Effect of season and trophic level on fatty acid composition and content of four commercial fish species from Krasnoyarsk Reservoir (Siberia, Russia)

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Abstract

Two groups of factors, phylogenetic and ecological, are presently regarded as controlling fatty acid composition of fish, including essential eicosapentaenoic (EPA) and docosahexaenoic (DHA) acids. Environmental effects, e.g., trophic position, temperature and/or seasonality, were previously studied using sums of fatty acids or only their level data. We tested the hypothesis that differences in trophic levels of piscivorous (pike and perch) and omnivorous (roach and bream) fish from a mesotrophic reservoir allow discriminating levels and contents of individual fatty acids, especially EPA and DHA. The more established measurements, i.e., stomach contents and carbon and nitrogen stable isotopes in fish muscles, were also carried out to provide linkages between the different ecological tracers, fatty acids versus stable isotopes, and matching the methods for long-term food sources (fatty acids and stable isotopes) and recent foraging (stomach content analysis). We also studied a putative influence of seasonality. Similar to other studies, there were seasonal changes in fatty acid composition and contents of two fish, perch and roach, due to direct and indirect effects of water temperature. Meanwhile, the piscivorous and omnivorous species captured in the same month, were explicitly differentiated on a base of stable isotopes and fatty acids. Significantly higher percentages and contents of DHA in piscivorous fish, perch and pike, relatively to those in roach and bream, likely indicated a higher trophic transfer efficiency for this essential fatty acid. All the fishes have commercial importance for regional fishery and are harvested from the studied reservoir for human nutrition. Regarding content of EPA+DHA ($mg \cdot g^{-1}$ fish) as the indicator of nutritive value for humans, pike had the highest nutritive value, roach and perch had intermediate overlapped values, and bream was of the least benefit.

Key words: piscivorous and omnivorous fish, trophic level, season, fatty acids, stable isotopes

1. Introduction

Consumption of fish is an important part of human diet, accounting for about 17 percent of the global population's intake of animal protein (FAO, 2016). In addition to protein, wild fish are unique and rich sources of such essential compounds as polyunsaturated fatty acids (PUFA), eicosapentaenoic acid (20:5n-3, EPA) and docosahexaenoic acid (22:6n-3, DHA), in human western diets (Robert 2006; Gladyshev et al., 2013, 2015*b*). EPA and DHA are biochemical precursors of important signaling molecules (prostaglandins, thromboxanes, leukotrienes, neuroprotectins) and on the base of over 30 years of human clinical trials and epidemiological surveys have been specifically recommended for prevention of cardiovascular diseases, psychiatric disorders and some other illnesses (Hibbeln et al., 2006; Plourde and Cunnane 2007; Bazan 2009; De Caterina 2011; Casula et al., 2013). Mechanisms underlying the cardioprotective effects of EPA and DHA as the signaling molecule (endogenous mediators) precursors, include arrhythmia prevention, vascular relaxation improvement, antiinflammatory responses, platelet aggregation inhibition and enhancement of plaque stability (e.g., Adkins and Kelley 2010). To reduce the risk of morbidity and mortality from cardiovascular disease, a number of international and national health organizations recommend personal intake of 0.5 – 1.0 g of EPA+DHA per day (Kris-Etherton et al., 2009; Adkins and Kelley 2010).

The main indicator of nutritive value of fish for humans, content of EPA+DHA (mg · g^{-1} of wet weight) in edible part, muscle tissues (filets), can vary among species and habitats by more than two orders of magnitude (Gladyshev et al., 2013). Exact causes of such great variations are unknown yet. Phylogenetic (species identity) factor may be of importance for fatty acid composition and content, i.e., some species contain extremely small amounts of EPA and DHA in their flesh (e.g., Kwetegyeka et al., 2008; Vasconi et al., 2015). In addition to phylogeny, fatty acid composition and PUFA supplies in fish may vary within a given species

65 due to various physiological and ecological factors (e.g., Ahlgren et al., 2009; Lau et al., 2012;
66 Vasconi et al., 2015).

67 Main ecological factors are believed to be food and water temperature, which are
68 determined by type of habitat and season (e.g., Ahlgren et al.,1996, 2009; Sushchik et al., 2006;
69 Czesny et al., 2011; Guler et al., 2011; Vasconi et al., 2015). In addition, seasonal changes of
70 reproductive phases, e.g. ripening, spawning and regeneration, which lead to the mobilisation
71 and re-allocation of endogenous reserves, also affect fatty acid composition both in reserve
72 somatic tissues, muscle and liver, and in gonads of fish (Mairesse et al., 2006; Perez et al., 2007;
73 Sushchik et al., 2007; Rojbek et al., 2014). A relative importance of the above ecological factor
74 is still unknown; moreover, results of experimental and field studies often are controversial
75 (Gribble et al., 2016). Recently, trophic position of fish, e.g., herbivorous, omnivorous
76 (invertivorous) or piscivorous, was shown to determine their FA composition (e.g., Ahlgren et
77 al., 2009; Czesny et al., 2011; Vasconi et al., 2015). For instance, in two freshwater studies
78 species that occupied higher trophic position, i.e. whose diet were part or all fish, contained
79 higher proportion of PUFA of n-3 and n-6 families (Williams et al., 2014; Vasconi et al., 2015).
80 The cited authors indicated that trophic position (food habits) was illuminating in
81 characterization of fatty acid composition compared to phylogenetic factor (taxonomic family).

82 As found recently, trophic transfer efficiency (TTE), measured as the ratio between
83 production of a trophic level and that of the previous level, was two-fold higher for long-chain
84 PUFA than for total organic carbon and short-chain PUFA (Gladyshev et al., 2011). The higher
85 TTE results in higher proportions (% of total fatty acids) and/or contents (mg/g tissue) in upper
86 levels of trophic chains: phytoplankton (seston) – zooplankton (e.g., Kainz et al., 2004),
87 phytobenthos – zoobenthos (Gladyshev et al., 2009*b*), fish – bird (Gladyshev et al., 2010*a*) and
88 plankton – fish (Strandberg et al., 2015). For fish of different trophic levels, there are pioneer
89 data of Ahlgren et al. (1996) on FA content of omnivorous and carnivorous species. However, in
90 the cited work sums of FAs of certain structural groups (saturated, mono- and polyunsaturated,

EPA+DHA) were compared. Meanwhile, as demonstrated by Strandberg et al. (2015), trophic transfer patterns of n-3 PUFA, including EPA and DHA, from food to fish related to their molecular structure. Thus, comparison of individual fatty acids, rather their sums, in biomass of fish of different trophic levels appears to be important.

