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Water moss as a food item of the zoobenthos in the Yenisei River

Research Article

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Abstract: Bryophytes are abundant in streams and are a habitat for many invertebrates, but their contribution to the diet of fluvial zoobenthos is still debated. To estimate the amount of bryophyte-derived organic matter assimilated by benthic invertebrates, we used a combination of fatty acid and stable isotope analyses during a four-year monthly study of a littoral site in the Yenisei River (Siberia, Russia). Acetylenic acids, which are highly specific biomarkers of the water moss *Fontinalis antipyretica*, were found in lipids of all dominant benthic animals: gammarids, ephemeropterans, chironomids and trichopterans. The dominant zoobenthic species, *Eulimnogammarus viridis*, had maximum levels of the biomarkers in its biomass during winter, and minimum levels in summer. The zoobenthos in the studied site regularly consume and assimilate bryophyte-derived organic matter as a minor supplemental food. This consumption increases in winter, when the main food source of the zoobenthos, epilithic biofilms, are probably scarce.

Keywords: *Bryophytes • Fontinalis • Acetylenic fatty acids • Gammarids • Trichopterans • Stable isotope analysis*

1. Introduction

Bryophytes often form dense beds in streams and are regarded as an important habitat for aquatic animals, but their importance as a food resource for benthic invertebrates is still unclear [1-5]. Phenolic compounds are widely thought to reduce the palatability of aquatic bryophytes, thus preventing their consumption [1]. However, a microscopic gut content analysis recently found that some zoobenthic taxa eat more bryophytes than previously expected [6,7]. Nevertheless, this method is known to have some shortcomings; for example, many ingested particles are not digested and assimilated (e.g., [8-10]).

In recent decades, biochemical markers and tracers – such as fatty acids (FA) and stable isotopes, which can indicate assimilated food – have been used to study the food spectra of aquatic invertebrates (e.g., [2,11-15]). The utility of FA as markers of particular groups of organisms results from taxonomic peculiarities of FA composition [11,12,15]. For instance, diatoms synthesize polyunsaturated eicosapentaenoic acid (20:5ω3), while green algae and cyanobacteria produce high amounts α-linolenic acid (18:3ω3), and odd-number and branched FAs are synthesized only by bacteria. If such biomarkers are found in the lipids of aquatic animals, the source of their diet can be definitively traced [11,12,15]. Bryophytes have highly specific biomarkers, acetylenic FAs, which are not synthesized by other aquatic plants [16-18].

Stable isotope analysis (SIA) is based on measuring the ratios of heavy and light isotopes of carbon, $^{13}C/^{12}C$, and nitrogen, $^{15}N/^{14}N$ [e.g., 2,14]. SIA of carbon allows the source of consumers' diet to be determined, because there is comparatively negligible fractionation of heavy and light isotopes of this element in processes of animal metabolism. In turn, SIA of nitrogen allows the trophic position of consumers to be calculated, because there is considerable fractionation (enrichment) of $^{15}N/^{14}N$ by an approximately constant value at each step of consumption [14].

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However, the isolated use of either SIA or fatty acid trophic markers often gives ambiguous results. For instance, in some rivers bryophytes and other potential food items have similar isotope signatures, and therefore cannot be distinguished by the stable isotope analyses [2]. In some studies the contribution of bryophytes to the diet of zoobenthos was estimated using fatty acids which could also be synthesized by other organisms, rather than on a basis of highly specific biomarkers [13]. Bryophytes have a very high biomass and production in stream ecosystems, and it is very important to trace by their contribution in aquatic trophic webs by the relevant modern methods or their combinations.

Thus, the main aim of our study was to trace bryophyte-derived material as assimilated food of river zoobenthos using a combination of stable isotope analysis (SIA) and fatty acid analyses, like some other studies [14]. In our fatty acid analyses we used biomarkers that were highly specific to bryophytes – acetylenic fatty acids [16-18], which are not synthesized by any other aquatic organisms, including microalgae and bacteria in the studied ecosystem. The main finding of our work is that the zoobenthos assimilated bryophyte-derived material. We hypothesize that bryophyte-derived materials are assimilated at least by some groups of zoobenthos at some times of year.

2. Experimental Procedures

2.1 Study site

The Yenisei River is the largest river in Russia, and the eighth largest in the world with respect to its flow rate, averaged over a year of 19 800 m^3 s^{-1}. The main hydroecological features of the river are given elsewhere [19]. Briefly, the main hydrochemical peculiarities of the Yenisei are a low turbidity, 100% saturation level of dissolved oxygen, and an organic carbon content of about 10 mg l^{-1}.

