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Stereospecific Analysis of Triacylglycerol and Phospholipid Fractions of Five Wild Freshwater Fish from Poyang Lake

Lin Lei,[†] Jing Li,[†] Guang-Yan Li, Jiang-Ning Hu, Liang Tang, Rong Liu, Ya-Wei Fan, and Ze-Yuan Deng*

State Key Lab of Food Science and Technology, Institute for Advanced Study, Nanchang University, Nanchang, Jiangxi, 330047, China

ABSTRACT: The fatty acids (FA) compositions and positional distributions in triacylglycerols (TAG) and phospholipids (PL) of five wild freshwater fish (*Squaliobarbus curriculus, Erythroculter ilishaeformis, Pseudobagrus fulvidraco, Bostrichthys sinensis,* and *Siniperca kneri Garman*) from Poyang Lake (the largest freshwater lake of China) were studied. For TAG, *S. kneri German* had the highest content (13.59%) of $n-3$ polyunsaturated fatty acids (PUFA) and *E. ilishaeformis* had the lowest ratio of $(n-6)/(n-3)$ (0.65). PL had a high content of PUFA, which declined in the order of phosphatidylethanolamine (PE) > phosphatidylcholine (PC) > TAG. 9c11t-18:2 accounted for 6.38−50.77% of total conjugated linoleic acids (CLA). The highest level of odd-branched chain fatty acids (OBCFA) was 26.7% in *B. sinensis*. The study revealed that the distribution of FA among the *sn* positions was not random: monounsaturated fatty acids (MUFA) and PUFA preferred positions 1 and 3 and saturated fatty acids (SFA) position 2 of TAG, while SFA and MUFA predominated over *sn*-1-PL and PUFA over *sn*-2-PL.

KEYWORDS: *Poyang Lake, wild freshwater fish, triacylglycerol, phosphatidylcholine, phosphatidylethanolamine, stereospecific analysis*

■ INTRODUCTION

Fish lipids have been under scientific research as a good source of $n-3$ and $n-6$ polyunsaturated fatty acids (PUFA), which have effects on retinal and neural development for infants, inflammation, and immunity processes as well as prevention of cardiac and circulatory disorders.[1−3] Although freshwater fish in general contain higher proportions of $n-6$ PUFA (2.42−21.92%) than marine species (0.43−14.2%), they also have excellent levels of $n-3$ PUFA, such as 20:5n-3 (1.15−13.8%)) and 22:6n-3 (0.94−24.8%), with a ratio of $(n-6)/(n-3)$ PUFA varing between 0.04 and 4.55.[4,5]

Fish PUFA may vary by differences in location, environment, season, gender, diet, and species. In fact, Jabeen and Chaudhry related the high amount of PUFA in freshwater fish with their feed preference to phytoplanktons, which are usually rich in essential fatty acids (FA), 18:2n-6 and 18:3n-3.[6,7] Compared with marine species, freshwater fish are more capable of desaturating and elongating 18:2n-6 and 18:3n-3 into long-chain PUFA (LCPUFA, $\geq C_{20}$) due to their higher activity of Δ6D (FA desaturase) gene expression.[8,9] Poyang Lake, as a main water source of the Yangtze River, is the largest freshwater lake in China and harbors about 133 species of fish. Thus, given the dietary and geographic effects on fish FA, the fish PUFA in Poyang Lake is distinguished from other regions studied before.

About 30 000 tons of fish in Poyang Lake have been caught annually in the 21st century and are commercially important for food in the Jiangxi province. However, little attention has been paid to the fish FA compositions of Poyang Lake. Currently, PUFA like 20:5n-3 and 22:6n-3 from deep-sea fish are developed as nutraceuticals, which are relatively expensive due to the great depth of the deep sea, making it hard to exploit. Since freshwater fish contain a good amount of $n-3$ and $n-6$ PUFA, how to adequately utilize freshwater fish lipids from Poyang Lake is highly important and promising.

On the other hand, the bioavailability of FA is affected by the structures of dietary triacylglycerols (TAG) and phospholipids (PL). Due to the specific hydrolysis of pancreatic lipase on TAG and phospholipase A_2 on PL, respectively, dietary lipids are mainly absorbed as *sn*-2-monoacylglycerols (*sn*-2-MAG), *sn*-1-lysophospholipids, and free FA split from the 1 and 3 positions of TAG or 2 position of PL, but absorption in the body gets more difficult for released long-chain saturated fatty acids ($\geq C_{14}$) because they tend to form insoluble soaps with magnesium and calcium.[10] The hydrolyzed products ($\geq C_{14}$) are absorbed, subjected to re-esterification, and then incorporated into the chylomicrometers as newly formed TAG and PL in the enterocytes. Therefore, FA of the *sn*-2-position and *sn*-1-position from dietary TAG and PL, respectively, are retained in plasma TAG, whereas FA split off may be partially replaced by endogenous FA.[10,11] The compositions, distributions, carbon chain length, and degree of unsaturation of FA vary significantly from species to species in fish lipids, which generally affect the nutritional, digestive, absorptive, and metabolic properties of dietary lipids.[12] Thus, to shed more light on the nutritional and biological effects of fish lipids, it is of significance to gain deeper understanding of structural FA distributions.

We have early shown that wild freshwater fish, namely, *Squaliobarbus curriculus, Erythroculter ilishaeformis, Pseudobagrus fulvidraco, Bostrichthys sinensis,* and *Siniperca kneri Garman* of Poyang Lake have higher total PUFA than freshwater species from other regions, and this value is similar to seawater fish.[13,14] For comprehensive details of the fish lipids as mentioned above, the aim of this study was to determine the FA profiles and stereospecific analysis of TAG, phosphatidylcholine (PC),

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Table 1. Total Lipid (% Wet Weight) and Lipid Classes (% Total Lipid) from Five Wild Freshwater Fish in Poyang Lake[a]

	TL	TAG	PC	PE
S. curriculus	2.10 ± 0.19[d]	80.86 ± 0.43[e]	3.75 ± 0.33[a]	2.04 ± 0.19[a]
E. ilishaeformis	1.73 ± 0.20[c]	65.69 ± 0.39[d]	4.06 ± 0.26[a]	2.80 ± 0.21[b]
P. fulvidraco	1.29 ± 0.22[b]	79.59 ± 0.50[c]	7.79 ± 0.19[b]	4.70 ± 0.23[c]
B. sinensis	0.51 ± 0.17[a]	59.48 ± 0.36[b]	11.16 ± 0.30[c]	5.07 ± 0.32[c]
S. kneri Garman	0.37 ± 0.08[a]	55.64 ± 0.44[a]	11.81 ± 0.45[d]	5.49 ± 0.18[d]

[a]Mean values ± SD (n = 3). Values in the same column with different letters show significant differences ($p < 0.05$). TL, total lipids; TAG, triacylglycerol; PC, phosphatidylcholine; PE, phosphatidylethanolamine.

and phosphatidylethanolamine (PE) of these five wild freshwater fish from Poyang Lake.

MATERIALS AND METHODS

Materials and Chemicals. Five wild freshwater fish, *S. curriculus, E. ilishaeformis, P. fulvidraco, B. sinensis, and S. kneri Garman*, were captured in May within Poyang Lake of Jiangxi province, China. Six individuals of each species were transported in ice to the laboratory. After removal of head, skin, viscera, and bone they were homogenized by a blender and then stored at −80 °C until analyzed. Fish lipids were extracted by a previous method.[15] PL standards from egg yolk including PC (purity > 99%), PE (purity > 98%), lysophosphatidylcholine (LPC, purity > 99%), and lysophosphatidylethanolamine (LPE, purity > 99%), lipase from porcine pancreas (Type II), phospholipase A_2 from porcine pancreas, and (trimethylsilyl) diazomethane solution were from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA). Standards of TAG, diacylglycerols, monoacylglycerols, and free FA were collected using modified Hita method from fish lipids.[16] Standard fatty acid methyl esters (FAME, #463) spiked with a mixture of four positional CLA isomers (#UC-59 M) were obtained from Nu-Chek Prep Inc. (Elysian, MN). Silica gel (ZCX-II, 54−74 μm) and silica gel GF254 TLC plates (20 × 20 cm) were obtained from Haiyang Chemical Group (Qingdao, China). *n*-Hexane used in GC was purchased from Merck (Darmstadt, Germany), and other solvents were analytical reagent grade.

