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Trophic Relationships between Macroinvertebrates and Fish in St. Petersburg Methane Seep Community in Abyssal Zone of Lake Baikal

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Abstract—Trophic relationships were examined for macroinvertebrates and fish inhabiting the St. Petersburg methane seep (central part of Lake Baikal, ~1400 m depth). The analyses of the values of carbon and nitrogen stable isotopes showed that all animals associated with the seep were heterotrophs with different feeding strategies; symbiotrophes were absent. Seep animals consumed combined food with different portion of methane-derived carbon ranged from 2.7 to 89%. The average $\delta^{13}C$ values varied in the range of –26.2‰ (in benthopelagic amphipods) to –64.5‰ (in gastropods). The trophic food web in the methane seep consists of filter feeders (pelagic amphipods, trophic position (TP) is 1.9), detritophages (gastropods, TP of 2.2, and burrowing amphipods, TP of 2.1), polyphages and necrophages (nectobenthic and benthopelagic amphipods of TP 2.8–3.2), and predators (planarians of TP 3.6–4.2 and cottoid fish of TP 3.0–3.8). Animals occupying similar trophic positions significantly differed in the $\delta^{13}C$ values and have partially overlapping components of food.

Keywords: methane seep, Lake Baikal, macroinvertebrates, cottoid fish, stable isotopes, trophic relationships

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INTRODUCTION

Lake Baikal is a single freshwater reservoir where deepwater fauna inhabits the maximum depth (1640 m). One of the important factors explaining the presence of the abissal fauna in the lake is a high concentration of oxygen dissolved in the water, the saturation level of which is not below than 74.5% even at the maximum depths (Vereshchagin, 1949). It has been previously revealed using the analysis of the carbon isotope signatures that the animals in the abyssal zone of Lake Baikal consume photosynthetic organic carbon from the plankton sunk to the bottom sediment (Yoshii, 1999).

Like in sea ecosystems, there are hydrothermal vents and cold methane seeps in Lake Baikal. For the past decades, more than 20 cold methane seeps and gas discharges have been discovered (Granin et al., 2010; Khlystov et al., 2013, etc.). The survey of the Lake Baikal seeps has shown that the bottom and the bottom water layers are characterized by high biological productivity when compared to the reference areas of the lake. It is well known that some animal species in the communities of the Frolikha hydrothermal vent and the methane seep (Gorevoy Utes) in Lake Baikal consume organic matter of chemosynthetic origin (Kuznetsov et al., 1991; Gebruk et al., 1993; Zemskaya et al., 2012; Sideleva and Fialkov, 2014).

Typical methane seep sites associated with gas-hydrate deposits are unknown in other freshwater ecosystems around the world. However, the significant contribution of biogenic methane to the energetic resources of the trophic chains is noted for some lakes. It is particularly indicated that the larva of some chironomidae feed on methanotrophic microorganisms (Kiyashko et al., 2001; Jones and Grey, 2011); in addition, the methane-derived carbon comprises approximately 60% of organic carbon in the tissues of chironomidae (Jones and Grey, 2011).

Numerous methane seeps in sea ecosystems are generally inhabited by heterotrophic animals, while symbiotrophs are rarely found. Symbiotrophic animals are characterized, as a rule, by a considerably lighter carbon and nitrogen isotope composition ($\delta^{13}C < -50‰$ and $\delta^{15}N < 3‰$, respectively, up to negative values) (Levin and Michener, 2002). Heterotrophic animals use different food resources; some of them consume phosynthetic organic matter and others digest the matter produced by methane- and/or chematrophic microorganisms. A range of organisms utilize the combined photo- and chemosynthetic resources of carbon (Levin and Michener, 2002; Zapata-Hernández et al., 2014; Grupe et al., 2015; etc.). In seep biocenoses, heterotrophic animals of different feeding strategies (filter feeders, gatherers, and scrapers) eat free-living chemoautotrophic and methanotrophic microorganisms. Methane-derived carbon in the primary consumers is transferred into the third and fourth trophic levels (Levin and Michener, 2002; Zapata-Hernández et al., 2014). Some animals utilize mixed food (plant and animal origin) and occupy an intermediate position between the trophic levels (TL); therefore, it is required to estimate the animal trophic position (TP) for describing the structure of the food webs (Vander Zanden and Rasmussen, 2001).

This article continues the investigation of the deepwater seep fauna communities of Lake Baikal. The carbon and nitrogen stable isotope analyses allowed us to estimate the trophic positions of animals inhabiting the St. Petersburg methane seep and characterize their trophic relations.

MATERIALS AND METHODS

Large macroinvertebrates and fish were collected with landing nets and a slurp gun (a suction device for trapping biological material) from the deepwater *Mir-1* and *Mir-2* manned submersibles (DMS) July 1–3, 2009, and August 15, 2010, respectively. The samples were taken from the soft bottom sediments, including the area close to the jelly-like microbial mats at a depth of 1365–1407 m in the St. Petersburg methane seep (E 52.87°, N 107.15°). Some of the amphipods were collected from the sample basket available on the DMS. In order to compare, the animals from the other deepwater regions of the lake were sampled from June 19, 2009 to August 17, 2009, with the same trapping supplies. The information on the material used for details is present in Tables 1 and 2.