The sampling site was situated in the middle section of the river, downstream of the Krasnoyarsk Hydroelectric dam and upstream Krasnoyarsk city, 55°58′ N and 92°43′ E, as depicted in Figure 1. The width of river at the sampling site is about 1 km. The river has a mountainous character; its banks are rocky and covered with taiga, *i.e.*, evergreen coniferous trees. Thus, there is no leaf litter in the river. With a high flow velocity at the site (about 2 m s^{-1}) there are no sediments (detritus) on the pebbly bottom. The surface of the river is ice-free throughout the winter because of the discharge of deep warm waters from the upstream reservoir. Water temperature ranged from 5–10°C in spring and summer and 0–5°C in autumn and winter.

Bottom pebbles are covered with epilithic biofilms, primarily composed of microalgae. The epilithic microalgae at the site are described in details elsewhere [20,21]; to summarize, the microalgal biomass was very high in spring and early summer, reaching *ca.* 1 000 g m^{-2} wet weight, at the expense of green algae. In summer, the phytobenthos consisted mostly of diatoms, whose biomass varied from about 5–50 g m^{-2}. In the late autumn and winter, the phytoperiphyton biomass varied from about 0.1 to 1 g m^{-2}, and cyanobacteria became the dominant species. Besides the microalgal phytobenthos, clumps of water moss, *Fontinalis antipyretica* L. ex Hedw., were characteristic of the sampling site. The study site is not shaded by riparian vegetation and the epilithic microalgae had a very high gross primary production, up to 95.1 g C m^{-2} day^{-1} [22].

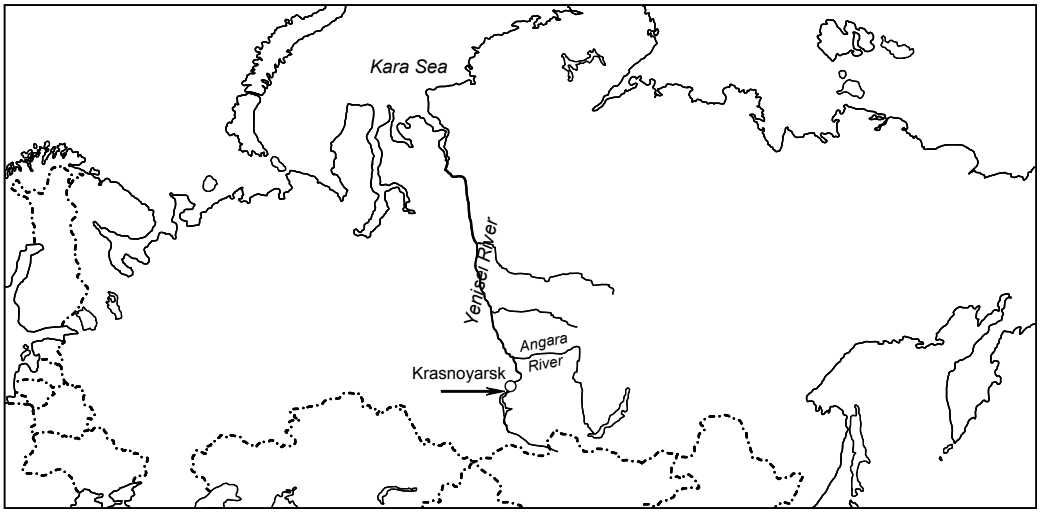

Figure 1. Map of the Yenisei River. The sample site is indicated by arrow.

The zoobenthos of the study site and their fatty acid composition is described in detail elsewhere [12,23,24]. Their biomass reaches up to *ca.* 40 g m^{-2} wet weight, and *Eulimnogammarus viridis* Dybowsky was by far the dominant species. The subdominant species were larvae of Trichoptera (*Apatania crymophila* McLachlan), Chironomidae (*Prodiamesa olivacea* Meiden, *Pseudodiamesa branickii* Nowicki, *Cricotopus algarum* Kieffer, *Orthocladius rhyacobius* Kieffer and others), and occasionally Ephemeroptera (*Ephemerella setigera* Bajkova, *Ephemerella ignita* Poda and *Ephemerella aurivillii* Bgtss); and sometimes Oligochaetes (*Lumbriculus variegatus* O.F. Muller, *Pristinella bilobata* Bretscher and *Stylaria lacustris* L.). The fatty acid composition of triacylglycerols indicate that the main part of diets of all the groups of zoobenthos originated from the epilithic diatoms, either directly or through a food chain [12].