Separation of Lipids by Adsorption Chromatography. Silica gel containing 5% water was prepared beforehand. A glass column containing silica gel was conditioned using 3 column volumes of petroleum ether (bp 40−60 °C)/ether (87:13, v/v), and then the fish lipids were applied onto the column according to the published method with modifications.[17] The neutral lipids tended to elute first with the solvents mentioned above (87:13, v/v) by 2 column volumes, followed by glycolipids with 2 column volumes of acetone and finally PL (methanol, 8 column volumes). Butylated hydroxytoluene (0.02%, BHT) was added to the solvents to prevent oxidation of PUFA. In each fraction about 1/3 of the column volume was collected into a tube, evaporated under vacuum (40 °C), and determined by TLC using *n*-hexane/ether/acetic acid (75:25:1, v/v/v) as developing solvent for neutral lipids while chloroform/methanol/water (65:25:4, v/v/v) was used for PL. The plates were allowed to dry under N_2 atmosphere and visualized by dichlorofluorescein. Lipids fractions were identified by comparison with TAG, PC, and PE standards. Fractions containing homogeneous TAG, PC, and PE were accumulated.

Stereospecific Analysis of TAG. The method of Hita et al. with modification was employed to determine FA in position 2 of TAG: an aliquot (10 mg) of purified TAG, 10 mL of buffer solution tris-HCl (1M, pH 7.6), and 2.5 mL of 0.05% bile salts solution.

One milliliter of 2.2% calcium chloride solution and 10 mg of pancreatic lipase were placed in a centrifuge tube and shaken vigorously for 1 min. Later, the mixture was incubated for 3 min at 37 °C and shaken intensively for 30 s, and then this procedure was repeated another two times. Afterward, 6 mL of diethyl ether was added to stop the reaction and the mixture was centrifuged at 4200 rpm for 5 min. The upper layer was dried under N_2 atmosphere and analyzed by TLC and GC.[16] *sn*-2-MAG obtained was subjected to methylation described by Ichihara et al.,[18] and another aliquot of purified TAG was methylated according to Cruz-Hernandez et al.[19]

The mean composition of each FA in positions 1 and 3 can be calculated from its concentrations in the intact TAG and *sn*-2-MAG according to the following relationship

$$\text{positions 1 and 3 mol \%} = (\text{TAG mol \%} \times 3 - sn\text{-2-MAG mol \%})/2$$

Stereospecific Analysis of PC and PE. The method of Robertson and Lands with little modification was employed to determine FA in positions 1 and 2 of PC (PE): an aliquot (5 mg) of purified PC (PE), 0.1 mL of phospholipase A_2 solution, and 2 mL of diethyl ether were placed in a centrifuge tube; then it was shaken vigorously for 3 h to be hydrolyzed thoroughly with 0.02% BHT under N_2 atmosphere at 25 °C. The mixture was analyzed by TLC and GC.[20] *sn*-1-LPC (*sn*-1-LPE) and intact PC (PE) were subjected to transesterification as described by Cruz-Hernandez et al.,[19] and free FA (in position 2) released from PC (PE) were methylated by the previous method.[21]

GC Analysis. The FAME were analyzed by a GC equipped with a flame ionization detector and a fused silica capillary column (100 m × 0.25 mm × 0.2 μm) coated with 100% cyanopropyl polysiloxane (CP-Sil 88, Chrompack; Middelburg, The Netherlands). The temperature program was 86 min in total: the initial temperature of the oven was 45 °C for 4 min, increased to 175 °C at a rate of 13 °C/min, maintained for 27 min, further raised to 215 °C at a rate of 4 °C/min, and finally kept at this temperature for 35 min.[19] Analysis of all peaks was accomplished by comparison of their retention time with FAME standards. All samples were carried out in triplicate.

Statistical Analysis. Fatty acid compositions were analyzed with one-way analysis of variance, and mean values were compared using Duncan's test. The significance level was set at $p < 0.05$. All statistical analyses were carried out using SPSS 13.0 software for Windows.

RESULTS

Lipid Contents. The lipid contents (%) of five wild freshwater fish are presented in Table 1. The results showed the total lipid contents differed among different species, and TAG composed the major part of the total lipids. The maximal values of total lipids (2.10%) and TAG (80.86%) were determined in *S. curriculus*, with a minimum in PC (3.75%) and PE (2.04%), whereas *S. kneri Garman* was lowest in total lipids (0.37%) and TAG contents (55.64%) but highest in PC (11.81%) and PE (5.49%). In general, an increase in the total lipids level was accompanied with an increase in TAG but a decrease in PL contents.

Total FA Compositions of TAG and PL in Five Wild Freshwater Fish. Table 2 illustrates the total FA profile in TAG, PC, and PE from *S. curriculus, E. ilishaeformis, P. fulvidraco, B. sinensis*, and *S. kneri Garman*. Generally, significant differences were observed for the majority of the FA among TAG, PC, and PE. In each species total saturated fatty acids (SFA) and total monounsaturated fatty acids (MUFA) mainly increased in the order PE < PC < TAG, whereas total PUFA declined as

Table 2. Total Fatty Acids Compositions (%) in TAG, PC, and PE of Five Wild Freshwater Fish from Poyang Lake[a]

