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Comparative study of the endemic freshwater fauna of Lake Baikal—V. Phospholipid and fatty acid composition of the deep-water amphipod crustacean *Acanthogammarus (Brachyuropus) grewingkii*

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Lipid and phospholipid compositions of an endemic deep-water freshwater gammarid, belonging to the subphylum Crustacea, *Acanthogammarus grewingkii* was studied. Content of alkenylacyl, alkylacyl and diacyl forms in the main phospholipid classes (phosphatidylethanolamine and phosphatydilcholine) were established using reaction micro-thin-layer chromatography. The fatty acids compositions of total lipids, neutral, glyco- and phospholipid fractions were investigated by capillary gas chromatography-mass spectrometry. Seventy-nine fatty acids were identified: 26 saturated (iso-, anteiso- and cyclo-), 26 monoenoic, 7 dienoic, 14 trienoic and 16 tetra-, penta- and hexaenoic. A number of demospongic fatty acids, such as 5,9–25:2, 5,9,19–26:3, 5,9,17–26:3, 5,9,23–28:3 and 5,9,21–28:3 acids, were found.

Key words: Lake Baikal; Phospholipids; Fatty acids; Amphipods.

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Introduction

Its natural conditions and the extraordinary specificity of its animal population combine to make Baikal the most remarkable lake in the world. Baikal is extraordinarily deep. Immediately beyond the island of Olkhon the shore slopes steeply to depth of 1620 m (Kozhov, 1962).

Amphipods are the most important food of the fish inhabiting Baikal. The gammarids of the family Gammaridae are of special interest to biologists as they have no close relatives in either fresh of seawater (Bazikalova, 1945). Deep-water gammarid species found in the Lake of Baikal usually live from 100 to 1300 m; their lipid biochemistry is very little studied.

Recently, we have investigated lipid composition of three gammarid species belonging to genus *Eulimnogammarus* (Dembitsky *et al.*, 1993) and Morris (1984) studied lipids from some Baicalian bathypelagic

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gammarids, mesopelagic copepods and four deep-water benthic gammarids *Paragarjajewia petersi*, *Ceratogammarus dybowski*, *Abyssogammarus* sp. and *Polycotilus* sp. Some authors reported the presence of carotenoids in Gammaridae of Lake Baikal (Czeczuga, 1975; Koluaev and Krekeshyva, 1987).

A comparative study of marine amphipod lipids has been reported by some investigators (Paradis and Ackman, 1976; Morris *et al.*, 1982; Clarke *et al.*, 1985; Saether *et al.*, 1986; Chapelle, 1986; Chapelle *et al.*, 1987; Fricke and Dehlenschlager, 1988; Pakrashi *et al.*, 1989; Webster and Lovell, 1991; Young *et al.*, 1992; Navarro *et al.*, 1993).

Although the lipid composition of marine crustaceans is well-documented, little information on the lipid composition of freshwater deep-living amphipods has been published.

The present work reports on phospholipids and fatty acids discovered in the deep-water amphipod *A. grewingkii* from Lake Baikal.

Material and Methods

Collection of samples

Acanthogammarus (*Brachyuropus*) *grewingkii* Dyb., belonging to the phylum Arthropoda, subphylum Crustacea, class Malacostraca, order Amphipoda, were sampled in the south-western part of Lake Baikal, in July 1992, at depths of 350–400 m. The animals were 7–9 cm in length and were put in tanks under water at the site of collection and transported to the laboratory (Biological station of Irkutsk University, Bolshye Koty).

Lipid analysis

The species were treated immediately in the laboratory as described previously (Dembitsky *et al.*, 1991). Lipids were extracted, separated by column and thin-layer chromatography, as described elsewhere (Dembitsky *et al.*, 1991). Alkenylacyl, alkylacyl and diacyl forms of phospholipids were detected by using micro-TLC method (Dembitsky, 1988).

Fatty acid ME esters were analysed by capillary gas–liquid chromatography–mass spectrometry using a Shimadzu QP-1000 instrument as described previously (Dembitsky *et al.*, 1993).

Results and Discussion

Among the members of the spiny gammarid group, the Baikal genus *Acanthogammarus* and related genera have a certain relationship with the relic species *Gammaracanthus loricatus* Bazikalova (1945).

Acanthogammarus (*Brachyuropus*) *grewingkii* is a large (6–9 cm long) spiny amphipod dwelling in deep waters (100–1000 m or even deeper). Its lifestyle is nectobenthic; the species is one of the most numerous in large-mesh trawl catches obtained from silt. In terms of biology, the species was studied in detail by Bazikalova (1954). According to her reports, their diet is a combination of predation, saprophagy and detritophagy. Bazikalova (1954) reported the presence of diatoms' shells, remains of amphipods, oligochaetes, copepods, cladocerans, individual spicules of Baikalian sponges and drowned imagoes in the stomachs of examined species.