2.2 Field sampling and sample pretreatment

2.2.1 Zoobenthos

Zoobenthos was collected monthly, from July 2006 to December 2009, (with a few exceptions; April 2007, Dec. 2007, Sept. 2009, Oct. 2009), using a Surber-type stream bottom sampler (mesh size 0.25 mm). Specimens of all groups were extracted from samples with forceps, within an hour of sampling; they were sorted by taxa and placed into beakers with filtered river water at a natural temperature. They were left for 15–20 h to allow them to empty their guts. Biochemical and elemental analyses were performed on gammarids (*E. viridis*), trichopterans (*A. crymophila*), chironomids (*P. olivacea* and *P. branickii*) and ephemeropterans (*E. setigera* and *E. ignita*). The animals were temporarily placed on filter paper to remove any surface moisture, then weighed (wet weight). Each sample included 6–20 specimens (the latter for chironomids), and was subdivided in two sub-samples for FA and SIA analyses. For fatty acid (FA) analyses animals were placed into a chloroform–methanol mixture (2:1, v/v). The samples were kept at −20°C and analyzed within one month. For SIA, animals were dried at 75°C for 24 h and were kept in a desiccator until the analysis.

2.2.2 Epilithic biofilms and water moss

For epilithic biofilms (phytoperiphyton) sampling, a 1 dm^2 frame was placed on the river bed; the pebbles inside the frame were withdrawn; and a toothbrush was used to brush the biofilms from the surface of stones, which were collected in a small volume of river water. Aliquots for subsequent analysis were centrifuged at 2,500 × g for 15 min, after which pellets were collected and pre-treated for FA analyses and SIA, performed as for the zoobenthos (see above).

Fresh upper shoots of the water moss *F. antipyretica* were collected from clumps and washed extensively under tap water to remove epiphytes. For fatty acid analyses, the pieces were briefly placed on filter paper to wipe of the surface moisture and moved to a chloroform–methanol mixture (2:1, v/v). For SIA the shoots of moss were dried at 75°C for 24 h and were kept in a desiccator until the analysis.

2.3 Fatty acid analyses

Detailed descriptions of the analyses of fatty acids (FA) of all the samples (animals, epilithic biofilms and moss) are given elsewhere [9,12,18]. In brief, lipids were extracted with three 5 ml portions of chloroform-methanol (2:1, v/v). To analyze the fatty acid composition of the total lipids, part of the lipid extract was methylated (see description below). Another part of the lipid extract was fractionated by thin layer chromatography on silica gel G with a solvent system for neutral lipids, as described in [12]. The lipid fractions containing triacylglycerols (TAG) and the polar lipids (PL) were scraped from the silica gel plate, and the solvent was evaporated prior to methylation. The methyl esters of fatty acids (FAMEs) of total lipids and of the two lipid classes were prepared in a mixture of methanol–sulphuric acid (20:1, v/v) at 85°C for two hours. Subsequently, the methanolysis was stopped by adding 2 ml of distilled water, and FAMEs were extracted with two 3 ml portions of hexane. The hexane lipid fraction was roto-evaporated until dry, and resuspended in 15–20 μl of hexane into which 1–2 μl of a sample was injected. FAMEs were analysed and identified using a gas chromatograph–mass spectrometer (GC/MS, model GCD Plus, Hewlett Packard, USA, or model 6890/5975C, "Agilent Technologies", USA), on a HP-FFAP capillary column (30 m length, 0.25 mm internal diameter). The conditions of chromatography were as follows: helium as a carrier gas with the flow rate of 1 ml min^{-1}; injector temperature of 220°C; initial temperature of 100°C; elevation of temperature to 190°C at a rate of 3°C min^{-1} with 5 min of isothermal regime and subsequent elevation of temperature to 230°C at a rate of 10°C min^{-1} with 20 min of isothermal regime; interface temperature of 260°C; ion source temperature of 165°C; electron impact at 70 eV; scanning of the fragments with atomic masses from 45 to 450 amu at 0.5 s $scan^{-1}$. Peaks of FAMEs were identified by their mass spectra, comparing to those in the database (Hewlett-Packard, USA; "Agilent Technologies", USA) and to those of available authentic standards (Sigma, USA). The location of double and triple bonds in unsaturated acids was confirmed after production of FA dimethyloxazoline derivatives (DMOX) [25] and their subsequent chromatography under

the same conditions as were used for the FAME. In order to produce DMOX, 0.2 ml of 2-amino-2-methyl-1-propanol (Sigma, USA) was added to the fraction of saponified lipids; then the solution was bubbled through with helium, tightly closed up, and heated to 180°C for 1.5 h. After cooling, the reaction mixture was diluted with distilled water, acidified, and derivatives (DMOX) were extracted with a hexane–acetone mixture (96:4).

The FAMES were quantified according to the peak of an internal standard, nonadecanoic acid, of which a 0.5 mg ml^{-1} solution was added in fixed volumes prior to the extraction.