In our work, we aimed to compare fatty acid composition and content using individual FA of two piscivorous and two planktivorous-benthivorous fish species from a large mesotrophic water body, Krasnoyarsk Reservoir, which is located in Central Siberia, Russia. The reservoir is one of the largest regional freshwater bodies and provides total amount of caught fish of nearly 1,500 metric tons per year (Analytic Reports.., 2016). The main commercial fish of interest are Eurasian perch (*Perca fluviatilis*), roach (*Rutilus rutilus*), bream (*Abramis brama*), and pike (*Esox lucius*), which average yearly harvests are of 940, 210, 170, and 15 metric tons, respectively. Most part of the caught fish is sold fresh, and some salted or dried. To take into account putative influence of seasonality, which may confound the comparison of trophic levels, we also studied seasonal dynamics of FA composition and content in two fish species, perch and roach, available during whole period of the study.

2. Methods

2.1. Study site

Krasnoyarsk Reservoir is a large water body that was created in the upper part of the Yenisei River during electric power station building (Fig. 1). It has previously been described in detail (Gladyshev et al., 1993, Ageev et al., 2008). The reservoir is deep (up to 110 m) and thermally stratified, and surface water temperature (0-10 m) varied from near zero (0.8 ^{o}C) under ice cover in March to 13 ^{o}C – 22 ^{o}C in June – August (Dubovskaya et al., 2004; Ageev et al., 2008). It is partly eutrophicated, and blooms of nuisance cyanobacteria species regularly occur in

bays and stretches. Zooplankton comprises mostly copepods, cladocerans and rotifers. The samples were taken in Ubei Bay, which is situated in the middle part of the reservoir (55° 06' 59" N, 91° 37' 44" E).

2.2. Sampling

Four fish species were caught in Ubei Bay of Krasnoyarsk Reservoir (Fig.1) in spring and summer months of 2014 and 2015 (Table 1). During the summer, the fish were caught using gill nets. Nets were set at a distance of 5 -50 meters from the shore, at a depth of 3 - 15 m. In March, perch was caught from under the ice using a hook fishing gear. Weather and variable catch rates resulted in incomplete sampling among the fish species and across each month and year (Table 1)**.** All caught fish were sexually mature. The ratio of males and females was approximately 1:1.

Fish were immediately brought to the nearby laboratory at the Biological station, School of Fundamental Biology and Biotechnology (Siberian Federal University, Krasnoyarsk, Russia). In the laboratory, fish were measured and weighed. Additionally, digestive tracts were removed for analysis of diet composition. For biochemical analyses, samples of the muscle tissues, weighing approximately 2-3 g, were taken from the dorsal side of fish individuals, 1 - 2 cm below the dorsal fin. When cutting the muscle samples, we avoided red muscles, skin and bones. The samples were divided into two subsamples: for fatty acid and stable isotope analyses. Stable isotope subsamples were additionally used for moisture measurements. For fatty acid analyses, ca. 1 g of muscle tissues were placed into chloroform : methanol mixture (2:1, volume/volume) and kept until further analysis at −20 °C. To measure moisture and stable isotope analyses, subsamples of ca. 1-2 g of wet weight were weighed, dried at 75 ^{o}C until constant weight, and weighed dry. Then, they were kept in a desiccator for further stable isotope elemental analysis.

2.3. Diet composition analysis

144 To characterize the diet of fish species, digestive tracts were removed through
145 longitudinal cuts in the abdomen using a scissor, scalpel and tweezers. For Cyprinidae species
146 (roach and bream), only content of the first 1/3 of intestine was analyzed, due to a high degree of
147 digestion in the final part. For piscivorous fish (perch and pike), the stomach contents were used
148 to analyze the diet composition. Food items were identified to the lowest practical taxonomic
149 level (order or class) and sorted under optical and stereoscopic microscopes. To tentatively
150 quantify the diet composition, we counted items, summed, and visually estimated their
151 approximate volumetric portion of the total content in each digestive tract analyzed. Then, the
152 diet items were divided into three groups based on their approximate percentage of the total
153 stomach volume (range of 30 – 60 %, 10 – 30 %, 1 – 10% of the total, respectively) for a given
154 species in a given month.
155
156 *2.4. Biochemical analyses*
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158 Lipid extraction and subsequent preparation of fatty acid methyl esters (FAMEs) were the
159 same as in our previous works (e.g. Gladyshev et al., 2015*c*). Briefly, lipids were extracted by a
160 modified Folch method with chloroform : methanol (2:1, v/v) three times, simultaneously with
161 mechanical homogenization of the tissues with glass beads. FAMEs were prepared in a mixture
162 of methanol-sulphuric acid (20:1, v/v) at 85 °C for 2 h. A gas chromatograph equipped with a
163 mass spectrometer detector (model 6890/5975C; Agilent Technologies, Santa Clara, USA) and
164 with a 30 m long, 0.25 mm internal diameter capillary column HP-FFAP was used for FAME
165 analysis. Detailed description of chromatographic and mass spectrometric conditions was given
166 elsewhere (Gladyshev et al., 2014). The FAMEs were quantified according to the peak area of
167 the internal standard, nonadecanoic acid, which we added to samples as a chloroform solution
168 prior to the lipid extraction.
169
170 *2.5. Stable isotope analyses*

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172 Detailed description of the measurement of stable carbon and nitrogen isotopes is given
173 elsewhere (Gladyshev et al., 2015*a*). Dried subsamples of fish muscles were homogenized using
174 a mortar and pestle and ~1 mg from each sample were analyzed with a continuous flow isotope
175 ratio mass spectrometer (CF-IRMS), model Delta V Plus (Thermo Scientific Corporation, USA)
176 interfaced with an elemental analyzer (Flash EA 1112 Series, Thermo Electron Corporation,
177 USA). Stable isotope data were expressed in the delta notation relative to Vienna Pee Dee
178 Belemnite (PDB) and atmospheric N_2 for $\delta^{13}C$ and $\delta^{15}N$, correspondingly. All samples were
179 analyzed in duplicate. The accuracy and precision of the measurement were verified twice or
180 triple per a day by secondary reference material USGS40 (L-glutamic acid) from International
181 Atomic Energy Agency. Analytical reproducibility was ±0.2‰ for C and ±0.3‰ for N.
182 As $\delta^{13}C$ values of aquatic animals could be biased due to variability in lipid content, we
183 recalculated total fatty acids in total lipid contents in the fish species using the conversion factor,
184 gram FA/gram lipid, which is reported as 0.7 for lean fish muscle (Greenfield and Southgate,
185 2003). Lipid contents for perch, roach, pike and bream caught in June ranged from 0.47 % to
186 0.81 % of wet weight. Because all lipid content values were much lower than 5 % (Post et al.,
187 2007), we did not normalize $\delta^{13}C$ values of the studied species.
188
189 *2.6. Statistical analyses*
190
191 Standard errors (SE), Kolmogorov-Smirnov one-sample test for normality $D_{K\text{-}S}$, one-way
192 ANOVA with Fisher's LSD *post hoc* tests and multivariate discriminant analysis (Legendre and
193 Legendre, 1998) were calculated conventionally, using STATISTICA software, version 9.0
194 (StatSoft Inc., USA).Only normally distributed variables (fatty acid percentages or contents)
195 were included in the analyses. Due to non-normal distribution, we removed 20:2n-6, 20:3n-3,
196 20:4n-3 and 24:1n-9 from ANOVA of seasonality for both perch and roach.