	S. curriculus			*E. ilishaeformis*			*P. fulvidraco*			*B. sinensis*			*S. kneri Garman*		
FA	TAG	PC	PE	TAG	PC	PE	TAG	PC	PE	TAG	PC	PE	TAG	PC	PE
12:0	0.15 ± 0.00	—	—	0.09 ± 0.00[b]	0.02 ± 0.02[a]	—	0.14 ± 0.00[b]	0.02 ± 0.01[a]	—	0.41 ± 0.01[b]	0.02 ± 0.01[a]	—	0.18 ± 0.01	—	—
iso 13:0	0.02 ± 0.00	—	—	0.02 ± 0.01	—	—	0.02 ± 0.00	—	—	0.04 ± 0.02	—	—	0.04 ± 0.00	—	—
13:0	0.08 ± 0.00	—	—	0.06 ± 0.00[b]	0.02 ± 0.01[a]	—	0.09 ± 0.00[b]	0.02 ± 0.01[a]	—	1.46 ± 0.05[b]	0.07 ± 0.00[a]	—	0.18 ± 0.00[b]	0.01 ± 0.01[a]	—
iso 14:0	0.08 ± 0.00	—	—	0.05 ± 0.01	—	—	0.06 ± 0.00	—	—	0.22 ± 0.01[b]	0.01 ± 0.01[a]	—	0.21 ± 0.01[b]	0.02 ± 0.01[a]	—
14:0	2.36 ± 0.04[c]	0.45 ± 0.00[b]	0.19 ± 0.01[a]	2.23 ± 0.05[c]	0.71 ± 0.01[b]	0.20 ± 0.00[a]	2.30 ± 0.06[c]	0.92 ± 0.02[b]	0.55 ± 0.00[a]	4.59 ± 0.15[c]	0.92 ± 0.04[b]	0.22 ± 0.01[a]	2.60 ± 0.07[b]	0.62 ± 0.07[a]	0.46 ± 0.01[a]
iso 15:0	0.42 ± 0.01[b]	0.07 ± 0.00[a]	—	0.43 ± 0.00[c]	0.11 ± 0.01[b]	0.05 ± 0.01[a]	0.45 ± 0.07[b]	0.12 ± 0.02[a]	—	1.25 ± 0.04[c]	0.22 ± 0.03[b]	0.09 ± 0.02[a]	1.41 ± 0.05[c]	0.22 ± 0.02[b]	0.12 ± 0.01[a]
anti 15:0	0.12 ± 0.00	—	—	0.09 ± 0.02[b]	0.01 ± 0.01[a]	—	0.17 ± 0.01[b]	0.02 ± 0.02[a]	—	0.22 ± 0.02[b]	0.03 ± 0.01[a]	—	0.61 ± 0.05[b]	0.05 ± 0.02[a]	—
15:0	0.79 ± 0.01[c]	0.43 ± 0.01[b]	0.13 ± 0.00[a]	0.82 ± 0.02[c]	0.57 ± 0.01[b]	0.14 ± 0.01[a]	1.13 ± 0.02[c]	0.95 ± 0.02[b]	0.16 ± 0.00[a]	4.55 ± 0.13[c]	2.40 ± 0.08[b]	0.44 ± 0.01[a]	1.58 ± 0.04[c]	0.88 ± 0.14[b]	0.52 ± 0.03[a]
iso 16:0	0.22 ± 0.00[c]	0.10 ± 0.00[b]	0.05 ± 0.00[a]	0.19 ± 0.05[b]	0.12 ± 0.01[ab]	0.05 ± 0.00[a]	0.41 ± 0.01[c]	0.18 ± 0.02[b]	0.07 ± 0.0[a]	0.52 ± 0.01[c]	0.26 ± 0.03[b]	0.02 ± 0.02[a]	1.10 ± 0.02[c]	0.27 ± 0.03[b]	0.10 ± 0.02[a]
16:0	19.68 ± 0.24[b]	22.36 ± 0.34[c]	9.61 ± 0.23[a]	21.04 ± 0.52[b]	24.41 ± 0.03[c]	10.21 ± 0.19[a]	21.10 ± 0.52[b]	21.52 ± 0.72[b]	6.33 ± 0.00[a]	18.91 ± 0.66[b]	20.65 ± 0.11[c]	8.82 ± 0.26[a]	16.29 ± 0.05[a]	26.00 ± 0.42[b]	12.52 ± 0.08[a]
iso 17:0	0.97 ± 0.02[c]	0.74 ± 0.01[b]	0.62 ± 0.02[a]	0.97 ± 0.03[c]	0.72 ± 0.01[b]	0.61 ± 0.02[a]	1.73 ± 0.03[c]	0.97 ± 0.01[b]	0.72 ± 0.03[a]	1.85 ± 0.04[c]	1.05 ± 0.01[b]	0.75 ± 0.02[a]	2.23 ± 0.02[c]	0.80 ± 0.14[a]	1.32 ± 0.08[b]
anti 17:0	0.24 ± 0.03	—	—	—	—	—	—	—	—	—	—	—	0.17 ± 0.02[b]	0.03 ± 0.01[a]	—
17:0	0.87 ± 0.05[b]	0.71 ± 0.00[a]	1.29 ± 0.03[c]	1.05 ± 0.03[b]	0.82 ± 0.02[a]	1.47 ± 0.02[c]	1.23 ± 0.03[c]	0.73 ± 0.01[a]	1.01 ± 0.01[b]	1.57 ± 0.05[ab]	1.88 ± 0.18[b]	1.37 ± 0.04[a]	1.83 ± 0.02[a]	1.77 ± 0.14[a]	2.64 ± 0.01[c]
iso 18:0	0.08 ± 0.00[b]	0.02 ± 0.00[a]	—	0.07 ± 0.03[a]	0.04 ± 0.00[a]	—	0.04 ± 0.01[a]	0.02 ± 0.01[a]	0.19 ± 0.01[b]	—	—	—	0.12 ± 0.01[b]	0.03 ± 0.00[a]	—
18:0	4.65 ± 0.08[a]	4.41 ± 0.02[a]	16.62 ± 0.26[b]	6.02 ± 0.11[a]	5.53 ± 0.14[a]	18.69 ± 0.33[b]	5.85 ± 0.11[b]	4.76 ± 0.03[a]	16.84 ± 0.20[c]	3.61 ± 0.07[a]	5.74 ± 0.15[b]	14.57 ± 0.47[c]	4.93 ± 0.12[b]	3.74 ± 0.21[a]	17.78 ± 0.30[c]
19:0	0.17 ± 0.05[a]	0.12 ± 0.01[a]	0.51 ± 0.00[b]	0.30 ± 0.00[b]	0.16 ± 0.01[a]	0.50 ± 0.01[c]	0.43 ± 0.06[b]	0.23 ± 0.00[a]	0.74 ± 0.07[c]	0.31 ± 0.01[a]	0.38 ± 0.01[b]	1.12 ± 0.01[c]	0.78 ± 0.02[b]	0.26 ± 0.02[a]	1.65 ± 0.01[c]
20:0	0.26 ± 0.00[c]	0.05 ± 0.00[a]	0.17 ± 0.02[b]	0.32 ± 0.00[c]	0.09 ± 0.01[a]	0.19 ± 0.02[b]	0.27 ± 0.00[c]	0.07 ± 0.01[a]	0.17 ± 0.01[b]	0.10 ± 0.01[a]	0.14 ± 0.03[a]	0.25 ± 0.11[a]	0.24 ± 0.01[a]	—	0.25 ± 0.06[a]
22:0	0.07 ± 0.01	—	—	0.13 ± 0.00[b]	0.05 ± 0.01[a]	—	0.09 ± 0.00[b]	0.03 ± 0.04[a]	—	—	—	—	0.13 ± 0.03	—	—
23:0	0.36 ± 0.00[c]	0.26 ± 0.00[a]	0.31 ± 0.00[b]	0.80 ± 0.03[b]	0.36 ± 0.03[a]	0.30 ± 0.03[a]	0.56 ± 0.00[b]	0.32 ± 0.03[a]	0.32 ± 0.05[a]	0.46 ± 0.08[a]	0.23 ± 0.02[a]	0.32 ± 0.11[a]	0.37 ± 0.03[b]	0.15 ± 0.04[a]	0.11 ± 0.01[a]
24:0	—	—	—	0.03 ± 0.00	—	—	0.13 ± 0.00	—	—	—	—	—	0.17 ± 0.03[b]	0.11 ± 0.03[b]	0.02 ± 0.01[a]
total SFA	31.59 ± 0.52[b]	29.72 ± 0.39[a]	29.50 ± 0.00[a]	34.79 ± 0.72[b]	33.71 ± 0.18[ab]	32.40 ± 0.13[a]	36.19 ± 0.78[c]	30.89 ± 0.68[b]	27.12 ± 0.17[a]	40.07 ± 0.97[c]	33.98 ± 0.06[b]	27.97 ± 0.39[a]	35.18 ± 0.86[a]	34.97 ± 0.09[a]	37.46 ± 0.18[b]
14:1n-5	0.06 ± 0.00	—	—	0.06 ± 0.00	—	—	0.19 ± 0.00	—	—	0.18 ± 0.02	—	—	0.07 ± 0.00	—	—