2.4 Stable isotope analyses

Samples of zoobenthos, phytoperiphyton and moss for stable carbon and nitrogen isotope ratios were analyzed with a continuous flow isotope ratio mass spectrometer (CF-IRMS), model Delta V Plus (Thermo Scientific Corporation, USA) interfaced with a elemental analyzer (Flash EA 1112 Series, Thermo Electron Corporation, USA). Dry helium of 5.5 grade was used as carrier gas for sample introduction. Reference tanks for N and C isotopes were made of pure N_2 (5.5 grade, 99.9995%) and CO_2 (4.5 grade; 99.995%).

The stable isotope ratios were given in the conventional differential δ-notation:

$$\delta R(‰) = [R_{sample}/R_{standard} - 1] \times 10^3 \qquad \text{(Eq. 1)}$$

where R=$^{13}C/^{12}C$ or $^{15}N/^{14}N$; δR=$\delta^{13}C$ or ^{15}N is the per mil (‰) deviation of that sample from the international isotope standard, Vienna PeeDee Belemnite (PDB) limestone for $\delta^{13}C$ and atmospheric N_2 for $\delta^{15}N$ (e.g., [2]). The accuracy and precision of the measurement was verified daily with the secondary reference material USGS40 from International Atomic Energy Agency (L-glutamic acid, $\delta^{15}N$=−4.5‰ and $\delta^{13}C$=−26.39‰). Analytical reproducibility was ±0.2‰ for C and ±0.3‰ for N. The laboratory standard (Urea, Thermo) was analyzed every 12 samples. Samples were analyzed in duplicate or triplicate when sufficient material was available.

Trophic position (TP) was calculated conventionally:

$$TP_x = (\delta^{15}N_x - \delta^{15}N_{base}) / \Delta\delta^{15}N + TP_{base} \qquad \text{(Eq. 2)}$$

where $\delta^{15}N_x$ is the isotope ratio of the taxon in question, $\Delta\delta^{15}N$ is the trophic enrichment (fractionation) constant, $\delta^{15}N_{base}$ and TP_{base} are the average $\delta^{15}N$ and trophic position of the baseline, respectively [26]. The constant $\Delta\delta^{15}N$=3.4‰ and TP_{base}=2 [14,26-28]. The taxonomic group of zoobenthos with the lowest $\delta^{15}N$ was selected as the baseline for estimating the TPs of other taxa [14].

2.5 Statistics

Standard errors, Student's *t*-test, Wilcoxon matched pairs test, Pearson product-moment correlation coefficients, Kolmogorov-Smirnov one-sample test for normality, one-way ANOVA and Fisher's LSD (least significant difference) *post-hoc* test were carried out conventionally [29], using STATISTICA software, version 9 (StatSoft Inc., Tulsa, OK, USA).

3. Results

Two acetylenic acids were found in most part of samples of the zoobenthos, except oligochaetes, and in epilithic biofilms. These acids were octadeca-9,12-dien-6-ynoic

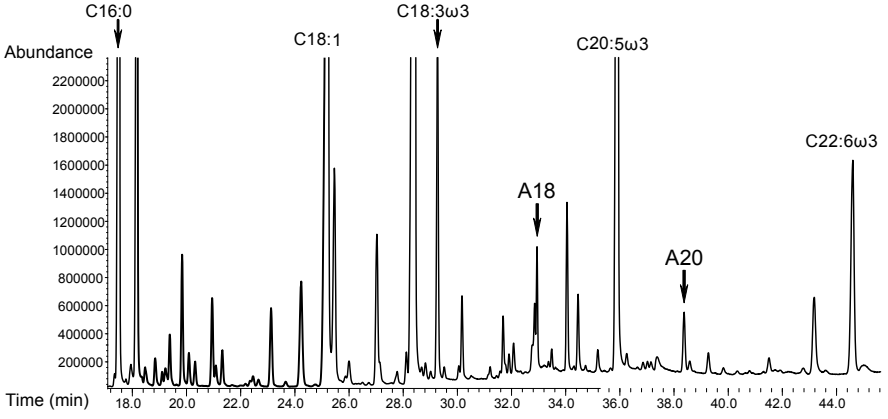

Figure 2. An example of chromatogram of methyl esters of fatty acids (FAMEs) including two acetylenic acids, octadeca-9,12-dien-6-ynoic (6a,9,12–18:3) designated as A18, and eicosa-11,14-dien-8-ynoic (8a,11,14–20:3), designated as A20. A sample of bodies of *Eulimnogammarus viridis* from the littoral site in the Yenisei River, March 20, 2008.

(6a,9,12–18:3) and eicosa-11,14-dien-8-ynoic (8a,11,14–20:3), designated in the following text as A18 and A20, respectively (Figure 2 and Figure 3).

