J Am Oil Chem Soc (2015) 92:1313–1320
DOI 10.1007/s11746-015-2692-6

ORIGINAL PAPER

Seasonal Variations in the Fatty Acid Composition of Phospholipid and Triacylglycerol in Gonad and Liver of *Mastacembelus simack*

Semra Kaçar[1] · **M. Başhan**[2]

Received: 11 February 2015 / Revised: 10 July 2015 / Accepted: 13 July 2015 / Published online: 23 July 2015

Abstract The seasonal effects on the fatty acid composition of triacylglycerol (TG) and phospholipid (PL) in the gonad and liver of *Mastacembelus simack* were determined using the gas chromatographic method. The most abundant fatty acids in the investigated seasons and tissues were palmitic acid (C16:0), stearic acid (C18:0), oleic acid (C18:1n-9), palmitoleic acid (C16:1n-7), arachidonic acid (C20:4n-6), eicosapentaenoic acid (C20:5n-3), and docosahexaenoic acid (C22:6n-3). The distribution proportions of ∑SFA (saturated fatty acids), ∑MUFA (monounsaturated fatty acids) and ∑PUFA (polyunsaturated fatty acids) were found to be different among PL and TG fractions in all seasons. The total lipid content of gonad and liver were 1.32 (November)–4.90 % (September) and 1.32 (September)–3.94 % (January), respectively. It was shown that the total lipid and fatty acid compositions in the gonad and liver of fish were significantly influenced by seasons.

Keywords *Mastacembelus simack* · Fatty acid · Phospholipid · Triacylglycerol

Abbreviations

AA	Arachidonic acid
DHA	Docosahexaenoic acid
EPA	Eicosapentaenoic acid
FA	Fatty acid(s)
GC	Gas chromatography
LA	Linoleic acid
ALA	Linolenic acid
MUFA	Monounsaturated fatty acid(s)
PUFA	Polyunsaturated fatty acid(s)
SFA	Saturated fatty acid(s)
PL	Phospholipid(s)
TG	Triacylglycerol(s)
EFA	Essential fatty acid
TLC	Thin layer chromatography
HUFA	Long-chain polyunsaturated fatty acid
FAME	Fatty acid methyl ester(s)

Introduction

Mastacembelus mastacembelus occurs in the Euphrates and Tigris River basin in the Middle East, Iraq, Iran, Syria and Turkey and it is known as the Mesopotamian spiny eel due to its habitat [1]. This species is also sole representative of the order Synbranchioformes in Turkish freshwaters and it was reported as *Mastacembelus simack* [2]. *M. simack* is a food fish and an important source of income for commercial fisheries in the region.

Fatty acids (FA), especially fish lipids, are important nutritional elements for human health. The beneficial effect of fish consumption on human health has been related, among other factors, to the high content of n-3 FA, especially EPA and DHA [3], which play an important role in the prevention of a wide variety of disorders, such as atherosclerosis, thrombosis, hypertension, autoimmune and inflammatory diseases, rheumatoid arthritis, eczema, osteoporosis, breast cancer, asthma and allergies [4].

The total lipids and FA composition of fish vary not only between species, but also between individuals depending on sexual cycle, age, feed, stage of maturity, environment,

✉ Semra Kaçar
semrakacar21@gmail.com

[1] Department of Nutrition and Dietetics, School of Health, Mardin Artuklu University, 47100 Mardin, Turkey

[2] Department of Biology, Faculty of Science, Dicle University, 21280 Diyarbakir, Turkey

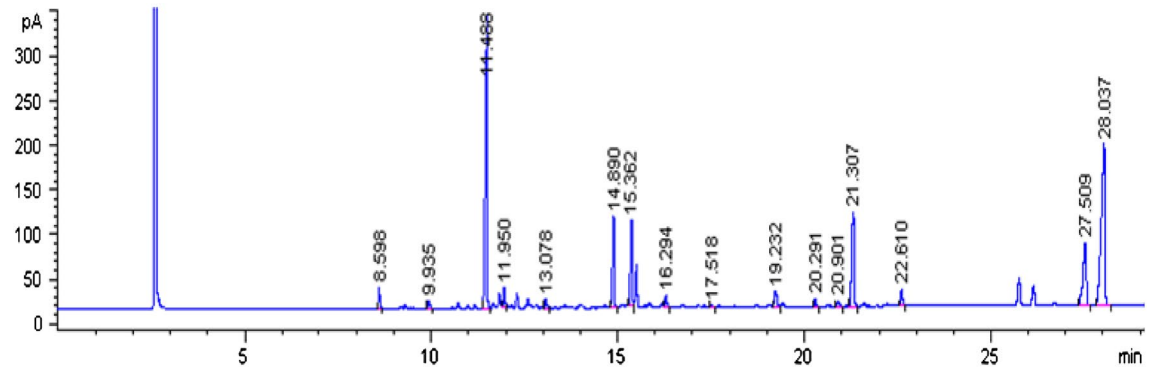

Fig. 1 The GC-chromatogram of PL fatty acids extracted from *M. simack* gonad in May

season and organs [5, 6]. The season can be the most important because it also affects the composition of the diets, which are available throughout the seasons [7].

Lipids are stored mainly in the liver and muscle of fish and transported together with protein to the ovaries during maturation of the gonad [8]. Fish liver oil has long been a preferred source of oil for the prevention of vision and growth problems. Liver is also the concentrated center of vitamins A, D, and n-3 FA [9].

Lipids and FA have a particularly important role in the reproductive parameters of fish, such as egg quality, spawning, hatching rate and survival of larvae [10]. Lipids are utilized as energy sources throughout embryogenesis. EPA and DHA are the major FA in the total lipid of eggs. In addition, AA as a major n-6 FA, stimulates ovarian and testicular steroidogenesis [11], steroid transport [12], or ovulation and oocyte maturation [13].

Lipids are an important component of diet, both as energy and EFA sources, which fish need for basic functions including growth and reproduction [14]. Significant changes and mobilizations of lipids take place during embryonic development [15]. The fatty acid composition of lipids from the gonads of fish reflects the fatty acid content of the lipid in the diet [16].

