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Comparison of fatty acid contents in major lipid classes of seven salmonid species from Siberian Arctic lakes

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Abstract: Long-chain omega-3 polyunsaturated fatty acids (LC-PUFA) essential for human nutrition are mostly obtained from wild fish catch. To sustain LC-PUFA supply from natural populations, one needs to know how environmental and intrinsic factors affect fish fatty acid (FA) profiles and contents. We studied seven Salmoniformes species from two arctic lakes. We aimed to estimate differences in FA composition of total lipids and two major lipid classes, polar lipids (PL) and triacylglycerols (TAG), among the species and to evaluate LC-PUFA contents corresponded to PL and TAG in muscles. Fatty acid profiles of PL and TAG in all species were characterized by prevalence of omega-3 LC-PUFA and C16-C18 monoenoic FA, respectively. Fish with similar feeding spectra were identified similarly in multivariate analyses of total lipids, TAG and PL, due to differences in levels of mostly the same FA. Thus, suitability both TAG and total lipids for identification of feeding spectra of fish was confirmed. All species had similar content of LC-PUFA esterified as PL, 1.9 - 3.5 mg · g^{-1}, while the content as TAG form strongly varied, from 0.9 to 9.8 mg · g^{-1}. The LC-PUFA-rich fish species accumulated these valuable compounds predominately in TAG form.

Keywords: Arctic; Salmoniformes; long-chain polyunsaturated fatty acids; polar lipids; triacylglycerols; eicosapentaenoic acid; docosahexaenoic acid

1. Introduction

Long-chain omega-3 polyunsaturated fatty acids (LC-PUFA), such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), are known to be essential compounds for human nutrition since they can modulate functioning of cardiovascular and neural systems and general metabolism being the precursors for synthesis of diverse lipid mediators and directly affecting membrane properties [1-5]. Most international and national health agencies and foundations recommended personal consumption of 0.5 – 1.0 g of EPA+DHA per day for reducing the risk of cardiovascular diseases and other metabolic disorders [6-8]. Although a lot of potential sources of food LC-PUFA are now considered, natural fish populations are still the major source of these compounds for human nutrition [9,10]. Recent reviews showed the deficiency of LC-PUFA supply with fish catches from natural populations and emphasized the potential negative influence of some global threats, like climate change, pollution, eutrophication, etc. [10-13]. To challenge above threats and to sustain LC-PUFA supply from natural populations, one need to know how environmental and intrinsic factors affect fish fatty acid profiles and contents, including those of EPA and DHA. Causes of

variations of fatty acid composition and content in wild fish are still incompletely understood [10, 14].

The ability of fish to deposit fat (lipids) in muscles vary from species to species and may be a crucial intrinsic factor [15]. According to their functions, lipids in fish, like in the most other animals, could roughly be divided into energy-reserve and membrane-structural groups [16, 17]. In fish, the reserve lipids are primarily represented by triacylglycerols (TAG) and include mostly fatty acids that come from food sources. Fatty acid profiles of TAG are generally considered as valuable trophic markers due to their resemblance with fatty acid profiles of particular food sources [18]. In addition, TAG fraction in fish can also contain high levels of monoenoic C16-C18 fatty acids that are intensively synthesized in so called "fatty" fish species to provide energy reserves. TAG molecules either accumulate directly in muscle cells as droplets or in specific adipocytes which may be integrated in muscle tissues or form separate layers of adipose tissue.

The structural polar lipids (PL) that form fish cellular and intercellular membranes mostly comprise phospholipids [19, 20]. As known, fatty acid composition of PL affect physico-chemical properties of cellular membranes. Hence, PL are considered to have conservative fatty acid profiles which slightly reflected that of diet. The essential omega-3 LC-PUFA are preferentially accumulated in PL fraction of muscle tissues due to their strong membrane-modulating properties. Thus, fatty acid profiles of the major lipid classes, TAG and PL, in fish muscles are different in general [19].

TAG content per mass unit of fish muscles is highly variable due to influence of many factors [15]. In contrast, PL content per mass unit of muscles is fairly–constant [21]. Thereby, we hypothesize that PL specific content has a putative upper threshold, because amounts of PL molecules in the tissue are likely determined by a volume of membranes.

Contents of omega-3 LC-PUFA in muscle tissue of diverse fish species greatly vary, approximately ~400-fold [22]. The question arises what part of this variation in total EPA and DHA contents is provided by TAG or PL variability? There is a basic assumption in current literature that a major part of omega-3 LC-PUFA presents as acyl groups of membrane phospholipid molecules [11].

To evaluate contribution of the two major lipid fractions in total content of LC-PUFA in edible muscle tissue (filets) we studied seven commercial species of order Salmoniformes that inhabit oligotrophic non-polluted lakes in Arctic Siberia. The fish species vary in their feeding habits and habitats and have different fat content in filets. Using data on these fish we aimed to compare distribution of fatty acids, including omega-3 LC-PUFA in total lipids and two major lipid classes: TAG and PL. Specifically, we aimed i) to check if the fish species with various feeding spectra can be differentiated basing on FA profiles of total lipids, TAG or PL, ii) to evaluate LC-PUFA content corresponded to TAG and PL classes in muscles, iii) to range species according to their nutritive value for humans in respect of LC-PUFA content.

2. Materials and Methods

2.1. Sampling

Fish specimen of commercial sizes were collected during July 2017, from catches of local authorized fishers. Following sampling was done in accordance with the BioEthics Protocol on Animal Care approved by the Siberian Federal University. The catches were from two oligotrophic arctic lakes, Sobachye and Pyasino. Sobachye Lake was previously characterised elsewhere [23]. Briefly, it is

86 located at 69^{0}01' N 91^{0}05' E, has maximum depth of 162 m and area equaled to 99 km^2. Pyasino Lake
87 situates at 69^{0}40' N 87^{0}51' E, has average depth of 4 m and area equaled to 735 km^2 [24].
88 Whitefish *Coregonus lavaretus* (Linnaeus, 1758), non-identified form of whitefish *C. lavaretus*,
89 round whitefish *Prosopium cylindraceum* (Pennant, 1784) and charr *Salvelinus drjagini* Logashev, 1940
90 were caught in Sobachye Lake; broad whitefish *Coregonus nasus* (Pallas, 1776), muksun *Coregonus*
91 *muksun* (Pallas, 1814) and inconnu *Stenodus leucichthys nelma* (Guldenstadt, 1772) were caught in
92 Pyasino Lake. Numbers of samples, average individual sizes and weights, and main food sources for
93 the studied fish species are given in Table 1. Stomach contents of all specimen were studied under a
94 light microscope, and main food items were identified to a possible taxon level.
95 For biochemical analyses, we cut slices of fish white muscles of approximately 2-3 g, 2-3 cm
96 below the dorsal fin. The samples were subdivided into two parts: for FA and moisture analyses. For
97 FA analyses, ca. 1 g of muscle tissues were immediately placed into a volume of 3 mL of
98 chloroform/methanol (2:1, by vol.) and kept until further analysis at −20 °C. Another subsample of
99 ca. 1-2 g of wet weight was weighed, dried at 105 ^{o}C until constant weight, and weighed dry for
100 moisture calculation.
101
102 *2.2. Lipid and Fatty Acid Analyses*
103
104 In laboratory, lipids were extracted with chloroform/methanol (2:1, by vol.) in triplicate,
105 simultaneously with homogenizing tissues with glass beads in a mortar. Prior to the extraction, an
106 aliquot of 19:0-fatty acid methyl ester (FAME) chloroform solution, as the internal standard, was
107 added to samples for quantification of chromatographic peaks. The extracts were combined and
108 dried with anhydrous Na_2SO_4 and the solvents were roto-evaporated under vacuum at 35 ^{o}C. The
109 extracted lipids was redissolved in a 1 ml portion of chloroform and separated in two equal parts. To
110 analyze the fatty acid composition of total lipids, one part of the lipid extract was methylated in the
111 following way. The lipids were hydrolysed under reflux at 90° C for 10 min in 0.8 ml of methanolic
112 sodium hydroxide solution (8 g/L). Then the mixture was cooled for 5 min at room temperature.
113 Next, 1 ml of methanol/sulphuric acid (97:3, by vol.) wad added and the mixture was heated under
114 reflux at 90° C for 10 min to methylate free fatty acids. At the end, 5 mL of saturated solution of NaCl
115 and 3 mL of hexane were added. The FAMEs were extracted for 1 min, the mixture was transferred
116 to a separatory funnel, and the lower aquatic layer was discarded. The hexane layer was additionally
117 washed once with an aliquot of the NaCl solution and twice with 5 mL of distilled water. Then the
118 hexane solution of FAMEs was dried with anhydrous Na_2SO_4, and hexane was removed by
119 roto-evaporating at 30 °C.
120 We fractionated another part of the lipid extracts by thin layer chromatography (TLC) on
121 silica gel G with the system hexane-diethyl ether-acetic acid mixture (85:15:1, v/v). We prepared a
122 reference mixture containing triolein, oleic acid, cholesterol, and phosphatidylcholine (Sigma, USA),
123 which was applied on side lanes of the silica gel plates. To identify lipid composition of fish species,
124 we applied aliquots of samples to the plates, then developed them as described above. After
125 developing the plates were sprayed with mixture of ethanol/sulphuric acid (90:10, by vol.) and
126 gently heated until grey spots of lipid classes appeared. To measure fatty acid profiles and quantity
127 of dominant lipid classes, we separated main portion of lipid extracts to the silica gel plates, then
128 visualized only side lanes corresponded to the reference-compound mixture by reaction with

phosphomolybdic acid ethanolic solution. Lipid spots of the fish samples on the plates were blind detected according to the positions of the reference compounds. The lipid spots containing TAG and PL fractions were scrapped off from the silica gel plate and placed in flasks. Aliquots of 19:0-FAME hexane solution (internal standard) were added into the flasks containing silica gel powder with the lipid fractions. To prepare FAMEs, 1ml of hexane and 0.2 mL of fresh 3 M methanolic sodium methoxide solution was added, and mixture was shaken vigorously for 1 min. Subsequently, the mixture was kept quiet at room temperature for 5 min, and finally 3 mL of hexane and 5 mL of a saturated solution of NaCl were added. The next procedure of FAME extraction and washing was the same as for those prepared from total lipids.