For the most abundant taxa of zoobenthos, the gammarids *E. viridis*, a comparison of the acetylenic acids levels in TAG and PL was done. A18 and A20 were constituents of TAG (0.58±0.14%), rather than polar lipids (0.07±0.03%), the difference was statistically significant (Wilcoxon matched pairs test T=0.0, P=0.00098, number of pairs N=17). There were no significant differences between the acetylenic acids level in TAG and that in total lipids (0.63±0.17%, T=61.0, P=0.36196). Thus, in the following analysis, the levels in total lipids were used.

On average, the ephemeropterans (*E. setigera* + *E. ignita*) had the highest value of level of A18+A20, while the lowest values of levels of these acids were in the chironomids (*P. olivacea* + *P. branickii*), the trichopterans (*A. crymophila*) and in the biofilms (Table 1). According to Kolmogorov-Smirnov one-sample test for normality, D-statistics for all the groups of zoobenthos and biofilms were lower than the critical values at P=0.05 (Table 1).

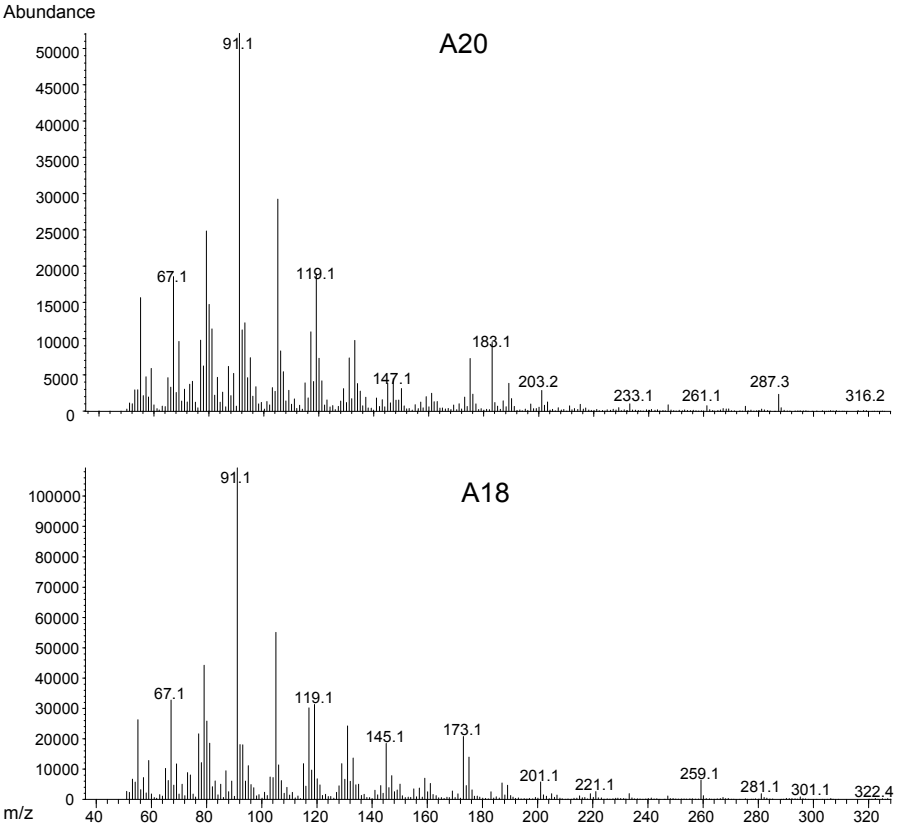

Figure 3. Mass spectra of two acetylenic acids, A18 (octadeca-9,12-dien-6-ynoic (6a,9,12–18:3)) and A20 (eicosa-11,14-dien-8-ynoic (8a,11,14–20:3)), from the chromatogram, depicted in Figure 1.

Taxon (group)	n	minimum	M ± SE	maximum	$D_{K\text{-}S}$
Gammarids	31	0.00	0.62^{a} ± 0.11	2.45	0.175
Trichopterans	17	0.00	0.15^{b} ± 0.05	0.77	0.273
Chironomids	22	0.00	0.05^{c} ± 0.01	0.24	0.253
Ephemeropterans	9	0.00	1.65^{d} ± 0.35	3.21	0.143
Biofilms	34	0.00	0.26^{b} ± 0.05	1.13	0.189

Table 1. Levels of sum of acetylenic fatty acids, octadeca-9,12-dien-6-ynoic (6a,9,12–18:3) and eicosa-11,14-dien-8-ynoic (8a,11,14–20:3) (% of the total sum of fatty acids, minimum, mean ± standard error, maximum) in zoobenthos and epilithic biofilms, number of samples, n, and results of Kolmogorov-Smirnov one-sample test for normality, $D_{K\text{-}S}$. Means, labeled with the same letter are not significantly different at $P<0.05$ after Student's t-test. The littoral site of the Yenisei River upstream Krasnoyarsk city, July, 2006 – December, 2009.