t16:1n-8	0.21 ± 0.00[c]	0.15 ± 0.00[b]	0.07 ± 0.00[a]	0.20 ± 0.02[b]	0.29 ± 0.02[c]	0.07 ± 0.01[a]	0.17 ± 0.03[b]	0.32 ± 0.00[c]	0.05 ± 0.01[a]	0.34 ± 0.02[ab]	0.38 ± 0.02[b]	0.15 ± 0.11[a]	0.25 ± 0.04[b]	0.25 ± 0.01[b]	0.07 ± 0.01[a]
t16:1n-7	0.05 ± 0.01[a]	0.06 ± 0.00[b]	—	—	0.79 ± 0.02[b]	0.60 ± 0.01[a]	—	—	—	—	—	—	—	0.11 ± 0.00	—
16:1n-9	0.49 ± 0.03[c]	0.25 ± 0.00[b]	0.15 ± 0.02[a]	0.43 ± 0.01[c]	0.20 ± 0.01[b]	0.09 ± 0.01[a]	0.66 ± 0.03[b]	0.74 ± 0.01[c]	0.18 ± 0.01[a]	—	—	—	0.54 ± 0.07[c]	0.11 ± 0.00[a]	0.41 ± 0.02[b]
16:1n-8	0.14 ± 0.02[b]	0.08 ± 0.00[a]	—	—	—	—	—	—	—	—	—	—	0.18 ± 0.06[b]	0.06 ± 0.00[ab]	0.03 ± 0.01[a]
16:1n-7	9.65 ± 0.06[c]	2.66 ± 0.04[b]	1.33 ± 0.02[a]	7.61 ± 0.12[c]	2.62 ± 0.01[b]	1.21 ± 0.01[a]	8.26 ± 0.20[c]	3.27 ± 0.00[b]	1.34 ± 0.00[a]	10.41 ± 0.22[c]	2.66 ± 0.02[b]	1.21 ± 0.11[a]	7.99 ± 0.08[c]	1.97 ± 0.00[b]	1.20 ± 0.04[a]
16:1n-6	0.35 ± 0.04[b]	0.29 ± 0.00[b]	0.06 ± 0.00[a]	0.27 ± 0.01[c]	0.20 ± 0.01[b]	0.06 ± 0.01[a]	0.29 ± 0.03[b]	0.32 ± 0.07[b]	0.05 ± 0.01[a]	0.56 ± 0.07[b]	0.37 ± 0.02[a]	0.37 ± 0.04[a]	0.52 ± 0.01[c]	0.43 ± 0.01[b]	0.27 ± 0.02[a]
17:1(a)	0.11 ± 0.01[a]	0.13 ± 0.00[a]	0.12 ± 0.03[a]	0.17 ± 0.05[a]	0.22 ± 0.02[a]	0.26 ± 0.01[a]	0.23 ± 0.01[b]	0.23 ± 0.01[b]	0.18 ± 0.01[a]	—	—	—	0.33 ± 0.09[a]	0.31 ± 0.04[a]	1.19 ± 0.07[b]
17:1(b)	0.05 ± 0.00[a]	0.04 ± 0.00[a]	—	0.08 ± 0.01[a]	0.05 ± 0.02[a]	—	0.12 ± 0.01[b]	0.09 ± 0.01[a]	—	—	—	—	0.14 ± 0.02	—	—
17:1n-12	0.20 ± 0.01[b]	0.03 ± 0.00[a]	—	0.11 ± 0.01	—	—	0.22 ± 0.01[b]	0.09 ± 0.01[a]	—	0.37 ± 0.04[b]	0.10 ± 0.02[a]	—	0.47 ± 0.01	—	—
17:1n-10	0.18 ± 0.02[b]	0.10 ± 0.02[a]	0.22 ± 0.01[b]	0.07 ± 0.00[a]	—	0.12 ± 0.00[b]	0.10 ± 0.01[b]	0.03 ± 0.00[a]	—	0.46 ± 0.03[b]	0.02 ± 0.01[a]	—	0.20 ± 0.01	—	—
17:1n-8	0.73 ± 0.04[c]	0.42 ± 0.02[b]	0.18 ± 0.00[a]	1.01 ± 0.02[b]	0.73 ± 0.29[ab]	0.24 ± 0.00[a]	0.99 ± 0.03[c]	0.52 ± 0.01[b]	0.30 ± 0.00[a]	1.20 ± 0.04[c]	0.68 ± 0.02[b]	0.52 ± 0.05[a]	0.86 ± 0.02[b]	0.45 ± 0.02[a]	0.25 ± 0.06[a]
t18:1n-9	0.27 ± 0.05[b]	0.19 ± 0.01[ab]	0.15 ± 0.01[a]	0.21 ± 0.02[b]	0.19 ± 0.04[ab]	0.13 ± 0.00[a]	0.33 ± 0.03[b]	0.33 ± 0.00[b]	0.23 ± 0.00[a]	0.76 ± 0.00[c]	0.31 ± 0.06[a]	0.15 ± 0.05[b]	0.36 ± 0.01[c]	0.28 ± 0.00[b]	0.20 ± 0.01[a]
t18:1n-7	0.20 ± 0.01[c]	0.06 ± 0.00[a]	0.10 ± 0.01[b]	0.08 ± 0.01[b]	0.03 ± 0.01[a]	0.07 ± 0.00[b]	0.10 ± 0.01[b]	0.05 ± 0.00[a]	0.10 ± 0.01[b]	—	—	0.09 ± 0.03	0.22 ± 0.00[c]	0.03 ± 0.01[a]	0.12 ± 0.01[b]
18:1n-12	—	—	—	—	—	0.15 ± 0.02	0.07 ± 0.00[a]	0.07 ± 0.00[a]	0.13 ± 0.01[b]	—	—	0.08 ± 0.04	0.17 ± 0.01[b]	0.08 ± 0.02[a]	0.12 ± 0.01[a]
18:1n-9	26.94 ± 0.10[c]	12.53 ± 0.57[b]	7.66 ± 0.19[a]	25.91 ± 0.52[c]	14.65 ± 0.29[b]	7.41 ± 0.12[a]	24.83 ± 0.58[c]	10.94 ± 0.04[b]	9.54 ± 0.47[a]	14.22 ± 0.45[c]	8.15 ± 0.28[b]	5.36 ± 0.12[a]	11.62 ± 0.44[c]	8.30 ± 0.10[b]	4.37 ± 0.03[a]
18:1n-7	4.08 ± 0.02[c]	2.58 ± 0.02[b]	2.38 ± 0.03[a]	3.60 ± 0.09[c]	2.58 ± 0.04[b]	2.07 ± 0.00[a]	3.90 ± 0.09[c]	3.04 ± 0.02[b]	2.26 ± 0.01[a]	3.25 ± 0.09[b]	3.16 ± 0.08[b]	2.63 ± 0.02[a]	5.38 ± 0.20[c]	2.49 ± 0.14[a]	3.29 ± 0.27[b]
18:1n-6	0.26 ± 0.03[c]	0.14 ± 0.03[ab]	0.17 ± 0.03[b]	0.12 ± 0.05[a]	0.30 ± 0.04[b]	0.14 ± 0.00[a]	0.25 ± 0.01[b]	0.08 ± 0.02[a]	0.16 ± 0.03[a]	0.13 ± 0.01[a]	0.10 ± 0.03[a]	0.15 ± 0.02[a]	0.22 ± 0.04[b]	0.07 ± 0.00[a]	0.16 ± 0.04[ab]
18:1n-5	0.19 ± 0.02[b]	0.13 ± 0.03[ab]	0.06 ± 0.03[a]	—	—	0.12 ± 0.01	0.05 ± 0.00[a]	0.08 ± 0.02[a]	—	—	—	—	0.25 ± 0.04[b]	0.07 ± 0.01[a]	0.11 ± 0.01[a]
19:1	0.24 ± 0.04	—	—	—	—	—	0.17 ± 0.01	—	—	0.25 ± 0.01	—	—	0.13 ± 0.04	—	—
20:1n-15	—	—	—	0.11 ± 0.00	—	—	0.10 ± 0.02	—	—	—	—	—	0.08 ± 0.04	—	—
20:1n-12	—	—	—	0.10 ± 0.00	—	—	1.26 ± 0.01[b]	0.19 ± 0.01[a]	0.13 ± 0.03[a]	—	—	—	—	—	—
20:1n-9	0.51 ± 0.03[b]	0.18 ± 0.00[a]	0.18 ± 0.00[a]	0.51 ± 0.01[b]	0.24 ± 0.02[a]	0.25 ± 0.02[a]	1.20 ± 0.01[c]	0.46 ± 0.01[a]	0.60 ± 0.04[b]	0.49 ± 0.02[b]	0.15 ± 0.06[a]	0.13 ± 0.04[a]	0.55 ± 0.03[b]	0.80 ± 0.00[c]	0.19 ± 0.01[a]
22:1n-9	—	—	—	0.07 ± 0.01	—	—	0.05 ± 0.00	—	—	0.07 ± 0.02[a]	0.23 ± 0.05[b]	0.46 ± 0.03[c]	0.18 ± 0.04	—	—
24:1n-9	0.12 ± 0.07[a]	0.08 ± 0.00[a]	0.05 ± 0.00[a]	—	—	—	—	—	—	—	—	—	0.07 ± 0.01	—	—
total cis MUFA	43.90 ± 0.14[c]	19.47 ± 0.48[b]	12.45 ± 0.28[a]	39.99 ± 0.78[c]	21.51 ± 0.05[b]	11.86 ± 0.15[a]	42.41 ± 0.86[c]	19.84 ± 0.01[b]	14.70 ± 0.58[a]	31.33 ± 0.78[c]	15.61 ± 0.19[b]	10.89 ± 0.15[a]	29.34 ± 0.86[c]	14.13 ± 0.08[b]	10.37 ± 0.36[a]

