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Contribution of Chemoautotrophic Production to Freshwater Macroinvertebrates in a Headwater Stream Using Multiple Stable Isotopes

key words: sulfur, food webs, aerobic habitat, microbial loop

Abstract

We estimated the food sources of macroinvertebrates using carbon, nitrogen, and sulfur stable isotopes in a headwater stream. Stream food webs, including macroinvertebrates, rely on production from autochthonous and allochthonous photosynthesis. We found that a freshwater grazer, the snail *Semisulcospira libertina*, may assimilate different food sources, based on stable carbon and nitrogen isotope evidence from snail muscle and the much lighter sulfur isotope signature than those of other associated macroinvertebrates. Previous studies have shown the importance of methanotrophic and sulfur bacteria in reductive environments (clay and organic-rich sediments) as food sources for macroinvertebrates. Our results show that the production by chemoautotrophic bacteria contributes to the food sources of a snail in a stream. Thus, the chemoautotrophic bacteria are important in the freshwater food webs, even in mostly aerobic habitats.

1. Introduction

In general, aquatic food webs rely on *in situ* algal production (i.e., macro-and microalgae) and allochthonous production (i.e., terrestrial litter) derived from photoautotrophy (e.g., FINLAY, 2001). In addition, many reports have shown the importance of food sources for the macroinvertebrate community from *in situ* chemoautotrophic sources, such as methanotrophic and sulfur-oxidizing bacteria, in deep-sea hydrothermal vents and cold seeps (e.g., LEVIN and MICHENER, 2002), lake bottoms (e.g., KIYASHKO *et al.,* 2001; GREY *et al*., 2004; HERSHEY *et al*., 2005, 2006), and backwater stream pools (KOHZU *et al*., 2004).

The food webs based on chemoautotrophy prevail in reductive habitats because sulfur-oxidizing and methanotrophic bacteria are commonly observed in and nearby anoxic condi-

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tions, respectively (see comprehensive review in KROUSE and GRINENKO, 1991). In freshwater ecosystems, such habitats are found in typical reductive environments of clay-rich sediment and large amounts of litter detritus (e.g., GREY *et al.,* 2004; KOHZU *et al*., 2004). In the gravel surface and water body of freshwater ecosystems, no studies have examined the contribution of chemoautotrophy to macroinvertebrate food sources. If chemoautotrophy is important in freshwater food webs, we must consider the link between aerobic and anaerobic habitats in these ecosystems.

Stable carbon isotope analysis is used to estimate the contribution of chemoautotrophy in food webs (e.g., KIYASHKO *et al*., 2001; KOHZU *et al*., 2004; HERSHEY *et al*., 2005, 2006). The $\delta^{13}C$ values of some macroinvertebrates in lakes and backwater pools vary from –65 to –30‰. Chemoautotrophic bacteria, such as methanotrophic and sulfur-oxidizing bacteria, have much lower $\delta^{13}C$ values than photoautotrophs. Thus, when their $\delta^{13}C$ values are markedly lower than those of particulate organic matter (POM) and periphyton, this should indicate that a possible contribution of chemoautotrophy to the macroinvertebrates (KOHZU *et al*., 2004).

Sulfur isotope signatures can be used to identify the sulfur nutrition in aquatic ecosystems in which sulfur-oxidizing bacteria are involved (FRY, 1986; GREY and DEINES, 2005). Incorporation of elemental sulfur into chemoautotrophs results in lower ^{34}S values, because ^{34}S-depleted sulfides are the likely source (FRY, 1986). In marine and saline habitats where reductive environments often develop, biomass with very low ^{34}S values, derived from the activity of sulfate-reducing and sulfur-oxidizing bacteria, contributes to the food webs (e.g., KHARLAMENKO *et al*., 2001). Nevertheless, the values of the sulfur isotope signature for understanding the role of chemoautotrophy has not been examined in stream food webs. Here, we investigated the contribution of chemoautotrophs (i.e., sulfur-oxidizing bacteria) to macroinvertebrates, especially freshwater snails, in a headwater stream using multiple isotope analysis, including carbon, nitrogen, and sulfur.

