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Comparison of fatty acid composition in major lipid classes of the dominant benthic invertebrates of the Yenisei river

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Abstract

The composition and content of fatty acids (FAs) in total lipids, triacylglycerols (TAG) and polar lipids (PL) in dominant groups of benthic invertebrates: gammarids (Gammaridae, Amphipoda), chironomid larvae (Chironomidae, Diptera), caddisfly larvae (Trichoptera) and mayfly larvae (Ephemeroptera) were studied in the Yenisei river. For the first time data on the FA composition of species belonging to Trichoptera (Insecta) are presented. The groups of aquatic insect larvae and gammarids weakly differed in total content of essential polyunsaturated fatty acids (PUFAs). Hence, the strong invasion of gammarids which occurred in the last decades in the Yenisei river should not result in a decrease in potential yield of essential PUFA in the ecosystem and corresponding decrease in food resource quality for fish in respect to PUFA content. Significant differences in biomarker FAs in TAG were found which correlated to specific food sources. Different levels of long-chain PUFA in PL of the invertebrates are discussed in relation to the genetic ability of particular taxa to form these FAs.

Keywords: Fatty acids; Triacylglycerols; Polar lipids; Gammarids; Aquatic larvae of insects

1. Introduction

The specificity of fatty acid (FA) synthesis and composition in different taxonomic groups is the basis for their wide use as biochemical markers of trophic and metabolic interactions in aquatic ecosystems (Desvilettes et al., 1997; Leveille et al., 1997). FA markers have been used to map the transfer of the organic matter through aquatic food webs and understand diet patterns of the aquatic animals (Ederington et al., 1995; Gladyshev et al., 1999, 2000).

Recently, along with biomarker significance of FA in aquatic ecosystems an important role of some polyunsaturated fatty acid (PUFA), which are essential components in nutrition of aquatic invertebrates and fish, are emphasized (Brett and Muller-Navarra, 1997; Muller-Navarra et al., 2000). It should be noted that the essential PUFA are of large physiological importance for animals of different taxonomic levels, including humans (Arts et al., 2001; Lauritzen et al., 2001; Broadhurst et al., 2002). Regular shortage of PUFA in the diet results in development and progression of cardiovascular disease in humans (Arts et al., 2001). In addition, the significance of docosahexaenoic acid (DHA) for normal brain/neural growth and development is intensively discussed (Lauritzen et al., 2001; Broadhurst et al., 2002). However, it has been pointed out (for instance by the British Nutrition Foundation) that consumption

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of essential PUFAs is insufficient even in Western developed countries (Arts et al., 2001). Fish and aquatic invertebrates are one of the main sources of the PUFA in human nutrition, therefore, aquatic ecosystems are considered as an important resource of essential PUFAs (Steffens, 1997). Such findings have resulted in the suggestion that researches should attempt to measure the total yield of PUFA in aquatic ecosystems (Arts et al., 2001).

Studies of yields of PUFA and their transfer within aquatic food webs must include an exact knowledge of FA contents in natural animal populations with careful consideration of taxonomic position, age and diet of each species.

Benthic invertebrates (zoobenthos) are one of the most important components of riverine ecosystems and a major food resource for fish. There are a number of reports of FA composition and content in freshwater zoobenthos in lakes, ponds and streams (Ghioni et al., 1996; Goedkoop et al., 1998, 2000; Gladyshev et al., 1999; Arts et al., 2001), whereas such data for large rivers are scarce.

In the middle and upper flow of the Yenisei river, the dominant groups of zoobenthos are crustaceae (gammarids) and the larvae of insects: chironomids, mayflies and caddisflies (Greze, 1957; Gladyshev and Moskvichova, 2002). The aim of present work was to carry out a comparative analysis of the FA composition and content in the dominant taxonomic groups of zoobenthos in the Yenisei river. It was planned to evaluate significance of the groups of zoobenthos as essential PUFA sources for fish. In addition, the FA composition of major lipid classes was studied to elucidate their potential food resources and to preliminarily estimate a genetic ability of these aquatic invertebrates to synthesize PUFA.

2. Investigated area, materials and methods

Samples of zoobenthos were collected at five stations during the ice-free season from the main channel of the Yenisei river (Siberia, Russia) alone approximately an 800 km reach extending from Krasnoyarsk city (56°00′N, 92°40′E) to the mouth of the Podkamennaya Tunguska river (60°57′N, 89°40′E). Organisms were sampled using a standard hydrobiological device—a Dulkate scraper hand-net taking bottom samples from the depth of 0.5–2.5 m. At each station several specimens belonging to four major taxonomic groups: gammarids (Gammaridae), mayfly larvae (Ephemeroptera), caddisfly larvae (Trichoptera) and chironomid larvae (Chironomidae), were collected. Specimens of different ages and species were pooled. Immediately after sorting, the live animals were placed into beakers with tap water for 2 h to empty their guts. Then the animal's body surfaces were gently wiped with filter paper and the animals were weighed; next, the animals were placed into 5 ml of chloroform/methanol (2:1, v/v). A fixed volume of an internal standard solution (C 19:0) was added. The samples were kept at −20 °C until further analysis.

Lipids were extracted with chloroform/methanol (2:1, v/v) 3 times simultaneously with mechanical homogenization of the tissues with glass beads. The combined lipid extracts were filtered, dried by passing through anhydrous Na_2SO_4 layer and evaporated at 35 °C. A part of the lipid extract was subjected to acidic methanolysis as described previously (Gladyshev et al., 2000). Another part of the lipid extract was separated on a TLC 6×6 cm microplate with solvent system for neutral lipids: hexane/diethyl ether/acetic acid (85:15:1, v/v/v). Lipid spots were identified by comparing their R_f with those of standards (Sigma, Serva) (Kalachova et al., 2001). Silica gel, containing triacylglycerols (TAG) and polar lipids (PL) was scraped from the plates and placed into 3 ml of diethyl ether (TAG) or of diethyl ether/ethanol (1:1, v/v) (PL) for 20 min. Then, to remove the silica gel, the solutions were filtered through a glassfiber GF/C filter and fixed volumes (20–100 μl) of internal standard (C 19:0) solution of 0.5 mg/ml were added. After solvent evaporation, the acidic methanolysis was carried out to prepare fatty acid methyl esters (FAME) of the FA in the two lipid classes.

