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Fatty Acid Content and Composition of Freshwater Planaria *Dendrocoelopsis* sp. (*Planariidae*, *Turbellaria*, *Platyhelminthes*) from the Yenisei River

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*For the first time the fatty acid content and composition of freshwater planarian *Dendrocoelopsis* sp. has been studied in a station of the large Siberian River, the Yenisei. The dominant fatty acids were palmitic, oleic, eicosapentaenoic and docosapentaenoic acids. The characteristic feature of planarian fatty acid composition was that ω 3 docosapentaenoic acid was 2-10 times higher than docosahexaenoic acid. The average content of ω 3 PUFA in the planarian was significantly higher than that of ω 6 PUFA, 7.20 ± 1.21 and 1.22 ± 0.22 mg/g of wet weight, respectively. The content of sum ω 3 PUFAs which are essential for the nutrition of aquatic organisms of the higher trophic levels in the studied planarian was comparatively high.*

Keywords: fatty acid, planaria, invertebrate, Turbellaria, Platyhelminthes

Introduction

Planarians are non-parasitic ancient flatworms, which are common representatives of benthic communities in both freshwater and brackish ponds and rivers worldwide. In rivers with high current velocity they occupy and attach on back sides of stones and pebbles, or on algae thallus. The size of the most of planarians specimen ranges from 3 to 12 mm. These flatworms have two or more eye-spots that can detect intensity of light and very simple nervous system (Dogel, 1975). The nervous system includes the ganglion, located at the head of the

planarian and two nerve cords which connect ganglion to the tail. There are many transverse nerves connected to the nerve cords. Digestive system consists of a mouth, located in the center of the underside of the body, pharynx and gastrovascular cavity. The pharynx connects the mouth to the gastrovascular cavity. The digestive system has three main branches throughout the body that increase assimilation and delivery of nutrients to all tissues (Dogel, 1975).

Regeneration processes are active in planarians due to simplicity of the organ systems, therefore, planarians have often been used as an

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object for morphological and cytological studies of regeneration (Politi et al., 1992; Cebria, 2007; Handberg-Thorsager et al., 2008). Planarians are primarily carnivorous consumers, but they can feed on dead organisms of invertebrates, detritus and decaying organic matter, and some species feed on diatoms (Dogel, 1975).

The planaria *Dendrocoelopsis* sp. (*Planariidae*, *Turbellaria*, *Platyhelminthes*) is a benthic habitant of the Yenisei River and one of the food sources for the dominant benthivorous fish *Thymallus arcticus* (Sushchik et al., 2006). The planaria's body is very soft that results in difficult distinguishing planarians in gut contents of the fish. We assume that the planarians are assimilated rather effectively and may be of importance in the fish nutrition.

Fatty acid contents are currently considered as the key indicator of biochemical food quality in aquatic food web studies (Gulati and DeMott, 1997). Fatty acid content and composition of freshwater planarians are almost unknown. The only data on fatty acid composition of phospholipids in this taxa are given in (Politi et al., 1992).

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 (Desvillettes 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 acids (PUFA), which are essential components in nutrition of aquatic invertebrates and fish, are emphasized (Brett and Muller-Navarra, 1997; Muller-Navarra et al., 2000). Studies of yields of PUFA and their transfer within aquatic food webs

must include an exact knowledge of FA contents in natural animal populations.

The aim of present work was to study fatty acid content and composition of freshwater planarian *Dendrocoelopsis* sp. from the Yenisei River: i) to specify its FA profile and find specific FAs or ratios, which can be used in trophic marker studies; ii) to estimate its potential as a source of essential PUFA for the higher trophic level.

Investigated area, materials and methods

Samples of planarians were collected in April and October of 2005, in January, October and December of 2006, January and September of 2007 from the Yenisei River (Siberia, Russia) in vicinity of Krasnoyarsk city (55° 58' N and 92° 43' E). Organisms were sampled from the littoral part at the site using a kick-bottom sampler by disturbing an area in frame 40×35 cm upstream of attached net (mouth 40×40 cm, mesh size 0.25 mm). Immediately after sorting, the live animals were placed into beakers with tap water for 24 h to empty their guts. Then the animal's body surfaces were gently wiped with filter paper and the animals (2-3 individuals) were weighed and placed in chloroform:methanol mixture (2:1, v/v) and kept until further analysis at -20 °C. Laboratory FA analyses and comprehensive identification of fatty acids are described in details elsewhere (Makhutova et al., 2003; Sushchik et al., 2003). Briefly, lipids from the samples were extracted with chloroform:methanol (2:1, v/v) 3 times simultaneously with mechanical homogenization of the tissues with glass beads. Before extraction, a fixed volume of an internal standard solution (19:0) was added to the samples. The combined lipid extracts were filtered, dried by passing through anhydrous Na₂SO₄ layer and evaporated at 35 °C. The lipid extract was subjected to acidic methanolysis as described previously (Gladyshev et al., 2000). Methyl