2. Methods

2.1. Study Area

The study site was in the Honzawa Stream, a headwater stream, on a small hill (Aobayama) in Sendai, northeastern Honshu, Japan (38°15′ N, 140°51′ E; 90 m above sea level). The area consists of Pliocene tuff of marine origin. All samples were collected within a 30 m length of riffle, and the streambed consisted of cobbles and sand with many litter packs. The stream width and water depth were 15–40 cm and 5–10 cm, respectively. The pH, water temperature, and electrical conductivity of the stream water were 6.2, 14.4 °C, and 25.2 $\mu S\ m^{-1}$, respectively, which were measured using a multiple water quality sensor (U-22; Horiba Co., Kyoto, Japan). The dissolved oxygen content of the surface water and the current velocity of surface flow were 7.1 ± 0.1 mg l^{-1} and 22.0 ± 0.3 cm s^{-1} (mean ± SD, $n = 3$), respectively. The SO_4^{2-} (sulfate) concentration of the water was estimated as 1.3 ± 0.1 mmol l^{-1} using ion chromatography (DX-120; Dionex Co., Sunnyvale, CA, USA). The chlorophyll *a* content of the biofilm brushed from the gravel surface was 0.88 ± 0.21 μg cm^{-2}, as extracted by *N,N*-dimethylformamide and measured using a fluorometer (AU-10; Turner Designs, Sunnyvale, CA, USA). All of these measurements were made on 29 June 2002.

2.2. Sample Collection and Preparation

We collected 3 replicates of dominant benthic macroinvertebrates from the streambed on 29 June 2002. Macroinvertebrate feeding groups were classified according to MERRITT and CUMMINS (1996). All macroinvertebrate tissues were subjected to stable isotope analysis with three individuals of each (several more for chironomid larvae). Muscle tissues was taken from *Semisulcospira libertina* (gastropods); lipids were removed using a chloroform-methanol mixture (2 : 1 by weight) because of the high lipid

content of the muscle. For sulfur isotope measurements, we collected 3 replicates of dominant invertebrates from each feeding group, including *S. libertina* (gastropod grazer), *Ephemera japonica* (mayfly and collector-gatherer), and *Anotogaster sieboldii* (dragonfly and predator). We did not observe any fishes during sampling, probably because of exclusion by a water gate at the downstream.

As potential food sources, we collected three replicates of suspended particulate organic matter (SPOM), benthic particulate organic matter (BPOM), and periphyton from the stream on 29 June 2002. However, for the periphyton samples two replicates could be measured for sulfur isotope. SPOM was collected by filtering the stream water through glass fiber filters (GF/F; Whatman Co., Florham Park, NJ, USA). BPOM was similarly collected using a 250 μm Surber net, and measured as two size fractions: >1 mm and <1 mm, using sieves. For sulfur isotope analysis, BPOM (>1 mm) was washed using deionized water to remove dissolved constituents, and combusted in a Parr Bomb (#1108; Parr Instrument Co., Moline, IL, USA), and the resulting sulfate was precipitated as $BaSO_4$. Periphyton was collected by scouring the gravel using a brush with three replicates. SPOM, BPOM, and periphyton samples were acidified with 1 mol L^{-1} HCl to remove any carbonate contaminants. All samples were freeze-dried and stored at –20 °C until stable isotope analysis.

We collected three replicates of surface stream water, filtered it using a Millipore filter (pore size 0.25 μm; Millipore, Billerica, MA, USA), and acidified it using 1 mol L^{-1} HCl to remove dissolved bicarbonates. The samples were boiled and sulfates were recovered as $BaSO_4$ by adding warm 10% $BaCl_2$ solution. The samples were then dried for isotope analysis.

2.3. Stable Isotope Analysis

The carbon and nitrogen isotope ratios of the samples were measured using a mass spectrometer (DELTA plus; Finnigan MAT, Bremen, Germany) connected to an elemental analyzer (NA-2500; Fisons Instruments, Beverly, MA, USA). For sulfur isotope measurements, the $BaSO_4$ isolates were converted to SO_2 gas via the thermal decomposition method of YANAGISAWA and SAKAI (1983). $^{34}S/^{32}S$ ratios were determined using a dual inlet mass spectrometer (SIRA-10; VG Isogas, Middlewich, UK) at the Institute for Study of the Earth's Interior, Okayama University, Japan. However, one of three periphyton replicate samples was not successfully measured for the sulfur isotope, thus we obtained only duplicate data of periphyton sample for the sulfur isotope.

