Chemistry of Natural Compounds, Vol. 46, No. 6, 2011

TRANSFORMATION THROUGH THE FOOD CHAIN OF LAKE BAIKAL HYDROBIONTS FATTY ACIDS

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UDC 547.915

The fatty-acid composition of tissues from Lake Baikal hydrobionts involved in the food chain such as Baikal seals, fish, and amphipods was studied. GC–MS detected more than 40 fatty acids with different degrees of unsaturation in tissues of Baikal hydrobionts. It was concluded from a comparison of the fatty-acid compositions of tissues from the studied animals that the formation mechanism of hydrobionts tissue is complicated and determined not only by the food composition but also the taxonomic formation specifics of the fatty-acid composition. Use of modern statistical data processing methods enabled food webs in the studied chain to be followed and the principal factors influencing the hydrobionts lipid composition to be determined.

Keywords: Lake Baikal endemics, fatty-acid composition, food chain, method of principal components.

Lake Baikal is unique not only for the huge reserves of freshwater, which make up about one fifth of the world's reserves and more than four fifths of Russia's, but also for the number of endemic organisms (endemics). Eighty percent of the lake's biota represent animals and plants with no analogs in any other freshwater bodies on the planet.

The principal factors in the existence of any ecosystem are the trophic or food webs consisting of several levels of primary producers and consumers of different levels. The complexity of food webs in aqueous ecosystems prompted a search for new effective study methods. It is known that the food base of aquatic mammals is the dominant source of lipids, the main energy resource for the growth and reproduction of hydrobionts, and that the lipid composition of food sources determines largely the lipid composition of animals at a higher trophic level [1–4]. Also, it was reported that the reserve lipid composition in tissues of aquatic animals is formed through more complicated mechanisms that are regulated by various metabolic processes and depend on the age, maturity, reproductive state, and external factors [5–9]. Thus, the principal parameters influencing the fatty-acid composition of hydrobionts must be elucidated and new approaches to studying the lipid composition of hydrobionts and methods for interpreting the resulting data must be sought.

Chemometric methods for analyzing fatty-acid (FA) composition, biochemical markers of hydrobionts, are promising for detailed studies of food webs of aquatic organisms [10]. This approach is based on the use of statistical methods for processing multi-dimensional data, e.g., for principal component analysis (PCA) and for studies of multi-component natural objects, e.g., mixtures of FAs in living organisms [11–13]. The traditional tools in this area, graphs and diagrams, are poorly suited for visualizing data where more than three interrelated quantities must be protrayed. We mean by "visualization of data" that approach to presenting a multi-dimensional distribution of data in a two-dimensional plane that displays at least qualitatively the principal trends inherent to the original distribution such as its topological features, internal dependencies among attributes, information about the location of data in the original space, etc. The first choice for visualizing most data is an orthogonal projection on the plane of the two principal components (PC1, PC2, or PC3). The projection plane is essentially a planar two-dimensional screen positioned such that the data are displayed with the smallest distortions [14–16].

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0009-3130/11/4606-0857

TABLE 1. Systematic Composition of Lake Baikal Fish

Taxon level	Taxon				
Order	*Salmoniforrnes*			*Scorpoeniformes*	
Suborder	*Salmonidei*			*Cottoidei*	
Family	*Coregonidae*		*Thymallidae*	*Comephoridae*	
Genus	*Coregonus*		*Thymallus*	*Comephorus*	
Species	*C. baicalensis* (Baikal whitefish)	*C. migratorius* (Baikal omul)	*Th. baicalensis* (Baikal grayling)	*C. baicalensis* (Greater livebearing sculpin)	*C. dybowski* (Lesser livebearing sculpin)