The lipid classes referred to as TG and PL have different functions in organisms. PL are important constituents of membranes and they function as precursors in eicosanoid metabolism, whereas the TG serve mainly as a depot of lipids used as an energy source [17].

However, no studies exist regarding fat content and the FA profile of liver and gonad of this species and the PL and TG fractions of these tissues. In the present study, monthly changes of fat content and FA composition of *M. simack* (liver and gonad) caught from Atatürk Dam Lake, Turkey, were investigated to determine which months contribute to the best source of fat and FA, and also to compare the distribution of such values in the investigated parts of *M. simack* (Fig. 1).

Materials and Methods

Experimental Material

Fishes were caught with casting nets from Atatürk Dam Lake in different seasons from May 2008 to January 2009. The geographic co-ordinates of the study area were 37°22′ 47.07″ North and 38° 33′ 8.56″ East (Bozova). Fish were killed with a sharp blow to the head; gonad and liver were excised. Fish sex was determined by their gonads. All representative fishes ($n = 3$ at each determination) used in the experiments were female. It is well known that female samples are to more prone to changes in biochemical composition due to gonad development and spawning. Lengths and weights of the fish used in the study were 48.2 ± 1.52 cm and 247.8 ± 60.71 g. The samples were stored at −30 °C in a freezer prior to analysis.

Lipid Extraction and Transmethylation of Fatty Acids

Gonad and liver tissues were homogenized in chloroform/methanol (2/1 v/v) solution in order to extract the gonad and liver tissue lipids [18]. The amount of lipids was determined gravimetrically. Then the total lipids were spotted on preparative thin layer chromatography (TLC) plates using commercial silica gel TLC plates (20 × 20 cm, 0.25 mm thick). After applying the lipid extracts, the TLC plates were developed in petroleum ether:diethyl ether:acetic acid (80:20:1 v/v). Lipid fractions were made visible by spraying 2′,7′-dichlorofluorescein (Supelco, Supelco Park, PA, USA) on TLC plates, and the PL and TG fractions were identified by corresponding standards. The fractions were scraped into reaction vials, and the associated FA were transmethylated by refluxing the fractions in acidified (sulfuric acid) methanol for 2 h at 85 °C [19]. The fatty acid methyl esters (FAME) were extracted with hexane. Autoxidation of unsaturated components was minimized by adding 50 μl of 2 % butylated hydroxytoluene (BHT) in chloroform to each sample.

Table 1 Total weight, length and total lipids of gonad and liver of female *M. simack*

	May (2008)	July (2008)	September (2008)	November (2008)	January (2009)
Mean total weight (g)	120 ± 0.25^{a}	260 ± 0.75^{b}	348 ± 0.46^{c}	376 ± 0.41^{c}	135 ± 0.62^{a}
Mean standard length (cm)	35.5 ± 0.12^{a}	51.0 ± 0.22^{b}	59.5 ± 0.34^{b}	57.5 ± 0.40^{b}	37.5 ± 0.31^{a}
Total lipids of gonad (%)	3.63 ± 0.10^{a}	1.68 ± 0.23^{b}	4.90 ± 0.05^{c}	1.32 ± 0.15^{b}	4.02 ± 0.22^{a}
Total lipids of liver (%)	2.19 ± 0.88^{a}	1.68 ± 0.61^{b}	1.32 ± 0.32^{b}	1.40 ± 0.11^{b}	3.94 ± 0.55^{c}

Values reported are means ± standard deviations; means followed by different superscript letters in the same line are significantly different ($p < 0.05$)

Gas Chromatography Analyses

FAME were separated and quantified by capillary GC using a Hewlett Packard (Wilmington, DE) GC (model 6890), a DB-23 capillary column (60 m × 0.25 mm i.d × 0.250 μm film thickness and Bonded 50 % cyanopropyl) (J & W Scientific, Folsom, CA), a flame ionization detector (FID) and Hewlett–Packard ChemStation software. The injection port and the detector temperatures were 270 and 280 °C, respectively. The split ratio was 1:20. The flow rates of compressed air and hydrogen were 300, 30 ml/min, respectively. Helium was used as the carrier gas (2.8 ml/min). The oven temperature was programmed at a rate of 6.5 °C/min from 130 °C (1 min hold) to 170 °C, then increased at a rate of 2.75 °C/min to 215 °C and held for 12 min, then again increased at a rate of 40 °C/min to 230 °C, which was held for 12 min. Total FA levels and spectra of FAME were obtained by HP 3365 ChemStation software. Chemical structures of the FAME were quantificated by analyses of spectra and by comparing obtained spectra with the spectra of authentic standards (Sigma-Aldrich Chemicals). Individual FAME were identified by comparison with the chromatographic behaviors of authentic standards (Sigma-Aldrich Chemicals). The amount of FA was given as a percentage.

Statistical Analysis

Statistical analyses were done by the SPSS (15.0) computer programme. Samples were analyzed in triplicate and the mean values were reported. The percentages of FA were tested by analyses of variance (ANOVA) and comparisons between means were performed with Tukey's test. Differences between means were considered to be significant at $p < 0.05$.

Results

Fat Content

The total lipid content of gonad and liver changed seasonally from 1.32 to 4.90 % and from 1.32 to 3.94 %, respectively (Table 1). The highest lipid content of the gonad tissue was found in the post-spawning stage (September). In liver tissue, the highest lipid content was in winter (January). In this study, the lipid content of gonad and liver tissue differed according to season.

Fatty Acid Composition of the TG and PL Fraction of Gonad Tissue

The fatty acid compositions of the TG and PL fractions of the gonad of *M. simack* are given in Tables 2 and 3, respectively.

In the TG fraction, the maximal ∑SFA contents were found in the September post-spawning period (44.45 %). Total MUFA were lowest in July (32.83 %). 18:1n-9 was the most abundant fatty acid in all seasons ranging from 24.83 to 30.41 %. The highest ∑PUFA amounts were found in the July spawning period (30.54 %). The lowest levels were found in the post spawning period during September (17.76 %). In the TG fraction, DHA was the major n-3 PUFA with levels of 5.37–13.68 %. C22:5n-3 was found to be at its highest level in May (5.64 %).