The results are presented using common delta notation calculated as $\delta^{13}C$, $\delta^{15}N$, or $\delta^{34}S = (R_{sample}/R_{standard} - 1) \cdot 1000$ (‰), where R is the $^{13}C/^{12}C$, $^{15}N/^{14}N$, or $^{34}S/^{32}S$ ratio for $\delta^{13}C$, $\delta^{15}N$, or $\delta^{34}S$, respectively. Pee Dee Belemnite (V-PDB), nitrogen in air, and Canyon Diablo Troilite were used as standards for $\delta^{13}C$, $\delta^{15}N$, and $\delta^{34}S$, respectively. Errors during the overall analyses were within ± 0.2‰ for $\delta^{13}C$, $\delta^{15}N$, and ^{34}S. Analysis of trophic relationships using stable isotope ratios is generally based on the premise that the enrichment of $\delta^{13}C$ and $\delta^{34}S$ during trophic transfer is slight (+0.4 ± 0.12‰ and +0.4 ± 0.52‰, respectively), while that of $\delta^{15}N$ is fairly large (+2.0 ± 0.20‰; MCCUTCHAN *et al.*, 2003).

3. Results

Most macroinvertebrates had similar $\delta^{13}C$ and $\delta^{15}N$ values (Fig. 1). However, the $\delta^{13}C$ value of *S. libertina* was significantly lower than those of other macroinvertebrates and potential food sources, i.e., BPOM, SPOM, and periphyton (one-way ANOVA, $F = 8.84$, $p < 0.01$, $n = 3$, Tukey multiple comparison, $p < 0.05$, $n = 3$), and two predatory species, *Parachauliodes japonicus* (Megaloptera) and *A. sieboldii* had higher $\delta^{15}N$ values than the other feeding groups, including grazers (*S. libertina*), caddisfly filter-feeders (*Diplectrona* spp., *Wormaldia* spp.), caddisfly shredders (*Goerodes* spp.) and collector-gatherers (*E. japonica, Amphinemura* spp., Chironomidae gen. spp). Except for *S. libertina*, the $\delta^{13}C$ values of macroinvertebrates (–28.0 to –26.2‰) were similar to those of BPOM, SPOM, and periphyton.

The $\delta^{13}C$ values of BPOM, SPOM, and periphyton ranged from –27.9 to –25.7‰. The $\delta^{13}C$ and $\delta^{15}N$ values of BPOM did not vary markedly with size. Moreover, the $\delta^{13}C$ value of BPOM was similar to that of C 3 terrestrial plant litter (–29 to –26‰; FRANCE, 1995).