To reveal putative differences in the FA composition of four fish species, multivariate discriminant analysis (MDA) was used. MDA was performed on the untransformed FA profile data; FA that had non-normal distribution were excluded from the analysis. The discriminant analysis is a method of linear modelling to classify observations into *a priori* known groups. MDA firstly tests for differences in the predictor variables among the pre-defined groups (i.e., it is identical to ANOVA for a single explanatory variable), and secondly finds the linear combinations (called discriminant functions) of the variables that best discriminate among the groups (Legendre & Legendre, 1998). Here predefined groups were fish species, i.e. perch, roach, pike and bream, caught in the same month, June, and their FA percentage were the variables.

3. Results

Proportions of food items found in stomachs of the studied fish species are presented in Table 2. Perch diet compositions switched from zooplankton items in spring to a mixed diet of invertebrates in early summer, and then to a diet including fish by late summer. In July and August, perch predominantly consumed fish, majority of that was roach. Roach diets included invertebrates in June, but primarily consisted of detritus, algae, and bacteria in July and August (Table 2). The diet of pike mostly included fish, primarily roach (Table 2), whereas bream consumed zooplankton, mostly copepods and cladocerans.

Results of stable isotope analyses are given in Fig. 2. Bream and roach had nearly equal mean $\delta^{15}N$ values, which indicated their similar trophic positions, and nearly equal mean $\delta^{13}C$ value, which indicated similar carbon sources. Perch had mean values of $\delta^{15}N$ higher than roach and bream by 2.7‰ and 2.6 ‰, respectively (Fig. 2); the differences were statistically significant (Student's *t*-test, $t = 4.36$, $P < 0.001$, degree of freedom, d.f = 20 and $t = 2.62$, $P < 0.05$, d.f. = 14, respectively). Similarly, pike had mean values of $\delta^{15}N$ higher than roach and bream by 3.2‰ and

223 3.0 ‰, respectively (Fig. 2).The difference of $\delta^{15}N$ between pike and roach was statistically
224 significant (t = 3.88, P< 0.01, d.f. = 14), but it was marginally insignificant for bream (t = 2.27,
225 P= 0.053, d.f. = 8). Mean values of $\delta^{15}N$ for pike and perch differed little and insignificantly.
226 Mean value of $\delta^{13}C$ for pike was significantly lower than that for perch (Fig. 2, t = 2.78, P< 0.05,
227 d.f. = 8).Mean values of $\delta^{13}C$ for bream and roach were not significantly different and evidently
228 overlapped with that of perch (Fig. 2). The difference of $\delta^{13}C$ mean values between pike and
229 roach, 2.0‰, was statistically significant (t = 2.72, P< 0.05, d.f. = 14), but it was insignificant for
230 bream (t = 1.61, P = 0.053, d.f. = 8).
231 Average moisture of the muscle tissue of the studied fish species varied from 70.2% to
232 72.7% (Table 3).
233 In March, percentages of 14:0, 15:0, 20:4n-6, 20:5n-3 and 22:5n-3 in perch biomass were
234 significantly higher than those in summer months (Table 4). In contrast, percentages of 16:1n-9,
235 18:0 and 22:6n-3 in March were significantly lower than those in summer months (Table 4).
236 Percentages of 16:1n-7, 17:0, 18:2n-6, 18:3n-3, 20:1n-9, and 22:5n-6 in perch biomass had no
237 any gradual seasonal trend, but varied significantly between months. Percentages of 16:0, 15-
238 17BFA, 18:1n-9, 18:1n-7 and 18:4n-3 in perch biomass had no significant differences between
239 the studied months (Table 4).
240 In roach biomass, percentages of 16:0, 20:4n-6, 22:5n-6 and 22:6n-3 decreased
241 significantly from June to August (Table 5). In contrast, percentages of 15:0, 15-17BFA, 17:0,
242 18:0, 18:1n-9, 18:1n-7 and 18:3n-3 increased significantly from June to August (Table 5).
243 Percentages of 18:2n-6, 18:4n-3, 20:1n-9, 20:3n-3 and 20:4n-3 in roach biomass had no any
244 gradual seasonal trend, but varied significantly between months. Percentages of 14:0, 16:1n-9,
245 16:1n-7, 20:5n-3, 22:5n-3, in roach biomass had no significant differences between the studied
246 months (Table 5).
247 Since we found significant seasonal trends and variation in FA composition of perch and
248 roach, we compared FA data for four fish species, caught in the studied reservoir in the same

month, June. Perch had the highest average percentage of 16:1n-9, 20:1n-9, and the lowest percentage of 18:3n-3 in biomass compared to those in the other species (Table 6). Perch had significantly lower percentage of 18:1n-9 than roach and bream (Table 6). Pike had the lowest mean percentages of 16:1n-7 and 20:4n-6 and the lowest ratio of n-6/n-3 (Table 6). Roach had the lowest percentage of 22:5n-6, but the highest value of 22:6n-3 percentage (Table 6). Bream had the highest average percentage of 15-17BFA in biomass (Table 6). The piscivorous species, perch and pike, had significantly lower percentages of 18:2n-6 and 20:5n-3, but significantly higher percentage of 22:6n-3 in their biomass, than the planktivorous and benthivorous species, roach and bream (Table 6). Percentages of 14:0, 16:0, 18:4n-3 did not differ significantly among the studied fish species (Table 6).

According to MDA, there were significant differences in the FA composition among the fish species. Both discriminant functions (Root 1 and 2) were high and statistically significant, and the cumulative proportion of variance explained by the two roots (discriminatory power) was 95.05%. Root 1 discriminated best the piscivorous species, perch and pike, from the planktivorous and benthivorous species, roach and bream (Fig. 3). The piscivorous species were separated due to higher DHA and lower EPA percentages than the lower trophic level fishes. In second discriminant function (Root 2), higher proportions of BFA separated bream and pike from roach and perch, which contained higher proportions of 20:1n-9 (Fig. 3).

Contents of 20:5n-3, sum 20:5n-3+22:6n-3 and sum of FA in perch biomass (mg g^{-1} of wet weight) were significantly higher in March, than in other months (Table 4). Contents of 22:6n-3 were relatively similar for perch in May and June, but declined significantly as summer progressed (Table 4). In contrast to perch, 20:5n-3, 22:6n-3 and sum of FA (mg g^{-1} of wet weight) in roach increased significantly from June to August (Table 5).