Analyses of all FAMEs were done with a gas chromatograph equipped with a mass spectrometer detector (model 6890/5975C; Agilent Technologies, USA) and with a 30-m long, 0.25-mm internal diameter capillary HP-FFAP column. Detailed descriptions of the chromatographic and mass-spectrometric conditions are given earlier [23].

2.3. Statistical Analysis

Kolmogorov-Smirnov one-sample test for normality $D_{K\text{-}S}$, standard errors (SE), Student's *t*-tests, one-way ANOVA with *post hoc* Tukey HSD test, Kruskal-Wallis test (in the absence of normal distribution) and canonical correspondence analysis (CCA) were calculated conventionally, using STATISTICA software, version 9.0 (StatSoft Inc., Tulsa, USA).

3. Results

According to gut content analysis, *C. lavaretus* in Sobachye Lake was benthivorous (Table 1). Whitefish of a non-identified form in this lake consumed mostly pupa and adult insects, i.e., foraged near the water surface. Round whitefish fed on benthic invertebrates and algae (Table 1). Both broad whitefish and muksun in Pyasino Lake consumed benthic food items, including detritus. Charr in Sobachye Lake and inconnu in Pyasino Lake were piscivorous (Table 1).

Average values of moisture content, lipid content and sum of fatty acid content for total lipids in the studied fish are given in Table 2. Lower moisture values were characteristic of the species with higher values of lipid and sum FA content, charr and whitefish (Table 2). In contrast, round whitefish and whitefish (non-identified form) had the maximum moisture content and the minimal contents of lipids and sum FAs. Sum FA content of total lipids significantly varied ~ 7-fold among the studied species (Table 2). Based on the averages of lipid and sum FA contents, charr and whitefish are further considered as "fatty" fish, muksun, inconnu and broad whitefish – as "medium fat" fish, and round whitefish and non-identified form of whitefish – as "lean" fish (Table 2).

Levels of 25 prominent individual FA and their structural groups in total lipids are showed in Table 3. Charr had the highest levels of 20:2n-6, 20:3n-3, 20:4n-3, 22:4n-3 and C24 PUFA among the studied species (Table 3). Whitefish had the significantly highest levels of 16:1n-7 and C16 PUFA, and tended to be higher in levels of 18:1n-9 and 20:5n-3. Muksun tended to have higher levels of 14:0, 20:1 and 22:5n-6 (Table 3). Broad whitefish had higher levels of 16:1n-9, C15-17 BFA (branched-chain fatty acids), 18:0, 18:1n-7, 18:2n-6, 18:3n-3 (Table 3). Whitefish of the non-identified form had higher levels of 16:0 and 22:6n-3 compared to those of the other fish. Inconnu and round whitefish had intermediate levels of all FA in total lipids (Table 3).

Table 1. The basic biological and sampling information on fish species from Siberian arctic lakes, 2017: *n* – number of sampled individuals; L - total length, cm (mean ± SE); W –total weight, g (mean ± SE); Food – items found in stomachs.

Common and species name	Lake	*n*	L	W	Food
Whitefish *Coregonus lavaretus*	Sobachye	7	480 ± 23	1153 ± 167	Amphipods, mollusks, chironomid larvae
Whitefish *Coregonus lavaretus* non-identified form	Sobachye	7	402 ± 12	568 ± 80	Chironomid and other insect pupa and adults
Round whitefish *Prosopium cylindraceum*	Sobachye	7	409 ± 7	488 ± 27	Caddisfly and chironomid larvae, filamentous algae
Charr *Salvelinus drjagini*	Sobachye	9	608 ± 17	2371 ± 271	Fish (Salmonidae)
Broad whitefish *Coregonus nasus*	Pyasino	10	563 ± 17	1916 ± 183	Gastropods, detritus
Muksun *Coregonus muksun*	Pyasino	8	492 ± 14	1271 ± 160	Ostracods, mollusks, chironomid larvae, detritus
Inconnu *Stenodus leucichthys nelma*	Pyasino	5	675 ± 86	3239 ± 1581	Fish

Table 2. Average (± SE - standard errors) moisture content (% wet weight), lipid content (mg · g^{-1} wet weight) and sum fatty acid content for total lipids (mg · g^{-1} wet weight) in muscle tissues of fish species caught in Siberian arctic lakes, 2017.

Common and species name	Moisture	Lipids	Total fatty acids
Charr *Salvelinus drjagini*	69.8 ± 1.3	155.8 ± 7.4	78.8 ± 5.1^{D}
Whitefish *Coregonus lavaretus*	69.8 ± 1.4	82.0 ± 1.9	62.7 ± 7.2^{CD}
Muksun *Coregonus muksun*	74.4 ± 1.0	n.d.	45.4 ± 8.7^{BC}
Inconnu *Stenodus leucichthys nelma*	72.1 ± 2.6	68.4 ± 11.4	36.5 ± 10.0^{ABC}
Broad whitefish *Coregonus nasus*	73.8 ± 1.4	n.d.	31.9 ± 5.1^{AB}
Round whitefish *Prosopium cylindraceum*	76.1 ± 0.6	39.1 ± 3.3	13.8 ± 1.3^{A}
Whitefish *Coregonus lavaretus* nonidentified form	76.0 ± 0.9	41.1 ± 2.1	11.5 ± 1.9^{A}

n.d. – no data

We performed CCA of FA profiles of total lipids in the fish species to find out their overall differences (Fig. 1). Along Dimension 1, most overall differences in FA composition were observed between charr, on the one hand, and whitefish, on the other hand. The differences were primarily caused by higher percentages of 22:4n-3, 20:3n-3 and C24 PUFA in charr, and higher percentages of C16 PUFA and 16:1n-7 in whitefish. In Dimension 2, whitefish of a non-identified form and round

Table 3. Mean levels of fatty acids (% of the total) in total lipids of Salmoniformes species: charr – *S. drjagini* from Sobachye Lake; whitefish – *C. lavaretus* from Sobachye Lake; muksun – *C. muksun* from Pyasino Lake; inconnu – *S. leucichthys nelma* from Pyasino Lake; broad whitefish – *C. nasus* from Pyasino Lake; round whitefish – *P. cylindraceum* from Sobachye Lake; whitefish nd – nonidentified form of *Coregonus lavaretus* from Sobachye Lake. Cases (fatty acids) with normal distribution are given in bold. Means labeled with the same letter are not significantly different at $P< 0.05$ after ANOVA *post-hoc* Tukey HSD test (cases with normal distribution) or Kruskal-Wallis test. If ANOVA is insignificant, letters are absent.