esters of fatty acids (FAMES) were analyzed on a gas chromatograph equipped with a mass spectrometer detector (GCD Plus, Hewlett-Packard, USA) and a 30 m long \times 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, USA) and to those of available authentic standards (Sigma, USA). Positions of double bonds in monoenoic acids were determined by GSMS of FAME dimethyldisulphide adducts prepared as described elsewhere (Christie, 1989). To determine double bond positions in polyenoic acids, GC-MS of dimethylloxazoline derivatives of FA was used (Sushchik et al., 2003).

Results

Samples of planarians were represented by *Dendrocoelopsis* sp. with individual's sizes ranged from 4 to 12 mm. More than 50 FA species were identified in the animal's bodies and contents of 45 prominent acids are given in Table 1.

Average content of saturated fatty acids (SFA) was 6.00 ± 0.96 mg/g of wet weight (~25 % of the total) (Fig. 1). Among them 16:0, 18:0 and 14:0 dominated. Monounsaturated fatty acids (MUFA) were in average 8.26 ± 1.34 mg/g of wet weight (~35 % of the total) (Table 1, Fig. 1). Monoenoic acids were primarily represented by 18:1 ω 9, 16:1 ω 7 and 18:1 ω 7 (Table 1).

Bacterial fatty acids, including those with odd straight and branched short chains were found. Their sum was in average 2.33 ± 0.3 mg/g of wet weight (~10 % of the total), and among them 18:1 ω 7 dominated (Table 1).

PUFA were in average 9.23 ± 1.59 mg/g of wet weight (~38 % of the total) (Table 1, Fig. 1). Fatty acids 20:5 ω 3, 22:5 ω 3, 18:3 ω 3, 18:2 ω 6 and 22:6 ω 3 had significant levels (Table 1). The planaria contained various PUFA with different chain lengths, from 16 to 22 carbon atoms. The groups of C20-PUFA and C22-PUFA had similar high values, 3.07 ± 0.75 (12.5 % of the total) and 2.80 ± 0.35 (12.3 % of the total) mg/g of wet weight, respectively. Note that the studied planaria was characterized by especially high concentrations of long-chain pentaenoates: 20:5 ω 3 and 22:5 ω 3 (Table 1). C16 PUFA in the animals presented in small quantities, approximately 4 % of the total. Among them, 16:2 ω 4, 16:3 ω 4, 16:3 ω 3 and 16:4 ω 1 dominated (Table 1, Fig. 1a). The percentage of C18 PUFA was in average 9.0 % of the total, fatty acids 18:3 ω 3, 18:2 ω 6 had significant levels (Table 1, Fig. 1a). The mean content of ω 3 PUFA was significantly higher than that of ω 6 PUFA, 7.20 ± 1.21 and 1.22 ± 0.22 mg/g of wet weight, respectively (Fig. 1b).

Total concentration of fatty acids was 23.69 ± 3.79 mg/g of wet weight. The minimum value of total concentration was found in April of 2005, and the maximum value – in October of 2006 (Table 1).

Many FA data in the literature are given in mg/g dry weight. Unfortunately, we didn't manage to directly measure moisture content of the studied planarians. In order to facilitate the comparison with such data of other types of fish food, we estimated the mean moisture content, using the mean content of several dominant benthic invertebrates from the Yenisei River (Gammaridae – 75.3 ± 0.78 %, Trichoptera – 83.8 ± 1.3 %, Chironomidae – 78.0 ± 0.54 and Oligochaeta – 78.1 ± 0.96), which accounted for 78.8 ± 1.79 %, $p < 0.01$. We extrapolated the mean moisture for benthos to the studied planaria and calculated the concentrations of dominant fatty acids in mg/g per dry weight (Table 2).