In the PL fraction, the seasonal variation of SFA in the gonad varies from 37.76 % during the pre-spawning period (May) to 59.08 % during the post-spawning period (September). The total MUFA were lowest in May (pre-spawning, 14.10 %) and highest in January (post-spawning, 24.63 %). Fluctuation in ∑PUFA of the gonad tissue ranged between 27.24 and 48.16 %, with the highest value found in the pre-spawning stage (May). It could be seen that DHA was the main PUFA in *M. simack* ovary and had the maximum percentage in the pre-spawning period during May (28.11 %). In September, the concentration of this acid decreased to its lowest value (8.22 %). The second major PUFA were C22:5n-3 and C20:4n-6 and their maximum concentration was 6.39 and 12.36 % in the pre-spawning period and spawning period (May and July), respectively. The n-3 FA content varied between 12.89 % (in January) and 36.58 % (in May). In addition, the high value of n-3/n-6 ratio was observed in May (3.16). The major components were C16:0, C16:1n-7, C18:1n-9, C18:2n-6, C18:3n-3, EPA and DHA in TG and PL extracted from the gonad of *M. simack* in all seasons.

Table 2 Fatty acid composition in TG fraction of gonad from female *M. simack* (% of total FA)

Fatty acids	May (2008)	July (2008)	September (2008)	November (2008)	January (2009)
C12:0	–	0.12 ± 0.01^{a}	–	–	–
C13:0	0.15 ± 0.08^{a}**	0.13 ± 0.01^{a}	1.62 ± 0.24^{b}	–	–
C14:0	2.58 ± 0.25^{a}	2.55 ± 0.36^{a}	4.92 ± 0.85^{b}	3.41 ± 0.56^{a}	1.52 ± 0.12^{c}
C15:0	0.62 ± 0.21^{a}	0.61 ± 0.05^{a}	1.52 ± 0.62^{b}	1.23 ± 0.38^{b}	0.67 ± 0.22^{a}
C16:0	24.53 ± 1.08^{a}	25.74 ± 1.38^{a}	30.27 ± 1.74^{b}	29.52 ± 1.33^{b}	24.69 ± 1.42^{a}
C17:0	0.66 ± 0.04^{a}	0.29 ± 0.02^{b}	1.05 ± 0.55^{c}	0.90 ± 0.20^{c}	0.99 ± 0.03^{c}
C18:0	5.36 ± 0.45^{a}	7.08 ± 0.77^{b}	5.07 ± 0.50^{a}	4.65 ± 0.35^{a}	3.70 ± 0.21^{a}
∑SFA	33.90 ± 2.20^{a}	36.52 ± 2.13^{a}	44.45 ± 1.44^{b}	39.71 ± 2.90^{b}	31.57 ± 2.21^{a}
C16:1n-7	10.53 ± 0.99^{a}	6.39 ± 0.66^{b}	11.44 ± 0.96^{a}	7.88 ± 0.54^{b}	9.88 ± 0.77^{a}
C18:1n-9	24.83 ± 1.90^{a}	24.98 ± 1.78^{a}	24.94 ± 1.66^{a}	30.11 ± 1.86^{b}	30.41 ± 1.94^{b}
C20:1n-9	0.88 ± 0.22^{a}	1.46 ± 0.78^{b}	1.31 ± 0.77^{b}	0.95 ± 0.40^{a}	1.14 ± 0.64^{b}
∑MUFA	36.24 ± 1.99^{a}	32.83 ± 1.81^{b}	37.69 ± 1.21^{a}	38.94 ± 1.22^{a}	41.43 ± 1.90^{c}
C18:2n-6	3.39 ± 0.33^{a}	2.47 ± 0.20^{a}	2.35 ± 0.22^{a}	5.78 ± 0.42^{b}	4.57 ± 0.58^{b}
C18:3n-3	1.77 ± 0.49^{a}	0.92 ± 0.22^{b}	0.75 ± 0.22^{b}	1.35 ± 0.86^{a}	2.65 ± 0.45^{c}
C20:2n-6	0.42 ± 0.05^{a}	0.43 ± 0.11^{a}	0.25 ± 0.06^{b}	0.55 ± 0.08^{a}	0.56 ± 0.01^{a}
C20:3n-6	0.42 ± 0.22^{a}	0.73 ± 0.21^{b}	0.09 ± 0.01^{c}	0.59 ± 0.05^{a}	0.57 ± 0.07^{a}
C20:4n-6	2.47 ± 0.48^{a}	4.92 ± 0.60^{b}	2.90 ± 0.34^{a}	3.04 ± 0.30^{a}	4.21 ± 0.87^{b}
C20:5n-3	2.71 ± 0.21^{a}	1.80 ± 0.33^{b}	1.89 ± 0.25^{b}	1.37 ± 0.81^{b}	0.99 ± 0.67^{c}
C22:5n-3	5.64 ± 0.55^{a}	5.59 ± 0.66^{a}	4.16 ± 0.44^{a}	1.49 ± 0.38^{b}	3.13 ± 0.83^{a}
C22:6n-3	12.96 ± 0.99^{a}	13.68 ± 0.87^{a}	5.37 ± 0.40^{b}	7.13 ± 0.88^{c}	10.24 ± 0.95^{a}
∑PUFA	29.78 ± 1.22^{a}	30.54 ± 2.21^{a}	17.76 ± 2.66^{b}	21.30 ± 2.10^{c}	26.92 ± 1.88^{d}
∑n-3	23.08	21.99	12.17	11.34	17.01
∑n-6	6.70	8.55	5.59	9.96	9.91
n-3/n-6	3.44	2.57	2.18	1.14	1.71

Means are the averages of three replicates

SFA saturated fatty acids, *MUFA* monounsaturated fatty acids, *PUFA* polyunsaturated fatty acids

** Values reported are means ± standard deviations; means followed by different superscript letters in the same line are significantly different ($p < 0.05$) by Tukey's test

Fatty Acid Composition of the TG and PL Fraction of Liver Tissue

Seasonal variations in the fatty acid compositions of TG and PL fractions of the liver of *M. simack* are given in Tables 4 and 5, respectively.