FAME were analysed on a gas chromatograph equipped with a mass spectrometer detector (GCD Plus, Hewlett-Packard, USA) and a 30-m long×0.32-mm internal diameter capillary column HP-FFAP. The column temperature programming was as follows: from 100 to 190 °C at 3 °C/min, 5 min isothermally, to 230 °C at 10 °C/min, and 20 min isothermally. Other instrumental conditions were as described elsewhere (Gladyshev et al., 2000). Peaks of FAME were identified by their mass spectra compared to those in the database (Hewlett-Packard) and to those of available

authentic standards (Sigma). Positions of double bonds in monoenoic acids were determined by GS-MS of FAME dimethyldisulphide adducts prepared as described elsewhere (Christie, 1989). To determine double bond positions in polyenoic acids, GC-MS of dimethyloxazoline derivatives of FA was used. The dimethyloxazoline derivatives of FA were prepared as follows (Spitzer, 1997): 0.2 ml of 2-amino-2-methyl-1-propanol (Sigma) was added to the saponified lipids, the flask was filled with helium, tightly closed and heated at 170–190 °C for 1.5 h. Then the reaction mixture was diluted with distilled water, acidified and the dimethyloxazoline derivatives of FA were extracted by hexane:acetone (96:4, v/v).

Mean contents of FA for each taxonomic group of animals and their standard errors were calculated summarizing all sampling stations along the river. Then single-factor ANOVAs for each FA per cent content according to (Campbell, 1967) were carried out as follow. Four taxonomic groups were taken as levels. Total number of samples was 20 (for TAG) and 19 (for PL). Total, residual and between levels sums of squares (s.s.) were calculated. Importance degree of the factor investigated,

$$f_x = (\text{between levels s.s.}/\text{total s.s}) \times 100\%,$$

and the variance ratio, F, were calculated. If $f_x > 50\%$ and $F > F_{st}$ (at 0.05 or 0.01 probability levels), it means that importance of the factor investigated (effect of the taxonomic group) was higher than that of all the other factors and statistically significant, i.e. variations of FA content within each of the groups were small and negligible, but differences of means amongst the groups were high and significant at P probability level.

3. Results

Samples of Gammaridae were represented by *Eulimnogammarus* (*Philolimnogammarus*) *viridis* Dybowsky and *Gmelinoides fasciatus* Stebb., larvae of Ephemeroptera by *Ephemera lineata* Eaton, *Ephemerela ignita* Poda, and *Pothamantus luteus* Linne, larvae of Trichoptera by *Apatania crymophila* McLachlan and *Aethaloptera evanescens* Brauer, and larvae of Chironomidae by *Diamesa baicalensis* Tshernovskij and by three other species which could not be identified.

Qualitative lipid composition of the taxonomic groups of animals was very similar. TLC revealed TAG as major lipid fraction and distinct fractions of PL and sterols in each animal group. Moreover, slight amounts of diacylglycerols and sterol esters were found in gammarids and caddisflies.

Fifty-three FA species were identified in zoobenthos samples (Table 1). Mean total FA content in biomass was rather variable with respect to taxonomic group, although there were no statistically significant differences amongst the groups of animals ($f_x = 17.3$, $F = 0.3$, $P > 0.05$, see Section 2 for details). The total content of long-chain highly unsaturated ω3 fatty acids (HUFA) (20:5ω3 + 22:5ω3 + 22:6ω3) in biomass was calculated. Maximum HUFA levels were found in mayflies and minimum levels in chironomids (Table 1), however, there were no significant differences of HUFA content amongst the groups ($f_x = 6.3$, $F = 1.0$, $P > 0.05$).

In each taxonomic group palmitic 16:0 was the most abundant (Table 1). Monoenes, 16:1ω9 + ω7 in mayflies and chironomids, and 18:1ω9 in gammarids and caddisflies, were the second dominant FA.

Caddisflies and chironomids were higher in 16-carbon PUFA of ω3, ω4 and ω6 type than the other two taxa (Table 1). It is interesting to remark that in two samples of caddisflies unusual FA, 14:2 and 14:3, were identified (Table 1), which were represented only in TAG. Unfortunately, determination of their double bond positions was not possible because of the trace amounts of these FA. The content of 18-carbon ω3 and ω6 PUFA was higher again in caddisflies and chironomids than in the other two taxa (Table 1). Non-methylene interrupted 18:2 FA, with double bonds in ω5 and ω9, was found and was the highest in mayflies (Table 1).

The content of 20-carbon monoenes was significantly higher in gammarids. Relatively high levels of 20-carbon PUFA of both ω3 and ω6, including essential eicosapentaenoic acid (EPA, 20:5ω3) and arachidonic acid (ARA, 20:4ω6), were characteristic of gammarids and mayflies (Table 1). Gammarids and caddisflies showed substantial level of 22-carbon PUFA, whereas the other two groups had only negligible amounts. In fact, gammarids had much higher level of the essential FA DHA (22:6ω3) and docosapentaenoic acid (DPA, 22:5ω3) as compared with the all insect larvae.

