39.50-44.04% in males. As can be seen from the data, SFA, MUFA, and PUFA percentages were found at similar rates in both sexes in all months. Similarly, the percentages of major components such as 16:0, 18:1n-9, 20:4n-6, 20:5n-3, and 22:6n-3 did not differ within months. In both sexes of *S. triostegus,* PUFA found most, SFA next, and MUFA least, in all seasons. In this fish, the ratio of n-3/n-6 was 1.75-2.97 in females and 1.85-2.76 in males. In both sexes, the n-3/n-6 rate increased in January (Table 4).

The SFA rate is reduced by exposure to low temperature, yet the proportion of unsaturated fatty acids increases (Jobling and Bendiksen 2003). The increased unsaturated fatty acids are either monoene or polyene (Wallaert and Babin, 1994, Fodor *et al.,* 1995, Logue *et al.*, 2000).

The increase of the ambient temperature reduces the accumulation of n-3 in PL (Delgado *et al.*, 1994). The change in the salinity and temperature of water affects the length and the degree of unsaturation of the fatty acids in the membrane PL in poikilotherms. (Cordier *et al.,* 2002).

In this study, it was observed that PUFA decreased in July and September when the temperature was high.

The 16:0 is dominant in SFA and its ratio in fish tissues is not affected by nutrition (Ackman *et al.*, 1975) Ackman *et al.*, (2002) detected that 16:0, 18:0, 18:1n-9, 16:1n-7, 20:4n-6, 20:5n-3, and 22:6n-3 were dominant in the PL fraction. The results are consistent with the results of the current study. Buzzi *et al.*, (1997) stated that in the liver of *Exos lucius*, linolenic acid was converted into EPA and DHA. This transformation is very important in fish physiology (Arts *et al.*, 2001).

AA was found as major n-6 PUFA in both sexes in all seasons. AA is the precursor molecule for the synthesis of thromboxane and prostaglandins (Bell *et al.*, 1994).

In the case of migration and gonad development, the amount of EPA and DHA is mostly maintained compared to MUFA. The fact that the percentages of these two components do not change much is necessary to maintain the cell membrane structure and function (Sargent *et al.*, 1995, Cejas *et al.*, 2004).

CONCLUSION

In the present study, it was found that the amount of DHA and EPA in both sexes did not differ much by season either. PUFA was detected most in the PL fraction in both sexes. According to these results, it can be said that the lipid metabolism of the fish changes depending on the spawning and season.

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Ethics committee approval:-

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