In the TG fraction, C16:0 was the major fatty acid of the SFA. C16:0 percentages in the liver tissue showed significant differences in all of the seasons and the highest level was 41.83 % in the post-spawning period. C18:0 was the second major fatty acid in the SFA in *M. simack.* However, there were no statistical differences between the values for the autumn and winter periods of C18:0. As can be seen in Table 4, other FA of the SFA were minor compounds, with the exception of C14:0 (myristic acid), C15:0 (pentadecanoic acid) and C17:0 (heptadecanoic acid). In the liver, the total SFA amounts were highest in September (53.29 %) and lowest in January (37.54 %). The percentages of C18:1n-9 acid ranged from 21.94 % (in May) to 26.98 % (in November). In the present study, C20:4n-6 was the major constituent of the n-6 PUFA. DHA was found to be at the highest level in the n-3 PUFA in the female *M. simack.* The ratio of PUFA was found to be lower than SFA and MUFA in all seasons.

In the PL fraction, total SFA was the highest in the spawning season (47.26 %). C16:0 and C18:0 were the major FA in SFA. Total MUFA was lower than SFA and PUFA in PL fraction. The highest PUFA was obtained in May (pre-spawning period) at 48.61 %, and the lowest was found in July at 37.07 % (spawning period). DHA was the most abundant n-3 PUFA, and its level showed the most variation during the season.

Discussion

Fat Content

The amount of lipids in the liver was at a maximum in January (3.94 %) and May (2.19 %), which is the pre-spawning period. In the gonad tissue, the lipid content was the highest in the post-spawning season (September, 4.90 %). The

Table 3 Fatty acid composition in the PL fraction of gonad from female *M. simack* (% of total FA)

Fatty acids	May (2008)	July (2008)	September (2008)	November (2008)	January (2009)
C14:0	$0.81 \pm 0.09^{a**}$	2.11 ± 0.23^{b}	1.91 ± 0.55^{b}	0.92 ± 0.45^{a}	1.00 ± 0.63^{a}
C15:0	0.15 ± 0.03^{a}	0.71 ± 0.18^{b}	1.41 ± 0.78^{c}	0.69 ± 0.21^{b}	0.79 ± 0.22^{b}
C16:0	22.02 ± 1.03^{a}	29.92 ± 1.66^{b}	41.17 ± 1.81^{c}	30.90 ± 1.00^{b}	37.69 ± 1.87^{c}
C17:0	0.09 ± 0.02^{a}	0.32 ± 0.16^{b}	0.32 ± 0.10^{b}	0.24 ± 0.07^{b}	1.08 ± 0.44^{c}
C18:0	14.69 ± 0.88^{a}	16.14 ± 0.69^{a}	14.27 ± 0.43^{a}	9.95 ± 0.77^{b}	6.81 ± 0.39^{c}
∑SFA	37.76 ± 0.99^{a}	49.20 ± 1.32^{b}	59.08 ± 3.23^{c}	42.70 ± 2.86^{d}	47.37 ± 3.34^{b}
C16:1n-7	2.51 ± 0.11^{a}	2.87 ± 0.35^{a}	1.48 ± 0.37^{b}	3.84 ± 0.55^{c}	4.84 ± 0.32^{c}
C18:1n-9	10.79 ± 0.85^{a}	15.85 ± 0.98^{b}	11.19 ± 0.79^{a}	16.10 ± 0.60^{b}	19.24 ± 0.57^{c}
C20:1n-9	0.80 ± 0.22^{a}	0.91 ± 0.38^{a}	0.91 ± 0.57^{a}	0.57 ± 0.53^{c}	0.55 ± 0.41^{c}
∑MUFA	14.10 ± 0.99^{a}	19.63 ± 0.89^{b}	13.58 ± 1.12^{a}	20.51 ± 1.22^{b}	24.63 ± 1.32^{b}
C18:2n-6	1.84 ± 0.32^{a}	1.53 ± 0.40^{a}	1.51 ± 0.12^{a}	4.15 ± 0.33^{b}	3.46 ± 0.78^{b}
C18:3n-3	0.29 ± 0.03^{a}	0.18 ± 0.01^{b}	0.61 ± 0.12^{c}	0.20 ± 0.02^{a}	0.55 ± 0.37^{c}
C20:2n-6	0.54 ± 0.12^{a}	0.38 ± 0.11^{b}	0.15 ± 0.02^{c}	0.78 ± 0.22^{d}	0.81 ± 0.07^{d}
C20:3n-6	0.79 ± 0.08^{a}	0.85 ± 0.33^{a}	0.51 ± 0.05^{b}	0.73 ± 0.66^{a}	0.89 ± 0.05^{a}
C20:4n-6	8.41 ± 0.51^{a}	12.36 ± 0.91^{b}	10.69 ± 0.67^{ab}	10.59 ± 0.88^{ab}	9.86 ± 0.71^{a}
C20:5n-3	1.79 ± 0.55^{a}	1.41 ± 0.84^{a}	0.56 ± 0.32^{b}	1.98 ± 0.81^{a}	0.48 ± 0.29^{b}
C22:5n-3	6.39 ± 0.78^{a}	3.34 ± 0.52^{b}	4.99 ± 0.44^{a}	2.80 ± 0.40^{b}	3.62 ± 0.81^{b}
C22:6n-3	28.11 ± 1.00^{a}	11.04 ± 0.90^{b}	8.22 ± 0.55^{c}	15.46 ± 0.63^{b}	8.24 ± 0.85^{c}
∑PUFA	48.16 ± 2.45^{a}	31.09 ± 2.20^{b}	27.24 ± 1.21^{c}	36.69 ± 1.77^{d}	27.91 ± 1.98^{c}
∑n-3	36.58	15.97	14.38	20.44	12.89
∑n-6	11.58	15.12	12.86	16.25	15.02
n-3/n-6	3.16	1.06	1.12	1.26	0.86

Means are the averages of three replicates

SFA saturated fatty acids, *MUFA* monounsaturated fatty acids, *PUFA* polyunsaturated fatty acids

** Values reported are means ± standard deviations; means followed by different superscript letters in the same line are significantly different ($p < 0.05$) by Tukey's test

study showed that the amount of total lipids in gonad tissue reached its maximum level at the end of spawning; but that the amount diminished during the reproduction season.

