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## Fatty Acid Composition Inherent in Deep-Water Baikal Amphipoda *Ommatogammarus Albinus*

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### Abstract

For the first time the fatty acid composition of deep-water Baikal amphipoda *Ommatogammarus albinus* sampled with the help of deep-water manned submersible units «Mir» was studied. Using the technique of chromatography–mass spectrometry, in the tissue of Baikalian amphipoda there have been 40 fatty acids observed those exhibit different unsaturation level with a high content of monounsaturated fatty acids, such as oleic acid, 18:1n9. A high ratio 18:1n9/18:1n7 and relatively low ratio values 16:1n7/16:0 and 20:5n3/22:6n3 indicate that deep-water amphipoda *Ommatogammarus albinus* belong to necrophages.

**Key words:** deep-water Baikal amphipoda, fatty acid composition

### INTRODUCTION

The Lake Baikal, the world natural heritage site, is the oldest (25 million years) and the largest (area of 23 000 km<sup>2</sup>) freshwater reservoir in the world. The Baikal is unique not only owing to the huge reserves of fresh water amounting to about 1/5 of the world reserves, and more than 4/5 of the freshwater reserves of our country, but also by a great number of endemic organisms. One of the most numerous groups (more than 272 species and 76 subspecies) characterized by an almost complete endemism in the Baikal Lake is presented by amphipoda (*Crustacea: Amphipoda*) [1, 2].

Deep-water amphipoda of the Baikal Lake dwell from the depth of 100 m down to the bottom. Despite the low relative biomass of amphipoda they represent an essential component in the exchange of matter and energy, because they serve as food for many aquatic organisms. [3] At the same time, the biochemistry

of lipids and fatty acid composition inherent in amphipoda are poorly studied until now.

The purpose of this work consisted in studying the fatty-acid composition of deep-water Baikal amphipoda *Ommatogammarus albinus*, dwelling near the hydrothermal unloading of the Frohlikh Bay (the Lake Baikal).

### EXPERIMENTAL

The samples of deep-water Baikal amphipoda *Ommatogammarus albinus* ( $n = 40$ ) were taken in August 2009, in the Frohlikh Bay (northern Baikal) from a depth of 430 m within the framework of International expedition with the participation deep-water manned submersible units “Mir”. In the Frohlikh Bay, there are areas where one can observe methane discharge of from the bottom sediments near the hydrothermal vents of underground sources. The source of carbon for the benthic

communities developing therein is presented by an organic substance that is formed on the basis of biogenic methane [4, 5].

#### *Preliminary treatment of the samples for chromatographic analysis*

To a weighed ample portion of the tissues of aquatic organisms (0.5–1.0 mg) was added 1 mL of 2 M HCl solution in methanol. Fatty acid methyl esters (FAME) were prepared using thick-walled tubes with Teflon caps during 2 h at 90 °C, in a muffle furnace. The resulting solution was evaporated using a flow of argon to a half of the initial volume to add then 0.5 mL of distilled water and 1 mL of hexane. The upper hexane layer was separated and the extraction procedure was repeated twice [6, 7].

#### *Analysis of fatty acid composition*

The composition of the methyl esters (ME) of fatty acids (FA) was studied with the help of a gas chromatography–mass spectrometry technique using an Agilent 6890 gas chromatograph with a quadrupole mass spectrometer MSD 5973N as a detector. We used a DB-Wax column having the inner diameter equal to 0.25 µm. Helium was used as the carrier gas (constant flow rate amounting to 1.5 mL/min). The column temperature was as it follows: 90 °C (4 min isothermal mode), 90–165 °C (heating rate 30 °C/min), 165–225 °C (heating rate 3 °C/

min, isothermal mode 10.5 min), and the evaporator temperature amounting to 250 °C. The sample volume was equal to 1 µL of solution with dividing the flow at a ratio of 40 : 1. The percentage composition of the mixture was calculated from the gas chromatographic peak areas. The qualitative analysis was based on the comparison of retention times and complete mass spectra of the corresponding pure compounds using a NIST02.L data library and GLC-68D standard mixtures (Nu-Chek-Prep Inc; Elysian, MN, USA).

#### RESULTS AND DISCUSSION

Fatty acid composition of deep-water Baikal amphipoda *Ommatogammarus albinus* is presented by more than 40 fatty acids with the carbon chain from C14 to C24 (Table 1 and Fig. 1). The content of the saturated fatty acid group ranges from 13.8 to 18.0 rel. %, the main saturated fatty acid is presented by palmitic acid 16:0, and its average content is equal to about 9.8 rel. %. Stearic acid 18:0 is contained in lesser amounts that is almost 2.7 rel. % of the total FA content, whereas the content of myristic acid 14:0 is approximately equal to 1 rel. % of the total FA content. For the previously studied deep-water Baikal amphipoda *Acanthogammarus (Brachyuropus) grewingkii* it as also found out that the main saturated FA are presented by 14:0 (6.2 rel. %), 16:0 (10.1 rel. %), and 18:0 (4.3 rel. %) [8].