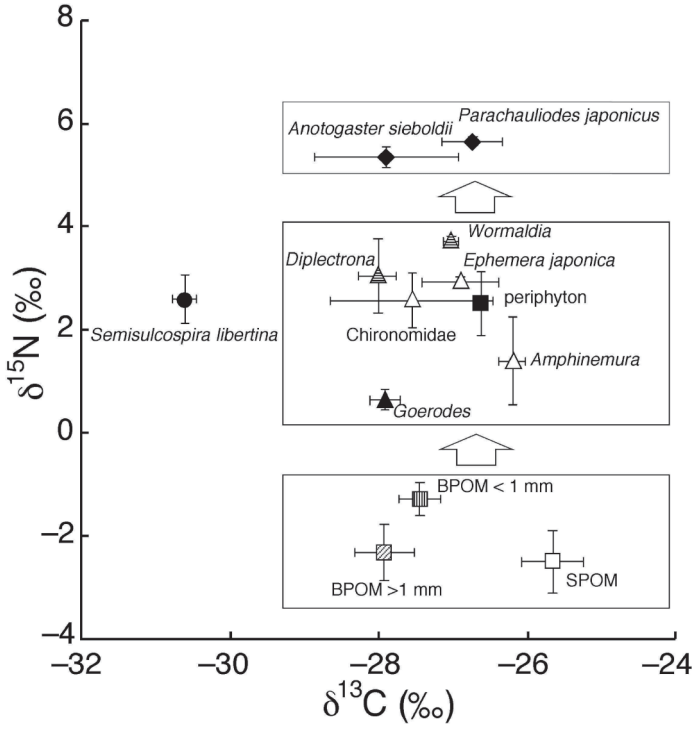

Figure 1. Plot of $\delta^{13}C$ and $\delta^{15}N$ values of macroinvertebrates and their potential food sources. BPOM (<1 mm and >1 mm mesh size) and SPOM indicate benthic and suspended particulate organic matter, respectively. Boxes indicate potential food sources. White, black, and hatched triangles indicate collector-gatherers, shredders, and collector-filterers, respectively. Black diamonds and circles indicate predators and grazers, respectively. The feeding groups were assigned according to MARRIOTT and CUMMINS (1996). Each symbol indicates mean ± SE (n = 3).

The $\delta^{13}C$ and $\delta^{15}N$ values of periphyton may be affected by current velocity and the concentration of dissolved inorganic carbon (FINLAY *et al*., 1999); however, we found a narrow range of $\delta^{13}C$ values for periphyton among replicate samples (–26.8 to –26.6‰).

The $\delta^{34}S$ values of a POM feeder (*E. japonica*) and a predator (*A. sieboldii*) were similar to those of dissolved sulfate in the stream water, periphyton and BPOM (Fig. 2). The $\delta^{34}S$ value of the snail (*S. libertina*; from –5.1 to –1.3‰) was significantly lower than those of other macroinvertebrates, BPOM, and dissolved sulfate (one-way ANOVA, $F = 6.19$, $p < 0.05$, $n = 3$, Tukey multiple comparison, $p < 0.05$, $n = 3$). Moreover, there was a significant correlation among the consumers (Pearson's correlation coefficient, $r = 0.940$, $p < 0.001$, $n = 9$, Fig. 2).

4. Discussion

The $\delta^{13}C$ and $\delta^{34}S$ values of *S. libertina* were consistently depleted relative to other macroinvertebrates inhabiting the streambed and their possible food sources. The food web of the macroinvertebrate community appears to rely on BPOM, SPOM, and periphyton as

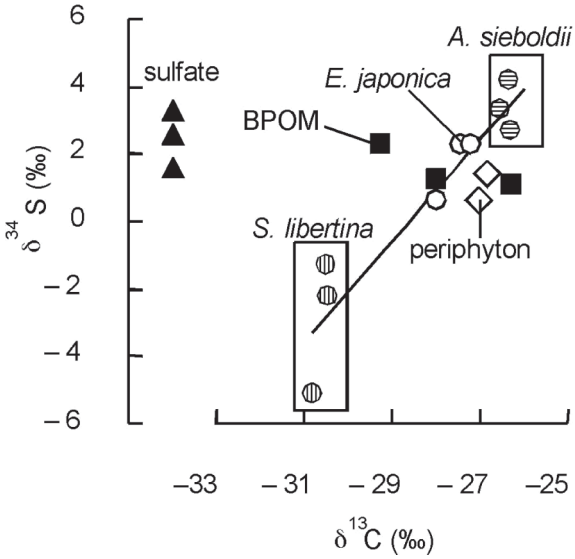

Figure 2. Plot of $\delta^{13}C$ and $\delta^{34}S$ values of sulfate in stream water (only $\delta^{34}S$), BPOM (benthic particulate organic matter, >1 mm mesh size), periphyton, and macroinvertebrates (snail, *Semisulcospira libertina*; POM feeder, *Ephemera japonica*; and predator, *Anotogaster sieboldii*). The line shows the significantly correlation among the consumers by Pearson's correlation coefficient ($r = 0.940$, $p < 0.001$, $n = 9$).

potential food sources (Figs. 1 and 2). Generally, macroinvertebrate species in streambeds are sustained by POM and autochthonous (periphyton) food sources as shown in previous studies using carbon and nitrogen stable isotope analyses (e.g., FINLAY *et al*., 2001). Nevertheless, the $\delta^{13}C$ value of *S. libertina* was significantly lower than those of other macroinvertebrates and potential food sources, suggesting that *S. libertina* assimilates different food sources from the rest of the macroinvertebrate community that inhabits the same streambed. However, *S. libertina* were observed not only on the stones, but also on the deposit terrestrial litters in the site. The food source of *S. libertina* may be organic matter on the stream gravel and litter because *S. libertina* generally feeds on these surfaces as a grazer.

