

# Low sulfur isotopic signatures ( $\delta^{34}\text{S}$ ) of macrozoobenthos from a brackish lagoon: contribution of microbially reduced sulfides

Gen KANAYA\*, Ei NOBATA\*\*, Yonosuke MAKI\*\*\*  
Eisuke KIKUCHI\*, Chitoshi MIZOTA\*\*\*\*

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Sulfate-reducing bacteria (SRB), Food source

## Abstract

Stable sulfur isotope ratios ( $\delta^{34}\text{S}$ ) of infaunal bivalves (*Macoma contabulata*, *Nuttallia olivacea*, and *Ruditapes philippinarum*) and polychaetes (*Hediste* spp.; consisted of *H. atoka* and *H. diadroma*) collected from two stations in the Gamo Lagoon (Japan) were determined to assess trophic pathways in highly reductive, estuarine soft-bottom habitats. The stations were characterized by distinctive sediment characteristics (Station A, sandy sediment with low sulfide content; Station B, muddy and sulfide-rich sediment). Soft tissues of the consumers exhibited much more depleted  $\delta^{34}\text{S}$  values (+3.2 to +12.1‰) than those of dissolved sulfate in the water column (+20.6 to +20.8‰). The value for each species was 1.6 to 5.3‰ lower at Station B than at Station A. These results suggest the assimilation of sulfides in the sediment ( $\delta^{34}\text{S}$ ; -23.2 to -22.7‰) via microbial trophic pathways. In this lagoon, benthic microalgae and/or other microbes in the sediment are the most probable  $^{34}\text{S}$ -depleted food source for the consumers. Interspecies variations in  $\delta^{34}\text{S}$  values are explained by the different dietary contribution of the  $^{34}\text{S}$ -depleted diets versus  $^{34}\text{S}$ -enriched phytoplankton and are closely related to different feeding habits (i.e., surface-deposit feeding, facultative suspension feeding, and obligatory suspension feeding).

## Introduction

Analysis of the natural abundance of stable isotopes of carbon, nitrogen, and sulfur has been employed in studies of estuarine ecosystems to trace the flow of primary production through the

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\* Center for Northeast Asian Studies, Tohoku University

\*\* Biological Institute, Graduate School of Science, Tohoku University

\*\*\* Faculty of Humanities and Social Sciences, Iwate University

\*\*\*\* Faculty of Agriculture, Iwate University

Low sulfur isotopic signatures ( $\delta^{34}\text{S}$ ) of macrozoobenthos from a brackish lagoon: contribution of microbially reduced sulfides food web [for review, see Peterson & Fry 1987]. In particular, stable carbon and sulfur isotope ratios ( $\delta^{13}\text{C}$  and  $\delta^{34}\text{S}$ ) are useful tools in evaluating assimilated diets of consumers, as the ratios are nearly constant during trophic transfer [e.g., +0.2‰ per trophic level for  $\delta^{34}\text{S}$  values; Peterson & Fry 1987]. Although the use of sulfur isotope signatures in an estuarine food web study has some problems (e.g., contamination of biotic samples by inorganic sulfides), it has apparent advantages in distinguishing several primary producers in estuaries that use different sulfur sources [e.g., Peterson et al. 1986; Sullivan & Moncreiff 1990; Currin et al. 1995; Chanton & Lewis 2002]. For example,  $\delta^{34}\text{S}$  ratios can distinguish benthic primary producers from phytoplankton. Marine phytoplankton generally exhibit  $\delta^{34}\text{S}$  values (+17 to +21‰) that are comparable to those of seawater sulfate (+21‰) [Peterson & Fry 1987]. In contrast, marine vascular plants exhibit much more depleted  $\delta^{34}\text{S}$  values [−10.7 to +12.2‰; Currin et al. 1995; Moncreiff & Sullivan 2001; Chanton & Lewis 2002; Yamanaka et al. 2003] since they incorporate a certain amount of  $^{34}\text{S}$ -depleted sulfides (<−20‰) in the reductive sediments via the root system [Fry et al. 1982; Peterson et al. 1986]. Reported  $\delta^{34}\text{S}$  values of marine microphytobenthos (+3.9 to +14.3‰) are intermediate between them [e.g., Sullivan & Moncreiff 1990; Currin et al. 1995; Stribling & Cornwell 1997; Moncreiff & Sullivan 2001]. Since the  $\delta^{13}\text{C}$  values of marine/estuarine primary producers sometimes overlap [e.g., Currin et al. 1995; Kanaya et al. 2007], a combined use of sulfur stable isotope analysis would help to reveal the trophic relationships in an estuarine ecosystem.