In various organs of the fish, the FA composition and lipid contents were affected by the species, sex, age, water temperature, nutritional condition, seasonal variation and fish origin [20]. Studies have shown that the lipid content is influenced by seasonal variations [21–23].

It was observed that, especially in the reproduction period, the lipids mobilized from the livers and muscles to the gonads for the development of the gonads [24]. In liver tissue, lipid content decreased during the reproduction period.

It was found that the total gonad lipid amounts became lower after the reproductive season. The reason that total lipids amounts decrease after the reproductive season, is that large amounts of lipids are stored in the ovaries during the development of embryos. These results are similar to those of Medford and Mackay [25], Newsome and Leduc [26] and Yılmaz *et al*. [27].

The amount of total lipids in the gonad and liver of *M. simack* shows different variations by months and parts of the body.

TG and PL Compositions in Liver and Gonad Tissue

The fatty acid composition is known to vary even between different tissues of the fish. The liver is an important organ in terms of lipid metabolism. Thus, analysis of the fatty acid profiles of the tissues, such as liver and gonad, from fish living in their natural ecosystem can yield valuable information [28].

Gonads synthesize sexual hormones and their lipid content is dependent upon the stage of the sexual cycle and the sex of the fish [29]. As a rule, the ovaries of fish accumulate many more stored lipids, particularly TGs, because of the need for provision of energy for the offspring and depends mainly on their way of life, feeding level and especially on the reproductive ecology of the species.

In the fatty acid analysis of liver and gonad tissue, C16:0, C18:1n-9, C22:6n-3 were the major components in PL and TG. The highest 16:0 level was found in liver and gonad tissue in TG and PL fractions in all seasons. This result is consistent with the anabolism of FA: 16:0 can be biosynthesized by fish through a conventional pathway catalyzed by cytosolic fatty acid synthetase [30]. In liver tissue, the ratio of SFA in TG fraction decreased to

Table 4 Fatty acid composition in the TG fraction of the liver from female *M. simack* (% of total FA)

Fatty acids	May (2008)	July (2008)	September (2008)	November (2008)	January (2009)
C10:0	0.10 ± 0.03^{a}**	0.94 ± 0.52^{b}	–	–	–
C12:0	0.13 ± 0.02^{a}	–	–	–	–
C13:0	0.31 ± 0.22^{a}	0.31 ± 0.05^{a}	–	–	–
C14:0	3.31 ± 0.38^{a}	1.89 ± 0.63^{b}	3.58 ± 0.85^{a}	2.71 ± 0.62^{a}	1.69 ± 0.74^{b}
C15:0	0.73 ± 0.20^{a}	0.59 ± 0.55^{a}	1.42 ± 0.63^{b}	1.02 ± 0.21^{b}	0.69 ± 0.44^{a}
C16:0	29.89 ± 1.08^{a}	32.72 ± 1.53^{b}	41.83 ± 1.44^{c}	33.70 ± 1.28^{b}	28.01 ± 1.84^{a}
C17:0	0.53 ± 0.04^{a}	0.35 ± 0.22^{b}	0.78 ± 0.65^{c}	0.36 ± 0.01^{b}	1.12 ± 0.40^{c}
C18:0	10.78 ± 0.41^{a}	10.00 ± 0.88^{a}	5.68 ± 0.56^{b}	5.53 ± 0.20^{b}	6.03 ± 0.42^{b}
∑SFA	45.78 ± 2.76^{a}	46.80 ± 2.34^{a}	53.29 ± 3.44^{b}	43.32 ± 2.78^{a}	37.54 ± 2.55^{c}
C16:1n-7	9.72 ± 0.78^{a}	5.08 ± 0.65^{b}	7.06 ± 0.39^{a}	9.36 ± 0.87^{a}	10.58 ± 0.88^{a}
C18:1n-9	21.94 ± 1.08^{a}	22.97 ± 1.58^{a}	22.42 ± 1.55^{a}	26.98 ± 1.86^{b}	26.33 ± 1.83^{b}
C20:1n-9	1.29 ± 0.11^{a}	1.75 ± 0.19^{a}	1.16 ± 0.88^{a}	1.74 ± 0.28^{a}	1.24 ± 0.45^{a}
∑MUFA	32.95 ± 1.66^{a}	29.80 ± 1.06^{a}	30.64 ± 1.23^{a}	38.08 ± 1.45^{b}	38.15 ± 1.98^{b}
C18:2n-6	2.41 ± 0.77^{a}	1.88 ± 0.81^{a}	3.30 ± 0.84^{a}	5.52 ± 0.41^{b}	4.42 ± 0.78^{b}
C18:3n-3	1.23 ± 0.55^{a}	0.64 ± 0.22^{b}	1.19 ± 0.50^{a}	1.18 ± 0.54^{a}	2.37 ± 0.79^{c}
C20:2n-6	0.87 ± 0.10^{a}	0.44 ± 0.20^{b}	0.49 ± 0.18^{b}	0.56 ± 0.52^{b}	0.48 ± 0.05^{b}
C20:3n-6	0.43 ± 0.08^{a}	0.56 ± 0.03^{a}	0.11 ± 0.01^{b}	0.42 ± 0.04^{a}	0.69 ± 0.55^{a}
C20:4n-6	3.80 ± 0.21^{a}	5.45 ± 0.62^{b}	3.03 ± 0.81^{a}	2.46 ± 0.98^{a}	5.62 ± 0.78^{b}
C20:5n-3	1.22 ± 0.21^{a}	0.92 ± 0.87^{a}	0.71 ± 0.63^{b}	1.19 ± 0.90^{a}	0.67 ± 0.44^{b}
C22:5n-3	2.53 ± 0.12^{a}	3.01 ± 0.52^{a}	1.86 ± 0.22^{b}	1.81 ± 0.85^{b}	2.33 ± 0.33^{a}
C22:6n-3	8.66 ± 0.81^{a}	10.41 ± 0.99^{a}	5.29 ± 0.42^{b}	5.39 ± 0.78^{b}	7.65 ± 0.38^{ab}
∑PUFA	21.15 ± 1.00^{a}	23.31 ± 1.45^{a}	15.98 ± 1.34^{b}	18.53 ± 1.09^{b}	24.23 ± 1.26^{a}
∑n-3	13.64	14.98	9.05	9.57	13.02
∑n-6	7.51	8.33	6.93	8.96	11.21
n-3/n-6	1.82	1.80	1.31	1.07	1.16