In each taxonomic group the specific bacterial marker FA, odd iso- and anteiso-acids and vaccenic

Table 1
Content of total FAs (mg/g of wet weight) of the taxonomic groups of zoobenthos in the Yenisei river (middle flow), June–August 2001

Fatty acids	Gammaridae	Ephemeroptera	Trichoptera	Chironomidae
12:0	0.07±0.02	0.07±0.03	0.40±0.29	0.22±0.07
12:1	nd	nd	0.04±0.04	nd
13:0	tr	0.01±0.01	0.01±0.01	nd
i14:0	0.01±0.01	nd	0.02±0.02	0.06±0.03
14:0	0.89±0.27	0.56±0.10	1.27±0.35	2.75±0.71
∑14:1ω7+14:1ω5	0.03±0.01	0.03±0.02	0.29±0.14	0.13±0.04
i15:0	0.08±0.03	0.32±0.12	0.11±0.03	0.27±0.08
ai15:0	0.04±0.01	0.09±0.02	0.03±0.01	0.15±0.05
14:2	nd	nd	0.06±0.05	nd
15:0	0.14±0.05	0.39±0.09	0.18±0.04	0.49±0.18
15:1	0.01±0.01	0.04±0.02	nd	0.09±0.07
14:3	nd	nd	0.06±0.04	tr
i16:0	0.02±0.01	0.03±0.01	0.02±0.02	0.07±0.05
16:0	4.00±0.84	5.04±0.53	6.00±1.36	8.46±1.83
16:1ω9+16:1ω7	1.99±0.54	2.96±0.50	2.80±1.16	3.97±1.27
16:1ω6	0.11±0.05	0.36±0.08	0.23±0.11	0.30±0.08
16:1ω5	0.05±0.02	0.03±0.03	nd	0.04±0.04
i17:0	0.07±0.02	0.07±0.01	0.13±0.07	0.17±0.07
16:2ω7	0.01±0.01	nd	nd	0.01±0.01
16:2ω6	0.01±0.01	tr	0.04±0.04	0.05±0.03
16:2ω4	0.22±0.09	0.23±0.05	0.49±0.33	0.55±0.26
16:3ω6	nd	nd	0.14±0.07	0.04±0.02
17:0	0.15±0.04	0.36±0.06	0.25±0.04	0.31±0.12
16:3ω4	0.24±0.12	0.03±0.01	0.27±0.12	0.41±0.19
16:3ω3	0.04±0.02	tr	0.35±0.16	0.12±0.08
17:1ω8+17:1ω6	0.05±0.01	0.11±0.05	0.02±0.02	0.04±0.03
16:4ω3	0.08±0.04	tr	0.90±0.71	0.14±0.06
16:4ω1	0.14±0.06	nd	0.14±0.10	0.23±0.13
18:0	0.78±0.15	1.56±0.18	2.17±0.36	2.49±1.01
18:1ω9	2.53±0.63	1.43±0.22	2.85±0.63	1.88±0.52
18:1ω7	0.63±0.20	1.81±0.22	0.36±0.25	1.57±0.51
18:1ω5	0.04±0.02	0.05±0.05	0.04±0.04	0.16±0.09
18:2ω6	0.50±0.11	0.61±0.11	0.89±0.51	0.90±0.32
18:2Δ9,13	0.02±0.01	0.10±0.03	0.02±0.02	nd
18:3ω6	0.05±0.02	0.08±0.03	0.09±0.06	0.09±0.04
18:3ω3	0.50±0.15	0.54±0.10	1.36±0.81	0.71±0.23
18:4ω3	0.33±0.11	0.20±0.04	0.50±0.23	0.50±0.24
20:0	0.08±0.02	0.14±0.02	0.16±0.04	0.28±0.16
20:1ω9	0.05±0.02	0.01±0.01	0.01±0.01	0.02±0.01
20:1ω7	0.04±0.01	0.02±0.01	0.02±0.02	nd
20:2ω6	0.05±0.02	0.02±0.01	nd	nd
20:3ω6	0.02±0.01	0.03±0.01	0.02±0.01	tr
20:4ω6	0.24±0.06	0.46±0.12	0.12±0.07	0.11±0.05
20:3ω3	0.06±0.02	0.02±0.01	nd	nd
20:4ω3	0.05±0.01	0.04±0.01	0.03±0.02	tr
20:5ω3	2.00±0.50	2.56±0.70	1.75±0.72	1.69±0.61
22:0	0.08±0.02	0.13±0.02	0.10±0.04	0.22±0.13
22:4ω6	0.01±0.01	nd	nd	nd
22:4ω3	0.01±0.01	nd	nd	nd
22:5ω6	0.04±0.01	nd	0.03±0.03	nd
22:5ω3	0.10±0.04	nd	0.01±0.01	tr
22:6ω3	0.30±0.11	0.01±0.01	0.13±0.09	0.01±0.01
HUFA	2.38±0.62	2.57±0.70	1.89±0.80	1.70±0.62
Total	16.91±3.73	20.55±2.45	24.88±7.45	29.71±6.68

Figures are means for six (Gammaridae), five (larvae of Ephemeroptera), four (larvae of Trichoptera) and six (larvae of Chironomidae) samples. HUFA, sum of 20:5ω3, 22:5ω3 and 22:6ω3; tr, values are <0.005 mg/g.

Table 2
Single-factor ANOVAs for per cent content of FAs (% of total; total, mg/g of wet weight) in TAG of the taxonomic groups of zoobenthos in the Yenisei river (middle flow), June–August 2001