In this paper, we report the  $\delta^{34}\text{S}$  values of infaunal bivalves and polychaetes collected in two neighboring locations in the highly eutrophic brackish Gamo Lagoon. Sediments at the two stations are characterized by contrasting sulfide contents. Here we discuss the possible trophic pathways from the  $^{34}\text{S}$ -depleted sulfide pool in the sediment to the benthic consumers based on stable isotope signatures ( $\delta^{34}\text{S}$ ,  $\delta^{13}\text{C}$ , and  $\delta^{15}\text{N}$ ) determined in the present and previous studies in the lagoon [Kanaya et al. 2005, 2007, this study].

## Materials and methods

**Study area:** The shallow and eutrophic brackish Gamo Lagoon (mean water depth: 0.8 m; area: 0.11 km<sup>2</sup>) is located at the north side of the Nanakita River, facing Sendai Bay, Japan (Fig. 1). A stone levee with three water gates (1.8 × 1.35-m opening) separates the lagoon from the river mouth. Lagoon water is tidally exchanged, and the average diurnal salinity ranges from 21 to 25 psu [Kurihara et al. 2000]. Marsh reeds (*Phragmites australis*) cover the lagoon edge. Macro red algae (*Gracilaria vermiculophylla*) densely vegetate the inner subtidal part of the lagoon, while seagrass is never present. In this study, two sampling stations were selected (Fig. 1). Station (St.)

A was located in a sandy-oxidized habitat near the lagoon mouth, whereas St. B was in a muddy, highly reductive, sulfide-rich habitat in the central lagoon.

**Sampling and preparation of biotic sam-**

**ples:** Four infaunal animal species with different feeding habits (*Macoma contabulata*, *Nuttallia olivacea*, *Ruditapes philippinarum*, and *Hediste* spp. [*H. atoka* and *H. diadroma*, not distinguished]) were collected by hand in October 1997. Their reported feeding habits are summarized in Table 3. In the laboratory, animals were provided with a continuous supply of fresh seawater for 1 day to eliminate sediment containing sulfides from the digestive tract. Soft tissues of three (*R. philippinarum*) to ten (*Hediste* spp.) individuals were dissected and soaked in deionized water (5°C, overnight) to remove seawater sulfate. The samples were freeze-dried (24 h) and powdered for stable isotope analysis. All samples for each species were mixed to obtain enough sulfur for the analysis (i.e. n = 1 for each species). The dominant macroalga *Gracilaria vermiculophylla* (aboveground parts) was collected at St. B in October 2003 (n = 3). In the laboratory, attached materials were carefully removed in deionized water. After freeze-drying (24 h), algal samples were ground into a powder and leached with deionized water (5°C, overnight) to remove seawater sulfate. Sulfur in the sample materials was converted to sulfate by combustion with a Parr Bomb #1108 and quantitatively recovered as BaSO<sub>4</sub> [Fry et al. 1982].

**Sampling and preparation of abiotic samples for isotopic analysis:** At both stations, sediment was collected using a set of cores (5 cm inner diameter × 30 cm length) in October 1997 and November 2003 to recover the sulfides and pore water sulfate for stable isotope analysis. A ~10-g aliquot of the wet sediment (1 to 3 cm deep) was centrifugally washed with 0.2 M NaCl to