Table 2. continued

FA	S. curriculus TAG	S. curriculus PC	S. curriculus PE	E. ilishaeformis TAG	E. ilishaeformis PC	E. ilishaeformis PE	P. fulvidraco TAG	P. fulvidraco PC	P. fulvidraco PE	B. sinensis TAG	B. sinensis PC	B. sinensis PE	S. kneri Garman TAG	S. kneri Garman PC	S. kneri Garman PE
total trans MUFA	0.72 ± 0.06^{c}	0.46 ± 0.01^{b}	0.32 ± 0.03^{a}	0.49 ± 0.01^{a}	1.29 ± 0.01^{c}	0.87 ± 0.00^{b}	0.61 ± 0.01^{b}	0.70 ± 0.00^{c}	0.38 ± 0.00^{a}	1.10 ± 0.03^{b}	0.70 ± 0.04^{a}	0.39 ± 0.20^{a}	0.83 ± 0.03^{c}	0.67 ± 0.01^{b}	0.38 ± 0.00^{a}
tt18:2n-6	0.14 ± 0.05	—	—	0.10 ± 0.02^{b}	0.01 ± 0.01^{a}	—	0.08 ± 0.00	—	—	0.21 ± 0.02^{b}	0.09 ± 0.00^{a}	—	0.07 ± 0.02	—	—
ct18:2n-6	0.11 ± 0.00^{b}	0.07 ± 0.00^{a}	—	0.09 ± 0.02	—	—	0.12 ± 0.01^{b}	0.06 ± 0.02^{a}	—	0.09 ± 0.03^{a}	0.08 ± 0.01^{a}	—	0.09 ± 0.02^{a}	0.13 ± 0.02^{a}	—
tc18:2n-6	0.11 ± 0.01^{b}	0.03 ± 0.00^{a}	—	0.07 ± 0.02	—	—	0.13 ± 0.02^{b}	0.05 ± 0.00^{a}	—	0.10 ± 0.03^{b}	0.03 ± 0.01^{a}	—	0.16 ± 0.02	—	—
total trans DUFA	0.36 ± 0.05^{b}	0.10 ± 0.00^{a}	—	0.26 ± 0.02^{b}	0.01 ± 0.01^{a}	—	0.34 ± 0.01^{b}	0.11 ± 0.02^{a}	—	0.40 ± 0.03^{b}	0.20 ± 0.01^{a}	—	0.32 ± 0.06^{b}	0.13 ± 0.02^{a}	—
9c11t-18:2	0.16 ± 0.01^{b}	0.09 ± 0.00^{a}	0.08 ± 0.01^{a}	0.03 ± 0.04	—	—	0.07 ± 0.03^{a}	0.03 ± 0.05^{a}	—	0.14 ± 0.03^{a}	0.12 ± 0.02^{a}	0.32 ± 0.11^{a}	0.19 ± 0.02^{b}	0.07 ± 0.00^{a}	—
11c13t-18:2	—	—	—	0.01 ± 0.02	—	—	0.05 ± 0.03	—	—	—	—	—	—	—	—
11t13t-18:2	0.03 ± 0.01	—	—	—	—	—	—	—	—	—	—	—	—	—	—
8t10t/9t11t/10t12t-18:2	0.29 ± 0.02	—	—	0.22 ± 0.02^{b}	0.12 ± 0.01^{a}	0.09 ± 0.04^{a}	0.38 ± 0.04^{b}	0.16 ± 0.00^{a}	0.14 ± 0.00^{a}	0.93 ± 0.14^{b}	0.17 ± 0.02^{a}	0.13 ± 0.05^{a}	0.55 ± 0.01^{b}	0.10 ± 0.01^{a}	—
total CLA	0.48 ± 0.04^{b}	0.09 ± 0.00^{a}	0.08 ± 0.00^{a}	0.26 ± 0.03^{b}	0.12 ± 0.01^{a}	0.09 ± 0.04^{a}	0.50 ± 0.10^{b}	0.19 ± 0.05^{a}	0.14 ± 0.00^{a}	1.07 ± 0.17^{b}	0.29 ± 0.03^{a}	0.45 ± 0.16^{a}	0.73 ± 0.01^{b}	0.17 ± 0.01^{a}	—
18:2n-6	8.28 ± 0.04^{c}	5.04 ± 0.08^{b}	2.95 ± 0.21^{a}	2.33 ± 0.05^{c}	1.49 ± 0.01^{b}	0.89 ± 0.01^{a}	3.20 ± 0.06^{c}	2.10 ± 0.03^{b}	1.23 ± 0.03^{a}	9.18 ± 0.21^{c}	3.23 ± 0.02^{b}	2.62 ± 0.09^{a}	5.97 ± 0.09^{c}	1.69 ± 0.03^{a}	3.78 ± 0.02^{b}
18:3n-6	0.21 ± 0.01^{b}	0.07 ± 0.00^{a}	—	0.10 ± 0.00^{b}	0.05 ± 0.00^{a}	—	0.13 ± 0.00^{b}	0.05 ± 0.00^{a}	—	1.01 ± 0.06^{b}	0.28 ± 0.01^{a}	0.18 ± 0.03^{a}	0.44 ± 0.00^{c}	0.12 ± 0.00^{b}	0.09 ± 0.01^{a}
20:2n-6	0.40 ± 0.00^{b}	0.37 ± 0.00^{a}	0.46 ± 0.01^{c}	0.31 ± 0.01^{c}	0.23 ± 0.00^{b}	0.21 ± 0.00^{a}	0.73 ± 0.01^{b}	0.80 ± 0.01^{c}	0.62 ± 0.02^{a}	0.29 ± 0.01^{a}	0.55 ± 0.01^{c}	0.41 ± 0.00^{b}	0.96 ± 0.00^{c}	0.24 ± 0.04^{a}	0.37 ± 0.01^{b}
20:3n-6	0.33 ± 0.01^{a}	0.71 ± 0.01^{b}	0.76 ± 0.02^{b}	0.50 ± 0.01^{b}	0.53 ± 0.00^{b}	0.41 ± 0.01^{a}	0.33 ± 0.01^{a}	0.60 ± 0.01^{b}	0.59 ± 0.00^{b}	0.28 ± 0.00^{a}	0.64 ± 0.02^{c}	0.42 ± 0.01^{b}	0.68 ± 0.01^{b}	0.45 ± 0.08^{a}	0.32 ± 0.01^{a}
20:4n-6	2.48 ± 0.10^{a}	15.27 ± 0.16^{b}	16.06 ± 0.03^{c}	3.62 ± 0.00^{a}	10.69 ± 0.24^{b}	14.16 ± 0.07^{c}	2.73 ± 0.05^{a}	13.84 ± 0.20^{b}	15.44 ± 0.90^{b}	2.14 ± 0.00^{a}	11.96 ± 0.49^{b}	19.13 ± 0.25^{c}	5.51 ± 0.11^{a}	14.11 ± 0.57^{c}	6.04 ± 0.05^{b}
22:2n-6	—	—	—	—	0.06 ± 0.01^{b}	0.03 ± 0.00^{a}	0.04 ± 0.00^{a}	0.05 ± 0.03^{a}	—	—	—	—	0.22 ± 0.00^{a}	0.24 ± 0.04^{b}	0.36 ± 0.04^{b}
22:4n-6	0.23 ± 0.00^{a}	0.77 ± 0.01^{b}	1.26 ± 0.07^{c}	0.56 ± 0.00^{a}	1.01 ± 0.01^{b}	1.93 ± 0.04^{c}	0.70 ± 0.00^{a}	1.59 ± 0.02^{b}	1.88 ± 0.03^{c}	0.31 ± 0.00^{a}	1.76 ± 0.01^{b}	1.76 ± 0.04^{b}	1.64 ± 0.01^{ab}	1.37 ± 0.17^{a}	1.97 ± 0.08^{b}
22:5n-6	0.53 ± 0.05^{a}	2.20 ± 0.09^{b}	4.87 ± 0.15^{c}	1.42 ± 0.03^{a}	4.23 ± 0.18^{b}	6.24 ± 0.21^{c}	0.91 ± 0.01^{a}	2.25 ± 0.01^{b}	2.46 ± 0.03^{c}	0.61 ± 0.17^{a}	5.50 ± 0.38^{b}	5.96 ± 0.03^{b}	2.11 ± 0.00^{a}	4.88 ± 0.00^{c}	3.47 ± 0.05^{b}
18:3n-3	4.27 ± 0.05^{c}	1.89 ± 0.00^{b}	1.09 ± 0.06^{a}	2.30 ± 0.04^{c}	0.86 ± 0.01^{b}	0.48 ± 0.01^{a}	2.15 ± 0.05^{c}	0.77 ± 0.01^{b}	0.44 ± 0.07^{a}	5.77 ± 0.17^{b}	1.21 ± 0.03^{a}	0.91 ± 0.02^{a}	4.55 ± 0.00^{b}	0.66 ± 0.02^{a}	0.64 ± 0.02^{a}
20:3n-3	0.34 ± 0.00^{b}	0.23 ± 0.01^{a}	0.40 ± 2.54^{c}	0.39 ± 0.01^{b}	0.21 ± 0.11^{a}	0.22 ± 0.00^{a}	0.34 ± 0.04^{a}	0.31 ± 0.01^{a}	0.32 ± 0.01^{a}	0.30 ± 0.00^{a}	0.33 ± 0.01^{b}	0.31 ± 0.00^{b}	0.38 ± 0.01^{c}	0.10 ± 0.00^{a}	0.19 ± 0.01^{b}
20:5n-3	2.10 ± 0.08^{a}	7.54 ± 0.12^{c}	3.15 ± 0.05^{b}	3.26 ± 0.05^{a}	6.12 ± 0.13^{b}	3.03 ± 0.05^{a}	1.80 ± 0.02^{a}	4.58 ± 0.10^{b}	1.84 ± 0.04^{a}	0.94 ± 0.01^{a}	1.66 ± 0.01^{c}	1.19 ± 0.04^{b}	2.50 ± 0.03^{b}	3.52 ± 0.12^{c}	1.31 ± 0.06^{a}
22:3n-3	—	—	—	0.01 ± 0.00	—	—	—	—	—	—	—	—	0.11 ± 0.01^{b}	0.05 ± 0.04^{ab}	0.03 ± 0.00^{a}
22:5n-3	0.63 ± 0.00^{a}	1.26 ± 0.05^{b}	2.33 ± 0.12^{c}	1.55 ± 0.01^{a}	1.54 ± 0.01^{a}	2.29 ± 0.06^{b}	1.31 ± 0.00^{a}	1.80 ± 0.01^{b}	2.01 ± 0.01^{c}	1.11 ± 0.01^{a}	2.99 ± 0.00^{b}	3.94 ± 0.17^{c}	1.92 ± 0.04^{a}	2.15 ± 0.04^{b}	2.90 ± 0.04^{c}
22:6n-3	2.24 ± 0.20^{a}	12.18 ± 0.30^{b}	22.93 ± 0.28^{b}	6.01 ± 0.13^{a}	15.44 ± 0.36^{b}	23.85 ± 0.31^{c}	3.17 ± 0.04^{a}	11.90 ± 0.17^{b}	28.26 ± 0.22^{c}	1.61 ± 0.05^{a}	14.18 ± 0.55^{b}	19.27 ± 0.06^{c}	4.14 ± 0.02^{a}	17.87 ± 1.41^{b}	26.79 ± 0.19^{c}
total PUFA	23.03 ± 0.62^{a}	47.81 ± 0.57^{b}	55.25 ± 3.06^{c}	22.89 ± 0.35^{a}	42.58 ± 0.90^{b}	53.84 ± 0.57^{c}	18.38 ± 0.33^{a}	40.94 ± 0.63^{b}	55.23 ± 0.74^{c}	25.02 ± 0.31^{a}	44.78 ± 0.65^{b}	56.55 ± 0.31^{c}	31.86 ± 0.25^{a}	47.69 ± 0.67^{b}	48.21 ± 0.56^{c}
total $n-6$ PUFA	12.45 ± 0.21^{a}	24.44 ± 0.19^{b}	25.36 ± 0.33^{c}	8.85 ± 0.10^{a}	18.29 ± 0.41^{b}	23.87 ± 0.21^{c}	8.78 ± 0.13^{a}	21.27 ± 0.24^{b}	22.22 ± 1.01^{b}	13.82 ± 0.01^{a}	23.92 ± 0.16^{b}	30.48 ± 0.11^{c}	17.54 ± 0.19^{b}	23.15 ± 0.64^{c}	16.38 ± 0.23^{a}
total $n-3$ PUFA	9.59 ± 0.33^{a}	23.10 ± 0.38^{b}	28.81 ± 2.71^{c}	13.52 ± 0.24^{a}	24.16 ± 0.49^{b}	29.88 ± 0.40^{c}	8.77 ± 0.08^{a}	19.36 ± 0.32^{b}	32.87 ± 0.26^{c}	9.73 ± 0.10^{a}	20.37 ± 0.53^{b}	25.61 ± 0.26^{c}	13.59 ± 0.07^{a}	24.36 ± 1.31^{b}	31.84 ± 0.33^{c}
$(n-6)/(n-3)$ PUFA	1.30 ± 0.64^{c}	1.06 ± 0.01^{b}	0.83 ± 0.06^{a}	0.65 ± 0.00^{a}	0.76 ± 0.00^{b}	0.80 ± 0.00^{c}	1.00 ± 0.01^{b}	1.10 ± 0.01^{c}	0.68 ± 0.04^{a}	1.42 ± 0.01^{b}	1.17 ± 0.02^{a}	1.19 ± 0.02^{a}	1.29 ± 0.01^{c}	0.95 ± 0.08^{b}	0.51 ± 0.01^{a}
total $((n-6)+(n-3))$/SFA	0.70 ± 1.05^{a}	1.60 ± 0.04^{b}	1.97 ± 0.10^{c}	0.64 ± 0.00^{a}	1.26 ± 0.02^{b}	1.66 ± 0.01^{c}	0.48 ± 0.00^{a}	1.32 ± 0.01^{b}	2.03 ± 0.01^{c}	0.59 ± 0.02^{a}	1.30 ± 0.02^{b}	2.01 ± 0.03^{c}	0.88 ± 0.01^{a}	1.36 ± 0.02^{c}	1.29 ± 0.01^{b}
total OBCFA	5.94 ± 0.19^{b}	3.16 ± 0.01^{a}	3.42 ± 0.08^{a}	6.29 ± 0.10^{b}	3.91 ± 0.29^{a}	3.73 ± 0.01^{a}	8.14 ± 0.03^{c}	4.53 ± 0.01^{b}	3.70 ± 0.02^{a}	14.74 ± 0.04^{c}	7.33 ± 0.15^{b}	4.63 ± 0.10^{a}	12.75 ± 0.41^{c}	5.46 ± 0.13^{a}	7.88 ± 0.12^{b}