Fatty acid	Gammaridae	Ephemeroptera	Trichoptera	Chironomidae	f_x	F
12:0	0.8±0.23	0.5±0.14	2.1±1.26	0.6±0.24	19.3	1.3
14:0	6.6±0.67	3.5±0.38	6.5±1.29	8.5±1.29	45.7	4.5
14:1ω7	0.2±0.10	0.1±0.07	1.0±0.10	0.4±0.18	68.0	11.3*
i15:0	0.7±0.25	2.3±0.73	0.5±0.14	0.6±0.23	44.2	4.2
ai15:0	0.4±0.06	0.6±0.18	0.4±0.19	0.3±0.13	11.5	0.7
15:0	1.4±0.42	2.3±0.44	1.7±0.80	1.2±0.52	11.7	0.7
15:1	0.1±0.06	0.2±0.07	0.2±0.20	0.2±0.09	3.7	0.2
16:0	25.2±2.00	30.6±1.99	30.0±2.95	24.7±1.65	26.0	1.9
16:1ω9+ω7	14.6±0.35	17.9±1.83	12.1±2.28	13.3±2.98	24.9	1.8
16:1ω6	0.9±0.25	2.0±0.34	1.5±0.51	1.6±0.76	15.7	1.0
16:1ω5	0.2±0.17	0.6±0.37	0.1±0.09	0.1±0.10	15.4	1.0
i17:0	0.4±0.11	0.4±0.06	0.3±0.12	0.3±0.06	7.3	0.4
16:2ω6	0.2±0.06	0.1±0.04	0.1±0.07	0.3±0.10	23.7	1.7
16:2ω4	1.4±0.34	1.4±0.17	1.3±0.62	2.2±0.68	11.1	0.7
16:3ω6	tr	nd	0.4±0.17	0.1±0.07	41.6	3.8
17:0	0.9±0.26	1.4±0.25	1.1±0.29	0.9±0.55	11.0	0.7
16:3ω4	1.5±0.59	0.3±0.10	0.7±0.33	1.6±0.62	25.9	1.9
17:1	0.2±0.08	0.4±0.11	nd	0.1±0.06	47.9	4.9
16:3ω3	0.2±0.08	tr	1.0±0.43	1.2±0.94	24.4	1.7
16:4ω3	0.5±0.21	tr	2.1±1.18	0.6±0.16	28.9	2.2
16:4ω1	0.7±0.43	tr	0.3±0.17	1.0±0.34	25.8	1.9
18:0	3.7±0.83	4.1±0.68	9.1±2.06	5.1±2.15	36.0	3.0
18:1ω9	13.1±1.87	5.6±0.46	9.5±0.69	7.6±1.68	51.9	5.8*
18:1ω7	3.7±0.32	7.8±0.71	2.6±0.91	4.1±1.06	64.1	9.5*
18:1ω5	0.3±0.13	0.4±0.27	nd	0.1±0.08	19.0	1.3
18:2ω6	2.8±0.44	2.0±0.14	3.3±0.73	3.8±1.11	20.8	1.4
18:2Δ9,13	0.2±0.04	0.5±0.10	tr	tr	69.2	12.0*
18:3ω6	0.4±0.08	0.4±0.10	0.2±0.12	1.4±0.86	27.1	2.0
18:3ω3	2.8±0.74	1.8±0.31	3.4±1.69	4.2±2.00	9.9	0.6
18:4ω3	2.1±0.57	0.8±0.15	1.4±0.59	2.4±0.91	20.6	1.4
20:0	0.3±0.11	0.3±0.12	0.6±0.26	0.5±0.30	8.0	0.5
20:1ω9	0.4±0.06	tr	tr	0.2±0.06	66.8	10.7*
20:1ω7	0.2±0.05	nd	tr	tr	47.2	4.8
20:2ω6	0.2±0.08	tr	nd	nd	36.2	3.0
20:3ω6	0.1±0.03	0.1±0.03	0.1±0.04	0.1±0.10	7.1	0.4
20:4ω6	0.8±0.15	1.4±0.30	0.4±0.18	0.2±0.15	53.2	6.1*
20:3ω3	0.3±0.10	tr	0.1±0.03	nd	47.2	4.8
20:4ω3	0.3±0.08	0.2±0.05	0.1±0.06	0.1±0.06	32.0	2.5
20:5ω3	9.3±1.47	9.3±1.54	4.6±1.47	9.5±2.45	26.5	1.9
22:0	0.2±0.08	0.1±0.09	0.3±0.21	0.4±0.42	6.1	0.3
22:3	0.2±0.06	tr	tr	0.1±0.05	38.9	3.4
22:5ω6	0.1±0.03	nd	0.1±0.06	tr	24.7	1.7
22:5ω3	0.3±0.08	nd	0.1±0.04	nd	60.1	8.0*
22:6ω3	0.7±0.18	0.1±0.05	0.3±0.18	0.1±0.11	37.5	3.2
Total	5.4±1.60	10.6±3.64	12.0±3.50	7.5±2.89	17.4	1.1

Figures are means for six (Gammaridae), five (larvae of Ephemeroptera), five (larvae of Trichoptera) and four (larvae of Chironomidae) samples. nd, not detected; tr, values are <0.05%.
* $f_x > 50\%$, $F > F_{st}$, and $P < 0.05$ (see text for details).

18:1ω7 (Desvilettes et al., 1997; Navarrete et al., 2000) were present, being more abundant in mayflies and chironomids than in the other two groups (Table 1).

3.1. FA composition of TAG and PL

FAs (% of the total) in the TAG and PL fractions are presented in Tables 2 and 3. The sum of FA

Table 3
Single-factor ANOVAs for per cent content of FAs (% of total; total, mg/g of wet weight) in PL of the taxonomic groups of zoobenthos in the Yenisei river (middle flow), June–August 2001