The characteristics of the biotopes were described using videos taken with the stationary cameras carried on the *Mir-2* DMS. The animals and the samples of the bacterial mats were washed with running water to remove the bottom-sediment particles. The samples were fixed in 4% formaldehyde to identify the species and were dried at a temperature of 60°C to define the isotopic composition values. Dry particulate organic matter weighed more than 30 mg.

The dried samples were ground with agate mortar and pestle and degreased with chloroform/methanol. The isotopic analyses were performed at the Stable Isotope Laboratory, Far Eastern Geological Institute, Far East Branch, Russian Academy of Sciences, Vladivostok). The sample particulate matter of 0.5 mg was weighed in the tinny capsules; then it was analyzed with the FlashEA 1112 Elemental Analyzer connected via a Conflo III Interface to a MAT-253 Isotope Mass Spectrometer (Thermoquest, Germany). The relative contents of the heavy ^{13}C and ^{15}N isotopes in the samples were determined in the common form as a δ deviations in ‰ from the relevant standard of the isotopic composition:

$$\delta X (‰) = [(R_{\text{sample}} - R_{\text{sample}})/ R_{\text{sample}}] \times 1000,$$

where X is ^{13}C or ^{15}N, while R is $^{13}C/^{12}C$ or $^{15}N/^{14}N$, respectively.

All of the $\delta^{13}C$ and $\delta^{15}N$ values are given according to the common international standards of isotopic compositions of PDB carbonate and atmospheric nitrogen. The CH-6, NBS-22, and N-1 and N-2 standards are used for calibration (International Atomic Energy Agency, Vienna). The accuracy of determining the $\delta^{13}C$ and $\delta^{15}N$ values comprised ±0.1‰.

After the extraction of lipids with hexane, the samples of the cottoid fish were reanalyzed for the $\delta^{13}C$ values because of the high levels of lipids and, consequently, high levels of C/N (Logan and Lutcavage 2008). The gastropod samples, which contained the shell carbonate remains, were placed into the silver bowls, treated with 1 M HCl, and reanalyzed for the content of carbon isotopes (Jaschinski et al., 2008). Gases were analyzed in cores sampled with the gravitational tubes.

A two-source mixing model was used to determine the proportion of methane-derived carbon in the animal tissues (Fry and Sherr, 1984):

$$\text{Fm} = (\delta_t - \delta_{\text{poc}})/(\delta_m - \delta_{\text{poc}}) \times 100\%,$$

where δ_t is a value for the carbon isotope in the animal tissue ($\delta^{13}C$) in ‰, δ_{poc} is a value for the carbon isotope of the particulate organic matter in ‰; the $\delta^{13}C$ -25.0 ± 1.3 value for the lake animals feeding on phytoplankton was used in the given case (Yoshi et al., 1999); δ_m is an average $\delta^{13}C$ value equal to 69.7‰ for the St. Petersburg seep microbial mats (Table 1); an average $\delta^{13}C$ value for methane (–61,7 ‰) releasing in the region of the St. Petersburg seep was used during the calculation of Fm for the organic matter in the surface layer of the sediment and the pelagobiont *Macrohectopus branickii* (Dybowsky) (Zemskaya et al., 2012). The use of different δ_m (–69.7 and –61.7‰) signatires in the calculations is explained by the fact that the methanogens of microbial mats inhabited by the animals lighten the $\delta^{13}C$ value compared with carbon derived from methane discharged from the sediments (Zemskaya et al., 2012; 2014).

Table 1. Ecological characteristics, $\delta^{13}C$ and $\delta^{15}N$ (‰) signatures, portion of methane-derived carbon (Fm, %), and trophic positions (TP) of samples collected in the St. Petersburg methane seep (central part of Lake Baikal)

Samples	Mode of life	Feeding strategy	$\delta^{15}N$	$\delta^{13}C$	Fm	TP
Total sediment organic matter ($n = 3$)			–	−37.6 ± 8.2 −45.3… − 29.0	34.3 ± 22.3 10.9 – 55.4	1
Jelly-like microbial mat ($n = 2$)			2.0 ± 0.5 1.7…2.4	−69.7 ± 5.5 −73.6… − 65.8		1
Amphipoda						
Macrohectopus branickii ($n = 1$)	Pelagobiont	Filter feeder, predator	6.7	−28.9	10.7	1.9
Polyacanthisca calceolata ($n = 9$)	Benthopelagic	Necrophage*	11.3 ± 0.5 10.6…12.0	−26.2 ± 0.5 −26.8… − 25.2	2.7 ± 1.1 0.4 – 4.40	3.2 ± 0.14 3.0 – 3.4
Acanthogammarus grewingkii ($n = 1$)	Nectobenthic	Polyphage	8.3	−52.5	61.6	2.6
Coniurus radoschkowskii ($n = 1$)	Benthic, unburrowing	"	7.1	−59.0	76.0	2.4
Echiuropus (*Asprogammarus*) sp. ($n = 1$)	Benthic, burrowing	Detritophage	5.9	−63.1	85.3	2.1
Unidentified ($n = 1$)	?	?	8.5	−61.8	82.2	2.8
Plathelminthes						
Bdellocephala bathyalis ($n = 5$)	Benthic, unburrowing	Predator	11.4 ± 0.8 10.7…12.4	−56.0 ± 3.2 −59.0… − 52.6	69.4 ± 7.1 61.7 – 76.1	3.6 ± 0.2 3.4 – 3.8
Atria cf. *kozhovi* ($n = 2$)	"	"	15.0 ± 0.1 14.9…15.03	−39.8 ± 0.2 −39.9… − 39.6	33.0 ± 0.5 32.7 – 33.3	4.5 ± 0.02 4.4 – 4.5
Sorocelis cf. *hepatizon* ($n = 1$)	"	"	15.1	−36.3	25.3	4.4
Gastropods						
Kobeltocochlea falsipumila ($n = 3$)	Benthic, capable of burrowing	Detritophage	6.3 ± 0.9 5.3…7.0	−64.5 ± 0.5 −64.8… − 63.9	88.3 ± 1.2 86.9 – 89.1	2.2 ± 0.3 1.9 – 2.4
Cottoidea						
Neocottus werestschagini ($n = 2$)	Benthic, unburrowing	Predator	10.0 ± 0.4 9.7…10.3	−63.0 ± 1.0 −63.7… − 62.3	85.0 ± 2.3 83.4 – 86.6	3.2 ± 0.2 3.0 – 3.3
Abyssocottus korotneffi ($n = 1$)	"	"	12.0	−56.7	70.8	3.8