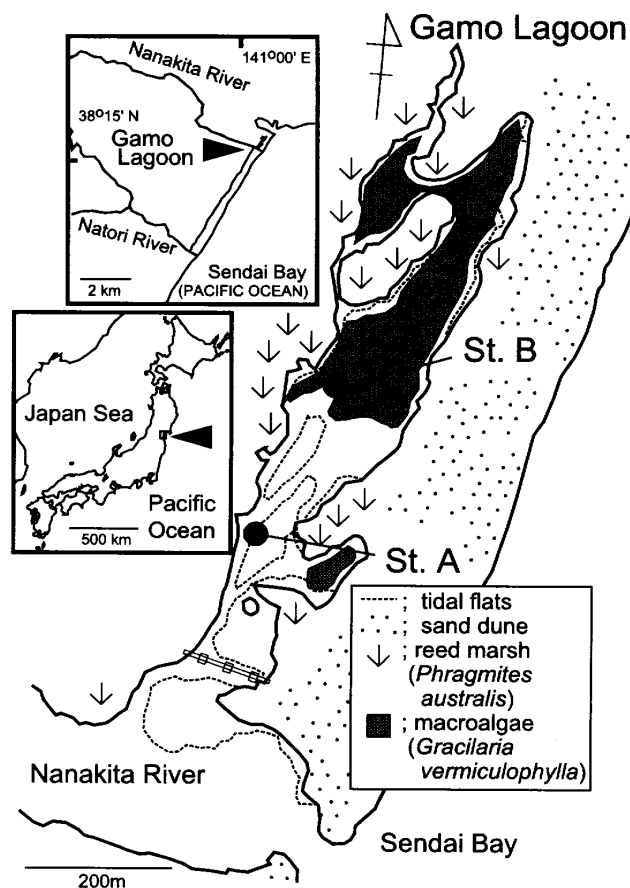


Fig. 1. Study site. Stations A (St. A) and B (St. B) were the sampling locations for benthic consumers and inorganic sulfur pools. St. A was situated in a sandy intertidal flat near the lagoon mouth, while St. B was in a muddy subtidal flat in the central lagoon. Areas covered with dense macroalgal patches (*Gracilaria vermiculophylla*) are shaded gray.

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Low sulfur isotopic signatures ( $\delta^{34}\text{S}$ ) of macrozoobenthos from a brackish lagoon: contribution of microbially reduced sulfides remove seawater sulfate. Subsequently, easily oxidizable sulfide (EOS)-sulfur in the sediment was converted to sulfate by a treatment with warm  $\text{H}_2\text{O}_2$  and precipitated as  $\text{BaSO}_4$ . Acid volatile sulfide (AVS)-sulfur was liberated from the sediment (5 to 6 cm deep) as hydrogen sulfide under an acidic condition [0.5 M  $\text{H}_2\text{SO}_4$ ; see Kanaya & Kikuchi 2004] while purging pure  $\text{N}_2$  gas into 5% cadmium acetate solution and then fixed as  $\text{CdS}$ . All precipitates were converted into  $\text{BaSO}_4$  as described above. Pore water sulfate was obtained by centrifuging the sediment (5 to 10 cm deep) with 0.2 M  $\text{NaCl}$  and precipitated as  $\text{BaSO}_4$ . Lagoon water was sampled at St. B in February 2000 and November 2003. Sulfate in the water sample was precipitated as  $\text{BaSO}_4$  after filtering through a 0.2- $\mu\text{m}$  polycarbonate membrane filter.

An additional sediment core was collected in February 2000 to determine sediment characteristics at the stations. After drying ( $60^\circ\text{C}$ , 48 h), silt-clay (particles  $< 0.063$  mm in diameter), total organic carbon (TOC), and total nitrogen (TN) contents of the sediment (0 to 3 cm deep) were determined as described by Kanaya & Kikuchi [2004]. A 5- to 6-cm-depth layer of the core was used for quantitative analysis of AVS (mostly  $\text{FeS}$ ), according to the method of Suzuki & Shiga [1953]. The oxidation-reduction potential (ORP) value of sediment at 5 cm depth was determined *in situ* in February 2000 using a handheld ORP meter (TOA RM-12P; Toa Electronics, Tokyo, Japan).