Fatty acid	Gammaridae	Ephemeroptera	Trichoptera	Chironomidae	f_x	F
12:0	0.3±0.13	0.4±0.16	0.6±0.22	0.7±0.25	15.3	0.9
13:0	tr	0.2±0.11	nd	nd	26.1	1.8
14:0	3.7±1.06	3.1±0.76	3.9±0.99	8.9±1.85	48.4	4.7
14:1ω7	0.3±0.11	0.3±0.10	0.5±0.13	0.3±0.11	9.8	0.5
i15:0	0.7±0.20	0.8±0.18	0.6±0.13	0.5±0.18	6.8	0.4
ai15:0	0.5±0.21	0.5±0.09	0.4±0.15	0.4±0.13	1.1	0.1
15:0	1.9±0.74	1.9±0.44	1.8±0.58	1.4±0.25	2.4	0.1
15:1	0.2±0.11	0.7±0.30	0.2±0.18	0.2±0.12	25.9	1.7
16:0	23.0±4.34	21.6±3.17	25.1±3.07	30.3±1.56	17.2	1.0
16:1ω9+ω7	5.0±0.85	7.7±0.76	5.7±0.65	8.7±1.26	42.7	3.7
16:1ω6	0.8±0.46	0.5±0.09	0.3±0.18	1.2±0.20	18.9	1.2
i17:0	0.3±0.12	0.3±0.04	0.3±0.06	0.3±0.09	2.0	0.1
ai17:0	0.2±0.15	0.2±0.13	0.4±0.14	0.1±0.09	11.1	0.6
16:2ω4	0.2±0.06	0.3±0.06	0.2±0.15	0.9±0.10	67.6	10.4*
17:0	1.6±0.43	2.0±0.19	1.7±0.28	1.2±0.20	17.5	1.1
16:3ω4	tr	nd	0.2±0.09	0.5±0.09	74.5	14.6*
17:1	0.1±0.07	0.2±0.15	nd	0.1±0.05	19.0	1.2
16:3ω3	nd	tr	0.2±0.06	0.1±0.06	43.9	3.9
16:4ω3	nd	nd	0.5±0.25	0.4±0.30	36.8	2.9
16:4ω1	nd	nd	nd	0.1±0.08	42.4	3.7
18:0	14.0±2.43	14.5±1.71	14.4±2.71	14.6±1.22	0.4	0.0
18:1ω9	12.1±1.99	12.0±1.31	14.5±1.82	6.6±0.88	39.9	3.3
18:1ω7	4.9±1.08	7.8±1.06	2.4±0.24	5.2±1.48	44.7	4.0
18:1ω5	0.4±0.28	0.6±0.30	nd	0.8±0.33	20.3	1.3
18:2ω6	4.5±1.09	4.7±0.81	3.7±0.81	4.5±1.04	3.4	0.2
18:2Δ9,13	0.2±0.08	tr	nd	0.2±0.11	22.5	1.5
18:3ω6	0.1±0.08	0.3±0.12	0.2±0.10	0.2±0.08	12.8	0.7
18:3ω3	2.4±0.78	3.1±0.74	5.9±2.29	2.4±0.73	25.9	1.7
18:4ω3	0.3±0.12	0.4±0.25	0.8±0.37	0.4±0.22	16.1	1.0
20:0	2.0±0.46	2.4±0.37	3.2±0.48	3.5±0.49	31.9	2.3
20:1ω9	0.3±0.15	tr	0.1±0.11	0.2±0.09	16.5	1.0
20:1ω7	0.1±0.12	0.1±0.04	tr	tr	5.8	0.3
20:2ω6	0.4±0.22	nd	0.1±0.14	nd	29.0	2.0
20:4ω6	2.5±0.80	2.1±0.42	0.9±0.39	nd	41.4	3.5
20:3ω3	0.4±0.19	tr	0.1±0.10	nd	29.4	2.1
20:4ω3	tr	0.1±0.04	tr	nd	13.0	0.7
20:5ω3	11.8±3.93	9.2±2.21	7.4±1.81	3.3±0.47	23.4	1.5
22:0	0.9±0.24	1.9±0.34	2.5±0.52	1.5±0.85	31.3	2.3
22:5ω6	0.3±0.19	nd	0.1±0.06	nd	26.7	1.8
22:5ω3	0.8±0.36	nd	0.2±0.19	nd	36.3	2.8
22:6ω3	2.5±1.11	nd	0.8±0.80	0.2±0.16	33.0	2.5
Total	0.9±0.14	1.3±0.18	1.3±0.43	0.8±0.04	23.4	1.5
TAG/PL	6.2	8.4	9.0	9.4		

Figures are means for six (Gammaridae), five (larvae of Ephemeroptera), four (larvae of Trichoptera) and four (larvae of Chironomidae) samples. nd, not detected; tr, values are $<0.05\%$.

* $f_x > 50\%$, $F > F_{st}$, and $P < 0.05$ (see text for details).

in TAG and PL was significantly lower than FA content in total lipids (compare Tables 1–3). We suspect that this was due to a loss of the material during sample processing.

As suggested, the loss was proportional to the total quantity of the material, therefore, ratio of FA in TAG and PL seems to be a more reliable estimation than their absolute amounts (Table 3). The TAG/PL FA ratio was $\sim 1.5\times$ higher in all insect larvae than that in gammarids (Table 3).

The FA composition of TAG was similar to that of total lipids (Tables 1 and 2). This was explained

by strong predominance of TAG in the lipids (Tables 2 and 3). The percentages of several FA, 14:1ω7, 18:1ω9, 18ω7, 18:2Δ9,13; 20:1ω9, 20:4ω6 and 22:5ω3, were significantly ($f_x > 50\%$, $F > F_{st}$, $P < 0.05$) different amongst the taxa. Note that almost all 20- and 22-carbon PUFA, including minor, were present in TAG (Table 2).

There were fewer FA in the PL than in the total lipids—41 FA (Table 3). Percentages of only two PL FA, 16:2ω4 and 16:3ω4, were significantly different amongst the animal groups in comparison to six significant FA of TAG (Tables 2 and 3). The differences in PL FA were the most prominent between gammarids and chironomids. The later group showed a lack of 20- and 22-carbon HUFA, except 20:5ω3, while gammarids were characterized by relatively high levels of these acids in PL (Table 3). Note that percentages of essential DHA, DPA in PL of gammarids were much higher than in the other taxa.