TABLE 1

Fatty acid composition of deep-water Baikal amphipoda *Ommatogammarus albinus*, rel. %±standard deviation

Acids	Content	Acids	Content	Acids	Content
14:0	0.97±0.25	16:1n7	6.60±1.31	18:2n4	0.13±0.04
i15:0	0.49±0.09	16:1n5	0.23±0.06	18:3n3	0.54±0.17
ai15:0	0.15±0.05	17:1n9	0.69±0.10	20:2n6	0.34±0.17
15:0	0.55±0.13	18:1n9	30.27±2.74	20:3n3	0.57±0.17
16:0	9.76±1.08	18:1n7	5.41±0.71	20:4n6	2.07±1.02
17:0	0.47±0.11	18:1n5	1.36±0.16	20:4n3	0.17±0.07
18:0	2.65±0.52	20:1n11	1.48±0.33	22:4n6	0.23±0.26
19:0	0.19±0.04	20:1n9	0.89±0.30	20:5n3	4.55±1.75
20:0	0.26±0.03	24:1n9	0.23±0.14	22:5n6	0.27±0.10
14:1n5	0.04±0.00	16:2n6	0.14±0.05	22:5n3	0.39±0.31
16:1n9	3.50±0.48	18:2n6	0.62±0.36	22:6n3	2.45±1.01

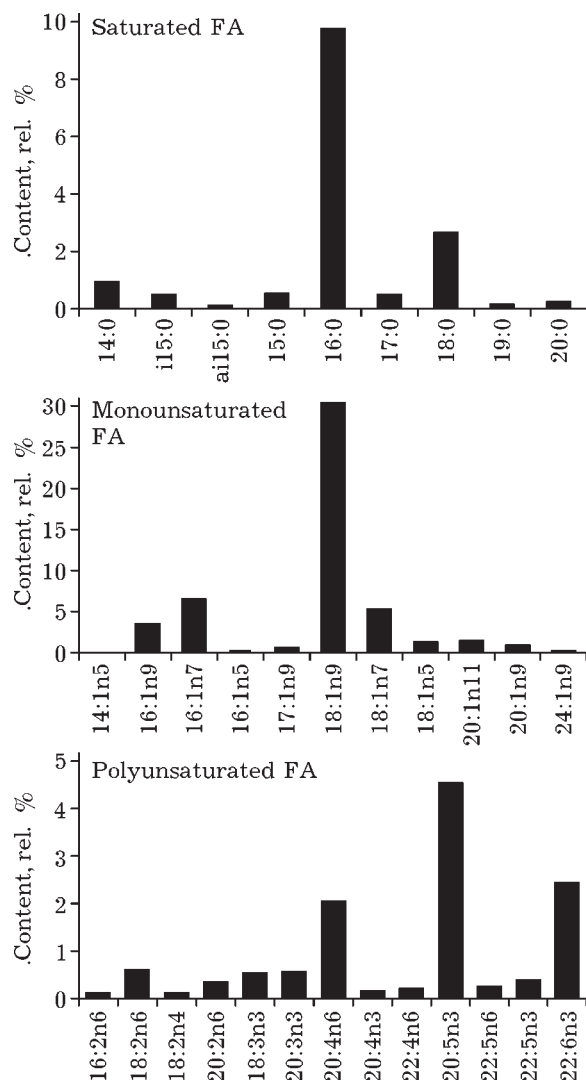


Fig. 1. Fatty acid composition inherent in amphipoda *Ommatogammarus albinus*.

The unsaturated FA dominate in the total fatty acid composition (about 65 % of total FA), the fraction of monounsaturated fatty acids (MUFA) therein amounts up to 52 rel. %, among those oleic acid, 18:1n9 is prevailing (almost 30 rel. % of the total FA content). Each of monounsaturated acids C16 and C18 is presented by three isomers (n9, n7 and n5), whereas acid C20 is presented by two isomers (n11 and n9).