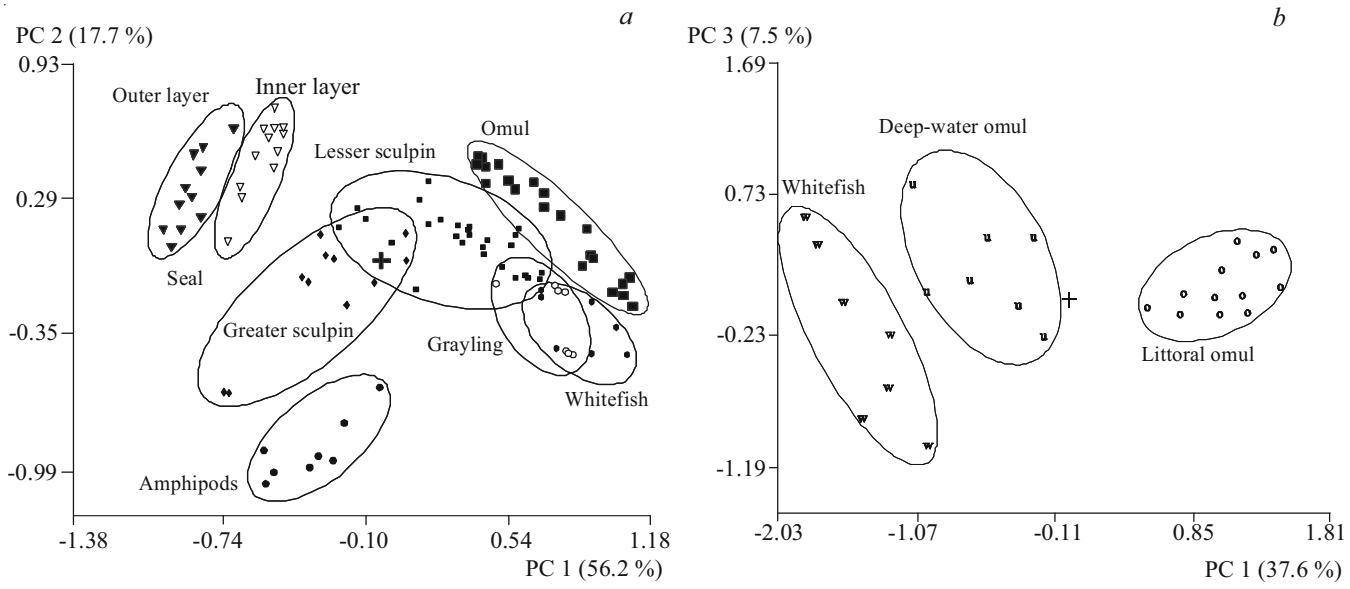

Fig. 1. Principal component analysis of fatty-acid composition in samples of subcutaneous fat of Baikal seal, fish, and amphipods (*a*) and the genus *Coregonus* (*b*).

The FA compositions of lipids from subcutaneous fatty tissue of Baikal seal *Phoca sibirica* G., the closing link of the Lake Baikal trophic chain, and muscle tissue of principal objects of the food chain, several lake fish such as Baikal sculpin (greater and lesser) *Comephorus baicalensis* P. and *C. dybowski* K., Baikal omul *Coregonus migratorius* G., whitefish *C. baicalensis* D., and grayling *Thymallus baicalensis* D. (Table 1) in addition to deep-water Baikal amphipods *Amphipoda* (Crustacea) were compared in order to study the FA transformation mechanism along the trophic chain. The fatty-acid composition was studied in layers in order to find trends in the FA distribution in the subcutaneous seal fatty tissue. The outer layer was the fat layer (3 mm) closest to the skin; the inner layer, that closest to muscle tissue.

GC–MS detected more than 40 FAs in tissues of Baikal hydrobionts (Table 2). Acids for which the relative amount was greater than 0.1% are listed. The composition of the outer layer in subcutaneous fatty tissue of Baikal seal had higher concentrations of short-chain monounsaturated FA with 14–18 C atoms than the inner layer. Monounsaturated 20–24 FA had a tendency to predominate in the inner layer. The highest concentrations of saturated FAs were found in the inner layer. The relative amounts of polyunsaturated FAs were practically the same through the whole fat layer.

The food base is the main source of FAs for aquatic mammals. Thus, practically all FAs of lipids from muscle tissue of the studied fish were detected in seal subcutaneous fat although at different concentrations (Table 1). Saturated acids (SFA) 16:0 (11.8–20.1%) and 18:0 (2.6-8.7); monounsaturated (MUFA) 16:1n7 (1.5–6.1) and 18:1n9 (4.7–22.4); and polyunsaturated 20:4n6 (2.6–6.1), 20:5n3 (3.8–15.3), and 22:6n3 (8.9–40.9) were present in the highest amounts. Furthermore, the animals themselves have the ability to synthesize SFAs with 14, 16, and 18 C atoms and MUFAs 16:1n7 and 18:1n9 [17]. Thus, the FA composition of mammal subcutaneous fatty tissue is the result of complicated processes involving lipid storage, mobilization, biosynthesis, and transport.