We compared contents of two essential FA, 20:5n-3 and 22:6n-3, their sum and total FA in four fish species, caught in the same month. June. Pike had the highest content of 22:6n-3 and sum 20:5n-3+22:6n-3 (Table 6). Roach had highest value of the content of 20:5n-3 in biomass

275 (Table 6). Sum of FA content did not differ significantly among the studied fish species (Table
276 6).
277
278 **4. Discussion**
279
280 Since sum of contents of EPA+DHA is used as the indicator of nutritive value of fish for
281 humans (Kris-Etherton et al., 2009; Adkins and Kelley 2010), pike from Krasnoyarsk Reservoir
282 had the highest nutritive value, while perch and roach had equal and intermediate value, and
283 bream had the lowest value, nearly half that of pike. Regarding another indicator of nutritive
284 value, ratio n-6/n-3, pike also had the lowest ratio, e.g., the highest nutritive value. However,
285 these ratios of all studied species, although significantly different, were far below threshold value
286 of any harmful effect for human nutrition. To avoid a possible effect of seasonality, we
287 compared nutritive values of the fish species of various trophic levels using individuals collected
288 for the same month, June. The same comparison for one month was done in the seminal work of
289 Ahlgren et al (1996).
290 To carry out more broad inter-species comparison, data for other seasons and water
291 bodies should be taken into consideration. Therefore, we took average data for all studied
292 months for perch and roach (for pike and bream only June samples were available), and
293 compared them with literature data on the same species, obtained by similar method, namely
294 using internal standard for FA quantification per a tissue mass unit (Table 7). In general, our data
295 fell within the known range of contents for most species, i.e., pike, roach and perch, or had very
296 similar values, i.e., bream (Table 7). Like in the other studies, bream had lowest nutritive value
297 regarding EPA+DHA content. The content of EPA and DHA for pike, perch and roach from
298 different populations overlapped, but pike tended to have the maximum nutritive value
299 (maximum sum of EPA+DHA), roach had intermediate value, and perch had a bit lower
300 EPA+DHA content than two above species (Table 7). In overall, relatively large ranges of EPA

and DHA content in some species, e.g., pike and roach, argue that more studies are still necessary to predict the causes of these variations and shifts in FA profiles.

Using the above data (Table 7), we can calculate the filet portions of the studied fish that could provide a daily dose of EPA and DHA recommended for healthy life. Approximately 220 g of Siberian pike, 333 g of perch, or 450 g roach and bream filets need to be consumed to meet the daily requirement of EPA+DHA of 0.5 g (Kris-Etherton et al., 2009; Adkins and Kelley 2010). We did not measure any contaminants, and therefore could not calculate benefit/risk ratio for consuming these fishes. Meanwhile, the studied reservoir is located in a pristine region, thus, risk for consuming these fishes would hardly exceed its benefits. For instance, a long-term study of PUFA and heavy metals in filets of Siberian grayling caught from the Yenisei River, in the section located just downstream the studied reservoir, showed that the fish intake was potentially very beneficial for human health, except on few occasions (Gladyshev et al., 2009*a*).

Using stable isotope and FA trophic markers and stomach content analyses, we intended to disclose how differences in trophic level among these four species might lead to differences in their supply of essential PUFA. Pike is evidently piscivorous species. Perch may be regarded as piscivorous-omnivorous species, since besides fish there were zooplankton and zoobenthos in their stomachs. Indeed, perch had a bit lower mean value of $\delta^{15}N$ than pike, although this difference was statistically insignificant. The differences of $\delta^{15}N$ mean values between perch and pike, on the one hand, and roach and bream, on the other, were 2.6-3.2‰.The conventional value of constant of fractionation between trophic levels, $\Delta\delta^{15}N$, for aquatic animals is known to be 3.4 ‰ (e.g., Vander Zanden and Rasmussen 2001; Barnard et al., 2006; Lau et al., 2009), and for fish muscle tissue it is 3.2‰ (Nilsen et al., 2008). On the other hand, using data generalized by Caut et al. (2009), the calculated fractionation factor was 2.0‰. Thus, according to nitrogen isotopic signatures, trophic positions of roach and bream differed from those of perch and pike by approximately one trophic level. Indeed, stomach content analyses indicated roach and bream as planktivorous-benthivorous species. In addition, we compared $\delta^{15}N$ values with ratios of

18:1n-9/18:1n-7, which increase was reported to trace higher trophic level animals (Kopprio et al., 2015; Kraft et al., 2015). However, abrupt increase in $\delta^{15}N$ in piscivorous perch and pike versus invertivorous species (roach and bream) was not accompanied by an apparent increase of these FA ratios (Fig. 4A). We suggested that although 18:1n-9/18:1n-7 ratio is an informative indicator for plankton communities, this ratio may be affected by more than just trophic position in freshwater fishes.

It is also important to emphasize, that according to $\delta^{13}C$ values, perch and roach obtained organic carbon from nearly the same basic source, while pike relied on some other base. Bream seems to have intermediate carbon sources relative to the two above bases. It is worth to note that pike and bream differed from perch and roach due to higher percentages of bacterial 15-17BFA, while two latter had higher 20:1n-9 levels (Fig.3, 4B), likely originated from copepods (Graeve et al., 2005). These results probably mean that pike and bream relied primarily on detritus (bottom and nearshore area) carbon sources, while roach and perch were incorporated mainly into food chain of offshore pelagic region. Although pike is known to be flexible in its feeding habits (e.g., Beaudoin et al., 2001), due to typical ambush hunting strategy, it prefers to feed in littoral or near bottom zones (e.g., Zambrano et al., 2006) that are enriched in detritus of both autochthonous and allochthonous origin. Although roach were common in stomach content analyses of pike, both stable isotope and fatty acid analysis suggest other items are important components of its diet. ~~e.g., detritus which was likely ingested accidentally~~. Concerning bream, its adult's diet is almost exclusively demersal, therefore, this species also benefits from bottom habitats (Michel and Oberdoff, 1995).

In any case, there was very good agreement between results of stable isotope and fatty acid biomarker analyses, e.g., carbon isotopic signatures and such FA-markers as BFA and 20:1n-9 (Fig.4B). These both analyses reflect the long-term carbon sources assimilated into the body tissues, in contrast to stomach content analysis providing information about recent foraging (Davis et al., 2012). In this study, stomach content analysis also partly contrasted with SI and FA