**Analysis of sulfur isotope ratios:** Dried  $\text{BaSO}_4$  was converted into  $\text{SO}_2$  gas via the thermal decomposition method of Yanagisawa & Sakai [1983].  $^{34}\text{S}/^{32}\text{S}$  ratios were determined using a dual inlet mass spectrometer (SIRA-10; VG Isogas, Middlewich, UK). The sulfur isotopic composition was expressed in conventional delta notation,

$$\delta^{34}\text{S} (\text{‰}) = (\text{R}_{\text{sample}}/\text{R}_{\text{standard}} - 1) \times 1000,$$

where R is the  $^{34}\text{S}/^{32}\text{S}$  ratio. Canyon Diablo Troilite (CDT) was used as a standard. Analytical error during the overall procedure was  $< \pm 0.2\text{‰}$ .

## Results and discussion

In marine and estuarine areas, anaerobic microbial decomposition in deeper sediment layers occurs mainly by sulfate reduction [Capone & Kiene 1988], resulting in the release of  $\text{H}_2\text{S}$ . At our study site, AVS contents were distinctively different between the two sampling stations (Table 1). St. A was characterized by sandy and more oxidized sediment with low sulfide contents (ORP: +274 mV; AVS:  $12.5 \mu\text{mol g}^{-1}$  dry weight). In contrast, St. B in the central lagoon was characterized by muddy and reductive sediment with high sulfide content (ORP: +44 mV; AVS:  $31.6 \mu\text{mol g}^{-1}$  dry weight). These results indicate the prevailing higher sulfate reduction at St. B

Table 1. Characterization of sediment collected in February 2000 at two stations. ORP: oxidation-reduction potential; AVS: acid volatile sulfide-sulfur.

	Mean (1SD)		n
	Station A	Station B	
AVS ( $\mu\text{mol g}^{-1}$ dry weight)	12.5 (2.5)	31.6 (8.1)	4
ORP (mV)	+274 (32)	+44 (58)	4
Total organic carbon (% dry weight)	0.82	3.46	1
Total nitrogen (% dry weight)	0.06	0.38	1
C/N ratio	13.2	9.2	1
Silt-clay content (% dry weight)	11	77	1

compared to St. A, as reported previously [Kanaya & Kikuchi 2004]. Free  $\text{H}_2\text{S}$  accumulates in the sediment around St. B especially in the warmer season, but not around St. A [Kanaya & Kikuchi 2004]. The different sediment conditions may alter the supply of reduced sulfides to microbes and microphytobenthos at the sediment surface of each station.

Table 2. Sulfur stable isotope ratios of inorganic sulfate/sulfide pools in Gamo Lagoon. EOS: easily oxidizable sulfide-sulfur; AVS: acid volatile sulfide-sulfur; n.d.: not determined.

Samples	Date	$\delta^{34}\text{S}$ (‰) $\pm$ SD		n
		Station A	Station B	
Water column sulfate	Oct. '97	n.d.	+20.6	1
	Nov. '03	+20.8 $\pm$ 0.1 <sup>*1</sup>	+20.8 $\pm$ 0.1 <sup>*2</sup>	3
Pore water sulfate	Nov. '03	+19.3 $\pm$ 0.5 <sup>*3</sup>	+17.9 $\pm$ 0.5 <sup>*4</sup>	3
Sediment EOS	Oct. '97	n.d.	-22.8	1
Sediment AVS	Nov. '03	-23.2 $\pm$ 0.7	-22.7 $\pm$ 0.7	3

\*1, \*2, \*3, and \*4; Salinity = 26.6, 22.4, 24.9, and 25.0 psu, respectively.

Bacterially reduced sulfur shows low  $\delta^{34}\text{S}$  values, less than  $-20\text{‰}$ , due to large isotopic discrimination by sulfate-reducing bacteria (SRB) [Peterson et al. 1986; Peterson & Fry 1987]. In Gamo, lagoon water  $\delta^{34}\text{S}$  values (+20.6 to +20.8‰; Table 2) were nearly identical to that of common seawater sulfate [+21‰; Peterson & Fry 1987]. In contrast, the sediment sulfide pool (i.e., EOS and AVS) had much more depleted  $\delta^{34}\text{S}$  values ( $-23.2$  to  $-22.7\text{‰}$ ) than the lagoon water sulfate; these values were similar to those reported previously from a salt marsh in the United States [pore water sulfides:  $-22\text{‰}$ ; Peterson et al. 1986]. At the two stations, the  $\delta^{34}\text{S}$  value of pore water sulfate ( $<+19.3\text{‰}$ ) was slightly depleted compared to that of lagoon water (+20.6 to +20.8‰) and was more depleted at St. B (+17.3‰) than at St. A (+19.3‰). This may have been due to reoxidation of the reduced sulfides in the sediment to pore water sulfate [Howarth & Stewart 1992].