In Tables 1 and 2 above the line: average value ± standard deviation; under the line: the minimum ... maximum value, n is the number of analyzed samples.
* Feeding pattern of this species is unknown; Takhteev (2000) indicates *P. calceolata* to the lifeform of the benthopelagic neophrons due to their morphological characteristics and the fact that the obtained specimens were caught with a plankton net and baited traps.

The trophic positions (TP) of animals consuming the food from the two sources were estimated according to Post (2002):

$$TP = \lambda + (\delta^{15}N_{sc} - [\delta^{15}N_{base\ 1} \times \alpha + \delta^{15}N_{base\ 2} \times (1 - \alpha)])/\Delta_n,$$

where $\lambda = 1$ is a base trophic level for the primary producers (plankton organic carbon (POC) and microbial mats); $\delta^{15}N_{sc}$ is an animal nitrogen isotope value in ‰; $\delta^{15}N_{base\ 1}$ is an average value for the microbial mat isotope equal to 2.0 ± 0.5 ‰; $\delta^{15}N_{base\ 2}$ is a mean of $\delta^{15}N_{POC}$ = 3.8 ± 0.4‰ for phytoplankton in the central depression of Lake Baikal (Yoshi et al., 1999); α is a portion of methane-derived carbon in the animal tissues (=Fm, expressed as a decimal); and $\Delta_{n =}$ 3.4 is fractionation at one trophical level. The trophic position (TP) is defined as noninteger value that represents the energy-weighed number of trophic energy transfers leading to a consumer (Vander Zanden and Rasmussen, 2001).

The Mann–Whitney test, providing the opportunity to compare samples of small size with the abnormal distribution, is used for pairwise comparisons of the values for carbon isotopes of animals belonging to one trophic level. Statistically significant differences ($p < 0.05$) in the mean values for the carbon isotope in

Table 2. The $\delta^{13}C$ and $\delta^{15}N$ (‰) isotope signatures of animals collecteded in Lake Baikal deeper than 100 m (literature and original data)

Samples	Region and sampling depth	Feeding strategy	$\delta^{15}N$	$\delta^{13}C$	Sources
Amphipoda					
Macrohectopus branickii (n = 1)	Chivyrkui Bay, ~300 m depth	Filter feeder, predator	8.3	–29.4	Original data
″(n = 86 for nitrogen (n = 91 for carbon	Lake Baikal middle and northern parts, Chivyrkui Bay, Barguzin Bay	″	7.8 ± 1.0	–25.8 ± 0.8	Yoshii et al., 1999
Polyacanthisca calceolata (n = 3)	Lake Baikal southern part, Begul Cape, 1100 m depth	Necrophage*	11.2 ± 0.4 / 10.7…11.4	–26.3 ± 2.0 / –26.8…–25.9	Original data
″(n = 1)	Lake Baikal middle part, Izhimei Cape, 1600 m depth	″	11.6	–25.5	″
″(n = 1)	Chivyrkui Bay, ~300 m depth	″	11.2	–26.3	″
Acanthogammarus grewingkii (n = 1)	Selenga Shallow, 200 m depth	Polyphage	11.6	–25.9	Yoshii, 1999
″(n = 2)	Barguzin Bay, 1150 m depth	″	11.3 ± 0.8 / 10.8…11.9	–27.2 ± 0.1 / –27.3…–27.23	Original data
Acanthogammarus reichertii (n = 1)	Selenga Shallow northern part, 100 m depth	″	9.9	–28.8	Yoshii, 1999
Ommatogammarus albinus (n = 1)	Lake Baikal unknown region, 130 m depth	Necrophage	12.8	–25.9	″
″(n = 3)	Lake Baikal middle part, oil-methane seep Gorevoy Utes, 897 m deep	″	13.1 ± 1.2 / 11.9–14.3	–26.6 ± 1.6 / –28.4…–25.4	Original data
″(n = 3)	Lake Baikal southern part, Marituy, 1232 m depth	″	12.8 ± 0.8 / 12.0…13.6	–25.5 ± 0.3 / –25.8…–25.2	Original data
″(n = 5)	Lake Baikal Middle part, Izhimei Cape, 1600 m depth	″	13.1 ± 0.4 / 12.7…13.8	–25.4 ± 0.7 / –26.7…–24.9	″
″(n = 3)	Chivyrkui Bay, ~300 m depth	″	12.8 ± 0.1 / 12.7…12.9	–25.3 ± 0.7 / –25.9…–24.6	″
Pachyschesis bazikalowae (n = 1)	Lake Baikal unknown region, 200–250 m depth	Oophage	11.7	–24.1	Yoshii, 1999
Abyssogammarus sarmatus (n = 3)	Selenga Shallow, Kukui underwater elevation, 580 m depth	Polyphage	9.1 ± 0.3 / 8.8…9.4	–26.5 ± 0.3 / –26.9…–26.3	Original data
Paragarjaewia petersii ″(n = 1)	Selenga Shallow, Kukui underwater elevation, 294 m depth	″	7.4	–27.3	″
″(n = 1)	Lake Baikal unknown region, 100 m depth	″	9.6	–27.9	Yoshii, 1999
Parapallasea lagowskii (n = 1)	Lake Baikal middle part, Turka, 600 m depth	Polyphage	8.1	–29.2	Original data
″(n = 1)	Selenga Shallow northern part, 100 m depth	″	10.4	–24.7	Yoshii, 1999
″(n = 3)	Lake Baikal middle part, oil-methane seep Gorevoy Utes, 897 m depth	″	8.5 ± 0.2 / 8.3– 8.7	–27.7 ± 0.6 / –28.3…–27.3	Original data
Macropereiopus florii (n = 1)	″	″	6.0	–42.3	″
″(n = 2)	Lake Baikal middle part, oil and methane seep Gorevoy Utes, 890 m depth	″	6.1 ± 0.2 / 6.0…6.3	–40.4 ± 1.8 / –42.3…– 38.7	Original data