353 markers, for instance, bream in June mostly consumed planktonic Cladocera and Copepoda,
354 although the long-term markers indicated its benthic feeding.
355 The multidimensional discriminant analysis revealed explicit differences between the
356 piscivorous and planktivorous-benthivorous fish, separated by the stable isotope analysis. The
357 piscivorous fish, perch and pike, had significantly higher percentages of DHA, but significantly
358 lower percentages of EPA, than those of the planktivorous-benthivorous fish, roach and bream.
359 Regarding transfer efficiency between trophic levels, or another words, selective accumulation of
360 PUFA, similar result was obtained by Stranberg et al. (2015), i.e., DHA had higher percentage in
361 planktivorous fish, than in zooplankton, while EPA had the same or even lower percentage, than
362 zooplankton. Thus, according to present data, only DHA, rather than EPA, is selectively
363 accumulated (more efficiently transferred) in organisms of higher trophic levels. However, as
364 mentioned above, we should not exclude a synthesis of certain amount of DHA by fish, at least
365 by perch. DHA is known to have a critical role in the functioning of neural tissue (brain and eye)
366 in fish and in their growth performance (Sargent et al., 1999; Tocher 2003; Trushenski et al.,
367 2012; Mozanzadeh et al., 2015; Rombenso et al., 2015). Hence, higher percentages of DHA in
368 piscivorous fish-species, pike and perch, may be related to their way of life, namely hunting large
369 motile prey, which demands more developed neural system. In the studies of Williams et al.
370 (2014) and Vasconi et al. (2015), predatory fish, including perch and pike, also were found to
371 accumulate especially high amounts of DHA in their muscles.
372 Seasonal dynamics of nutritive indicators (essential PUFA contents and n-6/n-3 ratio)
373 were revealed for the two studied species, perch and roach, inhabited Krasnoyarsk Reservoir.
374 Regarding nutritive value for humans, namely the spring perch had the highest content of
375 EPA+DHA per mass unit of the edible tissue, i.e. muscles (filets). Ratio of n-6/n-3 had
376 significant, but comparatively small variations in perch. In contrast to perch, content of
377 EPA+DHA in roach filets increased significantly from June to August, indicating the highest
378 nutritive value of this fish species at the end of summer. Variations of ratio n6/n3 were

379 statistically significant, but small, e.g., far below threshold value of any harmful effect for human
380 nutrition. Thus, peach caught in spring are best for human consumption, while roach are
381 nutritionally the most valuable in the late summer.

382 The above seasonal changes were believed to be driven by several ecological factors.
383 Numerous laboratory and field studies showed that fatty acid composition and content in tissues
384 are influenced in part by the food and water temperature (Gribble et al., 2016). The observed
385 significant decrease of percentage of 14:0 in conjunction with the significant increase of
386 percentage of 18:0 from March-June to July-August in perch biomass may be caused by a
387 homeoviscous adaptation. As known, the hypothesis of 'homeoviscous adaptation' suggests that
388 a decrease of a part of FAs with comparatively low melting point in response to a decrease of
389 ambient temperature maintains cell membrane fluidity (e.g., Arts and Kohler 2009). The same
390 adaptive changes of 14:0 and 18:0 levels in algae and zooplankton in response to an increase of
391 water temperature were reported in some other works (Dodson et al., 2014; Gladyshev et al.,
392 2015*c*).These two fatty acids, 14:0 and 18:0 can be synthesized by fish *de novo* (Tocher 2003).

393 However, other factors, such as diet or maturity related shift, were shown to overwhelm
394 temperature effect on FA in some fish (e.g., Uysal et al., 2006; Copeman et al., 2013). We also
395 find the significant seasonal changes in EPA and DHA percentages in the studied perch that
396 unlikely related to temperature adaptation. EPA was significantly higher in March than in the
397 summer months. This FA was likely originated from diatom algae, which may be abundant in
398 under-ice phytoplankton in spring (e.g., Katz et al., 2015) and thereby contributed substantially
399 to perch's trophic chain. Indeed, in a neighbor reservoir, a seasonal maximum of EPA in seston
400 occurred in spring and coincided with a peak of diatoms (Sushchik et al., 2003, 2004; Gladyshev
401 et al., 2010*b*). In contrast to EPA, DHA percentage was the lowest in perch biomass in spring,
402 but increased significantly in summer. However, in Krasnoyarsk Reservoir, there was no evident
403 source of DHA, for instance no abundant Dinophyceae or Euglenophyceae (Gladyshev et al.,
404 1993; Sushchik et al., 2004; Taipale et al., 2013). Hence, we speculate that DHA might be

405 synthesized in summer by perch from EPA, stored in spring. Indeed, there are some evidences of
406 an effective conversion of EPA into DHA by a freshwater fish (e.g., Sushchik et al., 2006).
407 The seasonal changes in EPA and DHA in the studied Siberian perch were contrasted
408 with those reported previously for perch from another lentic system, Lake Geneva (Mairesse et
409 al., 2006). In this lake, perch had a significantly lower proportion of DHA in summer than in
410 spring, while EPA showed a reversed tendency. The cited authors supposed a selective
411 mobilization and/or a specific retention of the PUFA during gonadal maturation and spawning.
412 In contrast, we consider that influence of reproductive stages was minimal in the studied Siberian
413 perch, while diet influence prevailed.
414 In roach, percentages of bacterial fatty acids, 15:0, 15-17BFA, 17:0 and 18:1n-7
415 (Napolitano 1999) increased significantly from June to August, indicating an increase of
416 contribution of bacterial matter to roach's food chain. In addition, percentage of 18:3n-3
417 increased in roach biomass, likely originating from some species of cyanobacteria (Sushchik et
418 al., 2004), which are dominant phytoplankton species in ~~the~~ Krasnoyarsk Reservoir in
419 midsummer (Gladyshev et al., 1993). Indeed, abundant detritus and cyanobacteria were found in
420 stomach contents of roach in July and August, while these food items were absent in June.
421 Percentage of 20:4n-6 in roach biomass decreased significantly from June to August. The
422 same seasonal decrease of this FA was also characteristic of perch. 20:4n-6 is regarded to be a
423 biomarker of allochthonous (terrestrial) organic matter (Gladyshev et al., 2015*a*). Probably, in
424 spring and early summer, when the reservoir was impounded and flooded adjacent territories, a
425 flux of allochthonous organic matter into aquatic food chains was higher, compared to that in
426 mid and late summer. It is worth to note, that similar increase of 20:4n-6 in fish muscle tissue in
427 spring was reported for two other water bodies (Karaçalı et al., 2011; Görgün et al., 2012). It was
428 also reported that *Daphnia* fed terrestrial particulate organic matter was 10-fold enriched in ARA
429 as compared to *Daphnia* fed algae (Taipale et al., 2015). A significant part of both roach's and

perch's diets in spring and early summer comprised Cladocera. Hence, these invertebrates may transfer allochthonous organic matter to planktivorous/piscivorous fishes in this season.

5. Conclusions

Like in many other studies, there were seasonal dynamics of fatty acid composition and contents of the studied fish. The seasonal changes of FA composition in fish were likely caused by direct and indirect effects of water temperature, which resulted in homeoviscous adaptation of fish cell membranes and in changes of base of food chains (phyto- and bacterioplankton, allochthonous organic matter), respectively. There were significantly higher percentages and contents of DHA in fish of higher trophic level, perch and pike, compared to those in roach and bream, which probably meant a higher trophic transfer efficiency (selective accumulation) of this PUFA in food chains. In contrast, percentages and contents of EPA were significantly higher in fish of the lower trophic level, roach and bream. Regarding sum of content of EPA+DHA (mg · g^{-1} WW) in fish as the indicator of their nutritive value for humans, pike in the Krasnoyarsk Reservoir had the highest nutritive value, roach and perch had intermediate overlapped values, and bream had comparatively lower nutritive value. This ranking of nutritive values of the studied fish species was generally supported by literature data from other water bodies.