TABLE 2. Fatty-Acid Content in Subcutaneous Fatty Tissue of Baikal Seals and Muscle Tissue of Baikal Fish and Amphopods, % ± SD

FA	Baikal seal		Whitefish	Omul		Grayling	Sculpin		Amphipods
	inner layer	outer layer		littoral	deep-water		greater	lesser	
14:0	4.24±0.65	5.9±0.87	1.34±1.05	3.01±0.99	1.4±0.48	0.98±0.17	2.54±0.92	2.32±1.25	0.97±0.25
14:1n5	1.75±0.54	0.8±0.22	0.11±0.08	0.09±0.03	0.13±0.03	0.01±0.00	0.2±0.05	0.15±0.11	0.04±0.00
i15:0	0.63±0.07	0.77±0.09	0.18±0.11	0.35±0.13	0.09±0.02	0.06±0.04	0.15±0.11	0.52±0.29	0.49±0.09
ai15:0	0.3±0.04	0.34±0.06	0.06±0.01	0.14±0.06	0.05±0.01	0.06±0.01	–	–	0.15±0.05
15:0	0.37±0.04	0.48±0.04	0.24±0.1	0.4±0.04	0.32±0.06	0.14±0.09	2.32±1.25	0.44±0.28	0.55±0.13
16:0	6.13±1.42	10.25±1.11	17.66±3.44	17.86±0.9	20.09±1.35	19.3±1.12	11.82±1.99	16.12±2.31	9.76±1.08
16:1n9	0.75±0.11	0.72±0.16	0.07±0.03	0.09±0.01	0.06±0.01	0.08±0.03	0.44±0.14	0.44±0.26	3.50±0.48
16:1n7	17.36±2.66	12.13±1.01	3.04±1.59	2.93±0.92	1.54±0.33	2.41±0.68	6.13±2.69	3.71±1.69	6.60±1.31
16:1n5	0.32±0.04	0.33±0.03	0.32±0.15	0.39±0.03	0.38±0.14	0.13±0.09	0.35±0.24	0.41±0.26	0.23±0.06
16:2n6	0.94±0.17	1.03±0.19	0.17±0.09	0.42±0.14	0.18±0.09	0.05±0.02	1.27±1.04	0.97±0.54	0.14±0.05
17:0	0.33±0.10	0.52±0.05	0.38±0.19	0.48±0.06	0.61±0.12	0.26±0.19	0.61±0.31	0.73±0.41	0.47±0.11
17:1n9	0.7±0.10	0.56±0.10	0.24±0.07	0.18±0.03	0.15±0.03	0.2±0.03	0.58±0.26	0.4±0.27	0.69±0.10
18:0	0.83±0.28	1.56±0.21	6.39±3.83	4.31±2.01	8.67±2.07	3.96±0.39	2.59±0.25	3.55±0.36	2.65±0.52
18:1n11	0.07±0.03	0.06±0.02	0.1±0.08	0.04±0.02	0.13±0.09	0.05±0.01	0.85±0.55	0.55±0.32	–
18:1n9	22.51±6.38	18.72±8.09	5.67±1.43	5.54±0.85	4.75±0.22	6.54±0.67	22.38±11.92	9.56±2.04	30.27±2.74
18:1n7	5.08±1.20	4.5±1.52	2.61±0.67	1.8±0.19	1.49±0.14	2.68±0.25	5.03±1.86	3.19±0.32	5.41±0.71
18:1n5	0.34±0.04	0.32±0.04	0.18±0.1	0.14±0.02	0.13±0.03	0.13±0.08	0.37±1.07	0.31±0.14	1.36±0.16
18:2n6	4.5±0.56	4.37±0.63	1.28±0.85	2.98±0.64	1.7±0.52	1.07±0.15	2.45±0.7	3.22±0.8	0.62±0.36
18:2n4	0.33±0.03	0.38±0.03	0.16±0.06	0.15±0.03	0.14±0.02	0.09±0.01	1.7±1.09	1.25±1.02	0.13±0.04
18:3n6	0.36±0.07	0.28±0.04	1±0.19	2.65±0.54	1.19±0.29	0.56±0.08	1.89±0.76	2.18±0.7	–
18:3n3	2.64±0.44	3.26±0.65	0.84±0.07	2.05±0.76	0.53±0.15	0.22±0.15	1.71±0.84	1.51±0.72	0.54±0.17
19:0	–	–	0.13±0.09	0.13±0.1	0.19±0.1	0.08±0.02	11.02±5.85	2.67±1.36	0.19±0.04
20:0	0.04±0.02	0.09±0.01	0.04±0.01	0.07±0.02	0.04±0.02	0.04±0.01	0.11±0.02	0.18±0.1	0.26±0.03
20:1n11	0.24±0.08	0.22±0.06	0.36±0.19	0.16±0.05	0.46±0.25	0.16±0.08	1.04±0.81	0.82±0.52	1.48±0.33
20:1n9	0.47±0.10	0.57±0.05	0.27±0.11	0.2±0.06	0.14±0.04	0.2±0.09	0.43±0.06	0.54±0.06	0.89±0.30
20:1n7	0.1±0.02	0.12±0.02	0.1±0.04	0.06±0.02	0.05±0.01	0.06±0.02	–	–	1.02±0.30
20:2n6	0.55±0.12	0.66±0.08	0.29±0.14	0.32±0.05	0.2±0.05	0.28±0.05	0.41±0.16	0.67±0.11	0.34±0.17
20:4n6	1.89±0.34	1.9±0.40	4.89±1.5	4.13±0.86	5.72±0.79	3.8±1.6	2.58±0.87	6.05±1.75	2.07±1.02
20:3n3	0.19±0.02	0.22±0.04	0.15±0.08	0.17±0.06	0.23±0.12	1.1±0.03	0.26±0.16	0.28±0.15	0.57±0.17
20:4n3	0.74±0.25	0.74±0.27	0.69±0.3	0.95±0.31	0.4±0.11	0.29±0.06	0.46±0.11	0.42±0.05	0.17±0.07
20:5n3	3.81±0.89	3.94±1.15	15.32±5.28	8.86±1.36	9.3±1.06	8.55±1.36	3.77±1.37	9.92±2.15	4.55±1.75
22:1n7	–	–	0.28±0.11	0.8±0.02	0.09±0.02	0.11±0.01	–	–	–
22:4n6	0.53±0.22	0.71±0.22	0.31±0.1	0.47±0.19	0.18±0.05	0.37±0.14	0.38±0.11	0.29±0.2	0.23±0.26
22:5n6	1.59±0.68	1.98±0.82	0.17±0.65	2.82±0.38	2.56±0.64	1.32±0.79	0.99±0.44	1.68±0.37	0.27±0.10
22:5n3	4.03±1.10	4.12±1.39	1.77±1.07	2.05±0.37	1.28±0.31	1.73±1.15	0.8±0.33	1.17±0.27	0.39±0.31
22:6n3	10.13±3.28	11.29±3.98	27.31±5.48	30.18±4.16	29.76±4.99	40.9±1.92	8.89±4.07	19.47±4.74	2.45±1.01
24:1n9	0.12±0.07	0.23±0.05	1.31±0.86	1.04±0.4	1.49±0.34	0.9±0.15	0.58±0.12	1.04±0.29	0.23±0.14