The total content of 18:1 acid isomers among the fatty acid composition of deep-water amphipoda *Ommatogammarus albinus* amounts almost up to 37 rel. %. For comparison, in the fatty acid composition of three species of deep-water Baikal gammarids (*Ceratogammarus dybowskii*, *Abyssogammarus* sp. and *Polycotilus* sp.),

sampled from the depth of 1000–1300 m, there are acid isomers prevailing such as 18:1 with the total content of 30.2 rel. % for *Abyssogammarus* sp. to 46.6 rel. % for *Polycotilus* sp. [9]. The deep-water amphipoda belonging to genus *Acanthogammarus* (*Brachyuropus*) *grewingkii* exhibit the total content of the isomers of acid 18:1 within the range of 12.7–26.1 % of the total FA content, but the dominant was isomer is presented by 18:1n7 [8].

Among the polyunsaturated fatty acids (PUFAs) there are essential acids prevailing such as eicosapentaenoic acid 20:5n3 (4.6 rel. %), docosahexaenoic acid 22:6n3 (2.5 rel. %) and arachidonic acid 20:4n6 (2.1 rel. %) (Fig. 2). The eicosapentaenoic acid 20:5n3 (12.4–17.8 rel. %) and docosahexaenoic acid 22:6n3 (5.8–20.9 rel. %) are also prevailing in the composition of deep-water Baikal gammarids *Ceratogammarus dybowskii*, *Abyssogammarus* sp. and *Polycotilus* sp. [9]. The high content of unsaturated fatty acids and the low content of saturated FA could be, to all appearance, caused by a low temperature and by need for maintaining a certain level of “liquid crystal” state in the membrane structures. So, in the course of studying the fatty acid composition of amphipoda dwelling in the Gibraltar Strait, it was established that the content of eicosapentaenoic acid 20:5n3 in the samples taken at 17 °C amounted to 18–22 rel. %, whereas at a higher temperature (25 °C) this value ranged within 11–17 rel. % [10, 11].

Alongside with the temperature, the fatty acid composition could be influenced by the type animals’ nutrition. The information concerning the trophic level, the composition and type of nutrition can be determined from the ratio be-

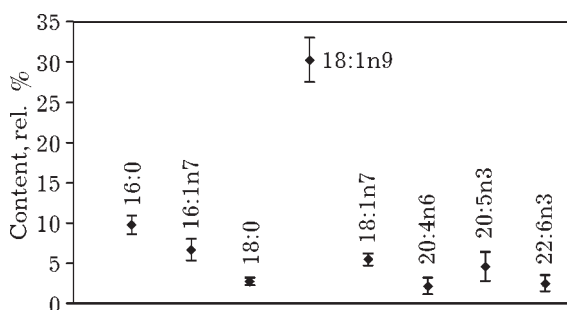


Fig. 2. Mean values and standard deviations of the content of the dominating fatty acids inherent in amphipoda *Ommatogammarus albinus*.

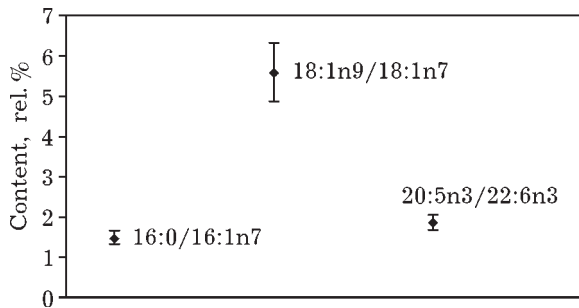


Fig. 3. Mean values and standard deviations of the content of the biomarker ratio values inherent in amphipoda *Ommatogammarus albinus*.

tween biomarker acids. A high content of specific lipid components such as acids 16:1n7, 18:1n7 and 20:5n3, those are used as trophic biomarkers are the indicators of consuming the diatoms [12–14]. On the contrary, flagellates contain large amounts of docosahexaenoic acid 22:6n3 [15–17], whereas arachidonic acid 20:4n6 comes from microalgae and it is consistently inherent in the amphipoda [12]. Oleic acid 18:1n9 is considered as an indicator of carnivorous nutrition [13], and according to these parameters the animals under investigation could belong to necrophages [12].

In the course of studying the fatty acid composition of deep-water Baikal amphipoda *Ommatogammarus albinus* we revealed a high biomarker ratio 18:1n9/18:1n7 and relatively low ratio values 16:1n7/16:0 and 20:5n3/22:6n3 (Fig. 3).

## CONCLUSION

Thus, for the first time the fatty acid composition of deep-water amphipoda *Ommatogammarus albinus* was studied to reveal that it is characterized by a high content of MUFA, in particular 18:1n9. Basing on the data con-

cerning a high ratio 18:1n9/18:1n7 and relatively low ratio values 16:1n7/16:0 and 20:5n3/22:6n3 it has been concluded that deep-water amphipoda *Ommatogammarus albinus* belong to necrophages [1, 18?20].

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