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671

672 **Figure legends**

673

674 **Fig.1** Map of the studied area. Asterisk indicates the sampling site in Ubei Bay of Krasnoyarsk
675 Reservoir (Siberia, Russia).

676

677 **Fig. 2** Average values of the isotope ratios in muscle tissue of four fish species from the
678 Krasnoyarsk Reservoir (Siberia, Russia), June 2014-2015. Bars represent standard errors.
679 Number of samples for each species is given in Table 1.

680

681 **Fig. 3** Scatterplot of canonical scores for the two discriminant functions, Root 1 (canonical R =
682 0.972, degree of freedom, d.f. = 104, $P < 0.001$) and Root 2 (canonical R = 0.947, d.f. = 84, $P <$
683 0.001), after multivariate discriminant analysis of the fatty acid percentages (% of total FAs) in
684 muscle tissue of four fish species (June 2014-2015, Krasnoyarsk Reservoir, Siberia, Russia), **a**;
685 factor structure coefficients showing the contribution of variables to the discriminant functions,
686 Root 1 and Root 2, **b**.

687

688 **Fig. 4** Average stable isotope signatures versus average values of FA markers (percentages of the
689 total FA or ratios) in muscle tissue of four fish species (June 2014-2015, Krasnoyarsk Reservoir,
690 Siberia, Russia). Nitrogen isotope ratios (black circles) versus 18:1n-9/18:1n7 (bars) as putative
691 markers of trophic level, **a**; carbon isotope ratio (black circles) versus 20:1n-9 and sum of
692 branched 15-17 fatty acids (bars) as putative markers of pelagic and detritus food sources, **b**.

693

Table 1. The basic biological and sampling information of four fish species from Krasnoyask Reservoir (Siberia, Russia).

Common name	Species name, Order	Food habits	Reproduction	Sampling period	Average fish total length, cm (mean ± SE)	Average fish total weight, g (mean ± SE)	Number of samples*
Eurasian perch	*Perca fluviatilis* (Linnaeus, 1758), Perciformes	Piscivorous-omnivorous	Spring-summer	June 2014, March, June - August 2015	21.9 ± 1.5	138.2 ± 21.8	37 (11)
Roach	*Rutilus rutilus* (Pallas, 1840), Cypriniformes	Omnivorous (planktivorous)	Summer	June 2014, June – August 2015	26.0 ± 1.2	183.7 ± 26.5	24 (11)
Pike	*Esox lucius* (Linnaeus, 1758), Esociformes	Piscivorous	Spring	June 2014	64.1 ± 3.4	526.3 ± 30.6	5 (5)
Bream	*Abramis brama* (Linnaeus, 1758), Cypriniformes	Omnivorous (bentivorous)	Summer	June 2015	44.2 ± 2.6	641.2 ± 53.5	5 (5)

* number of samples for fatty acid and moisture analyses, in brackets - number of samples for stable isotope analyses

Table 2. Food items in the stomach contents of fish caught in Krasnoyarsk Reservoir (Siberia, Russia) in 2014-2015: N - number of analyzed stomachs; n- number of empty stomachs.

Species, month	N/n	Mollusca	Plecoptera	Ephemeroptera	Copepoda	Cladocera	Detritus	Fish	Green algae	Diatom algae	Cyanobacteria
Eurasian perch											
March	7/0				+	+++	+			+	
June	15/3	+	++	++	+	++			+		
July	10/2	+			++			+++	+		+
August	5/0							+++	++		+
Roach											
June	14/1	+	++	++		++			+	+	
July	4/1					+	++		++	+	++
August	5/0						+++		++		+
Pike											
June	5/0	+					++	+++			
Bream											
June	5/0	+			++	+++			+	+	

+++food item comprising high proportion in all full stomachs of the specimens, i.e., approximately ranging of 30-60 % of the total volume;
++ food item comprising moderate proportion in all full stomachs of the specimens, i.e., approximately ranging of 10-30 % of the total volume;
+ food item comprising low proportion in all full stomachs of the specimens, i.e., approximately ranging of 1-10 % of the total volume.

Table 3. Average (± SE - standard errors) moisture co ntent (% of wet weight) in muscle tissues of fish species captured in Krasnoyarsk Reservoir (Siberia, Russia) in 2014-2015.

Species	Moisture		
Eurasian perch	72.7	±	0.6
Roach	70.2	±	0.6
Pike	72.0	±	0.6
Bream	71.6	±	1.1

Table 4. Results of one-way ANOVA comparing mean values (± SE) of levels (% of total FA) and contents (mg g^{-1} of wet weight) of fatty acids, responsible for differences among Eurasian perch captured in Krasnoyarsk Reservoir (Siberia, Russia)in different months of 2014-2015: *F*– Fisher's test and its significance, *P*(significant values are given in bold), *n* – number of samples; means labelled with the same letter are not significantly different at $P< 0.05$ after Fisher's LSD *post hoc* test. When ANOVA is insignificant, letter labels are absent.