Means are the averages of three replicates

SFA saturated fatty acids, *MUFA* monounsaturated fatty acids, *PUFA* polyunsaturated fatty acids

** Values reported are means ± standard deviations; means followed by different superscript letters in the same line are significantly different ($p < 0.05$) by Tukey's test

a minimum in January. In this period, the water temperature significantly decreases and gonad maturation begins. In both liver and gonad tissue, the most abundant SFA was C16:0, which is noted for being a predominant source of potential metabolic energy during the egg formation stage in female fish [31]. The dominance of C16:0 in fish lipid has been reported by other authors [17, 32]. C18:1n-9 is a characteristic MUFA in fish tissues [33]. Several studies report that C16:0 is the predominant source of potential metabolic energy in fish during the roe formation stage in female fish, mentioning the role of C18:1n-9 during the course of gonad development and the fact that PUFA are also an important source for metabolic energy of reproduction [34].

In gonad and liver tissue, AA is the main n-6 FA. AA has a vital function as the main precursor of eicosanoids [35]. Through its conversion to lipoxygenase metabolites, AA mediates a great variety of physiological functions including osmoregulation, cardiovascular functions, neural control and the functionality of reproductive systems [36]. DHA is the dominant n-3 FA in gonad and liver tissue. These are the roles that DHA play during vitellogenesis [15]. It has been assumed that PUFA such as C20:5 and C22:6 are involved in the physiological reproductive processes of fish [37]. Because of the specific role of (n-3) PUFA, especially DHA, in maintaining the structural and functional integrity in cell membranes, the relative percentage of this PUFA is expected to increase during the gonad development stage [38].

In the present study, the highest ovarian level of DHA was in May (pre-spawning) in the PL fraction. Specific the accumulation of C20 and C22 PUFA was more apparent in the fish gonads. Therefore, these results indicate that special FA are preferentially accumulated to the ovary to perform different physiological functions.

In the liver and gonad tissues, there were significant differences between TG and PL. There was a higher percentage of SFA and MUFA in the TG while PUFA was higher in the PL. MUFA and SFA constitute the main groups of FA in the neutral lipids of most fish studied [39]. The PL

Table 5 Fatty acid composition in the PL fraction of the liver from female *M. simack* (% of total FA)

Fatty acids	May (2008)	July (2008)	September (2008)	November (2008)	January (2009)
C14:0	0.79 ± 0.77[a]**	1.20 ± 0.21[b]	1.47 ± 0.81[b]	1.29 ± 0.32[b]	0.97 ± 0.80[a]
C15:0	0.25 ± 0.02[a]	0.45 ± 0.39[b]	0.58 ± 0.06[b]	0.62 ± 0.30[b]	0.58 ± 0.37[b]
C16:0	18.28 ± 0.55[a]	24.90 ± 1.06[b]	24.48 ± 1.08[b]	22.77 ± 1.22[b]	28.66 ± 1.80[c]
C17:0	0.20 ± 0.05[a]	0.11 ± 0.01[b]	0.34 ± 0.31[a]	0.18 ± 0.16[b]	1.14 ± 0.74[c]
C18:0	19.51 ± 0.22[a]	20.60 ± 0.98[a]	7.60 ± 0.87[b]	9.16 ± 0.38[b]	10.43 ± 0.99[b]
∑SFA	39.03 ± 2.65[a]	47.26 ± 2.09[b]	34.47 ± 2.39[c]	34.02 ± 1.88[c]	41.78 ± 1.07[a]
C16:1n-7	1.96 ± 0.83[a]	1.61 ± 0.36[a]	2.76 ± 0.19[b]	4.20 ± 0.88[c]	3.32 ± 0.58[b]
C18:1n-9	9.39 ± 0.32[a]	12.86 ± 0.78[b]	13.25 ± 0.25[b]	14.82 ± 0.80[b]	15.38 ± 0.99[b]
C20:1n-9	0.92 ± 0.85[a]	1.13 ± 0.84[a]	1.72 ± 0.39[b]	1.64 ± 0.83[b]	1.91 ± 0.65[b]
∑MUFA	12.27 ± 1.33[a]	15.60 ± 1.06[a]	17.73 ± 1.11[b]	20.66 ± 1.98[b]	20.61 ± 1.20[b]
C18:2n-6	1.95 ± 0.96[a]	1.98 ± 0.81[a]	1.32 ± 0.63[a]	4.18 ± 0.55[b]	3.37 ± 0.37[b]
C18:3n-3	0.29 ± 0.19[a]	0.33 ± 0.30[a]	0.76 ± 0.06[b]	0.76 ± 0.21[b]	0.70 ± 0.11[b]
C20:2n-6	0.50 ± 0.39[a]	0.46 ± 0.04[a]	0.22 ± 0.10[b]	1.01 ± 0.41[c]	0.69 ± 0.06[a]
C20:3n-6	0.82 ± 0.71[a]	0.83 ± 0.37[a]	0.35 ± 0.04[b]	0.88 ± 0.28[a]	0.93 ± 0.20[a]
C20:4n-6	9.12 ± 0.88[a]	13.16 ± 0.38[b]	12.52 ± 0.12[b]	11.00 ± 0.99[b]	12.68 ± 0.44[b]
C20:5n-3	1.01 ± 0.41[a]	0.53 ± 0.32[b]	0.36 ± 0.28[c]	0.94 ± 0.81[a]	0.69 ± 0.63[b]
C22:5n-3	5.23 ± 0.73[a]	3.04 ± 0.82[b]	6.67 ± 0.37[a]	5.00 ± 0.22[a]	3.41 ± 0.33[b]
C22:6n-3	29.69 ± 1.91[a]	16.74 ± 1.28[b]	25.50 ± 1.33[c]	21.47 ± 1.78[c]	15.05 ± 1.08[b]
∑PUFA	48.61 ± 2.22[a]	37.07 ± 2.09[b]	47.70 ± 1.23[a]	45.24 ± 1.90[a]	37.52 ± 1.21[b]
∑n-3	36.22	20.64	33.29	28.17	19.85
∑n-6	12.39	16.43	14.41	17.07	17.67
n-3/n-6	2.30	1.26	2.31	1.65	1.12