Fatty acid	charr	whitefish	muksun	inconnu	broad whitefish	round whitefish	whitefish nd
14:0	4.0±0.1^{A}	2.9±0.1^{B}	4.2±0.2^{A}	3.4±0.1^{AC}	2.5±0.2^{B}	2.2±0.2^{B}	2.3±0.2^{B}
15:0	0.3±0.0^{A}	0.2±0.0^{CD}	0.5±0.0^{B}	0.4±0.0^{A}	0.5±0.0^{B}	0.2±0.0^{D}	0.3±0.0^{C}
16:0	15.4±0.2^{C}	14.9±0.2^{C}	15.2±0.2^{C}	16.3±0.7^{AC}	18.2±0.4^{AB}	16.6±0.2^{AC}	18.9±0.8^{B}
16:1n-9	0.4±0.0^{ABD}	0.2±0.0^{C}	0.3±0.0^{CD}	0.4±0.0^{AC}	0.5±0.1^{B}	0.2±0.0^{C}	0.4±0.1^{ABD}
16:1n-7	6.6±0.2^{B}	17.4±0.5^{D}	10.1±0.3^{AC}	13.4±1.0^{ACE}	11.8±0.8^{C}	15.2±0.4^{DE}	9.7±1.4^{BC}
15-17BFA	1.8±0.0^{A}	1.0±0.0^{CD}	1.8±0.1^{A}	1.3±0.0^{AC}	2.5±0.2^{B}	0.6±0.0^{D}	1.0±0.1^{CD}
16PUFA	0.2±0.0^{B}	4.6±0.2^{C}	2.3±0.2^{DE}	1.5±0.2^{AD}	1.3±0.1^{A}	2.9±0.1^{E}	1.5±0.4^{AD}
17:0	0.2±0.0^{A}	0.3±0.0^{D}	0.4±0.0^{B}	0.2±0.0^{AD}	0.4±0.0^{B}	0.1±0.0^{C}	0.2±0.0^{A}
18:0	3.2±0.1^{C}	1.9±0.0^{D}	2.4±0.1^{A}	2.5±0.2^{AB}	3.0±0.1^{BC}	2.8±0.0^{AB}	2.5±0.2^{A}
18:1n-9	17.9±0.1^{AC}	19.8±0.2^{C}	16.1±0.4^{A}	16.8±0.8^{AC}	16.3±0.7^{A}	11.7±0.6^{B}	12.3±1.0^{B}
18:1n-7	3.1±0.0^{C}	3.9±0.1^{AC}	4.0±0.3^{AC}	4.3±0.2^{AB}	5.1±0.1^{B}	4.6±0.1^{AB}	3.7±0.5^{AC}
18:2n-6	3.0±0.1^{AC}	2.1±0.1^{A}	2.8±0.1^{AC}	2.7±0.2^{AC}	4.7±0.6^{B}	3.7±0.1^{BC}	2.7±0.3^{AC}
18:3n-3	2.6±0.1^{AC}	1.3±0.0^{A}	2.9±0.2^{BC}	2.2±0.2^{AC}	3.8±0.6^{B}	2.7±0.2^{AB}	1.6±0.1^{AC}
18:4n-3	1.7±0.0	1.8±0.1	1.8±0.1	1.6±0.1	1.5±0.2	1.4±0.2	1.3±0.02
∑20:1*	1.6±0.0^{AD}	1.2±0.1^{BCD}	2.3±0.1^{A}	2.1±0.9^{ABC}	1.7±0.3^{AB}	0.6±0.0^{C}	0.8±0.1^{BC}
20:2n-6	1.0±0.0^{C}	0.3±0.0^{A}	0.6±0.0^{B}	0.4±0.0^{A}	0.6±0.0^{B}	0.3±0.0^{A}	0.4±0.1^{A}
20:4n-6	1.9±0.0^{AC}	1.6±0.0^{CD}	2.4±0.1^{B}	2.3±0.3^{AB}	2.8±0.1^{B}	1.4±0.1^{D}	2.6±0.2^{B}
20:3n-3	1.5±0.0^{B}	0.2±0.0^{C}	0.6±0.0^{AB}	0.4±0.0^{ABC}	0.3±0.0^{AC}	0.2±0.0^{C}	0.4±0.1^{AC}
20:4n-3	2.9±0.0^{A}	0.7±0.0^{BC}	1.2±0.1^{AC}	1.1±0.1^{AC}	0.6±0.0^{B}	0.8±0.0^{BC}	0.8±0.1^{BC}
20:5n-3	4.8±0.1^{C}	10.4±0.2^{A}	9.6±0.2^{AB}	7.4±0.7^{ABC}	6.5±0.3^{BC}	10.2±0.1^{A}	10.3±0.3^{A}

22:5n-6	1.2±0.0^{B}	0.3±0.0^{C}	1.3±0.1^{B}	1.0±0.1^{AB}	0.8±0.1^{A}	0.3±0.0^{C}	0.8±0.1^{A}
22:4n-3	1.5±0.1^{C}	0.0±0.0^{AB}	0.1±0.0^{AC}	0.2±0.1^{AC}	0.0±0.0^{B}	0.0±0.0^{AB}	0.1±0.0^{ABC}
22:5n-3	3.0±0.1^{CD}	2.5±0.1^{AC}	2.5±0.1^{AC}	2.3±0.1^{A}	1.7±0.1^{B}	3.0±0.0^{D}	2.4±0.1^{A}
22:6n-3	12.1±0.1^{AC}	6.3±0.2^{D}	9.6±0.6^{ADE}	11.3±1.1^{ACD}	7.7±0.9^{AD}	14.4±1.5^{BCE}	20.1±3.0^{B}
24PUFA	4.3±0.3^{B}	1.0±0.1^{AB}	1.1±0.1^{BC}	1.0±0.2^{AC}	0.7±0.0^{A}	0.8±0.0^{A}	0.6±0.1^{AC}

190 * sum of 20:1n-11, 20:1n-9 and 20:1n-7

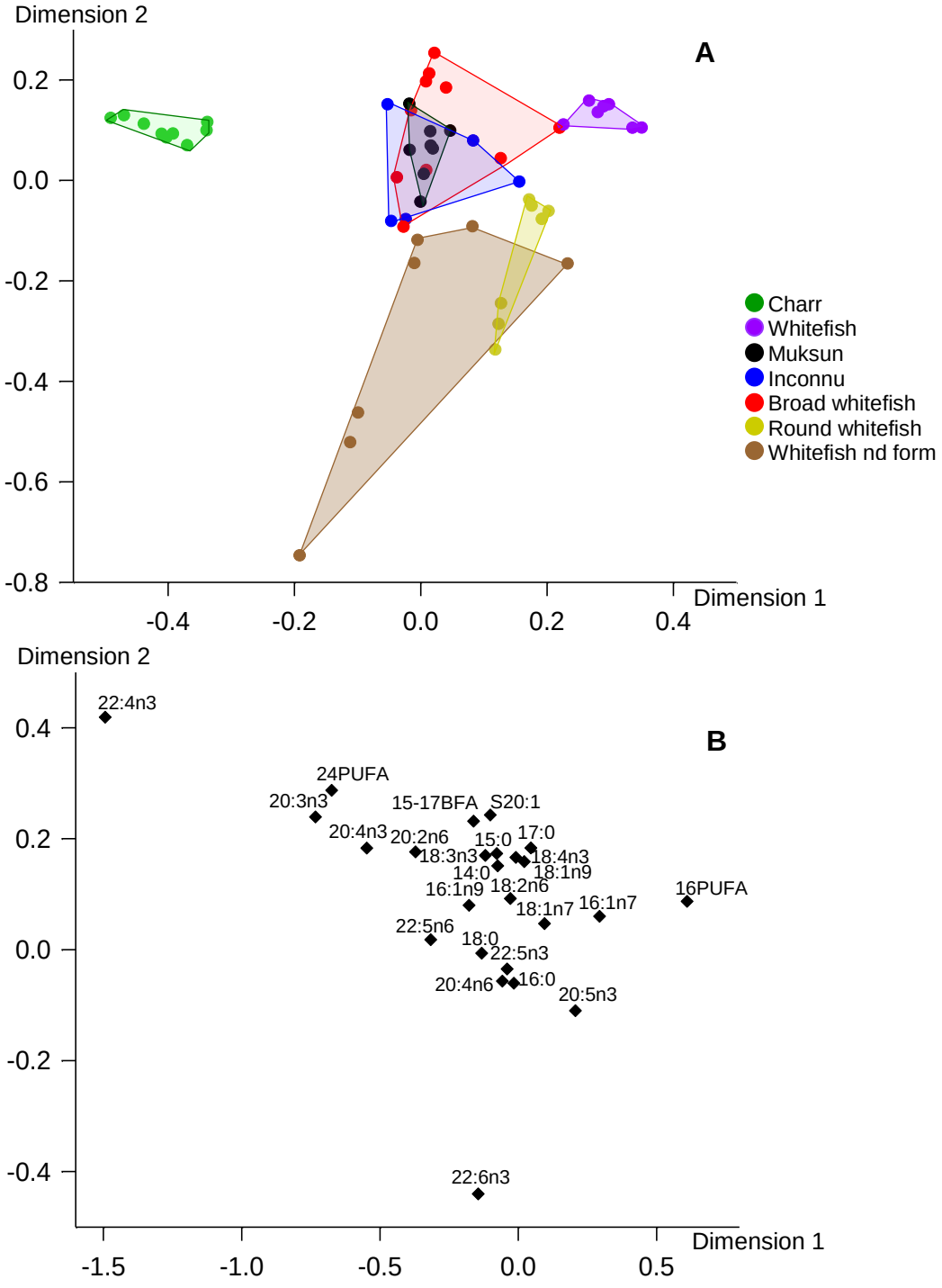

Figure 1. Canonical correspondence analysis of fatty acid percentages (% of FA sum) in total lipids of muscles of seven fish species from arctic lakes (Siberia, Russia). A – individual variables, B - factor structure coefficients for fatty acids. Dimension 1 and Dimension 2 represented 33.9% and 21.2% of inertia, respectively.

whitefish located at the one end and broad whitefish was at the another end (Fig.1). Whitefish of the non-identified form and round whitefish were separated due to higher levels of 22:6n-3 and 20:5n-3, and partial separation of broad whitefish was due to higher levels of C15-17 BFA (Table 3). Samples of the non-identified whitefish were markedly scattered (Fig.1), due to high variability in percentage of 22:6n-3 (Table 3).

In PL of all studied species, 22:6n-3, 16:0 and 20:5n-3 were dominant fatty acids (Table 4). Charr had the highest levels of 22:6n-3, 22:4n-3, 22:5n-6, 20:4n-3 among the studied species (Table 4). Whitefish had a significantly higher level of 20:5n-3 compared to that of the other fish. Inconnu tended to be higher in 18:1n-9 level. Broad whitefish had the highest levels of 20:4n-6 and 18:2n-6 (Table 4). Round whitefish had significantly higher levels of 16:1n-7, 18:1n-7 and C16 PUFA compared to those of the other fish. Muksun and whitefish of a non-identified form had intermediate levels of the most FA in PL (Table 4).