[a]Mean values ± SD (n = 3). Values in the same row with different letters for each species show significant differences ($p < 0.05$). TAG, triacylglycerol; PC, phosphatidylcholine; PE, phosphatidylethanolamine; total SFA, total saturated fatty acids; 17:1(a), 7-methyl-6-hexadecenoic acid; 17:1(b), 15-methyl-11-hexadecenoic acid; total *cis* MUFA, total *cis* monounsaturated fatty acids; total *trans* MUFA, total *trans* monounsaturated fatty acids; total *trans* DUFA, total *trans* diunsaturated fatty acids; total CLA, total conjugated linoleic acids; total PUFA, total polyunsaturated fatty acids; total $n-6$ PUFA, total $n-6$ polyunsaturated fatty acids; total $n-3$ PUFA, total $n-3$ polyunsaturated fatty acids; total OBCFA, total odd-branched chain fatty acids; —, not detected.

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PE > PC > TAG. The ratio of $(n-6)/(n-3)$ in all studied freshwater species ranged from 0.51 to 1.42; the ratio of $((n-6)+(n-3))$ PUFA/SFA was found to be lower than 1 for TAG (0.48–0.88)) and higher than 1 for PL (1.26–2.03) in our samples.

TAG. Among TAG of each selected fish, total SFA (31.59–40.07%), and total MUFA (30.17–44.62%) predominated over total PUFA (18.38–31.86%) (Table 2). The major SFA was 16:0 from 16.29% to 21.10% followed by 18:0. Total *cis* MUFA varied from 29.34% in *S. kneri Garman* to 43.90% in *S. curriculus,* among which 18:1n-9 was the most abundant. The levels of 18:2n-6 and 18:3n-3 in *B. sinensis* were higher than any other fish, with 9.18% and 5.77%, respectively. *E. ilishaeformis* predominated in the content of 20:5n-3 (3.26%) and 22:6n-3 (6.01%) and *S. kneri Garman* of 20:4n-6 (5.51%) and 22:5n-3 (1.92%). We also observed a considerable amount of total odd-branched chain fatty acids (OBCFA) ranging from 5.94% for *S. curriculus* to 14.74% for *B. sinensis.*

PL. On the contrary, the proportions of total PUFA had the maximum value (40.94–56.55%) and total MUFA (10.75–22.80%) showed the minimum in PL fractions of each sample (Table 2). Total SFA exhibited modest fluctuation between 27.12% and 37.46% in both PC and PE of all fish, with PC containing relatively higher and lower amounts of 16:0 and 18:0 than the PE fraction, respectively (Table 2). Total *cis* MUFA in PL were lower than TAG with 18:1n-9 constituting the major isomer. Total PUFA was the richest fraction among PL from 40.94% to 47.81% for PC and from 48.21% to 56.55% for PE. 20:4n-6 and 22:6n-3 in PL (>10%) exhibited exclusively dominance over other PUFA of all species, with the exception of *S. kneri Garman* for 20:4n-6 (6.04%) in PE. *S. curriculus* predominated in the content of 20:5n-3 (PC, 7.54%; PE, 3.15%) and *B. sinensis* of 22:5n-3 (PC, 2.99%; PE, 3.94%). The total OBCFA in PL were consistently lower than TAG, with contents varying from 3.16% for *S. curriculus* in PC to 7.88% for *S. kneri Garman* in PE.

FA Distributions in TAG and PL of Five Wild Freshwater Fish. Regarding positional FA distributions in TAG (Figure 1), 16:0, 22:5n-3, and 22:6n-3 were preferentially esterified in the *sn*-2-postion of all species while 18:0, 18:1n-9, 18:2n-6, 18:3n-3, 20:4n-6, and 20:5n-3 were mainly esterified in positions 1 and 3, with the exception of *E. ilishaeformis* and *B. sinensis* for 18:2n-6 and *P. fulvidraco* for 20:4n-6 (Figure 1b–d). As indicated in Figure 2, stereospecific analysis of the TAG fraction showed preferential association of total SFA to the *sn*-2-position whereas total MUFA and PUFA to positions 1 and 3 in all samples .