Since sulfur stable isotope ratios are nearly constant through a trophic chain [mean +0.2‰ per

trophic level; Peterson & Fry 1987], we can deduce the assimilated diet(s) of a consumer from the  $\delta^{34}\text{S}$  value. In general, marine offshore consumers exhibit similar  $\delta^{34}\text{S}$  values to that of seawater sulfate [Peterson et al. 1986; Chanton & Lewis 2002]. At our study site, however,  $\delta^{34}\text{S}$  values of the consumers (+3.2 to +12.1‰) were more depleted than those of sulfate in the water column and pore water (+17.3 to +20.8‰). The  $\delta^{34}\text{S}$  value for each consumer was 1.6 to 5.3‰ lower in the highly reductive habitat (St. B) than in the sandy oxidized habitat (St. A). Accordingly, a certain amount of sulfur in the animal tissue should be derived from the reduced sulfide pool ( $\delta^{34}\text{S}$  value; -23.2 to -22.7‰) via trophic transfer.

In this lagoon, carbon and nitrogen stable isotope studies have clearly demonstrated the dietary importance of benthic diatoms, autochthonous phytoplankton, and marine phytoplankton for macrozoobenthic animals, whereas detritus from terrestrial and marsh plants were scarcely assimilated [Kanaya et al. 2005, 2007]. Marine phytoplankton and macroalgae generally exhibit  $\delta^{34}\text{S}$  values (+17 to +21‰) similar to seawater sulfate (+21‰) [Peterson & Fry 1987]. In fact, the  $\delta^{34}\text{S}$

Table 3. Sulfur stable isotope ratio (‰) and total sulfur content (% dry weight) of macrozoobenthos and macroalgae at Stations A (St. A) and B (St. B). Samplings were conducted in October 1997 (animal samples) and October 2003 (macroalgae). SDF: surface-deposit feeder; OSF: obligatory suspension feeder; FSF: facultative suspension feeder; n.d.: no data.

Samples	$\delta^{34}\text{S}$ (‰)			Total sulfur (% dw)		n	Feeding habit
	St. A	St. B	$\Delta\delta^{34}\text{S}^{*1}$	St. A	St. B		
<b>Polychaeta</b>							
<i>Hediste</i> spp.	+11.4	+8.9	2.5	0.8	0.8	1	SDF <sup>(1), (2)</sup>
<b>Bivalva</b>							
<i>Macoma contabulata</i>	+8.5	+3.2	5.3	0.9	0.8	1	SDF <sup>(3)</sup>
<i>Nuttallia olivacea</i>	+11.6	+8.2	3.4	0.8	0.7	1	FSF <sup>(2), (3)</sup>
<i>Ruditapes philippinarum</i>	+12.1	+10.5	1.6	1.1	1.2	1	OSF <sup>(3)</sup>
<b>Macroalgae</b>							
<i>G. vermiculophylla</i>	n.d.	+19.3 <sup>*2</sup>		n.d.	n.d.	3	n.d.

(1) Kikuchi & Wada [1996]; (2) Tsuchiya & Kurihara [1980]; (3) Kanaya et al. [2005].