To reveal overall differences in PL FA, CCA was performed (Fig. 2). In the first dimension, a conspicuous difference of round whitefish versus charr was found. This difference was provided mostly by the greater levels of C16 PUFA, 16:1n-7 and 18:2n-6 in PL of round whitefish and by greater levels of C24 PUFA and 22:4n-3 in that of charr (Fig. 2). The variation in the second dimension of CCA was related to differences between whitefish and broad whitefish due to levels of 15:0 and 14:0 versus levels of C16 PUFA, 18:2n-6 and 18:3n-3.

In FA composition of triacylglycerols of all arctic fish, 18:1n-9, 16:1n-7, 16:0 and 20:5n-3 dominated (Table 5). Charr had the highest levels of 22:6n-3, C24 PUFA, 22:5n-3, 20:4n-3 and 20:3n-3 compared to that of the other studied species. Whitefish had the maximum levels of 18:1n-9 and 20:5n-3 (Table 5). Muksun had the significantly higher level of 14:0 than the other fish. Inconnu and whitefish of a non-identified form had intermediate FA levels in TAG (Table 5). Broad whitefish had the maximum levels of 16:0, C15-17 BFA, 17:0, 18:2n-6, 18:3n-3, and 20:4n-6 among the studied fish. Round whitefish had the significantly higher percentages of 16:1n-7 and C16 PUFA in TAG (Table 5).

To study differences in fish reserve lipids, we performed CCA of FA in TAG (Fig. 3). Like the multidimensional analysis for PL, dimension 1 showed a marked difference of round whitefish versus charr. This difference was provided mostly by the greater levels of C16 PUFA and 16:1n-7 in TAG of round whitefish and by greater levels of 22:4n-3 and 20:3n-3 in that of charr (Fig. 3). The second factor of CCA for TAG also showed a similar trend to that observed in CCA of PL (Fig.2, 3). In this dimension, differences were found between whitefish and broad whitefish due to variability in levels of C15-17 BFA, 17:0 and C16 PUFA (Fig.3).

In general, positioning of fish species in the biplot for reserve TAG well corresponded to that in biplot for structural PL (Fig.2, 3). It should be also noted that physiologically significant EPA and DHA, were not found among the FA markers responsible for separation of fish species in CCA for TAG and PL (Fig.2, 3). Positioning of fish species in the biplot for total lipids generally corresponded to that in biplots for the lipid classes, with exception of whitefish of the non-identified form (Fig. 1-3). The fatty acid markers responsible for separation of the fish samples in CCA were generally similar for total lipids, TAG and PL with exception of DHA and EPA in CCA of total lipids (Fig. 1-3).

A visual analysis of all thin-layer chromatograms showed a marked dominance of TAG, PL and sterols as major lipid fractions. Spots that corresponded to other lipid classes were negligible. Therefore, we considered TAG and PL as major acyl-containing fractions, summarized their FA

Table 4. Mean levels of fatty acids (% of the total) and total content of fatty acids (Sum FA, mg · g^{-1} wet weight) in polar lipids of Salmoniformes species: charr – *S. drjagini* from Sobachye Lake; whitefish – *C. lavaretus* from Sobachye Lake; muksun – *C. muksun* from Pyasino Lake; inconnu – *S. leucichthys nelma* from Pyasino Lake; broad whitefish – *C. nasus* from Pyasino Lake; round whitefish – *P. cylindraceum* from Sobachye Lake; whitefish nd – nonidentified form of *Coregonus lavaretus* from Sobachye Lake. Cases (fatty acids) with normal distribution are given in bold. Means labeled with the same letter are not significantly different at $P< 0.05$ after ANOVA *post-hoc* Tukey HSD test (cases with normal distribution) or Kruskal-Wallis test. If ANOVA is insignificant, letters are absent.

Fatty acid	charr	whitefish	muksun	inconnu	broad whitefish	round whitefish	whitefish nd
14:0	1.2±0.1^{AB}	0.9±0.0^{A}	1.1±0.1^{AB}	1.5±0.2^{B}	1.2±0.1^{AB}	0.9±0.1^{A}	1.4±0.1^{B}
15:0	0.2±0.0^{A}	0.2±0.0^{A}	0.4±0.0^{CD}	0.3±0.0^{BC}	0.5±0.0^{D}	0.2±0.0^{A}	0.3±0.0^{AB}
16:0	24.2±0.4^{A}	29.4±0.5^{BC}	26.3±0.6^{AB}	25.9±0.5^{AB}	27.3±0.8^{AB}	29.1±1.3^{BC}	31.7±1.1^{C}
16:1n-9	0.3±0.0^{AB}	0.1±0.0^{A}	0.3±0.0^{BC}	0.2±0.1^{AB}	0.5±0.1^{C}	0.2±0.0^{AB}	0.3±0.1^{BC}
16:1n-7	1.3±0.1^{A}	2.2±0.1^{BC}	1.9±0.2^{AB}	2.3±0.1^{B}	2.9±0.2^{B}	4.1±0.2^{D}	3.0±0.2^{C}
15-17BFA	0.6±0.0^{C}	0.1±0.0^{A}	0.4±0.0^{BC}	0.3±0.0^{AB}	0.6±0.1^{C}	0.2±0.0^{AB}	0.3±0.1^{AB}
16PUFA	n.o.^{A}	0.2±0.0^{B}	0.1±0.1^{AB}	0.1±0.0^{AB}	0.1±0.0^{AB}	0.3±0.0^{C}	0.1±0.0^{AB}
17:0	0.2±0.0^{A}	0.2±0.0^{AB}	0.3±0.0^{B}	0.2±0.0^{AB}	0.3±0.0^{B}	0.1±0.0^{A}	0.2±0.0^{A}
18:0	2.8±0.1^{AB}	2.1±0.1^{A}	3.0±0.1^{B}	2.9±0.3^{AB}	2.2±0.2^{A}	3.4±0.2^{B}	2.8±0.3^{AB}
18:1n-9	6.2±0.3^{AB}	6.5±0.2^{AB}	6.6±0.4^{AB}	7.8±0.4^{B}	6.1±0.5^{A}	5.6±0.3^{A}	6.9±0.3^{AB}
18:1n-7	1.6±0.1^{A}	1.7±0.1^{AB}	2.2±0.2^{BC}	2.4±0.1^{C}	2.5±0.1^{C}	3.3±0.2^{D}	2.4±0.3^{C}
18:2n-6	0.7±0.0^{A}	0.8±0.0^{A}	1.0±0.0^{A}	1.1±0.1^{A}	2.5±0.4^{B}	1.6±0.0^{A}	1.4±0.3^{A}
18:3n-3	0.6±0.0^{AB}	0.4±0.0^{A}	1.0±0.1^{ABC}	1.0±0.2^{ABC}	2.1±0.4^{C}	1.3±0.1^{BC}	0.7±0.1^{ABC}
18:4n-3	0.1±0.0^{AB}	n.o.^{A}	0.2±0.0^{B}	0.2±0.0^{AB}	0.1±0.0^{AB}	0.3±0.0^{B}	0.2±0.1^{AB}
∑20:1*	0.2±0.0^{BC}	0.1±0.0^{A}	0.3±0.0^{C}	0.1±0.0^{ABC}	0.1±0.0^{AB}	0.2±0.0^{ABC}	0.2±0.1^{AC}
20:2n-6	0.2±0.0^{BC}	n.o.^{A}	0.1±0.0^{AB}	0.1±0.0^{A}	0.2±0.0^{C}	0.1±0.0^{AC}	0.1±0.0^{AB}
20:4n-6	3.9±0.1^{BC}	3.0±0.1^{AB}	4.1±0.2^{C}	3.8±0.3^{BC}	5.6±0.3^{D}	2.4±0.1^{A}	3.2±0.2^{ABC}

20:3n-3	0.5±0.0^{C}	0.1±0.0^{A}	0.2±0.0^{B}	0.1±0.0^{AB}	0.2±0.0^{B}	0.1±0.0^{AB}	0.1±0.0^{AB}
20:4n-3	1.1±0.0^{B}	0.3±0.0^{A}	0.5±0.0^{AB}	0.5±0.1^{AB}	0.4±0.1^{A}	0.5±0.0^{AB}	0.4±0.0^{A}
20:5n-3	7.9±0.2^{A}	16.6±0.6^{D}	12.5±0.3^{BC}	11.2±1.1^{BC}	10.9±0.4^{B}	13.6±1.0^{C}	10.0±0.5^{AB}
22:5n-6	2.4±0.1^{C}	0.6±0.0^{A}	2.0±0.3^{C}	1.7±0.2^{BC}	2.2±0.2^{C}	0.4±0.0^{A}	0.9±0.1^{AB}
22:4n-3	0.2±0.0^{B}	n.o.^{A}	n.o.^{A}	n.o.^{A}	n.o.^{A}	n.o.^{A}	n.o.^{A}
22:5n-3	2.5±0.0^{A}	2.7±0.2^{AB}	2.5±0.1^{A}	2.4±0.3^{A}	2.6±0.1^{AB}	3.2±0.3^{B}	2.1±0.1^{A}
22:6n-3	39.9±0.5^{D}	31.1±0.7^{BC}	31.8±0.5^{C}	32.4±0.9^{C}	26.4±0.6^{A}	27.9±0.7^{AB}	30.1±1.1^{BC}
24PUFA	0.3±0.1	n.o.	n.o.	0.4±0.4	0.1±0.0	n.o.	n.o.
Sum FA	*3.0±0.2^{AB}*	*3.3±0.5^{AB}*	*3.2±0.9^{AB}*	*2.6±0.2^{AB}*	*3.4±0.2^{B}*	*3.2±0.7^{AB}*	*2.1±0.2^{A}*