Thus, the null hypothesis for normal distributions was accepted and parametric methods, Student's *t*-test and Pearson's correlation coefficient were used to compare the data. There were no significant pair correlations between the sum of A18 and A20 levels in the animals and biofilms.

Seasonal dynamics of the sum of acetylenic acids of the zoobenthos and the biofilms are depicted in Figure 4. To reveal possible differences among the levels of the acids in the four seasons one-way ANOVAR for each group was carried out (Table 2). According to ANOVA, levels of the acids in the gammarids significantly differed in the four seasons (Table 2). The highest levels of these acids occurred in winter, 0.95±0.28%, and the lowest in summer, 0.18±0.06% (Figure 4), with intermediate levels of A18+A20 in spring and in autumn (Figure 4). There were no significant seasonal differences in the levels of acetylenic acids in other zoobenthic taxa and biofilms (Table 2, Figure 4). Nevertheless, Fisher's LSD *post-hoc* test revealed significant differences between the levels of the acetylenic acids in the chironomids in winter (0.18±0.04%) and that in summer (0.02±0.01%): P=0.033, d.f.=19.

Results of SIA of the zoobenthos, the biofilms and the water moss, *F. antipyretica*, are given in Figure 3. The water moss was significantly more depleted in ^{13}C relative to biofilms and zoobenthos (Figure 5): Student's test t=6.66, P<0.001 and t=17.60, P<0.001, respectively. The chironomids, the gammarids and the ephemeropterans had significantly higher $\delta^{15}N$ ratios, than the trichopterans (Figure 5): t=2.78, P<0.05, t=5.30, P<0.001 and t=3.76, P<0.01, respectively. Thus, the trichopterans was selected as the baseline consumer with TP_{base}=2 (Equation 2). TP values for the gammarids, the chironomids and the ephemeropterans, calculated using Equation 2, were 2.9, 2.9 and 3.0, respectively.

Taxon (group)	*MSEf*	d.f.$_{MSEf}$	*MSEr*	d.f.$_{MSEr}$	*F*	*P*
Gammarids	1.0114	3	0.3245	27	3.12	0.043
Trichopterans	0.0100	2	0.0468	13	0.21	0.810
Chironomids	0.0098	3	0.0040	19	0.44	0.096
Ephemeropterans	1.2633	2	0.6133	5	2.06	0.223
Periphyton	0.0670	3	0.0904	30	0.75	0.520

Table 2. Results of one-way ANOVA comparing levels (% of the total sum of fatty acids) of sum of acetylenic fatty acids, octadeca-9,12-dien-6-ynoic (6a,9,12–18:3) and eicosa-11,14-dien-8-ynoic (8a,11,14–20:3) in four seasons (winter, spring, summer and autumn) in zoobenthos and biofilms in littoral of the Yenisei River in vicinity of Krasnoyarsk city (Siberia, Russia), July, 2006 – December, 2009: *MSEf* – mean square effect, *MSEr* – mean square error at degrees of freedom (d.f.) and the significance of differences according to Fisher's *F*-test.

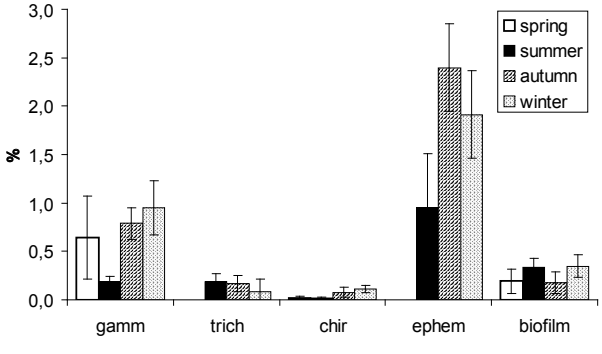

Figure 4. Seasonal dynamics of levels (% of total fatty acids) of two acetylenic acids, A18+A20, in epilithic biofilms and biomass of zoobenthos taxa (gamm – gammarids, trich – trichopterans, chir – chironomids, ephem – ephemeropterans) from the littoral site in the Yenisei River, July, 2006 – December, 2009. Significances of differences between seasons for each group are based on ANOVA and are given in Table 2.