Table 2. (Contd.)

Samples	Region and sampling depth	Feeding strategy	$\delta^{15}N$	$\delta^{13}C$	Sources
Macropereiopus albulus (n = 1)	″	″	6.5	–37.9	
Eulimnogammarus sp. 1 (n = 1)	Lake Baikal Southern part, Begul Cape, 1100 m	?	–1.0	–65.1	Original data
E. sp. 2 (n = 3)	Selenga Shallow, Kukui underwater elevation, 294 m depth	″	$\frac{9.0 \pm 0.1}{8.9...9.1}$	$\frac{-27.6 \pm 0.3}{-27.9...-27.4}$	″
Unidentified (n = 4)	Lake Baikal middle part, Izhimei Cape, 1600 m depth	?	$\frac{12.1 \pm 1.7}{9.5...13.1}$	$\frac{-26.7 \pm 0.6}{-27.5...-26.2}$	″
Unidentified (of average size) (n = 2)	Selenga Shallow, Kukui underwater elevation, 580 m depth	?	$\frac{9.3 \pm 0.7}{8.8...9.4}$	$\frac{-28.1 \pm 0.5}{-28.5...-27.8}$	″
Unidentified (of small size) (n = 2)	Selenga Shallow, Kukui underwater elevation, 294 m depth	?	$\frac{8.8 \pm 0.9}{8.1...9.4}$	$\frac{-27.4 \pm 0.3}{-27.6...-27.2}$	″
Plantelminthes					
Rimacephalus arecepta depigmentata (n = 1)	Lake Baikal southern part, Malen'kii mud volcano, 1320 m depth	Predator	14.5	–25.0	″
Unidentified (n = 1)	Lake Baikal middle part, oil-methane seep Gorevoy Utes, 850 m depth	″	12.5	–30.1	
″ (n = 3)	Lake Baikal northern part, Zavorotnyi Cape, 529 and 870 m depth	″	$\frac{15.2 \pm 1.1}{14.5...16.4}$	$\frac{-25.9 \pm 0.8}{-26.7...-25.2}$	Original data
″ (n = 1)	Barguzin Bay, 1216 m depth	″	13.9	–23.5	″
″ (n = 1)	Baikal Lake middle part, Turka, 680 m depth	″	14.0	–27.9	″
″ (n = 1)	Academic Ridge, 444 m depth	″	13.3	–24.6	″
Gastropods					″
Benedectia fragilis (n = 2)	Lake Baikal, Chivyrkui Bay, 248–205 m depth	Polyphage, scraper	$\frac{9.8 \pm 0.0}{9.8...9.7}$	$\frac{-25.4 \pm 0.2}{-25.6...-25.2}$	″
Benedictia maxima (n = 2)	″	Detritophage, scraper	$\frac{10.1 \pm 0.2}{9.9...10.2}$	$\frac{-25.7 \pm 0.1}{-25.8...-25.6}$	″
Cottoidei					
Abyssocottus korotneffi (n = 1)	Lake Baikal, Charauz-Krasnyi Yar Cape, 745 m depth	Predator	13.2	–26.3	Yoshii, 1999
Batrachocottus baicalensis (n = 2)	Lake Baikal southern part, Listvennichnoye, 120–200 m depth	″	12.4 ± 0.1	–17.2 ± 0.5	″
Batrachocottus multiradiatus (n = 4)	Lake Baikal, Kharauz-Krasnyi Yar Cape, 120–700 m depth	″	15.0 ± 1.6	–23.8 ± 2.1	″
Batrachocottus nikolskii (n = 2)	″	″	13.7 ± 0.2	–25.0 ± 0.1	″
Cottinella boulangeri (n = 1)	Lake Baikal, Kharauz-Krasnyi Yar Cape, 700 m depth	″	13.9	–25.9	″
Cottus kesslerii (n = 1)	Lake Baikal, Kharauz-Krasnyi Yar Cape, 120–200 m depth	″	11.2	–16.3	″