- sum of 20:1n-11, 20:1n-9 and 20:1n-7

Table 5. Mean levels of fatty acids (% of the total) and total content of fatty acids (Sum FA, mg · g^{-1} wet weight) in triacylglycerols of Salmoniformes species: charr – *S. drjagini* from Sobachye Lake; whitefish – *C. lavaretus* from Sobachye Lake; muksun – *C. muksun* from Pyasino Lake; inconnu – *S. leucichthys nelma* from Pyasino Lake; broad whitefish – *C. nasus* from Pyasino Lake; round whitefish – *P. cylindraceum* from Sobachye Lake; whitefish nd – nonidentified form of *Coregonus lavaretus* from Sobachye Lake. Cases (fatty acids) with normal distribution are given in bold. Means labeled with the same letter are not significantly different at $P < 0.05$ after ANOVA *post-hoc* Tukey HSD test (cases with normal distribution) or Kruskal-Wallis test. If ANOVA is insignificant, letters are absent.

Fatty acid	charr	whitefish	muksun	inconnu	broad whitefish	round whitefish	whitefish nd
14:0	4.4±0.1^{CD}	3.3±0.1^{B}	4.8±0.3^{D}	3.9±0.0^{BC}	2.7±0.2^{A}	3.1±0.2^{B}	3.5±0.4^{ABC}
15:0	0.3±0.0^{B}	0.2±0.0^{AB}	0.5±0.0^{C}	0.4±0.0^{BC}	0.5±0.1^{C}	0.2±0.0^{A}	0.3±0.0^{AB}
16:0	16.7±0.4^{C}	14.6±0.2^{AB}	14.6±0.8^{AB}	15.9±0.7^{B}	17.4±0.3^{C}	12.9±0.4^{A}	15.7±0.5^{B}
16:1n-9	0.8±0.3^{B}	0.3±0.0^{A}	0.4±0.0^{AB}	0.4±0.1^{AB}	0.8±0.1^{B}	0.3±0.0^{A}	0.5±0.1^{AB}
16:1n-7	7.9±0.4^{A}	19.4±0.8^{C}	12.6±1.3^{B}	15.9±1.4^{BC}	13.2±0.7^{B}	24.5±1.2^{D}	15.7±0.9^{BC}
15-17BFA	2.0±0.2^{BCD}	1.0±0.0^{AB}	1.9±0.1^{CD}	1.1±0.1^{ABC}	2.8±0.4^{D}	0.7±0.1^{A}	1.4±0.1^{AB}

16PUFA	0.4±0.1^{A}	4.8±0.2^{C}	3.0±0.6^{B}	1.6±0.4^{AB}	1.3±0.2^{AB}	5.4±0.7^{C}	2.2±0.5^{B}
17:0	0.2±0.0^{AB}	0.3±0.0^{BC}	0.3±0.0^{C}	0.2±0.0^{AB}	0.5±0.0^{D}	0.1±0.0^{A}	0.3±0.0^{BC}
18:0	3.4±0.1^{C}	1.9±0.0^{A}	2.4±0.1^{A}	2.5±0.3^{AB}	3.2±0.1^{BC}	2.5±0.1^{A}	2.6±0.3^{A}
18:1n-9	19.2±0.6^{BC}	21.5±0.4^{C}	16.8±1.0^{AB}	18.9±1.4^{B}	17.8±0.8^{AB}	14.6±0.8^{A}	18.3±0.6^{B}
18:1n-7	2.9±0.4^{A}	4.2±0.1^{AB}	4.6±0.4^{BC}	4.9±0.2^{BC}	5.9±0.3^{C}	5.9±0.4^{C}	4.8±0.5^{BC}
18:2n-6	3.2±0.1^{AB}	2.2±0.1^{A}	3.0±0.1^{AB}	3.0±0.4^{AB}	5.4±0.6^{D}	5.1±0.1^{CD}	3.9±0.3^{BC}
18:3n-3	2.6±0.1^{AB}	1.4±0.1^{A}	3.0±0.2^{BC}	2.4±0.3^{A}	4.0±0.5^{C}	3.6±0.4^{BC}	2.2±0.2^{AB}
18:4n-3	1.6±0.1	1.8±0.1	1.8±0.1	1.7±0.2	1.5±0.2	1.9±0.3	2.1±0.4
∑20:1*	1.3±0.0^{BC}	1.0±0.1^{AB}	1.9±0.2^{C}	2.0±0.9^{ABC}	1.7±0.3^{BC}	0.7±0.1^{A}	1.3±0.1^{ABC}
20:2n-6	0.9±0.0^{C}	0.2±0.0^{A}	0.6±0.0^{B}	0.4±0.0^{AB}	0.4±0.1^{AB}	0.3±0.0^{AB}	0.5±0.1^{B}
20:4n-6	1.7±0.0^{BC}	1.4±0.0^{B}	2.1±0.1^{D}	1.9±0.2^{CD}	2.3±0.1^{D}	0.6±0.0^{A}	1.7±0.1^{BCD}
20:3n-3	1.4±0.0^{C}	0.1±0.0^{A}	0.5±0.1^{BC}	0.4±0.0^{ABC}	0.3±0.0^{AB}	0.2±0.0^{AB}	0.4±0.1^{AB}
20:4n-3	2.7±0.1^{C}	0.7±0.1^{AB}	1.2±0.1^{BC}	1.2±0.1^{ABC}	0.6±0.0^{A}	0.9±0.1^{AB}	0.9±0.1^{AB}
20:5n-3	4.4±0.1^{A}	9.4±0.2^{D}	9.0±0.3^{D}	6.5±1.0^{BC}	5.3±0.4^{AB}	7.0±0.5^{BC}	8.6±0.5^{CD}
22:5n-6	0.9±0.0^{D}	0.2±0.0^{AB}	1.0±0.1^{D}	0.7±0.1^{CD}	0.5±0.1^{BC}	n.o.^{A}	0.4±0.1^{AB}
22:4n-3	1.3±0.0^{B}	n.o.^{A}	0.1±0.0^{A}	0.2±0.1^{A}	n.o.^{A}	n.o.^{A}	0.1±0.0^{A}
22:5n-3	2.7±0.1^{B}	2.2±0.1^{B}	2.4±0.2^{B}	2.1±0.2^{B}	1.4±0.1^{A}	2.3±0.1^{B}	2.1±0.2^{B}
22:6n-3	9.9±0.2^{C}	4.1±0.2^{B}	6.4±0.3^{C}	7.8±0.8^{C}	4.1±0.3^{B}	2.4±0.2^{A}	6.6±0.5^{C}
24PUFA	2.9±0.1^{B}	0.7±0.1^{A}	0.9±0.1^{AB}	0.6±0.3^{A}	0.7±0.0^{A}	0.6±0.1^{A}	0.7±0.1^{A}
Sum FA	*30.3±4.7^{AB}*	*33.2±6.9^{AB}*	*41.6±15.0^{B}*	*18.7±7.0^{AB}*	*19.5±7.0^{AB}*	*4.9±1.2^{A}*	*2.0±0.5^{A}*