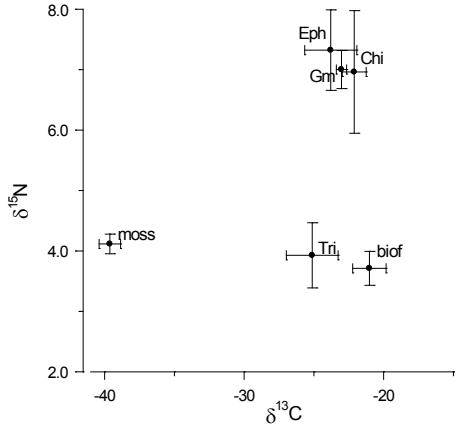

Figure 5. Average values of the isotope ratios (‰) in epilithic biofilms (biof), water moss *Fontinalis antipyretica* and zoobenthos taxa (Tri – trichopterans, Gm – gammarids, Chi – chironomids, Eph – ephemeropterans) from the littoral site in the Yenisei River, July, 2006 – December, 2009.

4. Discussion

The acetylenic fatty acids, which are highly-specific biomarkers of the water moss, *F. antipyretica* [18], were found in FA profiles of all the dominant zoobenthic taxa in the studied site of the Yenisei River. Thus, bryophyte-derived organic matter was definitely transferred through the trophic chain. Previous studies have found that bryophytes are likely to contribute substantially to the energetic balance of stream trophic chains [6,30]. In the studied site of the Yenisei River, the contribution of the water moss to energetic balance of the higher trophic levels seems to be comparatively small. Firstly, the levels of the acetylenic fatty acids in zoobenthos were on average about 0.1–1.7% (Table 1), while levels of sum of these acids in *F. antipyretica* varied from 14.8% to 31.8% and was on average 26.3% [18]. For comparison, levels of the FA markers of diatoms in zoobenthos and in epilithic biofilms at the studied site had practically the same value, around 30–50% [12,21,23,24]. Secondly, $\delta^{13}C$ signatures of the biofilms (comprised mostly by diatoms) and the zoobenthos were practically equal, while the moss had significantly lower values (Figure 5). These findings mean that biofilms are the principal mass and energy source for the zoobenthos. Our considerations on the minor part of the moss in the diets, confirmed indirectly by the isotope analyses, are based on the assumption that the acetylenic acids are retained in consumer lipids, like all other fatty acids. This assumption is used conventionally in all relevant FA-marker analyses [9,11-13,15].

Nevertheless, the presence of the acetylenic acids in FAs of zoobenthos indicated a consumption and assimilation of bryophyte-derived materials. Moreover, there was a tendency for zoobenthos to have a more depleted ^{13}C content than the biofilms (Figure 5). This shift of $\delta^{13}C$ values of zoobenthos seems to be due to a consumption of bryophyte-derived material, which had significantly lower $\delta^{13}C$ values than biofilms (Figure 5). Some other authors also reported similar differences in isotopic signatures of bryophytes and epilithic biofilms: $\delta^{13}C$ values of the liverwort *Porella pinnata* were about −38‰, while epilithon had $\delta^{13}C$ values about −34‰ [2]. McWilliam-Hughes *et al*. [30] found in Canadian rivers $\delta^{13}C$ signatures of *Fontinalis* sp. ranged from −38 to −33%, and the signatures also were lower, than that of biofilms. Moreover, scrapers, including ephemeropterans, in Canadian rivers were more depleted in ^{13}C than were the biofilm samples [30], just like zoobenthos in the studied site of the Yenisei River (Figure 5). Trophic fractionation is primarily an enrichment process, thus, the depletion of zoobenthos compared to biofilms indicates a contribution of water moss to the diet of the invertebrates [30].

The zoobenthic taxa with the highest acetylenic acid levels in their biomass, ephemeropterans and gammarids (Table 1), are predators. According to Eq. 2 with the constant $\Delta\delta^{15}N$=3.4‰, their trophic position TP=3 (Figure 5). However, although this value is conventionally modeled as a constant, the fractionation value between trophic levels can vary considerably [26]. A more precise designation for the studied ephemeropterans, gammarids, and chironomids is 'omnivorous'. The question arises: did the omnivorous zoobenthos taxa consume the water moss directly, or obtain the bryophyte-derived organic matter through a food chain? Many studies have reported the direct consumption of aquatic bryophytes, including *F. antipyretica*, by some taxa of benthic invertebrates [1,6,13]. Among the zoobenthos, gammarids are known to be highly opportunistic feeders capable of predation, and can also collect detritus, scrape periphytic microalgae and leaf litter, and graze on aquatic macrophytes [7,31-33]. Although gammarids, *E. viridis*, at the studied site evidently had a high degree of predation (Figure 5) and thereby might get the acetylenic acids through their prey, we suggest that they consumed bryophyte particles directly. First of all, organisms of the lower trophic level, trichopterans, the probable prey of gammarids [31], had significantly lower levels of the acetylenic acids than the gammarids (Table 1). Secondly, there were no correlations between A18+A20 levels in the zoobenthic taxa. We suppose that *E. viridis*, as well as the ephemeropterans and the chironomids, consumed the water moss as a minor supplemental food, albeit to a different extent.