The general lipid compositions from the *A. grewingkii* are given in Table 1. According to our results, TL in *A. grewingkii* amount to 16.0% dry wt (Table 1); phospholipids amount to 23.6%, and the major lipid was NL, making up 64.2% of the total lipids. In the gammarid examined, the major phospholipid classes were choline-containing lipids which constituted 57.5% of the total phospholipid (Table 1). The major phospholipid, PC, contained 16.3% of plasmalogen form, 35.2% of alkylacyl form, and 48.5% of diacyl form. In PE, the plasmalogen form is 2.8 times higher than in PC.

Small amounts of alkylacyl and alkenylacyl forms, commonly referred to as ether lipids, alkoxylipids or glyceryl ethers, are present in the lipids of many marine animals, including crustaceans (Dembitsky, 1985). Glyceryl ethers have been found in marine crustaceans (Isay *et al.*, 1976). Fricke *et al.* (1986) have studied 1-*O*-alkylglycerolipids in Antarctic krill *Euphausia superba*. They have found contents of 1-*O*-alkylglycerol in total lipids which vary from 0.43 (collected in December) to 0.65% on a wet wt basis (collected in March/April).

Table 1. Lipid composition of deep-water amphipod

Lipid classes		*A. grewingkii*	
Total lipids, % dry wt	(TL)	16.0	
Neutral lipids	(NL)	64.2	
Glycolipids	(GL)	12.2	
Phospholipids	(PL)	23.6	
Phosphatidylethanolamine	(PE)	22.0 ± 0.7	(100%)*
alkenylacyl form		10.1 ± 0.3	(45.9%)
alkylacyl form		5.8 ± 0.6	(26.4%)
diacyl form		6.1 ± 0.3	(27.7%)
Phosphatidylserine	(PS)	6.6 ± 0.2	
The sum of amino-containing lipids	(APL)	28.6	
Sphingomyelin	(SPH)	4.9 ± 0.2	
Phosphatidylcholine	(PC)	48.6 ± 1.1	(100%)*
alkenylacyl form		7.9 ± 0.5	(16.3%)
alkylacyl form		17.1 ± 0.9	(35.2%)
diacyl form		23.6 ± 0.7	(48.5%)
Lysophosphatidylcholine	(LPC)	4.0 ± 0.2	
The sum of choline-containing lipids	(CPL)	57.5	
Phosphatidylinositol	(PI)	6.3 ± 0.3	
Diphosphatidylglycerol	(DPG)	4.5 ± 0.3	
Phosphatidic acid	(PA)	3.0 ± 0.2	

*The sum (100%) of alkenylacyl, ackylacyl and diacyl forms of each of the phospholipid classes, namely: PE and PC.

2-*O*-Alkylglycerides could not be found in any samples. Plasmalogens have been found in marine (Dembitsky, 1979; Fricke *et al.*, 1986) and freshwater (Sugiura *et al.*, 1992; Dembitsky *et al.*, 1993) crustaceans.

There are some papers which describe high concentrations of alkylacyl forms in individual phospholipid classes of marine and freshwater Arthropoda. Sugiura *et al.* (1992) studied the levels of alkenylacyl, alkylacyl and diacyl subclasses of PC and PE in 28 species of various invertebrates, including 15 arthropod species. Plasmalogen forms in PE vary from trace amounts to 31.1% in the prawn *Penaeus japonicus*, and in alkylacyl form from trace to 23.2%.

The compositions of alkenylacyl and diacyl glycerophospholipids in the gills of two Pacific crabs, *Cancer antennariuus* and *Portunus xantusi*, were investigated (Chapelle *et al.*, 1987). Phospholipids from different organs and tissues of Crustacea were observed before 1985 by Chapelle (1986).

The fatty acid composition of the deep-water amphipod examined by capillary GC–MS is shown in Tables 2–4. We identified 79 fatty acids, including 26 saturated. The proportion of saturated fatty acids in the different fractions examined varied from 16.89% in the glycolipid fraction to 61.35% in neutral lipid fractions (Table 2). The major saturated acids were 14:0, 16:0 and 18:0. We found high levels of iso- (2.34%), anteiso- (0.87%) and cyclo-acids (1.14%) in neutral lipid fractions.