Monounsaturated and polyunsaturated 16-carbon acids of ω7, ω4 and ω1 types are considered typical biomarkers of diatom algae, polyunsaturated 16-carbon ω6, ω3 acids can be considered as markers of green algae (Cobalas and Lechado, 1989; Napolitano and Ackman, 1989; Leveille et al., 1997), and iso and anteiso 15, 17-carbon acids and vaccenic 18:1ω7 as bacterial markers (Navarrete et al., 2000). The totals of the percentages of FA, which are markers of diatoms, green algae and bacteria, for TAG and PL were calculated (Figs. 1 and 2). Calculations of the total were also made for the major structural groups of FA: saturated long-chain (18:0 + 20:0 + 22:0), 18-carbon PUFA (18:2ω6 + 18:3ω6 + 18:3ω3), 20-carbon ω3 PUFA, 22-carbon ω3 PUFA, 20- and 22-carbon ω6 PUFA, and short-chain acids (12:0 + 14:0 + 14:1) (Fig. 1). It should be remarked that FA in these groups showed similar distribution in the taxa.

Total relative content of 16-carbon FA which are markers for diatoms, was higher in TAG by a factor of two as compared with that in PL of each group, with no evident differences amongst the groups (Fig. 1). FA—markers for green algae were accumulated mainly in TAG. In contrast to the FA—diatom markers, their percentages varied considerably amongst the animal taxa, being much higher in caddisflies and chironomids (Fig. 1).

The relative content of 18-carbon PUFA was increased in PL as compared with the TAG in each group, except chironomids (Fig. 1). The levels of these FA in the groups were similar, excluding TAG of mayflies. Mayflies showed the lowest levels of both 18- and 16-carbon PUFA of ω3 and ω6 type, especially in TAG.

The total percentages of saturated 18–22-carbon FA of all the taxa were significantly higher in PL than in TAG (Fig. 1). Caddisflies showed the highest value of these FA. Levels of 20-carbon ω3 PUFA in both lipid fractions in each group were similar, except chironomids (Fig. 1). Percentage of these FA in PL of chironomids was lower by factor of 2× than in other taxa, whereas their percentage in TAG was of the same level (~10%).

PUFA of ω6 with 20- and 22-carbon accumulated mostly in PL of the groups, except chironomids. In chironomids long-chain ω6 PUFA were found only in TAG, and their percentages were much lower than in other groups (Fig. 1). 22-Carbon ω3 PUFA strongly accumulated in PL of gammarids and caddisflies, occurring only in trace amounts in chironomids and mayflies (Fig. 1).

Caddisflies and chironomids were characterized by relatively high contents of short-chain FA (Fig. 1). Caddisflies accumulated these FA mostly in TAG, whereas in chironomids there were similar percentages in both TAG and PL.

Maximum total content of bacterial marker FA was found in mayflies with strong enrichment of odd iso- and anteiso-acids in TAG and similar levels of vaccenic 18:1ω7 in both TAG and PL fractions (Fig. 2).

4. Discussion

It has been suggested that PUFA content in overwintering larvae of aquatic insects is considerably higher than in other aquatic invertebrates, e.g.—amphipods (Goedkoop et al., 2000). We also have found that larvae of *Chironomus plumosus* had an increased level of EPA in comparison to the gammarid *Gammarus lacustris*, inhabiting a small reservoir in a second tributary of the Yenisei (Gladyshev et al., 1999).

Long-term changes in zoobenthos species composition of the Yenisei has been occurring in part because of a natural invasion of gammarids from Baikal lake (through the Angara river). However, the total biomass of zoobenthos has not markedly changed. In 1940–1950, there was a strong dominance of overwintering insect larvae (mayflies and caddisflies) in the zoobenthos of the Yenisei (Greze, 1957), whereas now the major dominants