The dominant zoobenthic taxon, the gammarid *E. viridis*, might also consume moss particles from the biofilms. The biofilms evidently contained bryophyte particles (Table 1). These particles might be bryophyte litter. Nevertheless, it is not at all clear, how bryophyte tissues die, and stream bryophytes may not produce litter in the traditional sense of this word [1]. Bryophyte decomposition is generally quite slow and is retarded by low temperature [1]. Fragments of the bryophytes, indicated in the biofilms by the marker acetylenic acids (Table 1), were likely reproductive material. Fragmentation is probably the primary mechanism of reproduction and dispersal and it is well known that fragments of some bryophyte species remain viable after long periods of desiccation or freezing [1]. Nevertheless, there were no correlations between the acetylenic acids levels in the biofilms and in bodies of the zoobenthic taxa, including gammarids. Using the conventional assumption, mentioned above, that all consumed fatty acids are retained in consumer storage lipids roughly equally, one should suppose that there is correlation between levels of biomarker FAs

in consumed food and in consumers' bodies. Thus, we suppose that the zoobenthos consumed a small amount of the water moss as a supplemental food directly, grazing on the moss clumps, rather than moss particles in biofilms.

Amphipods generally switch to a low-calorie food when availability of more valuable food items becomes low [32]. Indeed, levels of the bryophyte markers, acetylenic FAs, peaked in *E. viridis* during winter (Figure 3, Table 2). Diatoms are high-quality food for invertebrate primary consumers (e.g., [13]), and in the studied site, the biomass of these microalgae peaked in summer and decreased in winter [21]. As evident from FA marker analysis [12] and SIA (Figure 5), gammarids derive most of their biomass and energy from biofilms, which are dominated by diatoms [21,23,24]. The gammarids likely increased consumption of the water moss, *F. antipyretica*, in the period when biomass of diatoms became insufficient to meet their food requirements. Thus, our data support the conclusion of Felten *et al*. [7] that bryophytes can be a food item of amphipods, but specify that the importance of bryophytes increases in periods of shortcoming of the main foodstuff, *i.e.,* diatoms. McWilliam-Hughes *et al*. [30] also concluded that bryophytes might be an important alternate (marginal) food source for aquatic macroinvertebrates when a preferred food (e.g., diatoms) is scarce. Torres-Ruiz *et al*. [13] found using nonmetric multidimensional scaling analyses of FA composition of a river zoobenthos (trichopterans, ephemeropterans and isopods) and their food sources (periphytic microalgae and water moss *Hygrohypnum luridum*), that in spring (March) the zoobenthos loaded in the analysis graph near the moss and the periphyton, while in summer (July) moss, *H. luridum*, separated from the groups of periphytic microalgae and zoobenthos. The separation likely indicated comparatively lower consumption of the water moss by the zoobenthos in summer. This finding [13] about the lower summer consumption of water moss agrees with our data. Indeed, in summer there were significantly lower levels of acetylenic FAs in *E. viridis* bodies, indicating comparatively lower consumption of the moss (Figure 4).

F. antipyretica can produce unpalatable phenolic compounds responding to grazer pressure [1]. An extract from the moss *Fontinalis novae-angliae*, which included C18 AcA, octadeca-9,12-dien-6-ynoic acid, deterred crayfish feeding, but the amphipod *Crangonyx gracilis* [34]. Probably this defense from herbivory by the secondary metabolites reduces the summer consumption of the moss by *E. viridis*, but in winter, under food limitation and likely lower metabolic activity of *F. antipyretica*, the gammarids seemed to consume more of the moss.

When we considered the gammarids and the trichopterans, we investigated only one species of each group: *E. viridis* and *A. crymophila*. In contrast, the chironominds and the ephemeropterans samples were mixtures of two species, *P. olivacea* and *P. branickii,* and *E. setigera* and *E. ignita*, respectively. Nevertheless, we suppose that such mixing did not affect the conclusions of our study, because the two species in each group have similar feeding behavior. *P. olivacea* feed on deposited detritus or small prey [35-37], and *P. branickii* is also omnivorous [38]. *E. setigera* consume detritus and periphyton [39], and food of *E. ignita* also consist of some unicellular and filamentous algae, detritus and occasionally invertebrates, as well as moss [40-44]. However, our data indicate that the chironomids and the ephemeropterans omnivorous species in the studied site of the Yenisei River had a comparatively high degree of predation (Figure 5).

Thus, the zoobenthos in the studied littoral site of the Yenisei River, especially ephemeropterans (*E. setigera* and *E. ignita*) and gammarids (*E. viridis*), regularly consumed and assimilate bryophyte-derived organic matter as a minor supplemental food. The consumption of bryophyte-derived material by the gammarids significantly increased in winter.

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