Most contents of the acids identified in the course of the present study were monoenoic, ranging from 14:1n-7 to 24:1n-9. C18, C20 and C22 acids, represented by five isomers of each. The 16:1 acids were also represented by three isomers, (n-5), (n-7) and (n-9). A wide range (23 acids from C14 to C24) of monoenoic acids was detected, in amounts of generally less than 1%. Several acids, 16:1n-9, 18:1n-9 and 18:1n-7 were present in amounts exceeding 1% in total lipids, and were characteristic of the studied amphipod (Table 3). Only seven fatty acids were identified as dienoic acids, among which was demospongic acid: 5.9–25:2, in amounts less than 0.5% in all lipid fractions (Table 3).

Analysis of total, neutral, phospho- and glycolipid fractions obtained through separation of the total lipid extract by column chromatography on silica gel revealed the small content 26:3 and 28:3 acids (Table 4). The most interesting are demospongic fatty acids, which frequently occur in marine (Dembitsky and Chelomin, 1985; Djerassi and Lam, 1991) and fresh-

Table 2. Saturated fatty acids from *A. grewingkii*

Fatty acids	TL	NL	PL	GL
13:0	0.07	0.23	0.04	0.00
14:0	6.23	9.92	7.47	4.04
15:0	0.22	0.52	0.18	0.13
16:0	10.07	22.86	9.74	8.56
17:0	0.56	1.15	0.10	0.05
18:0	4.25	9.25	2.87	1.64
19:0	0.67	1.70	0.18	0.24
20:0	1.05	2.30	0.45	0.20
22:0	1.78	5.56	0.24	0.17
24:0	0.65	1.45	0.19	0.14
i-13:0	0.02	0.01	0.00	0.00
i-14:0	0.04	0.18	0.02	0.01
i-15:0	0.08	0.40	0.05	0.11
i-16:0	0.03	0.41	0.00	0.00
i-17:0	0.02	0.05	0.00	0.00
i-18:0	0.47	0.73	0.09	0.06
i-19:0	0.34	0.56	0.05	0.07
ai-13:0	0.02	0.08	0.01	0.00
ai-15:0	0.10	0.56	0.04	0.05
ai-17:0	0.28	0.10	0.01	0.39
ai-19:0	0.10	0.13	0.09	0.08
c-9-17:0	0.14	0.88	0.21	0.04
c-9-19:0	0.4	0.26	0.01	0.47
Pristanic	0.29	0.28	0.02	0.08
Phytanic	0.30	0.81	0.02	0.27
4,8,12-triMe-13:0	0.33	0.97	0.17	0.09
The sum of saturated	28.60	61.35	22.25	16.89
The sum of iso-	1.00	2.34	0.21	0.25
The sum of anteiso-	0.50	0.87	0.15	0.52
The sum of cyclo-	0.63	1.14	0.22	0.51
The sum of branched-	0.92	2.06	0.21	0.44

water (Hahn *et al.*, 1989; Dembitsky *et al.*, 1991, 1993) sponges. The demospongic acids which were found in *A. grewingkii* have sponge originality (see Table 4). Sixteen Tetra-, penta- and hexaenoic acids were found mainly in phospho- (12.42%) and glycolipid (11.57%) fractions (Table 4); the major acids were 16:4n-1, 20:4n-6 and 22:5n-3. Small amounts of eicosapentaenoic acid were found.

Morris (1984) has studied the lipid and fatty acids of three deep-water gammarids, namely *Ceratogammarus dybowski*, *Abyssogammarus* sp. and *Polycotilus* sp., living in Lake Baikal. They were collected at depths of 1100–1300 m. High levels of total lipids (from 30 to 40%) and triglycerides (48–63%) were found in deep-water gammarids. Among phospholipid fatty acids three were major components: 18:1 (as a mixture of all isomers, varying from 22.4 to 45.2%), 20:5(n-3) (12.4–17.8%) and 22:6(n-3) (5.8–20.9%). 18:1 (all isomers) acid was a major component in triglycerides, varying from 30.2% in *Abyssogammarus* sp. to 46.6% in *Polycotilus* sp.

We found high levels of 18:1(n-7) isomers in all studied fractions (from 9.64% in phospholipids to 18.55% in neutral lipids).

The (n-3) long-chain polyunsaturated fatty acids [usually 20:5(n-3) and 22:6(n-3)] have been mainly associated with marine organisms and are not normally fatty acids of freshwater fauna (Ackman, 1989). The exception is the finding that the 20:5 and 22:6 acids make up 6–14% total lipids in fish from the Great Lakes (Ackman, 1967). He suggested that freshwater fish simply did not biosynthesize long-chain polyunsaturated acids, while Farkas (1971) suggested that dietary differences were responsible.