The results for the composition of seal fat and lipids from muscle tissue of the studied fish were processed by multivariant analysis of the principal components (PCs). This method of statistical analysis can integrate simultaneously information on all objects (in this instance tissue samples of the studied hydrobionts) and parameters (acid composition) and can produce PC plots (Fig. 1) that project all samples (n-dimensional space) onto a two-dimensional system. The system axes are the first and second (or third) PCs. The use of PCA to study trophic interrelationships enabled food webs in the lake ecosystem to be outlined in detail.

Figure 1 shows that the FA composition is different in the outer and inner fat layers. The difference between the FA compositions of seal subcutaneous fat and its food sources was the smallest for the FA composition of fish and the seal inner fat layer. This indicated that FAs from food are stored in the inner layer, which is the most informative with respect to studying food webs in the seal–food-base chain.

Figure 1 also suggests that the compositions of seal subcutaneous fat and Baikal sculpins are the closest. Therefore, Baikal sculpins are the principal seal food source. The results agree closely with literature data that were obtained by the traditional method of Ivanov (1936, 1938), which consists of rinsing out autolytes of consumed fish from the GI tract of the animals [18–20].

Moreover, food webs in the studied seal–fish–amphipods chain can be followed using the results. Thus, we established that the FA compositions were closest for amphipods and greater sculpin and for lesser sculpin and salmonids (whitefish, grayling, omul) in addition to the studied representatives of the order Salmoniformes (Table 1) among themselves. The FA compositions were different for the series amphipods–omul and amphipods–seal (Fig. 1). Despite differences in the concentrations of individual acids for the series amphipods–lesser sculpin, –grayling, and –whitefish (Table 2), processing the data by PCA enabled information about all FAs of the studied objects to be integrated simultaneously and a positive correlation to be found, i.e., a similarity in the compositions of the studied objects (Fig. 1).