At the oxic/anoxic interface with reductive habitats, some benthic macroinvertebrates assimilate bacterial production from methanotrophs and sulfur-oxdizing and sulfate-reducing bacteria (e.g., MIZOTA *et al*., 1999; KIYASHIKO *et al*., 2001; GREY *et al*., 2004; KOHZU *et al*., 2004; HERSHEY *et al*., 2005, 2006) On the gravel and litter surface, a thick biofilm may often be present, and this deep layer of biofilm is anoxic, as evidenced by very low amounts of dissolved oxygen (OKABE *et al*., 2005 in the wastewater habitat, NAKANO *et al*., 2006 in an experimental stream). Methane-oxidizing bacteria that preferentially consume ^{12}C are usually characterized by distinctly low $\delta^{13}C$ values (GROSSMAN *et al.,* 2002). As a consequence, the $\delta^{13}C$ values of the organisms that consume biomass derived from methanotrophs are expected to be low (KIYASHIKO *et al*., 2001; GREY *et al*., 2004; KOHZU *et al*., 2004). To date, researchers have empirically deduced that the extremely high depletion of $\delta^{13}C$ ($\ll -30‰$) in macroinvertebrates can be attributed to the incorporation of ^{13}C-depleted methane-derived carbon source via methane-oxidizing bacteria (KOHZU *et al*., 2004). Thus, the contribution of methanotrophs to the food source of the snail *S. libertina* appeared low because the $\delta^{13}C$ value of *S. libertina* was not extremely depleted ($-30.6 \pm 0.2‰$).

In addition, methanogen and sulfate-reducing bacteria grow on acetate or acetate plus sulfate, and at low acetate concentrations, the rate of acetate consumption by a sulfate-reducing bacteria approached 15-fold the rate of a methanogen (SCHÖNHEIT *et al*., 1982). Thus, at low acetate concentrations in the biofilm, sulfate-reducing bacteria might be more than methanogen.

Another possible food source for the snails is from chemoautotrophic bacteria, such as sulfur-oxidizing bacteria. Sulfur-oxidizing chemoautotrophic bacteria are the most ^{13}C-depleted primary producers in the lake sediment (KIYASHIKO *et al*., 2001) because sulfur-oxidizing chemoautotrophic bacteria assimilate light carbon isotopes to a greater degree than marine photoautotrophs (RUBY *et al*., 1987). Relatively low $\delta^{13}C$ values (–34.0 to –31.7‰) are reported for mats of sulfur-oxidizing bacteria from hydrothermal vents (CARY *et al*., 1989). For sulfur isotopes, sulfate-reducing bacteria produce sulfides that are depleted in ^{34}S during dissimilatory reduction (JONES and STARKEY, 1957; KAPLAN and RITTENBERG, 1964). The low $\delta^{34}S$ values of organisms relative to common seawater sulfates indicate the contribution of the reduced form of sulfur from the interstitial water of the anaerobic sediment to their biomass through assimilation of the symbiotic sulfur-oxidizing bacteria in their tissue (see CARY *et al*., 1989) and nutrition from sulfate-reducing and sulfur-oxidizing bacteria in clay-rich sediment (MIZOTA *et al*., 1999).

From the relevant evidence of the bacteria, we interpret that lower $\delta^{13}C$ associated with lower $\delta^{34}S$ values observed in the muscle of the grazer *S. libertine* reflect the reduced sulfur species (i.e., nutrition from sulfate-reducing and sulfur-oxidizing bacteria) in the body tissue, although the productivity of microalgae occurred on the surface of the river gravel (0.88 ± 0.21 μg chlorophyll *a* cm^{-2}). Deposit feeders are considered to selectively assimilate food sources from the sediment, and diatoms were not largely assimilated by chironomid larvae, despite their high productivity (DOI *et al*., 2006). Although snails would feed on the bulk biofilm on the stone and litter surfaces, they may selectively assimilate prey derived from bacterial production and/or heterotrophic protozoa, which feed on the bacteria, over periphytic diatoms. Unfortunately, the limitation of the present study is that samples were taken only once, but this study shows very clearly an estimation of the contribution of chemoautotrophic bacterial production for the snails. However, the importance of chemoautotrophs may vary seasonally.