Means are the averages of three replicates

SFA saturated fatty acids, *MUFA* monounsaturated fatty acids, *PUFA* polyunsaturated fatty acids

** Values reported are means ± standard deviations; means followed by different superscript letters in the same line are significantly different ($p < 0.05$) by Tukey's test

fraction contained a higher level of n-3 PUFA than the TG fraction. The DHA amounts were higher than the EPA amounts in all seasons. The PL fractions were composed of very high levels of PUFA, especially C22:6n-3, and their composition seemed to be better regulated than the composition of TG. In TG, the content of MUFA and SFA was higher than that of the PUFA. Total PL from fish tissues are characteristically rich in PUFA [40]. In the present study, the most abundant fraction of PL was both PUFA and SFA. In liver and gonad tissue, AA was more concentrated in the PL fractions than in the TG fractions, probably due to its functionality in cell membranes [41].

The FA composition of TG and PL fractions in gonad and liver tissues of *M. simack* showed different variations by season.

Conclusion

The findings of this study are useful for understanding the distribution of fat content and fatty acid composition in liver and gonad according to the season. The major FA in *M. simack* lipids were palmitic acid, stearic acid, oleic acid, palmitoleic acid, arachidonic acid, eicosapentaenoic acid and docosahexaenoic acid. The liver and gonad tissues, which have relatively higher eicosapentaenoic acid and docosahexaenoic acid can be utilized for the production of fish oil.

Acknowledgments This study was financed by the Dicle University Scientific Research Foundation (DUAPK-08-FF-07).

References

1. Oymak SA, Kırankaya ŞG, Doğan N (2009) Growth and reproduction of Mesopotamian spiny eel (*Mastacembelus mastacembelus* Banks and Solander, 1794) in Atatürk Dam Lake (Şanlıurfa), Turkey. J Appl Ichthyol 25(4):488–490
2. Geldiay R, Balık S (1988) Türkiye Tatlısu Balıkları, Ege Ünv. Fen Fak. Kitaplar Serisi No: 97, Ege Üniversitesi Basımevi, İzmir. 519 s
3. Zlatanos S, Laskaridis K (2007) Seasonal variation in the fatty acid composition of three Mediterranean fish–sardine (*Sardina pilchardus*), anchovy (*Engraulis encrasicholus*) and picarel (*Spicara smaris*). Food Chem 103:725–728
4. Uauy R, Valenzuela A (2000) Marine oils: the health benefits of n-3 fatty acids. Nutrition 16:680–684
5. Cengiz EI, Ünlü E, Başhan M (2010) Fatty acid composition of total lipids in muscle tissues of nine freshwater fish from the River Tigris (Turkey). Turk J Biol 34:433–438