Thus, the similarity in the tissue compositions suggests that Baikal amphipods are the principal food source for sculpins and a significant source for grayling and whitefish. Sculpins, in turn, are a food source for Salmoniformes salmonids. These conclusions are confirmed by hydrobiological research [21–23]. Thus, one of the principal food sources of omul *C. migratorius* is fry of fish from the genus *Comephorus*. Benthic amphipods play an insignificant role. Food sources of Baikal whitefish *C. baicalensis* are amphipods, mollusks, and larvae of midges and caddis-flies in addition to fish. The principal food bases of Baikal grayling *T. baicalensis* are amphipods, caddis-flies, mollusks, and cottoid fish. The somewhat indiscriminate diet of grayling and whitefish should be noted. This is also seen on the PCA plot of the FA compositions from specimens of these species.

Nevertheless, the species specificity of the FA compositions should also be noted, despite the clear interrelation of the FA compositions of the studied hydrobiont tissues in the series prey–predator. Thus, Fig. 1b shows that samples of Baikal omul and whitefish, representatives of the single genus *Coregonus*, and also of greater and lesser sculpin, representatives of the genus *Comephorus*, are grouped close to each other but do not completely overlap despite the similarity of the food base within the genera. This trend can be followed in a detailed comparison using the genus *Coregonus* as an example. Samples of Baikal omul from littoral and deep-water morphoecoforms were examined. The studied samples, belonging to a single genus, are distinctive even at the morphoecoform level. This confirms the species specificity in forming the FA composition. The results indicate that the formation mechanism of the FA composition of hydrobiont tissues is complicated and determined not only by the food source but also the taxonomic specificity.

Thus, the use of modern statistical data processing methods enabled the principal factors of lipid composition formation to be found. It is a promising method for studying the characteristics of hydrobiont feeding.

EXPERIMENTAL

Hydrobiont specimens were collected in Lake Baikal during 2005–2009 as a part of commercial harvesting of seal *Phoca sibirica* and fish and scientific expeditions, including the use of Mir deep-water manned probes.

The chemical composition of subcutaneous fat of seals was studied in layers. A fat sample (~20 mg) was taken from subcutaneous seal fat (n = 11) near the skin (outer layer) and immediately next to muscle tissue (inner layer).

Samples of fish muscle tissue were compared for *Comephorus baicalensis* and *C. dybowski* (n = 15); salmonids [benthic–deep-water and littoral morphoecoforms of Baikal omul (*Coregonus migratorius*, n = 14), grayling *Thymallus baicalensis* (n = 7), and lake whitefish *Coregonus lavaretus* (n = 10)]; and all biomass of Baikal amphipods Amphipoda and Crustacea (n = 40).

Preliminary Preparation of Samples for Chromatographic Analysis. An aliquot of total lipids (0.5–1.0 mg) was treated with HCl solution (1 mL, 2N) in MeOH. Methyl esters of fatty acids (MEFA) were prepared in thick-walled tubes with Teflon stoppers for 2 h at 90°C in a muffle furnace. The resulting solution was evaporated under Ar to half the volume and treated with distilled water (0.5 mL) and hexane (1 mL). The upper hexane layer was separated. The extraction was repeated twice more [24, 25].

Analysis of FA Composition. MEFAs were studied by GC–MS on an Agilent Packard HP 6890 gas chromatograph with a mass-spectrometric detector (HP MDS 5973). A CP-Wax column of inner diameter 0.20 μm was used. The carrier gas was He (constant flow rate 1.5 mL/min). The column temperature was 90°C (4-min isotherm), 90–165°C (30°C/min),

165–225°C (3°C/min, 10.5 min isotherm). The vaporizer temperature was 250°C. The sample volume was 1 μL with 40:1 stream division. The percent composition of a mixture was calculated from peak areas in the gas chromatograms. Qualitative analysis was based on a comparison with retention times and complete mass spectra of the corresponding pure compounds using libraries NIST02.L and standard mixtures GLC-68D (Nu-Chek-Prep, Elysian, Minnesota, USA).

Statistical Processing. The results were processed using PCA and the program set Sirius-7.0 [26].

ACKNOWLEDGMENT

The work was supported by an RFBR grant No. 08-05-98029-p_Sibir_a; RAS Presidium Program Project No. 20.9; and the Foundation for Conservation of Lake Baikal.

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