257 * sum of 20:1n-11, 20:1n-9 and 20:1n-7

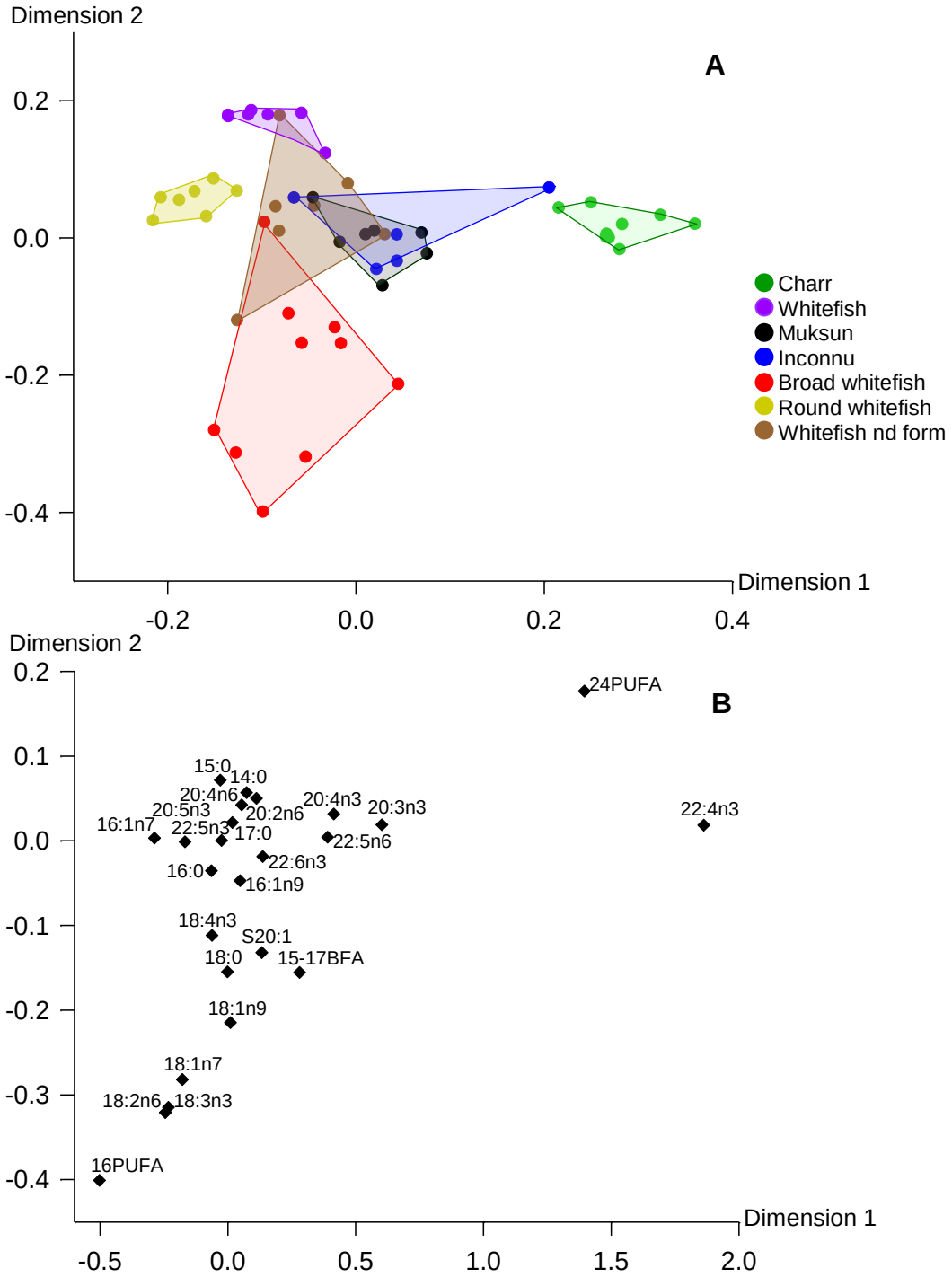

Figure 2. Canonical correspondence analysis of fatty acid percentages (% of FA sum) in polar lipids of muscles of seven fish species from arctic lakes (Siberia, Russia). A - Dimension 1 and Dimension 2 – individual variables, B - factor structure coefficients for fatty acids. Factor 1 and Factor 2 represented 30.9% and 22.5% of inertia, respectively.

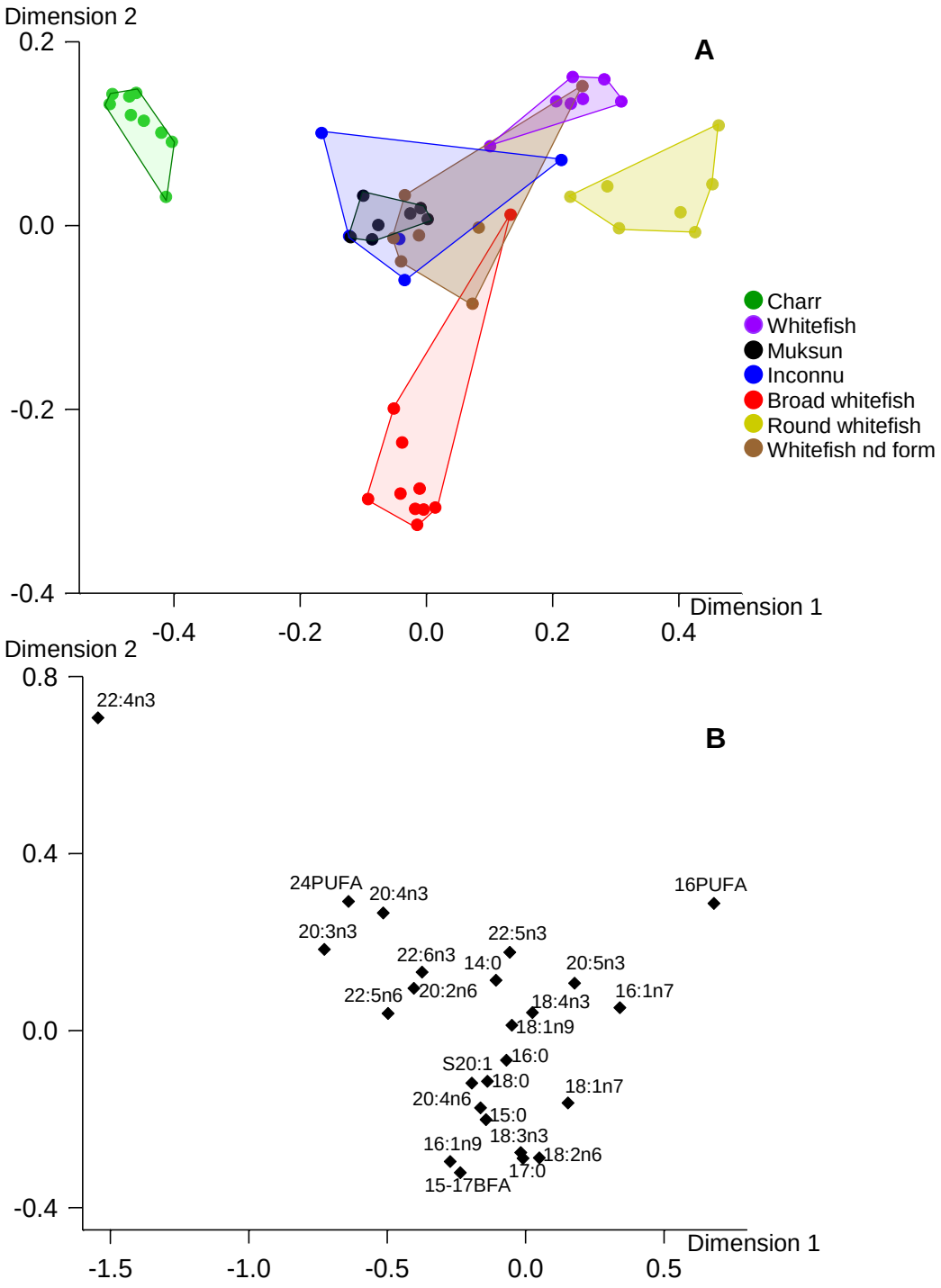

Figure 3. Canonical correspondence analysis of fatty acid percentages (% of FA sum) in triacylglycerols of muscles of seven fish species from arctic lakes (Siberia, Russia). A – individual variables, B - factor structure coefficients for fatty acids. Dimension 1 and Dimension 2 represented 51.1% and 15.6% of inertia, respectively.

277 contents per mass unit (Table 4, 5) and calculated their parts in the sum of FA in the fish muscles
278 (Fig.4A). Polar lipids constituted from 10.3 to 57.0 % of the acyl-containing lipid sum, being the
279 highest in whitefish of a non-indentified form (Fig.4A). Triacylglycerols were the dominant
280 acyl-containing lipid fraction in majority of the studied fish and exceeded 85% in charr, whitefish
281 and muksun (Fig.4A). Note that increase in lipid content and total fatty acids for the studied species
282 well corresponded with the increase in the TAG proportion of the acyl-containing lipids (Table 2,
283 Fig.4A).

284 Using the PL and TAG percentages of the acyl-containing lipid sum and content of EPA and
285 DHA of total lipids per mass unit, we calculated parts of EPA+DHA that provided by polar lipids
286 versus triacylglycerols and expressed them as mg · g^{-1} wet weight of muscle tissue (Fig.4B). Contents
287 of EPA+DHA provided by PL fraction varied from 1.9 to 3.5 mg · g^{-1} (Fig.4B). The average value for
288 the seven fish species was 2.4±0.2 mg · g^{-1}, and coefficient of variation, CV, was 8.7 %. Contents of
289 EPA+DHA in TAG were of a greater range, from 0.9 to 9.8 mg · g^{-1}; the average value was to 4.4±0.2
290 mg · g^{-1}, and CV was 28.9 % (Fig. 4B).

291

292 **4. Discussion**

293 *4.1. Main finding*

294

295 All the taxonomically related species of order Salmoniformes had nearly similar content of
296 EPA+DHA, 2.4±0.2 mg · g^{-1}, in PL. In contrast, content of EPA+DHA esterified as TAG varied
297 ~10-fold among the studied salmonids. Thus, all variations of nutritive value, i.e., EPA+DHA content
298 per mass unit of filet, were caused by TAG fraction, while PL had a constant species (taxon) –
299 specific physiologically optimum content. As a result, fatty fish, charr, whitefish and muksun,
300 contained most amounts of nutritionally valuable EPA and DHA in TAG fraction of muscles.
301 Conversely, the lean species, like round whitefish and whitefish of the non-identified form, had the
302 omega-3 LC-PUFA contained mostly in polar lipids. Regarding nutritive value, the fatty species with
303 higher proportion of TAG in muscles, charr, whitefish and muksun, appeared to be most valuable
304 and had 13.3 ± 0.8, 10.4 ± 1.1 and 8.4 ± 1.4 mg · g^{-1}, respectively.