	March	June	July	August	*F*	*P*
n	7	15	10	5		
14:0, %	1.2 ± 0.1^{A}	1.0 ± 0.0^{AB}	0.9 ± 0.1^{B}	0.8 ± 0.2^{B}	3.5	**0.0258**
15:0	0.4 ± 0.0^{A}	0.3 ± 0.0^{B}	0.3 ± 0.0^{B}	0.3 ± 0.0^{B}	7.1	**0.0008**
16:0	20.3 ± 0.5	20.0 ± 0.4	20.8 ± 0.4	20.0 ± 0.2	1.0	0.4117
16:1n-9	0.5 ± 0.0^{A}	1.1 ± 0.0^{B}	1.2 ± 0.1^{BC}	1.3 ± 0.1^{C}	25.1	**0.0000**
16:1n-7	2.2 ± 0.1^{A}	3.5 ± 0.2^{B}	2.9 ± 0.4^{AB}	3.6 ± 0.5^{B}	3.4	**0.0292**
15-17BFA*	0.7 ± 0.0	0.9 ± 0.1	1.0 ± 0.1	0.9 ± 0.0	1.8	0.1735
17:0	0.5 ± 0.0^{A}	0.4 ± 0.0^{B}	0.6 ± 0.0^{C}	0.6 ± 0.0^{A}	45.5	**0.0000**
18:0	5.6 ± 0.1^{A}	5.1 ± 0.2^{A}	7.6 ± 0.1^{B}	7.4 ± 0.1^{B}	45.7	**0.0000**
18:1n-9	6.1 ± 0.2	6.7 ± 0.3	7.0 ± 0.3	7.3 ± 0.4	1.7	0.0000
18:1n-7	2.8 ± 0.1	2.9 ± 0.1	3.0 ± 0.2	3.2 ± 0.2	1.2	0.0000
18:2n-6	2.6 ± 0.1^{A}	1.8 ± 0.1^{B}	2.7 ± 0.3^{BC}	3.1 ± 0.4^{AC}	7.0	**0.0009**
18:3n-3	2.0 ± 0.0^{A}	1.1 ± 0.1^{B}	1.9 ± 0.1^{BC}	2.0 ± 0.1^{AC}	6.4	**0.0015**
18:4n-3	1.0 ± 0.0	0.5 ± 0.1	0.6 ± 0.0	0.6 ± 0.0	0.4	0.7651
20:1n-9	0.3 ± 0.0^{A}	0.6 ± 0.0^{B}	0.3 ± 0.0^{B}	0.1 ± 0.0^{B}	4.6	**0.0085**
20:4n-6	9.9 ± 0.3^{A}	8.5 ± 0.2^{B}	6.5 ± 0.2^{C}	6.1 ± 0.4^{C}	31.0	**0.0000**
20:5n-3	14.2 ± 0.3^{A}	7.6 ± 0.3^{B}	9.1 ± 0.3^{C}	9.5 ± 0.5^{C}	70.4	**0.0000**
22:5n-6	1.5 ± 0.1^{A}	2.3 ± 0.1^{B}	1.3 ± 0.1^{A}	1.2 ± 0.1^{A}	24.7	**0.0000**
22:5n-3	3.0 ± 0.1^{A}	2.1 ± 0.1^{B}	2.3 ± 0.1^{B}	2.1 ± 0.1^{B}	8.8	**0.0020**
22:6n-3	18.8 ± 0.1^{A}	28.1 ± 0.8^{B}	25.2 ± 1.1^{C}	24.3 ± 1.2^{C}	17.6	**0.0000**
20:5n-3, mg g^{-1}	0.8 ± 0.0^{A}	0.3 ± 0.0^{B}	0.3 ± 0.0^{B}	0.4 ± 0.0^{B}	68.2	**0.0000**
22:6n-3	1.1 ± 0.1^{AC}	1.2 ± 0.1^{AB}	0.9 ± 0.0^{C}	0.9 ± 0.0^{C}	4.5	**0.0097**
20:5n-3 +22:6n-3	2.0 ± 0.1^{A}	1.5 ± 0.1^{B}	1.2 ± 0.1^{C}	1.3 ± 0.0^{BC}	9.0	**0.0002**
ΣFA	6.0 ± 0.2^{A}	4.4 ± 0.3^{B}	3.5 ± 0.2^{C}	3.8 ± 0.2^{BC}	9.4	**0.0001**
n6/n3	0.4 ± 0.0^{A}	0.4 ± 0.0^{A}	0.3 ± 0.0^{B}	0.3 ± 0.0^{B}	8.2	**0.0003**

* 15-17BFA, sum of branched fatty acids with 15 and 17-carbon atom chains.

Table 5. Results of one-way ANOVA comparing mean values (± SE) of levels (% of total FA) and contents (mg g^{-1} of wet weight) of fatty acids, responsible for differences among roach captured in Krasnoyarsk Reservoir (Siberia, Russia)in different months of 2014-2015: *F*– Fisher's test and its significance, *P*(significant values are given in bold), *n* – number of samples; means labelled with the same letter are not significantly different at *P*< 0.05 after Fisher's LSD *post hoc* test. When ANOVA is insignificant, letter labels are absent.

	June	July	August	*F*	*P*
n	15	5	4		
14:0, %	1.4 ± 0.1	0.8 ± 0.0	1.0 ± 0.0	3.0	0.0707
15:0	0.3 ± 0.0^{A}	0.4 ± 0.0^{B}	0.4 ± 0.0^{B}	6.2	**0.0075**
16:0	20.1 ± 0.7^{A}	18.9 ± 0.5^{AB}	16.5 ± 0.3^{B}	4.3	**0.0266**
16:1n-9	0.4 ± 0.1	0.2 ± 0.1	0.5 ± 0.1	1.3	0.2979
16:1n-7	3.0 ± 0.2	4.3 ± 0.5	4.4 ± 1.4	3.0	0.0707
15-17BFA*	0.9 ± 0.1^{A}	1.8 ± 0.0^{B}	2.3 ± 0.2^{C}	33.1	**0.0000**
17:0	0.4 ± 0.0^{A}	0.7 ± 0.0^{B}	0.6 ± 0.0^{B}	55.9	**0.0000**
18:0	5.2 ± 0.1^{A}	6.2 ± 0.2^{B}	5.7 ± 0.1^{AB}	7.8	**0.0028**
18:1n-9	8.1 ± 0.5^{A}	10.6 ± 0.3^{B}	13.7 ± 0.6^{C}	18.7	**0.0000**
18:1n-7	3.0 ± 0.1^{A}	4.0 ± 0.3^{B}	4.6 ± 0.1^{B}	23.2	**0.0000**
18:2n-6	3.1 ± 0.1^{A}	2.6 ± 0.2^{A}	3.7 ± 0.1^{B}	5.1	**0.0153**
18:3n-3	1.6 ± 0.1^{A}	3.3 ± 0.1^{B}	3.1 ± 0.1^{B}	92.0	**0.0000**
18:4n-3	0.5 ± 0.0^{A}	0.8 ± 0.1^{B}	0.6 ± 0.0^{AB}	5.1	**0.0158**
20:1n-9	0.3 ± 0.0^{A}	0.1 ± 0.1^{B}	0.3 ± 0.1^{AB}	5.9	**0.0090**
20:2n-6	0.7 ± 0.1^{A}	0.4 ± 0.0^{B}	0.4 ± 0.1^{B}	8.0	**0.0026**
20:4n-6	9.0 ± 0.5^{A}	5.4 ± 0.1^{B}	5.0 ± 0.2^{B}	14.5	**0.0001**
20:3n-3	0.7 ± 0.0^{A}	1.2 ± 0.0^{B}	0.8 ± 0.0^{A}	20.8	**0.0000**
20:4n-3	1.8 ± 0.1^{A}	1.9 ± 0.0^{A}	1.3 ± 0.1^{B}	3.9	**0.0375**
20:5n-3	11.9 ± 0.6	12.3 ± 0.2	10.1 ± 0.2	2.0	0.1546
22:5n-6	1.0 ± 0.1^{A}	0.4 ± 0.0^{B}	0.7 ± 0.0^{AB}	4.6	**0.0214**
22:5n-3	3.6 ± 0.2	3.1 ± 0.1	3.3 ± 0.1	1.3	0.3048
22:6n-3	19.7 ± 0.7^{A}	16.0 ± 0.6^{B}	15.1 ± 0.6^{B}	9.1	**0.0015**
20:5n-3, mg g^{-1}	0.6 ± 0.0^{A}	1.4 ± 0.2^{B}	1.4 ± 0.2^{B}	24.6	**0.0000**
22:6n-3	0.9 ± 0.1^{A}	1.8 ± 0.2^{B}	2.1 ± 0.3^{B}	20.7	**0.0000**
20:5n-3 +22:6n-3	1.5 ± 0.1^{A}	3.2 ± 0.3^{B}	3.5 ± 0.5^{B}	23.4	**0.0000**
ΣFA	4.9 ± 0.4^{A}	11.3 ± 1.4^{B}	14.2 ± 2.4^{B}	26.0	**0.0000**
n6/n3	0.4 ± 0.0^{A}	0.2 ± 0.0^{B}	0.3 ± 0.0^{B}	12.5	**0.0003**

* 15-17BFA, sum of branched fatty acids with 15 and 17-carbon atom chains.