\*1: Difference of the  $\delta^{34}\text{S}$  values at the two stations; \*2: 1 SD = 0.1.

value of the macroalga *G. vermiculophylla* (+19.3‰, Table 3) was close to that of lagoon water sulfate (> +20.6‰; Table 2). Autochthonous phytoplankton in this lagoon should also take up lagoon water sulfate and may have  $\delta^{34}\text{S}$  values similar to that of *G. vermiculophylla*. In contrast, benthic diatoms may have depleted  $\delta^{34}\text{S}$  values relative to lagoon water sulfate. Currin et al. [1995] compiled literature values and reported a mean  $\delta^{34}\text{S}$  value of +10‰ for benthic microalgae (diatoms and cyanobacteria) in intertidal habitats. Stribling & Cornwell [1997] reported a  $\delta^{34}\text{S}$

value of +5.4‰ for benthic diatoms in an estuarine salt marsh. These data imply that benthic microalgae incorporate a significant amount of sedimental sulfides, and hence, they would be sensitive to the reducing capabilities of the sediments. At the two stations, we previously determined the  $\delta^{34}\text{S}$  value of benthic diatom samples collected in March 2004. The values were much more depleted than those of seawater sulfate [St. A:  $-4.6 \pm 2.1\text{‰}$ ; St. B:  $-6.1 \pm 1.6\text{‰}$ ; G. Kanaya unpubl. data]. Although the low  $\delta^{34}\text{S}$  values may be partly attributable to contamination of resuspended sediment rich in inorganic sulfides, benthic diatoms are the most probable  $^{34}\text{S}$ -depleted food source for the consumers in this lagoon.

Similar to the report by Chanton & Lewis [2002], we observed depleted  $\delta^{34}\text{S}$  values for deposit-feeding consumers relative to those of suspension feeders (Table 3). At each station, *M. contabulata* exhibited the most depleted  $\delta^{34}\text{S}$  value among the consumers (St. A: +8.5‰; St. B: +3.2‰), while the suspension-feeding bivalve *R. philippinarum* had the highest values (St. A: +12.1‰; St. B: +10.5‰). The values for *Hediste* spp. and *N. olivacea* were intermediate. These interspecies differences would be explained by their feeding habits. *M. contabulata* is a typical surface-deposit feeder that ingests fine organic particles (e.g., benthic diatoms) around the burrow using a long inhalant siphon, while *N. olivacea* conducts both suspension and deposit feeding [Tsuchiya & Kurihara 1980; Kanaya et al. 2005]. Although *Hediste* spp. is considered a surface-deposit feeder [Tsuchiya & Kurihara 1980; Kikuchi & Wada 1996], some nereidid polychaetes are suspension feeders [Riisgard 1991]. In contrast, *R. philippinarum* is an obligatory suspension feeder that ingests fine organic particles (e.g., phytoplankton) in the water column, although it assimilates a certain amount of resuspended benthic diatoms [Kanaya et al. 2005, 2007].

Observed differences in the  $\delta^{34}\text{S}$  value would thus reflect the different dietary intake of  $^{34}\text{S}$ -depleted diets including benthic diatoms versus  $^{34}\text{S}$ -enriched phytoplankton (see Fig. 2). The  $\delta^{34}\text{S}$  value of *M. contabulata* at St. B (+3.2‰) was lower than the reported  $\delta^{34}\text{S}$  values of benthic microalgae [ $> +3.9\text{‰}$ ; Currin et al. 1995; Stribling & Cornwell 1997; Moncreiff & Sullivan 2001]. This may be attributable to the assimilation of other unsampled chemoautotrophs or microheterotrophs by the bivalve. Peterson et al. [1986] reported the  $\delta^{34}\text{S}$  value of purple sulfur bacteria collected from the sediment surface in a reductive salt marsh as  $-6.5\text{‰}$ . Around St. B, white or purple mats of sulfur-oxidizing bacteria are often found during warmer seasons [G. Kanaya pers. obs.]. Such microbes are the other likely trophic pathway from the reduced sulfides to the benthic consumers at St. B.