With respect to positional FA distributions in PC (Figure 3) and PE (Figure 4), 16:0, 18:0, 18:1n-9, and 18:3n-3 were preferentially esterified in the *sn*-1-position, followed in sequence by *sn*-2-position. 20:4n-6, 20:5n-3, 22:5n-3, and 22:6n-3 preferentially distributed in the *sn*-2-position of PL in all freshwater fish. Figure 2 shows that the levels of total PUFA were much higher in the *sn*-2-position (59.88–87.26%) than the *sn*-1-position (15.78–33.96%), whereas total SFA and total MUFA mainly occupied the *sn*-1-position (46.71–66.57% and 13.94–25.43%, respectively).

■ DISCUSSION

PUFA. Table 2 shows that the total PUFA characterized the lowest and highest contents in TAG and PL among the fish selected, respectively, which were in good agreement with Simonetti et al., who reported the most notable PUFA were found in PL of cultured freshwater fish.[22] It is interesting to

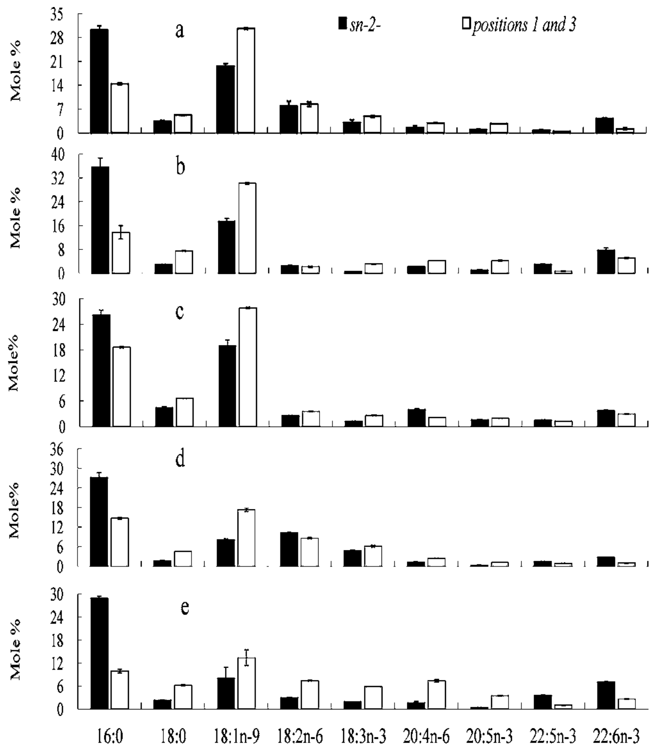

Figure 1. Positional distribution of major fatty acids in TAG of five wild freshwater fish from Poyang Lake (mol %, $n = 3$). Mean values with standard deviations plotted as bars: (a) *S. curriculus*; (b) *E. ilishaeformis*; (c) *P. fulvidraco*; (d) *B. sinensis*; (e) *S. kneri Garman*; TAG, triacylglycerol.

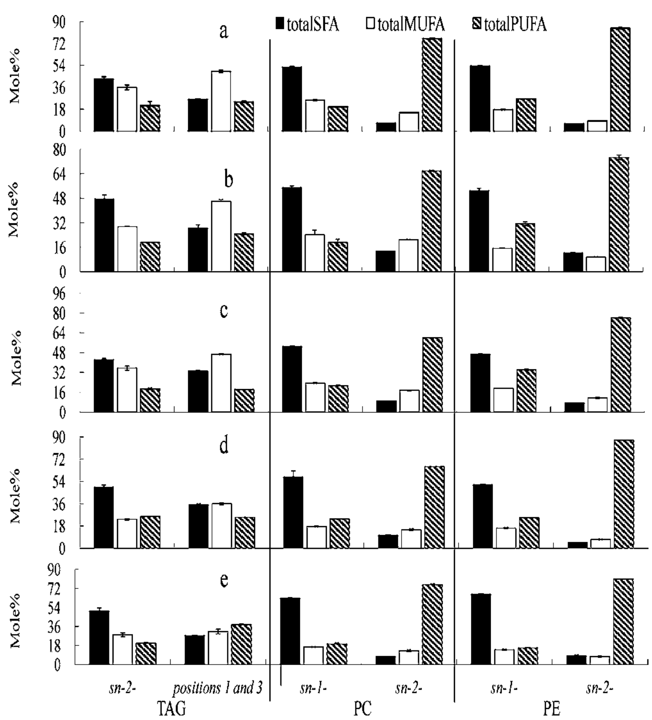

Figure 2. Positional distribution of total SFA, total MUFA, and total PUFA in TAG, PC, and PE fractions of five wild freshwater fish from Poyang Lake (mol %, $n = 3$). Mean values with standard deviations plotted as bars: (a) *S. curriculus*; (b) *E. ilishaeformis*; (c) *P. fulvidraco*; (d) *B. sinensis*; (e) *S. kneri Garman*; total SFA, total saturated fatty acids; total MUFA, total monounsaturated fatty acids; total PUFA, total polyunsaturated fatty acids; TAG, triacylglycerol; PC, phosphatidylcholine; PE, phosphatidylethanolamine.

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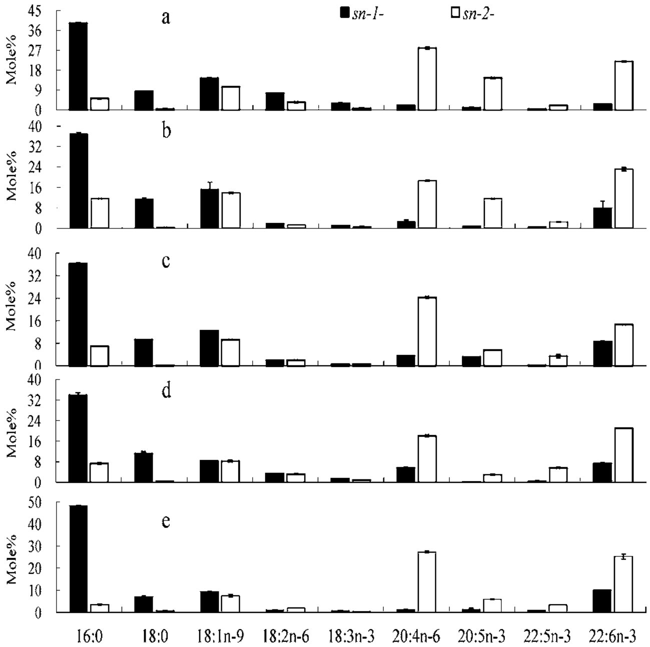

Figure 3. Positional distribution of major fatty acids in PC of five wild freshwater fish from Poyang Lake (mol %, $n = 3$). Mean values with standard deviations plotted as bars: (a) *S. curriculus*; (b) *E. ilishaeformis*; (c) *P. fulvidraco*; (d) *B. sinensis*; (e) *S. kneri Garman*; PC, phosphatidylcholine.

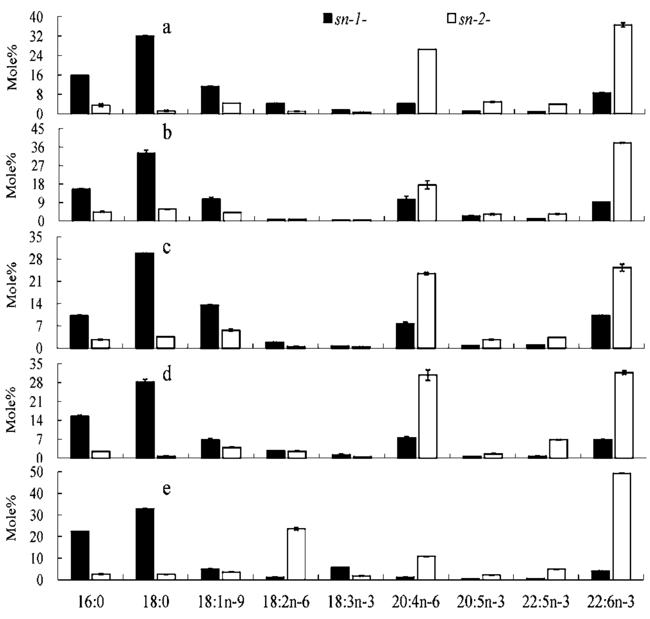

Figure 4. Positional distribution of major fatty acids in PE of five wild freshwater fish from Poyang Lake (mol %, $n = 3$). Mean values with standard deviations plotted as bars: (a) *S. curriculus*; (b) *E. ilishaeformis*; (c) *P. fulvidraco*; (d) *B. sinensis*; (e) *S. kneri Garman*; PE, phosphatidylethanolamine.