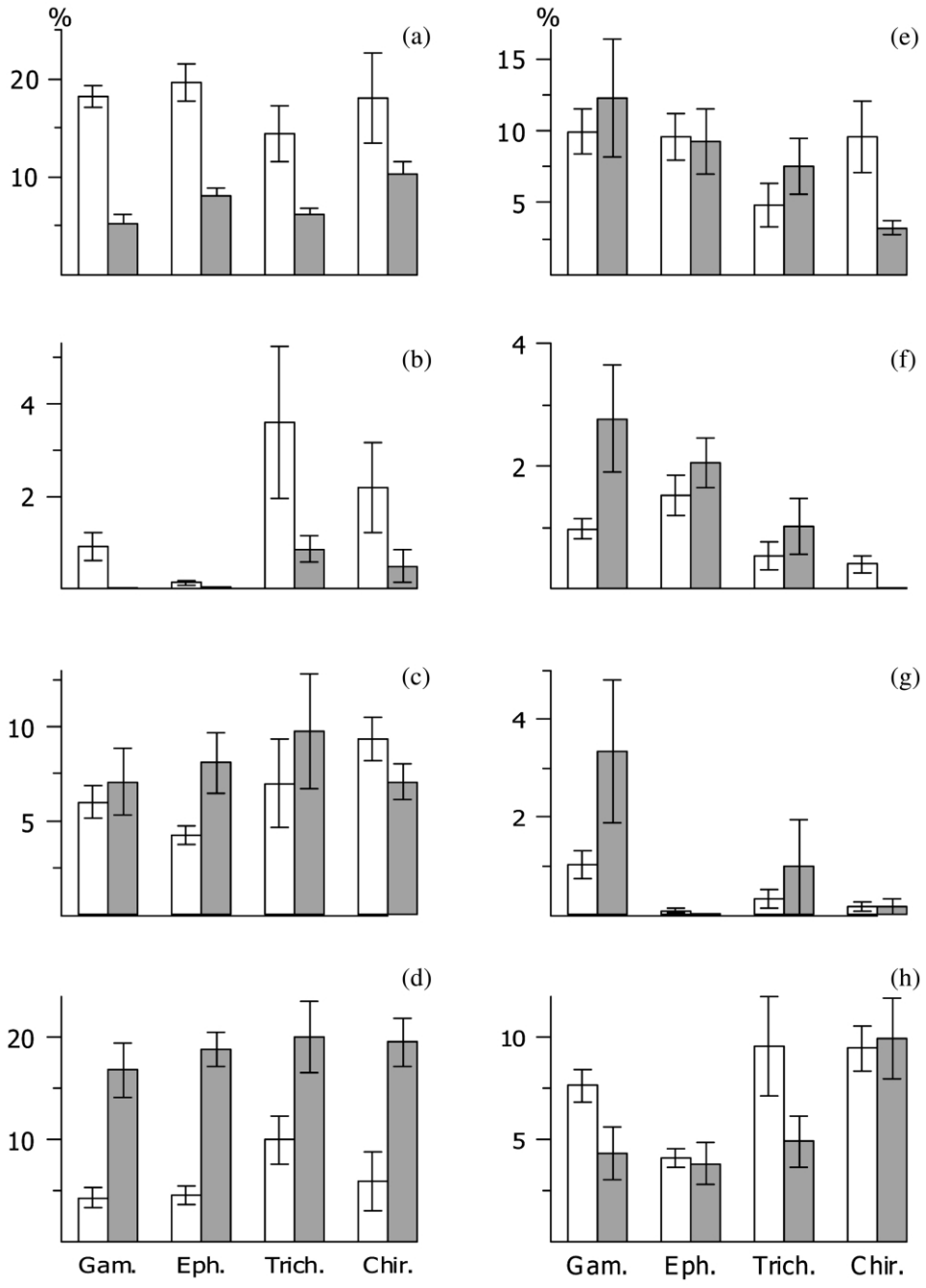

Fig. 1. Mean content of FA groups (% of total) in TAG (light bars) and in PL (dark bars) of benthic invertebrates in the Yenisei river, 2001. (a) 16-Carbon specific diatom marker FA (16:1ω7+16:2ω4+16:3ω4+16:4ω1); (b) 16-carbon specific green alga marker FA (16:2ω6+16:3ω6+16:3ω3); (c) 18-carbon PUFA (18:2ω6+18:3ω6+18:3ω3); (d) saturated 18–22-carbon FA (18:0+20:0+22:0); (e) 20-carbon ω3 PUFA (20:3ω3+20:4ω3+20:5ω3); (f) 20- and 22-carbon ω6 PUFA (20:3ω6+20:4ω6+22:4ω6+22:5ω6); (g) 22-carbon ω3 PUFA (22:3ω3+22:4ω3+22:5ω3+22:6ω3); (h) short-chain FA (12:0+14:0+14:1).

are the gammarids *E. viridis* and *G. fasciatus* (Gladyshev and Moskvichova, 2002). Evidently, if aquatic insect larvae in all places of the river have increased levels of essential PUFA, their exclusion by gammarids may lead to a substantial decrease in quality of food resources for fish. To

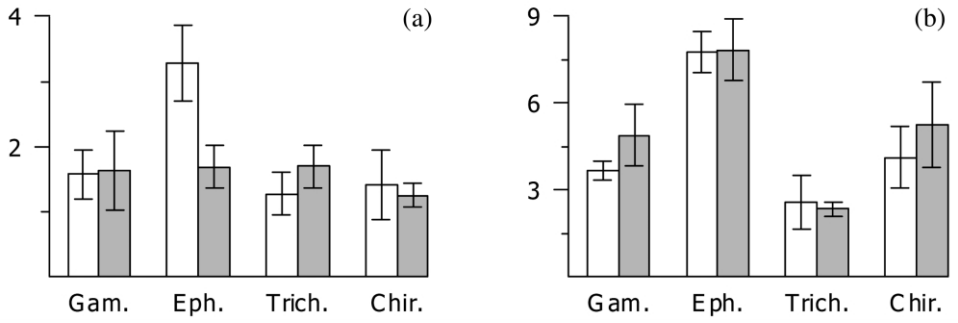

Fig. 2. Mean content (% of total) of specific bacterial marker FA in TAG (light bars) and PL (dark bars) of benthic invertebrates in the Yenisei river, 2001. (a) Branch-chain odd FA (i15:0 + ai15:0 + i17:0); (b) 18:1ω7.

substantiate this suggestion, one needs exact data on FA content and composition in the different zoobenthos taxa.

However, literature data on FA in freshwater amphipods and aquatic insect larvae are very scarce. Moreover, data for taxonomic group such as Thichoptera are absent in available literature. In the present study the invader species, the group of gammarids, showed relatively high levels of essential EPA and ARA, and much higher levels of essential DHA and DPA than all the groups of aquatic insect larvae. Therefore, it can be concluded that the invasion of gammarids occurring in the river might not result in a considerable decrease in quality of food resources for fish in respect to essential PUFA content. Nevertheless, it should be emphasized that PUFA content is only one aspect for an estimation of the food quality. Gammarids could be less available for fish and may be predators toward other macroinvertebrates including Ephemeroptera and Trichoptera thereby decreasing the total food sources (MacNeil et al., 2000). Our conclusion on high food quality of the invasive gammarids is evidently very limited and cannot represent a complete estimation of effect of the invasion on the food sources for fish in the investigated area. Moreover, all insect larvae had somewhat higher content of total lipids and TAG in biomass than gammarids. This is in accordance with literature indicated more intensive accumulation of lipids during juvenile growth stages of invertebrates (Bychek and Gushchina, 1999; Cripps et al., 1999).

FA composition in TAG of aquatic animals is mostly related to input of dietary FA, while FA composition of PL is more strongly determined genetically by species-specific biosynthesis (Napolitano and Ackman, 1989; Falk-Petersen et al., 2001). This information coupled with data on specific biomarker FA might help to elucidate diet preferences in different taxonomic groups as well as their capacity for PUFA biosynthesis, although, such conclusions are tentative. All studied groups of zoobenthos accumulated 16-carbon PUFA, 16:1ω7 and 14:0 in TAG, hence, a significant part of their diet is likely to comprise diatoms. Higher content of green alga FA biomarkers (16- and 18-carbon ω3 and ω6 PUFA) in TAG of caddisflies and chironomids may be considered as an evidence for abundant greens in their diet. Previously, we have demonstrated that chironomids in a small reservoir also preferred the food particles with high content of ω6 and ω3 PUFA, originating from green algae and cyanobacteria (Gladyshev et al., 1999).