The  $\delta^{34}\text{S}$  values of consumers in the present study (+3.2 to +12.1‰) are comparable to those in other reductive tidal flats and salt marshes [Peterson et al. 1986; Mizota et al. 1999;

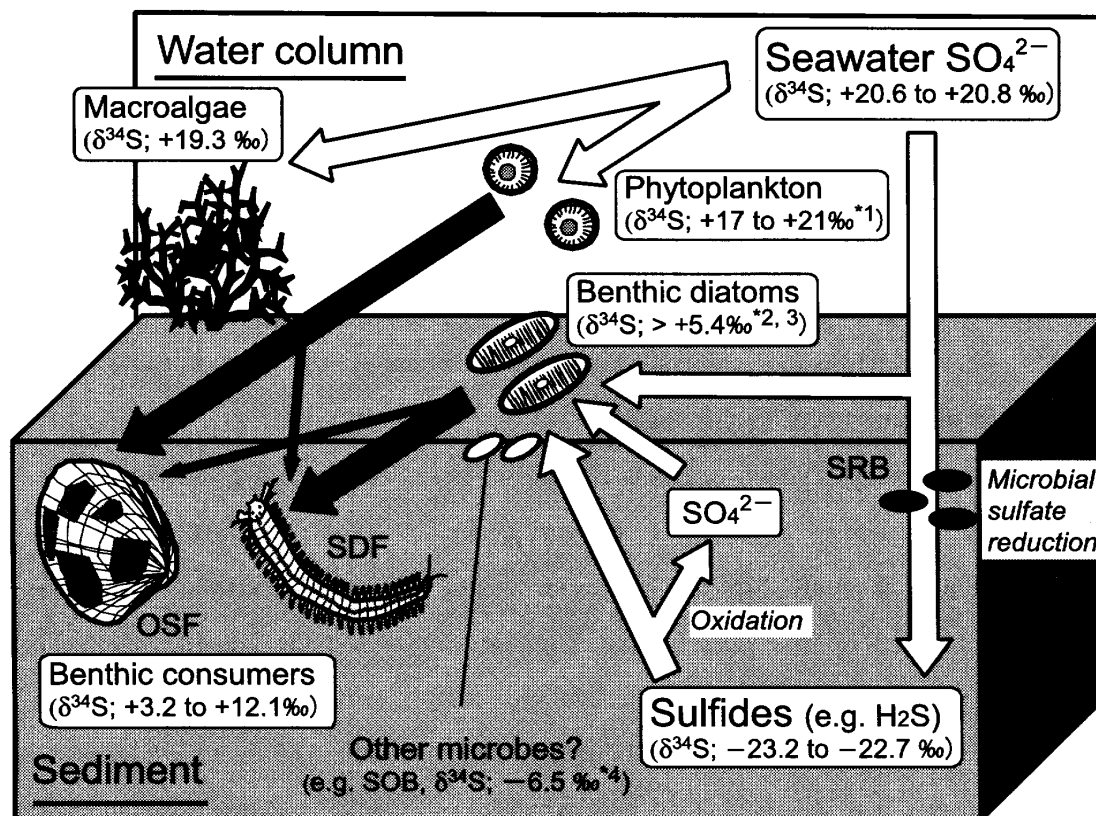


Fig. 2. Schematic illustrations of the sulfur cycle in the muddy and highly reductive habitat in Gamo Lagoon. White arrows show the sulfur flows among the inorganic sulfur pools, primary producers, and microbes. Black arrows denote the sulfur uptake by the benthic consumers from their diets. Widths of the black arrows indicate the relative dietary contribution of phytoplankton and benthic diatoms (and/or other benthic microbes). \*1: Peterson & Fry [1987]; \*2: Currin et al. [1995]; \*3: Stribling & Cornwell [1997]; \*4: Peterson et al. [1986]. SRB: sulfate-reducing bacteria; SOB: sulfur-oxidizing bacteria; OSF: obligatory suspension feeder (*Ruditapes philippinarum*); SDF: surface-deposit feeder (*Hediste* spp.).

Yamanaka et al. 2003]. This implies that microbially reduced sulfides in the sediment become a major sulfur source for macrozoobenthos in a soft-bottom habitat where sulfate reduction takes place (see Fig. 2). At our study site, benthic diatoms and/or other microbes are the most probable  $^{34}\text{S}$ -depleted food sources for consumers (see previous section). Since  $\delta^{34}\text{S}$  analysis can distinguish  $^{34}\text{S}$ -depleted benthic microbes from  $^{34}\text{S}$ -enriched food sources (e.g., macroalgae and marine/estuarine phytoplankton), this method has great advantages in evaluating the food web structure in reductive soft-bottom environments.



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