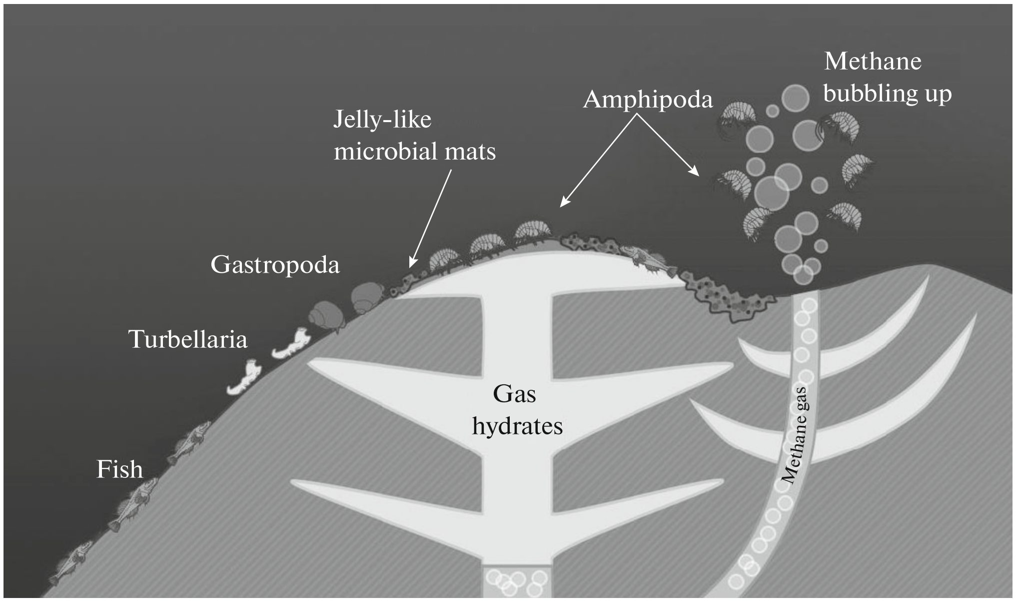

Fig. 1. Diagram of St. Petersburg methane seep geomorphologial structure and distribution of macroinvertebrates and cottoid fish.

the tissues of two animal groups to be compared can be evidence of different food resources. The calculations are performed using the Statistica 6 program package and the Excel for Windows program.

RESULTS AND DISCUSSION

Description of a biotope and distribution of macroinvertebrates and fish. The bottom landscape of the lake in the area of the St. Petersburg methane seep is made up of hills of various sizes and a height of 4 to 6 m. The hilltops had rounded profiles; the top part of the high hills looked like a long mountain ridge. The hills were formed through a rise of gas hydrates; gas discharged bubbling from the bottom sediments of two hills during sampling (Fig. 1). Flowing methane had a mixed biogenic and thermogenic origin with a small proportion (0.23–0.33%) of ethane (Zemskaya et al., 2012; Khlystov et al., 2013).

The bottom sediment upper layers with a thickness of several millimeters to 1 cm were nonuniform in color; the light brown tints typical for the oxic sediments were predominant. The black color spots typical for the anoxic sediments were indicated. The upper sediment layer (approximately 7 cm thick) was represented by fine-dispersed components (less than 30 μm) generally composed of diatom silts. In addition, mica flat crystals with impurity of fine silica sand occurred; the threads of sulfur bacteria were found in some sediment samples. The jelly-like microbial mats including methanotrophic bacteria, methanogenic archea, and cyanobacteria were usually located between the spots of the oxic and anoxic sediments on the hills (Zemskaya et al., 2015). A similar composition of the microorganisms was registered in the surface sediment layer (Kadnikov et al., 2012). The organic matter in the upper layer of the bottom sediments in this region contained 10.9 to 55.4% methane-derived carbon (Table 1).

The specimens of some species of benthic amphipods (Crustacea: Amphipoda) and gastropods *Kobeltocochlea falsipumyla* Sitnikova (Caenogastropoda: Benedictiidae) were found in the microbial mat zone. Amphipods were predominant among the macroinvertebrates by the number of species; they were evenly distributed and the density of the visibly distinct amphipods comprised approximately 100 specimens per m^{-2}. In addition, amphipods were indicated at the bottom with a number of tiny pores through which the gas-containing and mineralized fluids flowing out happened. Benthopelagic amphipods (*Polyacanthisca calceolata* Bazikalova) generally floated in the bottom water; their maximal concentration was registered at the points of bubbling gas releases. Gastropods in the small groups of 5 to 10 specimens per m^{-2} were found to occur at the bottom. Large planarians (Turbellaria: Dendrocoelidae) of the *Bdellocephala, Atria*, and *Sorocelis* genera were found lying on the side slopes of the hill ridges often at some distance from the microbial mats. The cottoid fish localization was nonuniform. The bottom cottoid fish were lying still and immovable on the surface of the silt bottom sediments and the exposed gas hydrates. The pelagic fish were actively moving in the bottom strata waters. Eight species of the cottoid fish were found at the St. Petersburg methane seep; six and two species of them were indicated to the demersal and pelagic fish, respectively. The demersal species were represented by *Neocottus werestschagini* (Taliev), *Abyssocottus korotneffi* Berg, *A. gibbosus* Berg, *Cottinella boulengeri* (Berg), *Asprocottus abyssalis* Taliev et Korjakov, and *Batrachocottus*