6. Kayhan H, Başhan M, Kaçar S (2014) Seasonal variations in the fatty acid composition of phospholipids and triacylglycerols of brown trout. Eur J Lipid Sci Technol. doi:10.1002/ejlt.201400152
7. Szlinder-Richert J, Usydus Z, Wyszynski M, Adamczyk M (2010) Variation in fat content and fatty-acid composition of the Baltic herring *Clupea harengus membras*. J Fish Biol 77:585–599
8. Cejas JR, Almansa E, Jerez S, Bolaños A, Samper M, Lorenzo A (2004) Lipid and fatty acid composition of muscle and liver from wild and captive mature female broodstocks of white sea bream, *Diplodus sargus*. Comp Biochem Physiol B 138:91–102
9. Hui YH (2006) Food biochemistry and food processing. Wiley-Blackwell, Iowa
10. Pickova J, Brannas E, Andersson T (2007) Importance of fatty acids in broodstock diets with emphasis on Arctic char (*Salvelinus alpinus*) eggs. Aquac Int 15:305–311
11. Henrotte E, Milla S, Mandiki SNM, Kestemont P (2011) Arachidonic acid induces production of 17,20β-dihydroxy-4-pregnen-3-one (DHP) via a putative PGE2 receptor in fish follicles from the Eurasian perch. Lipids 46:179–187
12. Hwang JH, Parton A, Czechanski A, Ballatori N, Barnes D (2008) Arachidonic acid induced expression of the organic solute and steroid transporter-beta (Ost-beta) in a cartilaginous fish cell line. Comp Biochem Physiol C 148:39–47
13. Lister A, Van Der Kraak G (2008) An investigation into the role of prostaglandins in zebrafish oocyte maturation and ovulation. Gen Comp Endocrinol 159:46–57
14. Sargent JR, Henderson RJ, Tocher DR (1989) The lipids. In: Halver JE (ed) Fish nutrition, vol 2. Academic Press, New York, pp 154–218
15. Sargent JR (1995) Origin and functions of eggs lipids: nutritional implications. In: Bromage NR, Roberts RJ (eds) Broodstock management and egg and larval quality. Blackwell Science, London, pp 353–372
16. Fernandez-Palacios H, Izquierdo M, Robaina L, Valencia A, Salhi M, Vergara JM (1995) Effect of n-3 HUFA level in broodstock diets on egg quality of gilthead seabream *Sparus aurata L.* Aquaculture 132:325–337
17. Suloma A, Ogata HY (2012) Lipid and fatty acid composition of commercially important tropical freshwater fish gonads: guidelines for specific broodstock diet. Turk J Fish Aquat Sci 12:743–749
18. Folch J, Lees M, Stanley A (1957) Simple method for the isolation and purification of total lipids from animal tissues. J Biol Chem 226:497–509
19. Stanley-Samuelson DW, Dadd RH (1983) Long-chain polyunsaturated fatty acids: patterns of occurrence in insects. J Food Sci Technol 13:549–558
20. Lathi E (1987) Total lipid and cholesterol of liver and muscle in some fish species, especially Vendace (*Coregonus albula* L.) in Filland. Arch Hydrobiol 110:133–142
21. Agren J, Mute P, Hanninen O, Harranen J, Pentila I (1987) Seasonal variations of lipid fatty acids of Boreal freshwater fish species. Comp Biochem Physiol 88B:905–909
22. Konar V, Canpolat A, Yılmaz Ö (1999) *Capoeta trutta* ve *Barbus rajanorum mystaseus*'un kas dokularındaki total lipid ve yağ asidi miktar ve bileşimlerinin üreme peryodu süresince değişimi. Turk J Biol 23:319–331
23. Kandemir Ş, Polat N (2007) Seasonal variation of total lipid and total fatty acid in muscle and liver of rainbow trout (*Oncorhynchus mykiss* W., 1792) reared in Derbent Dam Lake. Turk J Fish Aquat Sci 7:27–31
24. Castell JD, Sinnhuber RO, Wales JH, Lee DJ (1972) Essential fatty acids in the diet of rainbow trout (*Salmo gairdnerii*): growth, feed conversion and some gross deficiency symptoms. J Nutr 102:77–86
25. Medford BA, Mackay WC (1978) Protein and lipid content of gonads, liver and muscle of Northern pike (*Esox lucius* L.) in relation to gonad growth. J Fish Res Board Can 35:213–219
26. Newsome EG, Leduc G (1975) Seasonal change of fat content in the yellow perch (*Perca flavescens*) of two Laurentian lakes. J Fish Res Board Can 32:2214–2221
27. Yılmaz Ö, Konar V, Çelik S (1995) Elazığ Hazar Gölü'ndeki *Capoeta capoetaumbla*'nın dişi ve erkek bireylerinde bazı dokularının total lipit ve yağ asidi bileşimleri. Biyokimya dergisi 20:31–42
28. Rodriguez C, Acosta C, Badia P, Cejas JR, Santamaria FJ, Lorenzo A (2004) Assessment of lipid and essential fatty acids requirements of black seabream (*Spondyliosoma cantharus*) by comparison of lipid composition in muscle and liver of wild and captive adult fish. Comp Biochem Physiol B 139:619–629
29. Vuorela R, Kaitaranta J, Linko RR (1979) Proximate composition of fish roe in relation to maturity. Can Inst Food Sci Technol J 12:186–188
30. Sargent JR, Tocher DR, Bell JG (2002) The lipids. In: Halver JE (ed) Fish nutrition, Chap 4, 3rd edn. Academic Press, San Diego, pp 181–257
31. Henderson RJ, Sargent JR, Hopkins CCE (1984) Changes in the content and fatty acid composition of lipid in an isolated population of the capelin, *Mallotus villosus*, during sexual maturation and spawning. Mar Biol 78:255–263. doi:10.1007/BF00393011
32. Passi S, Cataudella S, Di Marco P, De Simone F, Rastrelli L (2002) Fatty acid composition and antioxidant levels in muscle tissue of different Mediterranean marine species of fish and shellfish. J Agric Food Chem 50:7314–7322
33. Steffens W (1997) Effects of variation in essential fatty acids in fish feeds on nutritive value of freshwater fish for humans. Aquaculture 151:97–119
34. Huynh MD (2007) Comparison of fatty acid profiles of spawning and non-spawning Pacific herring, *Clupea harengus pallasi*. Comp Biochem Physiol B 146:504–511
35. Bell JG, Tocher DR, McDonald FM, Sargent JR (1994) Effect of supplementation with (20:3n-6), (20:4n-6) and (20:5n-3) on the production of prostaglandin-e and prostaglandin-f on the 1-series, 2-series and 3-series in turbot (*Scophthalmus maximus*) brain astroglial cells in primary culture. Biochim Biophys Acta 1211:335–342
36. Sorbera LA, Zanuy S, Acrrillo M (1998) A role for polyunsaturated fatty acids and prostaglandins in oocyte maturation in the sea bass (*Dicentrarchus labrax*). Ann N Y Acad Sci 839:535–537
37. Almansa E, Pérez MJ, Cejas JR, Badia P, Villamandos JE, Lorenzo A (1999) Influence of broodstock gilthead seabream (*Sparus aurata* L.) dietary fatty acids on egg quality and egg fatty acid composition throughout the spawning season. Aquaculture 170(3–4):323–336
38. Mourente G, Tocher DR, Sargent JR (1991) Specific accumulation of docosahexaenoic acid (22:6n-3) in brain lipids during development of juvenile turbot *Scophthalmus maximus* L. Lipids 26:871–877
39. Ackman RG (1967) Characteristics of the fatty acid composition and biochemistry of some fresh-water fish oils and lipids in comparison with marine oils and lipids. Comp Biochem Physiol 22(3):907–922
40. Gunstone FD, Wijesundera RC, Scrimgeour CM (1978) Component acids of lipids from marine and freshwater species with special reference to furan-containing acids. J Sci Food Agric 29:539–550
41. Fountoulaki E, Alexis MN, Nengas I, Venou B (2003) Effects of dietary arachidonic acid (20:4n-6), on growth, body composition, and tissue fatty acid profile of gilthead bream fingerlings (*Sparus aurata L.*). Aquaculture 225:309–323. doi:10.1016/S0044-8486(03)00298-9