Table 1. Content of FAs (mg/g of wet weight) of the planaria *Dendrocoelopsis sp.* from the Yenisei River

Fatty acids	2005		2006			2007		M±SE
	April	October	January	October	December	January	September	
1	2	3	4	5	6	7	8	9
12:0	0.02	0.01	0.02	0.08	0.03	0.01	0.22	0.06±0.03
14:0	0.16	0.34	0.37	1.53	0.62	0.39	0.90	0.62±0.18
15:0	0.03	0.04	0.06	0.15	0.09	0.05	0.27	0.10±0.03
16:0	1.87	2.49	3.92	7.46	4.75	2.86	4.09	3.92±0.70
17:0	0.06	0.06	0.16	0.21	0.13	0.15	0.18	0.14±0.02
18:0	0.63	1.09	0.85	1.07	0.98	1.16	1.22	1.00±0.08
20:0	0.05	0.07	0.08	0.09	0.08	0.07	0.09	0.08±0.01
22:0	0.06	0.06	0.10	0.13	0.13	0.08	0.14	0.10±0.01
i14:0	0.01	0.01	0.01	0.03	0.02	0.01	0.05	0.02±0.01
ai15:0	0.04	0.06	0.07	0.14	0.09	0.06	0.15	0.09±0.02
i15:0	0.02	0.02	0.02	0.06	0.02	0.02	0.12	0.04±0.01
ai17:0	0.02	0.03	0.03	0.06	0.05	0.03	0.05	0.04±0.01
14:1ω7+14:1ω5	0.01	0.02	0.07	0.07	0.23	0.02	0.11	0.07±0.03
16:1ω9	0.09	nd	nd	nd	0.12	nd	0.20	0.06±0.03
16:1ω7	0.61	1.21	1.73	4.60	3.20	1.35	2.02	2.10±0.52
16:1ω5	nd	0.03	0.08	0.08	0.05	0.03	0.05	0.05±0.01
17:1	0.03	0.01	nd	0.04	0.07	0.03	0.02	0.03±0.01
18:1ω9	2.59	2.99	3.83	8.23	3.37	3.99	2.79	3.97±0.74
18:1ω7	1.61	1.38	1.24	2.23	2.44	1.22	2.02	1.73±0.19
18:1ω5	0.02	0.03	nd	0.08	nd	0.03	nd	0.02±0.01
20:1ω9	0.01	0.19	0.12	0.20	0.02	0.18	0.13	0.12±0.03
20:1ω7	0.15	0.04	nd	0.13	0.09	0.03	0.05	0.07±0.02
16:2ω7	0.03	0.01	0.03	0.06	0.02	0.02	0.02	0.03±0.01
16:2ω6	0.01	0.02	nd	0.04	0.11	0.02	0.03	0.03±0.01
16:2ω4	0.07	0.17	0.14	0.75	0.34	0.20	0.44	0.30±0.09
16:3ω4	0.06	0.10	0.10	0.36	0.33	0.19	0.32	0.21±0.05
16:3ω3	0.05	0.08	0.19	0.15	0.67	0.13	0.14	0.20±0.08
16:4ω3	0.03	0.04	0.10	0.08	0.38	0.06	0.09	0.11±0.05
16:4ω1	0.02	0.07	0.07	0.28	0.35	0.12	0.43	0.19±0.06
18:2ω6	0.31	0.39	0.44	1.11	0.74	0.51	0.62	0.59±0.10
18:2Δ9,13	0.04	0.09	0.05	0.15	0.07	0.09	0.13	0.09±0.02
18:3ω6	0.03	0.08	0.05	0.19	0.10	0.09	0.08	0.09±0.02
18:3ω3	0.59	0.54	0.75	1.18	2.67	1.04	0.88	1.09±0.28
18:4ω3	0.15	0.23	0.16	0.78	0.45	0.28	0.43	0.35±0.08
20:2ω6	0.05	0.04	0.07	0.26	0.12	0.12	0.07	0.10±0.03
20:3ω6	0.01	0.02	0.01	0.06	0.02	0.03	0.02	0.03±0.01
20:3ω3	0.07	0.06	0.04	0.19	0.10	0.10	0.07	0.09±0.02
20:4ω6	0.10	0.08	0.08	0.34	0.15	0.23	0.09	0.15±0.04
20:4ω3	0.04	0.06	0.04	0.15	0.06	0.07	0.08	0.07±0.01