Biofilm consists of various microorganisms such as bacteria, algae, and fungi, and these microorganisms create a microbial exopolysaccharide matrix (LOCK *et al*., 1984). The deep layer of biofilm on the stones is anoxic, as shown by very low levels of dissolved oxygen and high H_2S concentrations (OKABE *et al*., 2005). Thus, the heterotrophic protozoa that can assimilate the ^{34}S-depleted sulfur from the reductive layer of the biofilm, including benthic microalgae and bacteria, on the stones and litters. Bacteria and heterotrophic protozoa, such as ciliates and flagellates, are highly abundant in biofilm, and microbial loops in streambeds may be as active as those in lakes and oceans (FUKUDA *et al*., 2004). So we can assume that the protozoa and bacteria were the probable sources of ^{34}S-depleted organic matter for the snail. Grazers, such as snails, may serve as an important connection between the photoautotrophic and chemoautotrophic components of the food web in stream ecosystems.

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6. References

CANFIELD, D. E., B. THAMDRUP and S. FLEISCHER, 1998: Isotope fractionation and sulfur metabolism by pure and enrichment cultures elemental sulfur-disproportionating bacteria. – Limnol. Oceanogr. **43**: 253–264.

CARY, S. C., R. D. VETTER and H. FELBECK, 1989: Habitat characterization and nutritional strategies of the endosymbiont-bearing bivalve *Lucinoma aequizonata*. – Mar. Ecol. Prog. Ser. **55**: 31–45.

DOI, H., E. KIKUCHI, S. TAKAGI and S. SHIKANO, 2006: Selective assimilation of the deposit feeders: experimental evidence using stable isotope ratios. – Basic Appl. Ecol. **7**: 159–166.

FINLAY, J. C., M. E. POWER and G. CABANA, 1999: Effects of water velocity on algal carbon isotope ratios: implications for river food web studies. – Limnol. Oceanogr. **44**: 1198–1203.

FINLAY, J. C., 2001: Stable carbon isotope ratios of river biota: implications for energy flow in lotic food webs. – Ecology **84**: 1052–1064.

FRANCE, R. L., 1995: Critical examination of stable isotope analysis as a means for tracing carbon pathways in stream ecosystems. – Can. J. Fish. Aquat. Sci. **52**: 651–656.

FRY, B., 1986: Sources of carbon and sulfur nutrition for consumers in three meromictic lakes of New York State. – Limnol. Oceanogr. **31**: 79–88.

FUKUDA, M., A. ASHIDA, Y. TOMARU and S. NAKANO, 2004: An improved method for collecting heterotrophic microorganisms inhabiting on pebbles in streams. – Limnology **5**: 41–46.

GREY, J., A. KELLY and R. I. JONES, 2004: High intraspecific variability in carbon and nitrogen stable isotope ratios of lake chironomid larvae. – Limnol. Oceanogr. **49**: 239– 244.

GREY, J. and P. DEINES, 2005: Differential assimilation of methanotrophic and chemoautotrophic bacteria by lake chironomid larvae. – Aquat. Microb. Ecolol. **40**: 61–66.

GROSSMAN, E. L., L. A. CIFUENTES and I. M. COZZARELLI, 2002: Anaerobic methane oxidation in a landfill-leachate plume. – Environ. Sci. Technol. **36**: 2436–2442.

HERSHEY, A. E., S. BEATY, K. FORTINO, S. KELLY, M. KEYSE, C. LUECKE, and W. J. O'BRIEN, 2005: $\delta^{13}C$ signatures of chironomids in arctic lakes: Role and direction of benthic-pelagic coupling. – Verh. Internat. Verein. Limnol. **29**: 92–96.