Mayflies accumulated bacterial marker FA in their TAG, suggesting a more intensive ingestion of detritus and sediment particles enriched in bacteria. Moreover, there was rather large content of vaccenic acid, 18:1ω7, which is also a typical bacterial marker (Navarrete et al., 2000), in PL of each zoobenthic group. This might reflect intensive exchange of acyl groups between TAG and PL or the possibility of biosynthesis of this FA by the aquatic invertebrates. It should be noted that in the literature there is no evidence for existence of desaturases converting 18:0 into 18:1ω7 in animals (Tocher et al., 1998). However, in some terrestrial insects desaturase Δ11 using 16:0 as a substrate has been found (Svatos et al., 1999), but the possibility of use of 18:0 as a substrate by this enzyme has not been considered yet.

Strong variation in FA composition in TAG is likely related to different selective feeding of the

groups of zoobenthos. Despite the relative similarity in FA composition of PL amongst the taxonomic groups, considerable differences in contents of 20- and 22-carbon PUFA, especially minor, should be noted (Table 3, Fig. 1). We suggest this might reflect taxonomic peculiarities of each group's biosynthetic capacity for long-chain PUFA from dietary 18:2ω6 and 18:3ω3.

Conversion of PUFA in animal tissues may be traced by detecting FA, which are intermediates in biosynthesis of the final products—22:5ω6 and 22:6ω3 (Sprecher, 2000). According to recent studies (Sprecher, 2000) the biosynthesis of ω3 PUFA in animals is as follows:

$$18{:}3\omega3 \rightarrow 18{:}4\omega3 \rightarrow 20{:}4\omega3 \rightarrow 20{:}5\omega3 \rightarrow 22{:}5\omega3 \rightarrow C24\ PUFA \rightarrow 22{:}6\omega3;$$

and biosynthesis of ω6 PUFA is as follows:

$$18{:}2\omega6 \rightarrow 18{:}3\omega6 \rightarrow 20{:}3\omega6 \rightarrow 20{:}4\omega6 \rightarrow 22{:}4\omega6 \rightarrow C24\ PUFA \rightarrow 22{:}5\omega6.$$

In gammarids all the FA-intermediates in the biosynthesis of 22-carbon PUFA of both types (Tables 1–3) were present, additionally, 22:4ω6 (~0.1%) was found in some samples. We suggest that relatively high content of 22-carbon PUFA in these invertebrates might reflect higher activity of their biosynthetic system. As can be concluded from the content of FA-intermediates in the groups of mayflies and caddisflies, these animals were capable to synthesize 20:5ω3 and 20:4ω6 actively, whereas biosynthesis of 22-PUFA was not so active, especially in mayflies.

Contents of long-chain PUFA in PL of animals are known to be higher than those in TAG. Chironomids showed unusual reverse PUFA distribution: percentages of 20:4ω6 and 20:5ω3 were considerably higher in TAG than in PL. Besides this, in chironomids some FA-intermediates were found, therefore, ability for 20-carbon PUFA biosynthesis de novo can not be excluded.

Total FA content of gammarids from the Yenisei river was significantly higher than gammarids from two small eutrophic freshwater ponds (Arts et al., 2001), whereas the percentages of main essential PUFA (18:2ω6, 18:3ω3, 20:5ω3, 22:6ω3) in gammarids from the Yenisei were relatively lower. Gammarids in the Yenisei were characterized by higher content of polyenoic and monoenoic 16-carbon FA, especially diatom markers. These acids accumulated mainly in TAG, hence, they likely originated from the diet enriched in diatoms. It should be noted that gammarids in the freshwater ponds showed higher levels of 18:2ω6 and 18:3ω3 as compared with gammarids in the Yenisei (Gladyshev et al., 1999; Arts et al., 2001). This indicates that food resources were more abundant in these FA in ponds than in the Yenisei river. This is most likely be related to the strong dominance of green, eugleniod algae and cyanobacteria in limnetic waters.

High content of FA, which are markers for diatoms, in TAG has also been reported for the marine benthic amphipod *Corophium volutator* (Napolitano and Ackman, 1989). However, marine and saltwater amphipods were higher in levels of long-chain ω3 PUFA, in comparison to gammarids in the Yenisei river (Napolitano and Ackman, 1989; Gladyshev et al., 2000); this being a typical difference between freshwater and saltwater animals (Steffens, 1997).

Mayflies in the Yenisei and those (although, adults) in work of Ghioni et al. (1996) were similar in high levels of 18:1ω7 and 20:5ω3, and trace levels of 22:6ω3. The 16-carbon PUFA were more abundant in mayflies of the Yenisei river, reflecting the strong diatom input to their diet.

Comparison of total FA composition in chironomids from the Yenisei river and three species of *Chironomus* and *Procladius* genera from lake Erken (Goedkoop et al., 2000) showed that chironomids in both aquatic ecosystems contain very little 22-carbon PUFA, whereas other benthic invertebrates in both ecosystems accumulated them, especially DHA. Chironomids in the Yenisei were higher in 16-carbon PUFA, 14:0 and 16:1ω7, while relatively lower in 18-carbon PUFA than *Chironomus* species in lake Erken. These data indicate that a marked part of chironomid diet in the Yenisei comprised diatoms. The lack of 22-carbon PUFA was likely related to weak enzymatic ability of this taxonomic group to convert 20:5ω3 to 22:6ω3. The suggestion is in a good accordance with the Goedkoop et al. (2000) data.

Thus, despite diverse species and age composition of samples and collecting them on stations situated in long-distance places and in different months of the vegetation season, evident differences in FA composition amongst the zoobenthos taxonomic groups of the Yenisei were found. Different FA composition in studied animals was likely explained by two main reasons: (1) genetic capacities in PUFA biosynthesis, and (2) different

food sources due to selective feeding of these groups.

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