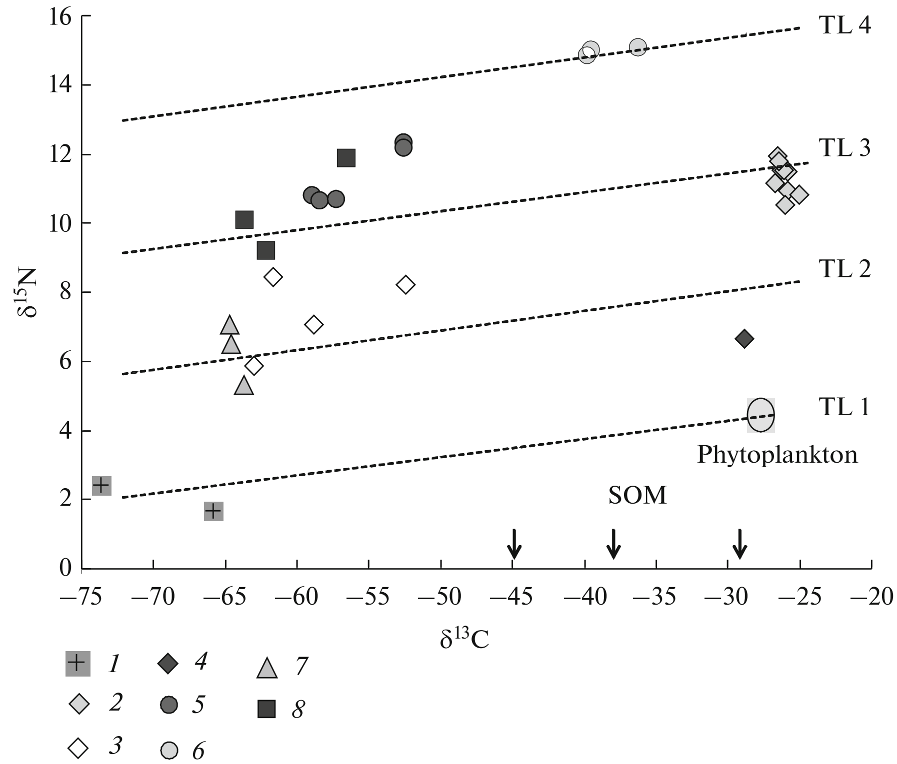

Fig. 2. The $\delta^{13}C$ and $\delta^{15}N$ values and trophic levels (TL) for animals in the St. Petersburg methane seep. (*1*) Microbial mats, (*2*) benthopelagic amphipods, (*3*) benthic amphipods, (*4*) planctonic amphipods, (*5*) turbellaria (*Bdellocephala*), (*6*) turbellaria (*Atria, Sorocelis*), (*7*) gastropods, and (*8*) cottoid fish species; SOM—sediment organic matter.

nikolskii (Berg); the pelagic species were as follows: *Comephorus baicalensis* (Pallas) and *Comephorus dybowskii* Korotneff (Sideleva and Fialkov, 2014).

The carbon and nitrogen isotope values and trophic positions of macroinvertebrates and fish at the St. Petersburg methane seep. The values of the stable isotopes of carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) varied from –25.2‰ (amphipoda *P. calceolata*) to –64.8‰ (gastropods) and from 5.3‰ (a specimen of *K. falsipumyla*) to 15.1‰ (planarians of the *Sorocelis* genus), respectively (Table 1, Fig. 2). Negative $\delta^{15}N$ values were not revealed.

The animals are divided into two groups according to the isotope composition of carbon; the first group includes the animals characterized by the heavier $\delta^{13}C$ values (>–40‰). This group unites pelagic (*M. branickii*) and benthopelalgic (*P. calceolata*) amphipods ($\delta^{13}C$ –29.0 and –26.8‰, respectively) and planarians of the *Atria* and *Sorocelis* genera ($\delta^{13}C$ –36.3 and – 39.9‰, respectively). The proportion of methane-derived carbon comprised 4.4% in *P. calceolata*, 10% in *M. branickii*, 25% in *Atria,* and 33% in *Sorocelis* (Table 1). These data indicate that the photosynthetic organic matter was predominant in the food of the above-listed animals. The animals of this group occupy different trophic positions: pelagobiont *M. branickii*—TP 1.9; benthopelagic amphipods *P. calceolata*—TP 3.2; and benthic planatians of *Sorocelis* and *Atria* genera—TP 4.4 and 4.5, respectively (Table 1); therefore, both the primary and the secondary consumers are present in this group.