305

306 *4.2. Fatty acid markers in fish total lipids, TAG and PL*

307

308 In our snap-shot field study of salmonids from arctic lakes we found significant differences
309 between fatty acid profiles of the main lipid classes, with prevalence of n-3 LC-PUFA and
310 monoenoic 16-18 FA in structural PL and storage TAG, respectively. In addition, both FA profiles of
311 TAG and PL, as well as profiles of total lipids, had distinct peculiarities among the studied fish that
312 allowed separating the most species in the CCA biplots (Fig.1-3). It should be emphasized the
313 separations of the fish in the multivariate analyses of all three lipid fractions were provided mainly
314 by the same marker FA: C24 PUFA, 22:4n-3, 20:3n-3, C16 PUFA, 16:1n-7 and C15-17 BFA. The
315 conspicuous exception was 22:6n-3 and 20:5n-3 in total lipid CCA that separated whitefish of the
316 non-identified form and round whitefish from the other species (Fig.1). PL had a predominant
317 contribution in total lipids of these lean fish (Fig.4A). Therefore, FA profiles of total lipids of
318 whitefish of the non-identified form and round whitefish mostly reflected FA composition of PL

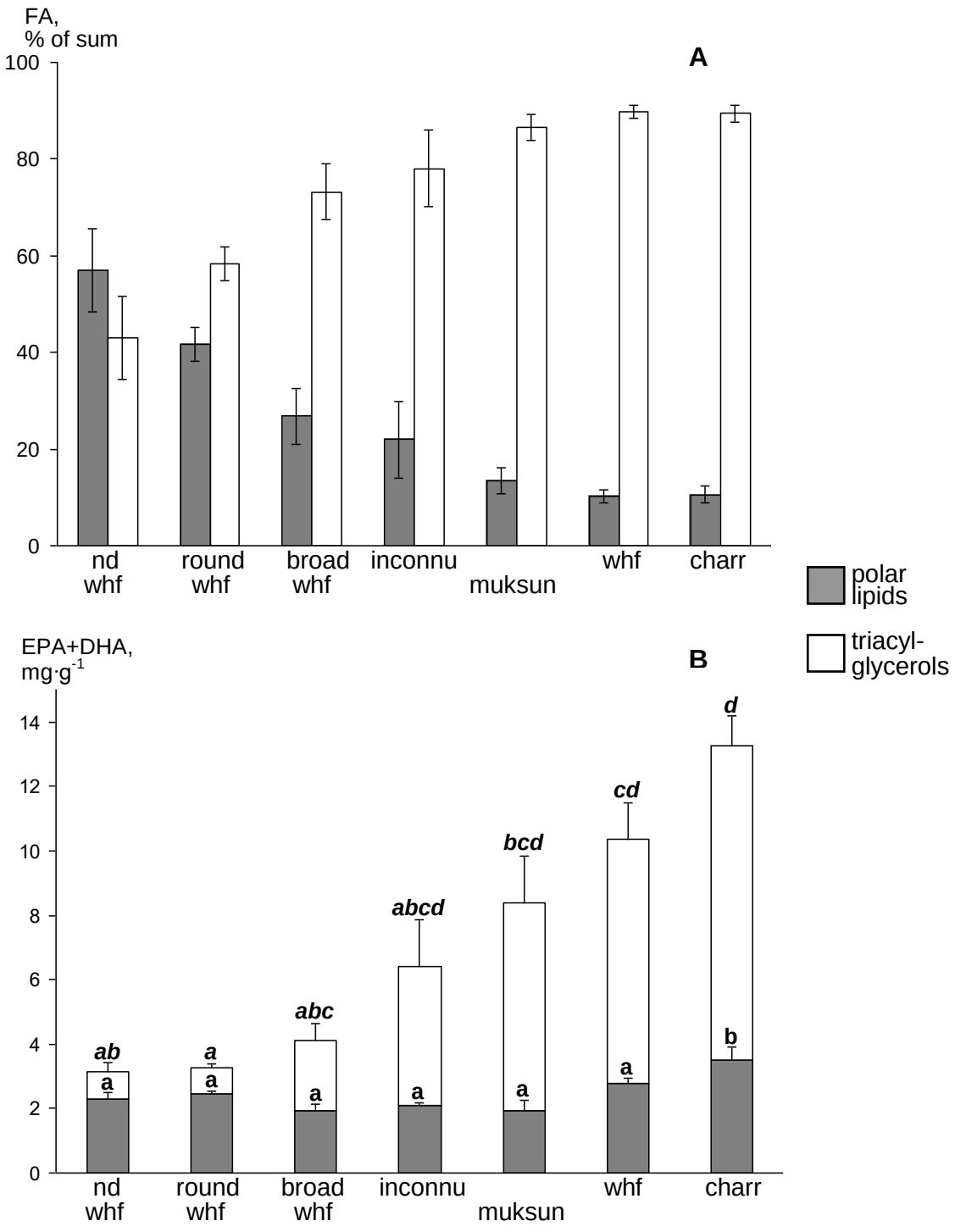

Figure 4. Mean percentages of polar lipids and triacylglycerols of their sum – A, and mean contents (mg · g^{-1} wet weight) of sum of eicosapentaenoic acid and docosahexaenoic acid that corresponded to polar lipids and triacylglycerols – B, in muscles of seven fish species from Arctic lakes (Siberia, Russia). Bars represent standard errors. Means labelled with the same letter are not significantly different at $P < 0.05$ after Tukey HSD *post hoc* test.

which were considerably rich in DHA and EPA (Table 4). As a result, DHA and EPA, dominant FA of PL, played as markers for the lean fish in CCA of total lipids.

Similar patterns of biomarker FAs, characteristic of zoobenthic, algal, terrestrial and other food sources, within TAG and PL fractions of the studied species allowed to use both these fractions, as well as their sum, total lipids, for identification of food sources of wild fish (Fig.1-3). In ecological studies, analysis of FA trophic markers of various consumers are often performed for TAG assuming that they generally deposit fatty acid molecules coming from food assimilation [18, 25, 26]. Meanwhile, many studies used FA composition of total lipids to elucidate trophic relations of various fish species [e.g., 27-31]. In overall, both approaches base on a premise that biochemical composition of food sources is reflected in FA profiles of TAG or total lipids.

Another lipid class of high concern is polar lipids comprised mostly phospholipids and glycolipids that are main constituents of cell membranes. As known, the specific FA composition of PL provides proper membrane structure and functions. As a result, FA composition of PL are considered to be highly conserved relative to diet and tended to reflect FA biosynthetic capacities of an organism [18].

In contrast to the common opinion on conserved PL composition, in some studies FA profiles of PL were successfully used as trophic markers. For instance, fatty acid profiles of both polar and neutral lipids conspicuously differed among three species, benthivorous whitefish *Coregonus clupeaformis*, and piscivorous walleye *Sander vitreus* and northern pike *Esox lucius*, due to different feeding habits of the fish [32]. We also confirmed that PL FA profiles of muscles allow to identify feeding spectra of fish similarly that TAG profiles do (Fig. 2, 3). For instance, round whitefish was one of the most separated species in the both multivariate analyses of TAG PL due to the greatest levels of C16-PUFA and 16:1n-7 which originated from diatoms and green algae [33]. Indeed, the algae were one of the dominant items in stomach content of this species (Table 1).

In CCA biplots of total lipids, PL and TAG, charr had a particular position due to higher levels of minor n-3 PUFA, like 22:4-3, C24 PUFA and 20:3n-3. These FA were not assigned as trophic markers, whereas some of them, C24 PUFA, were considered as intermediate compounds indicative for conversion of C20 to C22 PUFA [34, 35]. In TAG of charr the percentage of 22:4n-3 accounted for 1.3% of FA sum, being absent or found in traces in other studied fish. The presence of this PUFA was previously reported for least cisco *Coregonus sardinella*, small-sized pelagic fish inhabited Sobachye Lake [23]. The studied charr from Sobachye Lake was piscivorous (Table 1), thus, it could obtain this PUFA from the consumed least cisco. Like in our study, species of the same genus and its prey, lake trout *Salvelinus namaycush* and cisco from Great Bear Lake, were together separated from other hydrobionts in a multivariate analysis due to higher levels of 22:4-3 and 20:3n-3 [31]. Alternative explanation based on coincidence between 22:4n-3 and C24 PUFA levels is that the fatty acid 22:4n-3 may be a marker of LC-PUFA conversion in fish. Anyway, we suppose that considerable levels of 22:4n-3, 20:3n-3 and C24 PUFA might be a characteristic feature of FA profiles of *Salvelinus* genus.