note that the fish TAG and PL of Poyang Lake were rich in PUFA (18.38–31.86% and 40.94–56.55%, respectively) (Table 2), which were similar to PUFA from marine fish (23.75–22.44% for TAG; 49–52% for PL)[23,24] and higher than wild freshwater trout from Turkey (20.48–34.38% for NL; 16.65–35.14% for PL).[25] The relative abundance of 20:4n-6 (2.48–19.13%, Table 2) in species selected was much higher than wild freshwater species (0.14–0.42%) from the Indus River[6] and marine species (0.19–0.68%) from Malaysia.[26] As a precursor for prostaglandin and thromboxane, 20:4n-6 could facilitate wound healing by influencing the process of the blood clot and attachment to the endothelial cells.[27] Table 2 shows that the levels of 20:5n-3 and 22:6n-3 in TAG (3.26% and 6.01%, respectively), PC (6.12% and 15.44%, respectively), and PE (3.03% and 23.85%, respectively) from *E. ilishaeformis* were close to data (4.25–11.6% for 20:5n-3; 6.15–16.1% for 22:6n-3) obtained from the Mauritanian coast.[28] As reported in the Introduction, the variability of the fish fatty acid profile is due to location, environment, season, gender, diet, and species. Benedito-Palos et al.[29] and Suzuki et al.[30] proved that the high PUFA levels of fish depends on their diet rich in essential FA. A considerable amount of 18:2n-6 (17.26–27.88%), 18:3n-3 (5.51%), 20:5n-3 (2.03–2.95%), and 22:6n-3 (2.48–3.64%) were found in planktons of Poyang Lake (Lei et al., unpublished work). As a main water source of the Yangtze River and the largest freshwater lake in China, the unique location of Poyang Lake has a significant effect on its biodiversity. Hence, the high ratio of PUFA of wild freshwater fish in the study may be attributed to the distinctive environment of Poyang Lake.

The physiological effects of $n - 3$ PUFA on the human body have been well documented;[2,3] however, consumption (<100 mg/d) of $n - 3$ PUFA for Chinese continental residents is much lower than the international recommended daily allowance (250 mg/d). Therefore, the dietary intake of freshwater fish ($n - 3$ PUFA, 8.77–32.87%) from Poyang Lake could validly increase the $n - 3$ PUFA requirement for inland residents. Besides, the balanced intake of $n - 6$ and $n - 3$ PUFA functions is more important for human health. An inappropriate ratio of $(n - 6)/(n - 3)$ may cause inflammation, dysfunction of the immunological system, and cancer as well as a greater chance for cardiovascular disease.[3,31] Hence, FAO/WHO has recommended that the ratio of $(n - 6)/(n - 3)$ PUFA should be less than 4.[32] The ratio of $(n - 6)/(n - 3)$ PUFA in all studied freshwater species (0.51–1.42) would be beneficial for improving human nutrition. The ratio of $((n - 6) + (n - 3))$ PUFA/SFA (0.48–2.03) in our samples is in accordance with FAO/WHO, who suggests that the ratio should be above 0.4–0.5 (Table 2).[32]

Conjugated Linoleic Acids (CLA). Ruminant products are the major sources of CLA in the diet. Interestingly, a good level of CLA was found in fish lipids, with 9c11t-18:2 constituting between 6.38% for *E. ilishaeformis* and 50.77% for *S. curriculus* of total CLA in fish lipids. CLA is desirable for atherosclerosis-reducing, anticarcinogenic, and body fat-lowering properties.[33–35] However, whether fish could serve as one of the natural CLA supplements for the human diet should be given careful consideration since there is a large amount of tt-CLA (accounting for 66.67–91.49% of total CLA) in studied fish lipids (Table 2).

OBCFA. It is interesting to note the considerable amount of three patterns of OBCFA in fish lipids, which usually originate from plankton or bacteria.[36] The total proportions of iso- and anti-FA, so-called branched chain fatty acids (BCFA), in TAG (1.75–5.89%) were consistently higher than PL (1.6–2.96%) in each species (Figure 5), which was in contrast to results obtained by Hauff and Vetter,[37] with 0.01–0.3% for NL and 0.01–0.77% for PL; however, the dominance of iso15:0 and iso17:0 in BCFA from all fish (0.07–2.23%) was in accordance with Hauff and Vetter.[37] These BCFA function as antitumor agents against human cancer cells.[38] Saturated odd carbon fatty acids (SOCFA), such as 15:0 and 17:0, were the most abundant (2.27–8.35%) among the three patterns of OBCFA, with the

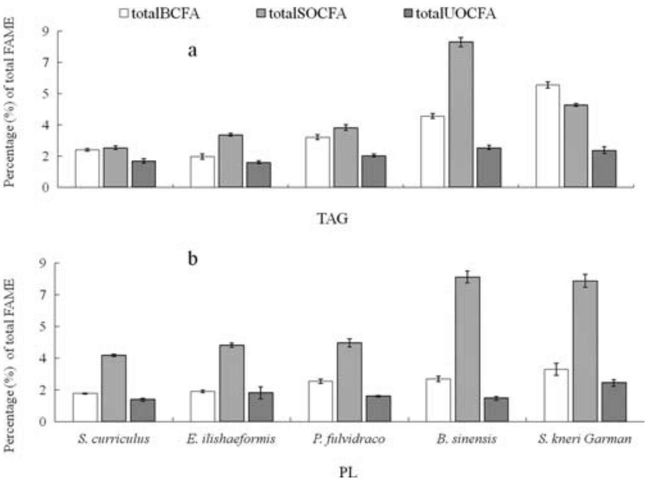

Figure 5. Content (%) of total BCFA, total SOCFA, and total UOCFA in TAG (a) and PL (b) of five wild freshwater fish from Poyang Lake (n = 3). Mean values with standard deviations plotted as bars; total BCFA, total branched chain fatty acids; total SOCFA, total saturated odd carbon fatty acids; total UOCFA, total unsaturated odd carbon fatty acids; TAG, triacylglycerol; PL, phospholipids.

exception of *S. kneri Garman* for TAG (Figure 5). SOCFA play a role in counteracting the decreases in liver glycogen and serum glucose during starvation.[39] Unsaturated odd carbon fatty acids (UOCFA), mainly composed of 17:1n-8, may act as modulators of neutrophil functions including degranulation and superoxide generation.[40]

Stereospecific Analysis of TAG and PL. It is well known that the bioavailability of FA is significantly affected by dietary lipid structure; as a general rule, absorption of FA in the *sn*-2-position in TAG is preferred.[41] Christensen et al. found that the digestion, absorption, and metabolism of 20:5n-3 and 22:6n-3 esterificated in the *sn*-2- position were much more efficient.[42] Thus, the preferential distribution of 22:5n-3 and 22:6n-3 in the *sn*-2-position of glycerol could enhance its fat absorption and physiological response in vivo, whereas the bioavailability of 18:2n-6, 18:3n-3, 20:4n-6, and 20:5n-3 that were exclusively present in positions 1 and 3 may be impaired (Figure 1).[43] However, to shed comprehensive light on the biological response of fish lipids from Poyang Lake, FA in positions 1 and 3 of TAG should be further studied.

Figure 2 shows our results are well confirmed by previous reports, which indicate that there is a preferential location of the *sn*-2-position with PUFA, while both SFA and MUFA occupy the *sn*-1-position of PL.[22] The general trend is attributed to the theory that the PUFA (more prone to oxidation) on the *sn*-2-postion of PL could be protected against oxidative damage.[44] Yoshida et al. found that 18:2 distributed in the *sn*-2-position of PC in sesame seeds was significantly protected from oxidation during the roasting process, whose content remained at about 42% of total lipids, whereas the content of 18:2 in the *sn*-1-position of PC declined from about 30% to 12% of total lipids.[45] On the other hand, absorption of dietary PL may be superior to TAG. Carnielli et al. studied four groups of preterm infants fed different diets (Group 1, preterm breast milk; Group 2, a formula without LCPUFA; Group 3, a formula with LCPUFA from egg yolk PL; Group 4, a formula with LCPUFA from unicellular microorganisms TAG) and found that absorption of 22:6n-3 was better in Group 3 (88.3%) than in any other group (78.4% for Group 1, 0 for Group 2, and 80.6% for Group 4).[46]

In summary, in view of the high content of PUFA and the appropriate ratio of $(n - 6)/(n - 3)$ PUFA, consumption of wild freshwater fish in Poyang Lake could be a good alternative to the marine fish for improving human health and nutrition. The study also reveals that the distribution of FA among the *sn* positions was not random: the preferential distributions of PUFA in position 2 of TAG and PL could facilitate themselves to be better utilized by humans. Nevertheless, the physiological effects and larger presence of OBCFA found in fish lipids need further investigation.

■ AUTHOR INFORMATION

Corresponding Author

*Phone/fax: +86 791 88304402. E-mail: zeyuandeng@hotmail.com.

Author Contributions

†These authors are considered as cofirst authors.

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Notes

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