The second group involves animals characterized by the lighter $\delta^{13}C$ values (<–40‰) and a high methane-derived carbon portion of 61.6 to 89%. The second group unites planarians (*Bdellocephala bathyalis* Timoshkin and Porfirieva), gastropods (*K. falsipumyla*), benthic (*Coniurus radoschkowskii* (Dybowsky) and *Echiuropus* sp.), and nectobenthic amphipods (*Acanthogammarus grewingkii* (Dybowsky) and the demersal cottoid fish (Table 1). The results (Table 1) indicate the food of these animals has contained more than 60% of the methanotrophic organic matter produced by the microorganisms. The animals of this group also occupy different trophic positions: gastropoids—TP 2.2; burrowing amphipods *Echiuropus*—TP 2.1, *Coniurus*—TP 2.4; and nectobenthic amphipods *A. grewingkii*—TP 2.8 (Table 1). There is a significant difference in the $\delta^{13}C$ values ($p < 0.05$) between the primary consumers, such as the gastropods and the burrowing amphipods, that indicates the different food composition in these macroinvertebrates, despite the partially overlapping. The $\delta^{13}C$ values were an indication of the significant difference in the secondary consumers too, such as planarians of the *Bdello-*

cephala genus (TP 3.4–3.8) and the *N. werestschagini* demersal cottoid fish (TP 3.0–3.3). Therefore, the food composition of these animals was different, despite the overlapping ranges of their prey.

The lighter isotope carbon ($\delta^{13}C$ from –52 to –64.8‰) along with the positive $\delta^{15}N$ > 5.9‰ (Table 1) is evidence of the absence of symbiotrophy in animals associated with the St. Petersburg methane seep.

The values for heavy weight $\delta^{13}C$ (>–30 ‰) and $\delta^{15}N$ (>6.0 ‰) (Table 2) are typical for most animals inhabiting other regions, including the background deepwater areas of Lake Baikal.

Modes of life and feeding strategies. The two analyzed species of amphipods (*P. calceolata* and *M. branickii)* consuming the photosynthetic organic carbon differ in feeding strategies. In the pelagial trophic web of Lake Baikal, *M. branickii* is both a filter feeder consuming phytoplankton (a primary consumer) and a predator (a second consumer) eating planktonic crustaceans and probably the larvae of the golomyanka (*Comephorus baicalensis*) (Mel'nik et al., 1995). Previous surveys showed that the *Macrogectopus* trapped in the middle and northern parts of the lake consumed mainly the photosynthetic organic matter ($\delta^{13}C$ –25.8 ± 0.8‰), despite of 2 or 3 positions in the trophic web (Yoshii et al., 1999).

The amphipods *M. branickii* trapped in the water bottom of the St. Petersburg methane seep filtered the seston with a number of methanotrophic bacteria, judging by the trophic position (TP 1.9) and a portion of methane-derived carbon (Fm = 10.7%) (Table 1). This is quite apparent, since the increased amount of metanotrophic bacteria and the increased concentrations of methane were revealed in the water column in the methane seeps of the lake (Zakharenko et al., 2015). It is well known that under an ice cover, methane accumulation occurs in the water column of Lake Baikal, resulting in lightening the isotopic composition of the inorganic carbon dissolved in the water ($\delta^{13}C_{DIC}$ to –35.6‰) and the organic carbon of phytoplankton ($\delta^{13}C_{poc}$ up to –33.5‰) (Prokopenko and Williams, 2005). The impact of methane on the primary production and its involvement in the pelagic food webs of Lake Baikal remain to be seen.

P. calceolata are identified to the mode of life of benthopelagic neophrons according to the functional and morphological specificities, while according to the feeding strategy they are assigned to necrophages (Takhteev, 2000). This species is caught in a trap with dead fish bait, which indicates the food reaction of the *P. calceolata* species to the bait smell. Obligate necrophages of the *Ommatogammarus* genus have a bait reaction similar to *P. calceolata* and are trapped in large amounts. The $\delta^{15}N$ values in *Ommatogammarus albinus* (Dybowsky) comprised 12.8‰ (TP 3.6) (Yoshii, 1999); i.e., they are close to that in *P. calceolata* (TP 3.2) trapped in the water area of the St. Petersburg methane seep (Table 1). The *P. calceolata* specimens trapped both at the Begul Cape, where the methane outlets might exist, and in the reference areas of the lake (Chivyrkui Bay and Izhimei Cape) had a heavier carbon isotope signature (Table 2), which indicates their consumption of photosynthetic food, regardless of whether they inhabit the area of methane releases or not.

Nectobenthic amphipods *A. grewingkii* are omnivorous opportunists and feed on any available food items: detritus, invertebrates, and carrion. They pick up the algae that settled to the bottom from the surface of the sediment in periods of mass developments of planktonic diatoms (Mekhanikova, 2010). The *A. grewingkii* in the reference areas of the lake (Barguzin Bay and Selenga Shallow) consumed food of photosynthetic origin (Table 2), unlike the specimens inhabiting the St. Petersburg methane seep, which food consisted of ~60% of the methanesynthetic organic matter (Table 1).

The benthic amphipods *C. radoschkowskii* and *Echiuropus* (*Asprogammarus*) sp. differ in the modes of life and food composition. V.V. Takhteev (2000) classifies *C. radoschkowskii* as carapace pelophylous. The feeding strategy of this species is unknown; however, by analogy with the other similar species of Baikal large amphipods inhabiting the bottom sediment surface, it can be supposed that *C. radoschkowskii* has a wide trophic niche. Those species amphipods living in the St. Petersburg methane seep exhibited approximately 76% of methane-derived carbon.