Table 3. Mono- and dienoic fatty acid composition from *A. grewingkii*

Fatty acids	TL	NL	PL	GL
14:1n-7	0.39	0.27	0.53	0.53
14:1n-5	0.67	0.41	0.65	0.68
16:1n-9	3.56	4.28	2.15	4.68
16:1n-7	0.85	1.29	1.13	1.11
16:1n-5	0.40	0.38	0.47	0.49
17:1n-8	0.16	0.16	0.18	0.25
18:1n-13	0.10	0.03	0.04	0.31
18:1n-11	0.16	0.09	0.24	0.18
18:1n-9	4.57	6.39	1.83	2.47
18:1n-7	15.36	18.55	9.64	14.62
18:1n-5	0.92	1.07	0.95	0.77
19:1*	0.10	0.18	0.16	0.11
19:1n-8	0.25	0.15	0.15	0.12
20:1n-13	0.03	0.00	0.01	0.15
20:1n-11	0.09	0.00	0.10	0.06
20:1n-9	0.27	0.26	0.28	0.27
20:1n-7	0.56	0.10	0.76	0.59
20:1n-5	0.17	0.31	0.13	0.09
21:1n-9	0.19	0.14	0.26	0.28
22:1n-15	0.68	0.41	0.94	1.57
22:1n-13	0.03	0.00	0.07	0.04
22:1n-11	0.01	0.00	0.00	0.64
22:1n-9	0.57	0.10	0.71	0.42
22:1n-7	0.19	0.13	0.24	0.27
23:1n-9	0.17	0.02	0.09	0.21
24:1n-9	0.88	0.08	0.94	1.13
The sum of monoenoic	31.33	34.80	22.65	32.04
16:2n-4	0.34	0.00	0.34	0.41
18:2n-9	0.83	0.13	0.19	0.86
18:2n-6	7.67	0.39	13.61	9.41
20:2n-9	0.35	0.00	0.25	0.34
20:2n-6	0.08	0.00	0.30	0.09
22:2n-6	0.15	0.00	0.18	0.47
5,9-25:2a	0.35	0.12	0.48	0.31
The sum of dienoic	9.77	0.64	15.35	11.89

*These fatty acids are probably from sponge food.

Table 4. Polyenoic fatty acids from *A. grewingkii*

Fatty acids	TL	NL	PL	GL
16:3n-4	0.76	0.00	0.29	0.61
16:3n-6	0.78	0.00	1.44	1.04
18:3n-6	9.79	2.08	12.51	9.78
18:3n-3	8.77	0.93	8.48	11.97
20:3n-9	0.43	0.00	0.50	0.44
5,11,14–20:3	0.39	0.00	0.66	0.71
20:3n-6	0.07	0.00	0.10	0.12
20:3n-3	0.37	0.00	0.86	0.55
22:3n-9	0.06	0.00	0.09	0.59
22:3n-6	0.21	0.00	0.53	0.26
5,9,19–26:3*	0.13	0.07	0.52	0.39
5,9,17–26:3*	0.24	0.05	0.71	0.31
5,9,23–28:3*	0.16	0.02	0.29	0.58
5,9,21–28:3*	0.21	0.00	0.35	0.26
The sum of trienoic	22.74	3.15	27.33	27.61
16:4n-1	1.21	0.00	1.38	0.99
18:4n-3	0.11	0.00	0.17	0.24
20:4n-5	2.48	0.06	5.49	3.95
5,11,14,17–20:4	0.59	0.00	0.73	0.78
20:4n-3	0.11	0.00	0.23	0.26
22:4n-6	0.23	0.00	0.16	0.11
22:4n-3	0.24	0.00	0.27	0.62
24:4n-6	0.38	0.00	0.47	0.54
18:5n-3	0.33	0.00	0.43	0.56
20:5n-3	0.07	0.00	0.10	0.15
21:5n-3	0.01	0.00	0.01	0.01
22:5n-6	0.12	0.00	0.13	0.16
22:5n-3	1.16	0.00	1.70	2.24
24:5n-6	0.44	0.00	0.38	0.26
22:6n-3	0.11	0.11	0.31	0.16
24:6n-3	0.34	0.00	0.46	0.54
The sum of tetra-, penta- and hexaenoic	7.93	0.06	12.42	11.57
The sum (n-3) acids	11.71	0.93	13.02	17.30
The sum (n-6) acids	14.51	2.14	21.21	16.22

*See footnote in Table 3.

We found that the sum (n-3) and (n-6) fatty acids in the different lipid fractions are different, being, in neutral lipid fraction (n-3), 0.93% and in (n-6), 2.14%; in glycolipids, 13.02 and 21.21%; and phospholipids 17.30 and 16.22%, respectively.

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