Table 1. (continuation)

	1	2	3	4	5	6	7	8	9
20:5 ω 3	1.63	1.88	0.94	6.25	2.54	2.60	2.56	2.63 \pm 0.65	
21:5 ω 3	0.04	0.05	0.04	0.14	0.12	0.08	0.06	0.08 \pm 0.02	
22:4 ω 6	0.20	0.11	0.12	0.25	0.34	0.30	0.12	0.21 \pm 0.04	
22:5 ω 6	0.02	0.02	0.02	0.05	0.03	0.02	nd	0.02 \pm 0.01	
22:5 ω 3	1.89	2.21	0.93	2.60	2.36	2.39	2.02	2.06 \pm 0.21	
22:6 ω 3	0.48	0.22	0.15	1.08	0.73	0.73	0.20	0.51 \pm 0.13	
Total	14.07	16.80	17.26	43.22	29.49	21.22	23.77	23.69 \pm 3.79	
ω 3 PUFA	4.97	5.35	3.33	12.61	10.07	7.48	6.54	7.20 \pm 1.21	
ω 6 PUFA	0.73	0.75	0.78	2.29	1.63	1.30	1.03	1.22 \pm 0.22	
ω 3/ ω 6	6.83	7.10	4.26	5.50	6.18	5.74	6.32	5.99 \pm 0.36	

n.d. – not detected

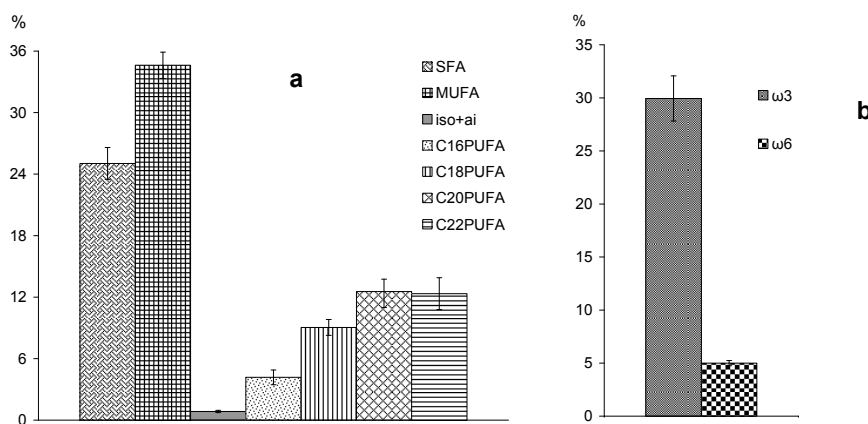


Fig. 1. Average levels (M, %) of FA groups in the planaria *Dendrocoelopsis sp.* from the Yenisei River, bars represent standard errors. **a)** – SFA – saturated fatty acids (sum of 12:0, 14:0, 15:0, 16:0, 17:0, 18:0, 20:0, and 22:0); MUFA – monounsaturated fatty acids (sum of 14:1 ω 7, 14:1 ω 5, 16:1 ω 9, 16:1 ω 7, 16:1 ω 5, 17:1 ω , 18:1 ω 9, 18:1 ω 7, 18:1 ω 5, 20:1 ω 9, 20:1 ω 7, and 24:1 ω 9); iso+anteisoacids (i14:0, ai15:0, i15:0, i15:1, ai17:0); C16-PUFA (16:2 ω 7, 16:2 ω 4, 16:3 ω 4, 16:4 ω 1, 16:2 ω 6, 16:3 ω 3, 16:4 ω 3); C18-PUFA (18:2 ω 6, 18:2 Δ 9,13, 18:3 ω 6, 18:3 ω 3, 18:4 ω 3); C20-PUFA (20:2 ω 6, 20:3 ω 6, 20:4 ω 6, 20:3 ω 3, 20:4 ω 3, 20:5 ω 3); C22-PUFA (22:4 ω 6, 22:5 ω 6, 22:5 ω 3, 22:6 ω 3). **b)** – ω 3 – sum of ω 3 PUFA; ω 6 – sum of ω 6 PUFA

Table 2. Content of dominant FAs (mg/g of dry weight) of the planaria *Dendrocoelopsis sp.* from the Yenisei River.