HERSHEY, A. E., S. BEAT Y, K. FORTINO, S. KELLY, M. KEYSE, C. LUECKE, W. J. O'BRIEN and S. C. WHALEN, 2006: Stable isotope signatures of benthic invertebrates in arctic lakes indicate limited coupling to pelagic production – Limnol. Oceanog. **51**: 177–188.

JONES, G. E. and R. J. STARKEY, 1957: Fractionation of stable isotopes of sulfur by microorganisms and their role in deposition of native sulfur. – J. Appl. Microbiol. **5**: 111–115.

KAPLAN, I. R. and S. C. RITTENBERG, 1964: Microbiological fractionation of sulfur isotopes. – J. Gen. App. Microbiol. **15**: 96–101.

KHARLAMENKO, V. I., S. I. KIYASHKO, A. B. IMBS and D. I. VYSHKVARTZEV, 2001: Identification of food sources of invertebrates from the seagrass *Zostera marina* community using carbon and sulfur stable isotope ratio and fatty acid analyses. – Mar. Ecol. Prog. Ser. **220**: 103–117.

KIAYSHKO, S. I., T. NARITA and E. WADA, 2001: Contribution of methanotrophs to freshwater macroinvertebrates: evidence from stable isotope ratios. – Aquat. Microb. Ecol. **24**: 203– 207.

KOHZU, A., C. KATO, T. IWATA, D. KISHI, M. MURAKAMI, S. NAKANO and E. WADA, 2004: Stream food web fueled by methanederived carbon. – Aquat. Microb. Ecol. **36**: 189–194.

KROUSE, R. H. and V. A. GRINENKO, 1991: Stable Isotopes: Natural and Anthropogenic Sulphur in the Environment. – *In*: SCOPE 43, John Wiley and Sons Ltd: West Sussex.

LEVIN, L. and R. H. MICHENER, 2002: Isotopic evidence for chemosynthesis based nutrition of macrobenthos: the lightness of being at Pacific methane seeps. – Limnol. Oceanogr. **47**: 1336– 1345.

LOCK, M. A., R. R. WALLACE, J. W. COSTERTON, R. M. VENTULLO and S. E. CHARLTON, 1984: River epilithon: toward a structural-functional model. – Oikos **42**: 10–22.

MERITT, W. R. and W. K. CUMMINS, 1996: An Introduction to the Aquatic Insects of North America. 3rd ed., Kendall/Hunt Publishing Company, USA.

MIZOTA, C., S. SHIMOYAMA and T. YAMANAKA, 1999: An Isotopic characterization of sulfur uptake by benthic animals from Tsuyazaki inlet, northern Kyusyu, Japan. – Benthos Res. **54**: 81–85.

MCCUTCHAN, J. H., W. M. LEWIS, C. KENDALL and C. C. MCGRATH, 2003: Variation in trophic shift for stable isotope ratios of carbon, nitrogen, and sulfur. – Oikos **102**: 378–390.

NAKANO, S., A. TAKESHITA, T. OHTSUKA and D. NAKAI, 2006: Vertical profiles of current velocity and dissolved oxygen saturation in biofilms on artificial and natural substrates. – Limnology in press.

OKABE, S., T. ITO, K. SUGITA and H. SATOH, 2005: Succession of internal sulfur cycles and sulfur-oxidizing bacterial communities in microaerophilic wastewater biofilms. – Appl. Environ. Microbiol. **71**: 2520–2529.

RUBY, E. G., H. W. JANNASCH and W. G. DEUSER, 1987: Fractionation of stable carbon isotopes during chemoautotrophic growth of sulfur-oxidizing bacteria. – Appl. Environ. Microbiol. **53**: 1940–1943.

SCHÖNHEIT, P., J. K. KRISTJANSSON and R. K. THAUER, 1982: Kinetic mechanism for the ability of sulfate reducers to out-compete methanogens for acetate. – Arch. Microbiol. **132**: 285–288.

YANAGISAWA, F. and H. SAKAI, 1983: Thermal decomposition of barium sulfate-vanadium pentaoxide silica glass mixtures for preparation of sulfur dioxide in sulfur isotope ratios measurements. – Anal. Chem. **55**: 985–987.

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