Table 6. Results of one-way ANOVA comparing mean values (± SE) of levels (% of total FA) and contents (mg g^{-1} of wet weight) of fatty acids, responsible for differences among fish species captured in Krasnoyarsk Reservoir (Siberia, Russia) in June of 2014-2015: *F*– Fisher's test and its significance, *P* (significant values are given in bold), *n* – number of samples; means labelled with the same letter are not significantly different at *P*< 0.05 after Fisher's LSD *post hoc* test. When ANOVA is insignificant, letter labels are absent.

	perch	pike	roach	bream	*F*	*P*
n	15	5	15	5		
14:0, %	1.0 ± 0.0	1.0 ± 0.2	1.4 ± 0.1	1.2 ± 0.3	1.4	0.2478
15:0	0.3 ± 0.0^{AB}	0.4 ± 0.0^{B}	0.3 ± 0.0^{A}	0.4 ± 0.0^{B}	3.5	**0.0255**
16:0	20.0 ± 0.4	19.9 ± 0.5	20.1 ± 0.7	18.0 ± 0.4	1.6	0.2090
16:1n-9	1.1 ± 0.0^{B}	0.5 ± 0.1^{A}	0.4 ± 0.1^{A}	0.5 ± 0.1^{A}	16.1	**0.0000**
16:1n-7	3.5 ± 0.2^{B}	1.8 ± 0.3^{A}	3.0 ± 0.2^{B}	2.8 ± 0.5^{B}	5.5	**0.0032**
15-17BFA*	0.9 ± 0.1^{A}	1.2 ± 0.1^{A}	0.9 ± 0.1^{A}	1.9 ± 0.4^{B}	7.6	**0.0004**
17:0	0.4 ± 0.0^{B}	0.4 ± 0.0^{AB}	0.4 ± 0.0^{B}	0.5 ± 0.1^{A}	3.3	**0.0319**
18:0	5.1 ± 0.2^{A}	5.9 ± 0.4^{BC}	5.2 ± 0.1^{AB}	6.2 ± 0.3^{C}	4.0	**0.0150**
18:1n-9	6.7 ± 0.3^{A}	7.7 ± 0.4^{AB}	8.1 ± 0.5^{B}	9.1 ± 0.9^{B}	3.5	**0.0247**
18:1n-7	2.9 ± 0.1^{A}	2.4 ± 0.1^{A}	3.0 ± 0.1^{AB}	3.4 ± 0.4^{B}	3.6	**0.0229**
18:2n-6	1.8 ± 0.1^{A}	2.2 ± 0.2^{A}	3.1 ± 0.1^{B}	2.9 ± 0.4^{B}	15.9	**0.0000**
18:3n-3	1.1 ± 0.1^{A}	1.7 ± 0.3^{B}	1.6 ± 0.1^{B}	1.7 ± 0.3^{B}	4.1	**0.0140**
18:4n-3	0.5 ± 0.1	0.9 ± 0.2	0.5 ± 0.0	0.5 ± 0.1	2.7	0.0621
20:1n-9	0.6 ± 0.0^{A}	0.2 ± 0.0^{B}	0.3 ± 0.0^{C}	0.2 ± 0.1^{B}	13.2	**0.0000**
20:4n-6	8.5 ± 0.2^{BC}	5.2 ± 0.4^{A}	9.0 ± 0.5^{C}	7.2 ± 0.5^{B}	9.2	**0.0001**
20:5n-3	7.6 ± 0.3^{A}	7.0 ± 0.3^{A}	11.9 ± 0.6^{B}	9.9 ± 0.7^{C}	21.7	**0.0000**
22:5n-6	2.3 ± 0.1^{B}	2.0 ± 0.2^{BC}	1.0 ± 0.1^{A}	1.5 ± 0.1^{C}	23.7	**0.0000**
22:5n-3	2.1 ± 0.1^{A}	2.1 ± 0.2^{A}	3.6 ± 0.2^{B}	2.6 ± 0.2^{A}	18.2	**0.0000**
22:6n-3	28.1 ± 0.8^{A}	32.9 ± 1.8^{B}	19.7 ± 0.7^{C}	22.9 ± 1.6^{C}	31.9	**0.0000**
20:5n-3, mg g^{-1}	0.3 ± 0.0^{A}	0.4 ± 0.0^{A}	0.6 ± 0.0^{B}	0.3 ± 0.0^{A}	9.0	**0.0001**
22:6n-3	1.2 ± 0.1^{A}	1.9 ± 0.1^{B}	0.9 ± 0.1^{C}	0.7 ± 0.1^{C}	13.7	**0.0000**
20:5n-3 + 22:6n-3	1.5 ± 0.1^{A}	2.3 ± 0.1^{B}	1.5 ± 0.1^{A}	1.1 ± 0.1^{C}	7.2	**0.0007**
ΣFA	4.4 ± 0.3	5.7 ± 0.3	4.9 ± 0.4	3.3 ± 0.6	2.8	0.0556
n6/n3	0.4 ± 0.0^{A}	0.2 ± 0.0^{B}	0.4 ± 0.0^{A}	0.3 ± 0.0^{A}	8.7	**0.0002**

* 15-17BFA, sum of branched fatty acids with 15 and 17-carbon atom chains.

Table 7. Content of eicosapentaenoic and docosahexaenoic acids (mg g^{-1}, wet weight) in four fish species.

Species	EPA	DHA	EPA+DHA	Source
Eurasian perch (*Perca fluviatilis*)	0.27	0.91	1.18	Ahlgren et al., 1994*
	0.35	1.34	1.69	Vasconi et al., 2015**
	0.43	1.07	1.49	present data
Roach (*Rutilus rutilus*)	0.56	0.98	1.54	Ahlgren et al., 1994*
	0.93	2.42	3.36	Vasconi et al., 2015**
	0.88	1.32	2.20	present data
Pike (*Esox lucius*)	0.31	1.19	1.50	Ahlgren et al., 1994*
	0.21	1.13	1.34	Neff et al., 2014
	0.32	1.12	1.44	Williams et al., 2014
	0.74	3.97	4.72	Vasconi et al., 2015**
	0.40	1.88	2.28	present data
Bream (*Abramis brama*)	0.37	0.60	0.97	Ahlgren et al., 1994*
	0.32	0.74	1.06	present data

*The data were recalculated from mg $g^{-1}$of dry weight (DW) to mg g^{-1} of wet weight (WW) using DW/WW (%) ratios given in Table 1 of the reference.
**Recalculated from Table 5 of the reference.