Fatty acids	2005		2006			2007		M±SE
	April	October	January	October	December	January	September	
14:0	0.75	1.60	1.75	7.22	2.92	1.84	4.25	2.9±0.83
16:0	8.82	11.75	18.49	35.19	22.41	13.49	19.29	18.49±3.31
18:0	2.97	5.14	4.01	5.05	4.62	5.47	5.75	4.72±0.36
16:1ω7	2.88	5.71	8.16	21.70	15.09	6.37	9.53	9.92±2.43
18:1ω9	12.22	14.10	18.07	38.82	15.90	18.82	13.16	18.73±3.47
18:1ω7	7.59	6.51	5.85	10.52	11.51	5.75	9.53	8.18±0.88
16:2ω4	0.33	0.80	0.66	3.54	1.60	0.94	2.08	1.42±0.42
16:3ω4	0.28	0.47	0.47	1.70	1.56	0.90	1.51	0.98±0.23
16:4ω1	0.09	0.33	0.33	1.32	1.65	0.57	2.03	0.9±0.29
18:2ω6	1.46	1.84	2.08	5.24	3.49	2.41	2.92	2.78±0.48
18:3ω3	2.78	2.55	3.54	5.57	12.59	4.91	4.15	5.15±1.31
20:5ω3	7.69	8.87	4.43	29.48	11.98	12.26	12.08	12.4±3.05
22:5ω3	8.92	10.42	4.39	12.26	11.13	11.27	9.53	9.7±0.98
22:6ω3	2.26	1.04	0.71	5.09	3.44	3.44	0.94	2.42±0.62
Total	66.37	79.25	81.42	203.87	139.10	100.09	112.12	111.8±17.87
ω3 PUFA	23.44	25.24	15.71	59.48	47.50	35.28	30.85	33.93±5.7
ω6 PUFA	3.44	3.54	3.68	10.80	7.69	6.13	4.86	5.73±1.03

Discussion

The study is focused on description of fatty acid content and composition of a freshwater planaria. The main characteristic of the studied planaria *Dendrocoelopsis sp.* is comparatively high level of ω3 docosapentaenoic acid (ω3DPA, 22:5ω3) which is 2 – 10 times higher than level of docosahexaenoic acid (DHA, 22:6ω3). In the work by L. Politi and coauthors (1992) the similar result for fatty acids in phospholipids has been showed. However, the planaria species from the cited work has been characterized by a low percentage of eicosapentaenoic acid (EPA, 20:5ω3), while in our study both pentaenoic long-chain acids, 22:5ω3 and 20:5ω3, showed similar high values. We suppose that high concentration of ω3DPA is a specific feature of fatty acid composition of *Planariidae* family.

According to data of Politi et al. (1992), planaria *Dugesia anceps* contained higher levels of ω6 PUFA than the planaria *Dendrocoelopsis*

sp. in our study. Among ω6 PUFA 20:4ω6, 18:2ω6 and 22:5ω6 dominated in *Dugesia anceps*, while *Dendrocoelopsis sp.* contained mainly 18:2ω6, 22:4ω6 and 20:4ω6 (Table 1). Unfortunately, there is no other data on fatty acid composition in planarians in the known literature. Hence, we compare our data with available values for different species of worms. Schlechtriem et al. (2004) has studied fatty acid composition of free-living nematode *Panagrellus redivivus* grown on various culture media. Among PUFA of *P. redivivus* 18:2ω6, 20:4ω6, 20:3ω6 dominated. The percentage of 20:5ω3 varied from 1.7 % to 6.1 % in *P. redivivus*, compare to content of this FA in *Dendrocoelopsis sp.* ranged from 5.4 % to 14.3 %. FA 22:5ω3 has not been detected in *P. redivivus* bodies. In contrast to our data, the content of ω6 PUFA in nematode *P. redivivus* was significantly higher than that ω3 PUFA (Schlechtriem et al., 2004). In another study of nematode *P. redivivus* similar FA profiles have been reported, including