In both CCA analyses of TAG and PL, broad whitefish well separated from the other species due to higher levels of C15-17 BFA, 17:0, 18:2n-6 and 18:3n-3. Two former FA are known to be markers of bacterial organic matter, while two latter are considered as markers of terrestrial organic matter [31, 33]. Broad whitefish is a typical benthivorous species that likely got these marker fatty acids from detritus enriched with bacterial and terrestrial organic matter. The species had the

405 highest levels of 18:2n-6 in TAG, and 20:4n-6 in PL, relatively. This finding likely indicates for the
406 initial storage of dietary 18:2n-6 in TAG and its consequent conversion to 20:4n-6 with further
407 transfer to PL.
408 Similarity of FA sets that are markers for food sources between TAG and PL classes likely
409 indicates that the studied wild fish are able to directly incorporate dietary biochemical components,
410 i.e., fatty acyl groups, into membrane PL. Besides FA originated from food assimilation, fish are
411 able to include in lipid molecules fatty acyl groups obtained due to biosynthesis or conversion from
412 precursors. Freshwater fish are known to have capacity to synthesize LC-PUFA from the shorter
413 chain precursors [35, 36]. Indeed, some studied fish, e.g., charr, contained in TAG and PL certain
414 amounts of C24 PUFA and 20:4n-3 that likely were intermediates of DHA and EPA synthesis.
415
416 *4.3. Content of essential LC-PUFA in fish PL and TAG*
417
418 The studied seven salmonid species varied ~ 7-fold in total lipid and fatty acid contents per a
419 mass unit of muscle tissues. Most of this variation was related with different TAG content in muscles
420 (Table 5, Fig.4), whereas PL contents evaluated as their FA sum varied slightly (Table 4). The
421 observed variation in lipid class contents in the studied fish is in agreement with well-known notion
422 that polar lipids comprise cellular membranes and, as a result, have a relatively constant content in
423 muscle cells, in contrast to that of triacylglycerols [19, 21, 37]. For instance, an absence of relation
424 between total lipid and phospholipid contents and a strong relation between total lipid and
425 triacylglycerol contents expressed as percentages of muscle mass were previously shown for a
426 number of marine myctophid species [38].
427 Fish polar lipids are commonly considered as a physiologically crucial lipid class that are
428 rich in LC-PUFA, mostly in DHA and, to a lesser extent, in EPA [19, 37, 39]. Indeed, percentages of
429 DHA and EPA in PL of various wild marine and freshwater species ranged as 11.5 – 55.7 % and
430 2.6-14.6 %, with average values of 31.4 % and 7.4 %, respectively [40-46]. The average levels of EPA
431 and DHA in the fish species from our study coincided with the above ranges, except the EPA value
432 of whitefish, 16.6%, which was a bit higher than the known values.
433 Triacylglycerols are considered to be relatively poor in LC-PUFA and preferably accumulate
434 monoenoic C16-22 FA [18, 19, 37]. According to the available data, levels of DHA and EPA in fish
435 TAG varied in intervals of 2.3-23.3 % and 1.1-14.1 %, with average values of 8.8 % and 5.3 %,
436 respectively [40-46]. The percentages of both EPA and DHA of TAG in the fish species from our
437 study well coincided with the above ranges (Table 5).
438 Triacylglycerols commonly comprise a large part of total lipids in muscles of medium-fat and
439 fatty fish species. For instance, TAG achieved 80 %, 90 % and 51.5 % in marine species arrow-tooth
440 flounder (*Atheresthes stomias)* and golden pompano (*Trachinotus blochii*) and freshwater whitefish
441 (*Coregonus lavaretus*), respectively [43, 47, 48]. In the studied freshwater salmonids TAG percentages
442 varied from 43.4 to 89.7% of the sum of two acyl-containing lipid classes (Fig.4). Such high TAG
443 levels may be explained by adaptation of the fish species to low-temperature conditions in the
444 studied arctic lakes [49, 50].
445 Regarding the relatively high contents of TAG per mass unit and percentages of EPA and
446 DHA in TAG, we hypothesized that content of EPA and DHA in TAG would appreciably contribute
447 to total content of EPA and DHA and would increase with lipid content in muscles of the studied

448 fish. Hence, we compared content of EPA and DHA esterified as TAG versus that esterified as PL.
449 Content of EPA+DHA in PL per mass unit of muscle tissues were similar among the studied
450 salmonids, moderately varying in the interval of 1.9-3.5 mg · g^{-1} (Fig.4B). In contrast, values of
451 EPA+DHA in TAG of the fish species greatly varied, ~10-fold. Lean fish, i.e., whitefish of the
452 non-identified form, round whitefish and broad whitefish, contained only 25-47 % of EPA+DHA of
453 total content of these PUFA in the muscles esterified as TAG molecules. In contrast, medium-fat and
454 fatty fish, like inconnu, muksun, whitefish and charr, had more than half of their muscle EPA and
455 DHA content in form of TAG molecules, up to 72 %. Thus, the wild salmonids that had relatively
456 high content of n-3 LC-PUFA in muscles (~ > 5 mg · g^{-1}) contained the major portion of these
457 nutritionally valuable compounds in the storage lipids. Our finding evidently contradicts a common
458 notion that lean and medium-fat fish that have PL as a main lipid class in the muscles are the best
459 dietary sources of n-3 LC-PUFA for humans [19, 51]. Wild fatty fish which are able to deposit large
460 amounts of storage lipids in their muscles appear to be the most valuable sources of n-3 LC-PUFA in
461 human diet.

462 Further, our results are in a good accordance with many studies showed strong relationship
463 between total lipid and EPA, DHA or their sum contents in fish muscles. Such relation was found for
464 marine species, sprat *Sprattus sprattus* and herring *Clupea harengus* from Baltic sea [52], for five
465 marine species from the northeast Pacific [38] and for several freshwater species from a subalpine
466 lake [53]. The significant relation was also found across farmed families of Atlantic salmon *Salmo*
467 *salar* [54].

468 It is interesting to note that percentages of the n-3 LC-PUFA and lipid (or total FA as their
469 proxy) content were negatively correlated in aforementioned and other studies [10]. The reported
470 negative correlation was explained by the fact that total lipids increase preferably at the expense of
471 TAG, whereas content of the membrane phospholipids, which are rich in n-3 PUFA remains fairly
472 constant [21, 37]. As a result, the proportions of EPA and DHA in muscle total lipids become diluted
473 by the accumulation of neutral lipids, which have high levels of monounsaturated FA. Although
474 increase of total lipids content at the expense of TAG in fish muscles, as a rule, leads to decrease of
475 n-3 PUFA percentage, this does not mean that a concomitant decrease of nutritional quality of a fish
476 occurs. Nutritional quality of fish products must be estimated on quantitative base expressed as mg
477 FA per gram of tissue rather than percentage base [10].

478 Quantitative (mg per gram of tissue) measurements of TAG vs PL contribution in n-3
479 LC-PUFA of fish muscles are very scarce. Some studies gave indirect evidence of significant TAG
480 contribution. For instance, among four fish species commercially harvested in Alaskan waters,
481 arrow-tooth flounder *Atheresthes stomias* had maximum contents of EPA and DHA, 7.0 mg · g^{-1}, as
482 well as maximum levels of TAG, 80% of total lipids in edible muscles [47]. Myctophid fish species
483 with higher total lipid content (proxy for TAG content) also had higher contents EPA and DHA
484 esterified as TAG [38].

485 Some direct measurements showed that lean fish contained less than half of EPA and DHA
486 in their muscles esterified as TAG, like wild white seabream *Diplodus sargus* [40], whitefish *Coregonus*
487 *lavaretus* [43], six commercial Chilean marine species [46]. In contrast, the only studied medium-fat
488 fish (2–4% lipid content of wet mass), Pacific sandperch *Prolatilus jugularis*, had approximately 60%
489 of EPA+DHA esterified as TAG [46]. Similar to latter finding, farmed *C. lavaretus* which had one of
490 the highest known values of EPA and DHA in muscles, 18.6 mg · g^{-1} wet weight, had 61% of that

value in TAG [43]. In our study, fish species were strongly variable in lipid and total FA content and, as a result, in n-3 LC-PUFA content esterified as TAG. Like in abovementioned studies, the fatty fish, muksun, whitefish and charr, had relatively higher content of EPA+DHA per mass unit that were mostly esterified as TAG (Fig.4B).

If to take the threshold of the recommended personal daily dose of EPA + DHA as 1 g and the average per serve portion of fish as 200 g [55, 56], a fish of proper nutritional value should contain EPA + DHA nearly or more than 5 mg · g^{-1} of filet [57]. The obtained data on lipid classes composition and content mean that when such fish is consumed, nearly or more than half of the essential n-3 LC-PUFA come as TAG form. Recent studies showed that bioavailability of FA, including LC-PUFA esterified as TAG may be lower than that esterified as PL [58, 59; but see 60]. Thus, distribution of LC-PUFA in major lipid classes should be further addressed in studies of nutritional quality of various fish products.

5. Conclusions

The studied fish with similar feeding spectra were identified similarly by a multivariate analysis of FA profiles of total lipids, TAG and PL. Marker FA characteristic of diverse food sources (benthic, terrestrial, etc.), accumulated in nearly similar proportions within TAG and PL, and thereby allow to use both these fractions, as well as total lipids, for identification of food sources of wild fish. The found incorporation of the food FAs same fatty acid markers in structural polar lipids deserves further studies. Regarding contribution of TAG and PL into content of essential LC-PUFA of the taxonomically closely related fish species of order Salmoniformes, we found that content of EPA+DHA esterified as PL was nearly invariable, presenting presumably a species- (taxon-) specific physiologically optimal level. In contrast, content of EPA+DHA esterified as TAG greatly varied among the studied fish and provided most contribution to total EPA+DHA content in the fatty fish species, charr, whitefish and muksun. We can conclude that EPA+DHA-rich fish species likely accumulate these nutritionally valuable compounds predominately in TAG form.

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