Bazikalova (1975) suggested small amphipods belonging to the *Echiuropus* genus *(Asprogammarus* subgenus) are capable to burrow into the sediments. *Echiuropus* (*Asprogammarus*) *pulchellus* (Dybowsky) and *E. (A.) pulchelliformis* (Bazikalova) were found in the benthic samples from the St. Petersburg methane seep. The exemplars of amphipods used for the isotope analysis were possibly one of these species. Fine detritus and planktonic diatoms *Aulacoseira baicalensis* (Meyer) and *Cyclotella* sp. are detected in the gut contents of one specimen of *E.* (*A.*) *pulchellus* from the St. Petersburg methane seep. The food of *Echiuropus* (*Asprogammarus*) sp. inhabiting the methane seep consisted of 85% of methane-derived carbon. (Table 1).

Issues of the other amphipods possibly belonging to species *Poekilogammarus* (*Onychogammarus*) *megonychoides* Bazikalova had a lighter carbon isotope composition ($\delta^{13}C$ –61.8‰) (Table 1). This species was often found in the benthic samples of the survey region. *P.* (*O.*) *megonychoides* is a nectobenthic species (Tachteew, 1995). A bolus of one of the *P.* (*O.*) *megonychoides* species from the St. Petersburg methane seep consisted of fine detritus and planktonic diatom algae (*Aulacoseira* sp.); a share of the consumed photosynthetic carbon percentage comprised less than 20% (Table 1).

Gastropods of the *Kobeltocochlea* genus are detritophags grazing on the substrate. The bolus composition in gastropods sampled at the St. Petersburg methane seep included detrius and planktonic diatom algae. However, the photosynthetic organic matter made up a small portion (1–13%) in food of gastropods *K. falsipumyla* upon analyzing the $\delta^{13}C$ values (–64.5 ± 0.5‰) and the methane-derived carbon content (Fm 88.3 ± 2%) (Table 1).

The animals of these groups from the nonseep areas of Lake Baikal, unlike amphipods and gastropods inhabiting the St. Petersburg methane seep, consume planktonic detritus. An exclusion can be made for amphipods of the *Eulimnogammarus* genus trapped at 1100-m depth at the Begul Cape (Table 2); they have the lightest isotope values of $\delta^{13}C$ (–65.1‰) among the analyzed amphipods.

The information on the food composition in deep-water planarians in Lake Baikal is sparse. Porfir'eva (1977) has supposed that planarians tend to occupy higher positions in the trophic chains, using oligochaeta, amphipods, and weakened fish as food items. Cannibalism is quite common among some planarian species of Lake Baikal (O.A. Timoshkin, personal communication).

With regard to the trophic position and the methane-derived carbon percentage (TP 3.4–3.6; Fm 61.7–76.1%) in planarians of the *Bdellocephala* genus at the St. Petersburg methane seep, they fed on the gastropods and benthic amphipods, which consumed food containing 60–87% of the methanotrophic origin. Planarians of the *Sorocelis* and *Atria* genera, occupying higher trophic positions (TP 4.4–4.5) (Fig. 2) and consuming mixed food with the predominant photosynthetic organic matter (Fm 25–33%), might have some other preys, as compared to that in *Bdellocephala*. They could feed on turbellaria and cottoid fish, which consumed, in turn, the invertebrate species absorbing more than 60% of methane-derived carbon (Table 1) and the other animals mostly feeding on the photosynthetic organic matter.

The planarians sampled in the reference areas of the Lake Baikal abyssal zone and the area of the Malen'kii mud volcano and at the thermal anomaly close to the Zavorotnyi Cape and the gas hydrate deposits of the Academic Ridge mountain range had a heavier carbon isotope signature ($\delta^{13}C$ > –23.5–27.9‰) (Table 2). These data indicate that their prey were animals mostly consuming the photosynthetic organic matter.

Cottoid fish are carnivorous; their food in Lake Baikal is generally based on amphipods. The amphipod proportions in the bolus reach 96.0 and 92.4% in the *A. korotneffi* and *N. werestschagini* abyssal species, respectively (Sideleva and Mekhanikova, 1990). No shared species was found in the feeding components of these amphipods; *A. korotneffi* feeds on mobile nectobenthic forms, while *N. werestschagini* uses small burrowing forms (Sideleva and Mekhanikova, 1990). The $\delta^{13}C$ values for six cottoid fish, including *A. korotneffi* trapped in the abyssal zone of Lake Baikal (Table 2), vary in the range of –26.3 to –16.3‰, which indicates the involvement of carbon of photosynthetic origin (Yoshii, 1999).

CONCLUSIONS

The analyzed macroinvertebrates and fish inhabiting the St. Petersburg methane seep in Lake Baikal occupy different trophic positions in the mixed chemo- and photosynthetic web. The animals feed on combined food, which differed in the portion of methane-derived carbon in the range of 2.7–33% (in pelagic and benthopelagic amphipods and planarians of the *Bdellocephala* genus) to 60–89% (in nectobenthic and benthic burrowing amphipods, demersal gastropods, planarians of the *Atria* and *Sorocelis* genera, and the cottoid fish). The organic matter of photosynthetic origin occurs at the bottom as sunken phytoplankton- and zooplankton-derived compounds. Methane is involved into the food chain through microorganisms; it can be traced for all of the chains in the food web of the seep biocenosis. The community of invertebrates and fish associated with the St. Petersburg methane seep area is composed of animals typical for the benthal of the lake, which consume the photosynthetic organic matter out of the methane discharge areas.

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