the lack of 22:5 ω 3 (Chamberlain et al., 2005). In contrast, in biomass of nematode *Caenorhabditis elegans* 22:5 ω 3 was detected in small quantities; however, ω 3/ ω 6 ratio was much lower than in the studied planaria (Kang et al., 2001). We have also compared our data with fatty acid composition of different oligochaetes. L. Sampedro and coauthors (2006) have found that PUFA in *Lumbricus terrestris* dominated by 20:5 ω 3 and 20:4 ω 6. The percentage of 22:5 ω 3 in this species accounted for 2.66 \pm 0.17 % while 20:5 ω 3 and 20:4 ω 6 accounted for 21.46 \pm 1.0 % and 15.86 \pm 0.96 % respectively (Sampedro et al., 2006). In our previous work, oligochaetes from the Yenisei River (*Lumbriculus variegatus*, *Pristinella bilobata*, and *Stylaria lacustris*) contained low percentage of 22:5 ω 3 (in average 2.2 %) (Sushchik et al., 2007). Among PUFA, EPA dominated in oligochaetes, accounting for about 20 %. The ratio ω 3/ ω 6 in oligochaetes of the Yenisei River was in 2 times lower compare to that in the planaria (Sushchik et al., 2006).

In general, ω 3DPA is not unique FA for most aquatic organisms, and have been found in significant quantities in fish (Ahlgren et al., 1994; Zenebe et al., 1998, 2003). Meanwhile, the percentages of ω 3DPA were close to that of EPA, but markedly lower than that of DHA for the all studied species of omnivorous, carnivorous and herbivorous fish (Zenebe et al., 1998). Significant amounts of ω 3DPA have been found in seal adipose tissues, however its percentage also didn't exceed that of DHA (Shahidi et al., 1994; Brox et al., 2001).

On the basis of our finding the question arises why namely ω 3DPA is build up in significant amounts and become the keystone ω 3 PUFA in planarians? The physiological role of this PUFA almost is unknown, and we can just speculate. The flatworm planarians are known to have ability of fast regeneration and to have stem cells which allow the complete restoration of the nervous system in just a few days. In addition, it is known that the regeneration process requires

the inactivation of apoptosis in the newly formed cells. The ω 3 PUFA, DHA, has recently been shown to prevent neuronal apoptosis (Rotstein et al., 1997; German et al., 2006). Several FA have been tested, including DHA, ω 6DPA, ARA, oleic and palmitic acids, with DHA being the most effective in promoting photoreceptor survival and in decreasing the number of apoptotic nuclei (Rotstein et al., 1997; Kim et al., 2003). Omega-3DPA has not been tested; however, it might have the similar neural properties as DHA in planarians. However, DHA is considered nowadays as the unique fatty acid with specific properties. The sixth ethylenic bond in DHA changes the character of the fatty acid compare to those with five bonds (ω 3 and ω 6 DPA), completing the methylene-interrupted sequence along the carbon chain and conferring a folded, slightly spiral nature to the molecule (Crawford et al., 1999). Hence, ω 3DPA is unlikely able to function as DHA in neural membranes.

Besides, ω 3DPA is an intermediate product in synthesis of DHA from EPA, and its accumulation might be used as a reserve for DHA synthesis.

The studied planaria contained typical bacterial fatty acid markers: i14:0, ai15:0, i15:0, i15:1, ai17:0, 15:0, 17:0, 17:1 and 18:1 ω 7, and fatty acid markers of diatoms: 16:1 ω 7, 16:2 ω 7, 16:2 ω 4, 16:3 ω 4, 16:4w1, 20:5 ω 3 (Erwin, 1973; Claustre et al., 1988/1989; Reemtsma et al., 1990; Parrish et al., 1992; Dzierzewicz et al., 1996; Leveille et al., 1997; Shin et al., 2000; Saliot et al., 2001; Makhutova and Khromeck, 2008). We suppose that this finding indicates feeding the planaria on the epiphytes of stones and periphytic algae. Periphyton from the Yenisei River collected in the littoral from stones contained diatoms mainly in autumn, winter and early spring (Gladyshev et al., 2005; Sushchik et al., 2007).

In conclusion, the main characteristic of FA composition of the studied freshwater representative of *Planariidae* is an extremely high

ratio (2 – 10) of ω 3DPA to DHA, that is unusual for most hydrobionts of different organizational levels. The causes of accumulation of ω 3DPA in planarians stay unknown and we hypothesise on its involvement in regeneration processes of nervous system. The content of sum ω 3 PUFAs which are essential for the nutrition of aquatic organisms of the higher trophic levels was high.

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