Spatial dietary shift of macrozoobenthos in a brackish lagoon revealed by carbon and nitrogen stable isotope ratios

Gen Kanaya[1,*], Shigeto Takagi[1], Ei Nobata[2], Eisuke Kikuchi[1]

[1]The Center for Northeast Asian Studies, Tohoku University, Sendai 980-8576, Japan
[2]Biological Institute, Graduate School of Science, Tohoku University, Sendai 980–8578, Japan

ABSTRACT: The food sources of 14 benthic consumer species (polychaetes, bivalves, amphipods and decapods) were investigated at 2 locations in a brackish lagoon (Gamo Lagoon, Japan) using carbon and nitrogen stable isotope ratios. Consumer diets were elucidated from the $\delta^{13}C$ values of food sources, including riverine and terrestrial materials ($< -25.6‰$), autochthonous particulate organic matter (POM, $-23.6‰$), the macroalga *Gracilaria vermiculophylla* ($-20.4‰$), marine POM ($-19.9‰$), benthic and epiphytic diatoms (-16.5 to $-15‰$) and the macroalga *Enteromorpha prolifera* ($-13.1‰$). *G. vermiculophylla* was distinguished from marine POM by a high $\delta^{15}N$ value. Consumers showed dietary shifts on a small scale (~200 m) corresponding to changes in the food supply. At the lagoon mouth (Site A) benthic diatoms were the major diet for deposit feeders with enriched $\delta^{13}C$ and/or $\delta^{15}N$ values. Suspension feeders also assimilated a certain amount of benthic diatoms as well as marine POM due to current induced resuspension at this site. In contrast, autochthonous POM (phytoplankton) was an important diet component in the inner lagoon (Site B-bare) where chlorophyll *a* concentration was high (110 to 113 µg l^{-1}). In macroalgal patches (Site B-algae), epiphytic diatoms were a major dietary component of consumers. Based on the $\delta^{13}C$ range of consumers (-21.5 to $-13.5‰$), the benthic food web was primarily based on estuarine and marine microalgal production, whereas riverine and terrestrial materials were of minor importance.

KEY WORDS: Food source analysis · Spatial change · $\delta^{13}C$ · $\delta^{15}N$ · Benthic consumers · Phytoplankton · Benthic diatoms · Epiphytic diatoms

INTRODUCTION

Estuaries receive large amounts of organic matter from a variety of sources: allochthonous materials advected by marine and riverine inflow and autochthonous materials including phytoplankton, microphytobenthos, macroalgae and marsh plants (Heip et al. 1995). Organic materials are assimilated by benthic consumers directly or after bacterial trophic mediation and incorporated into the food web (Peterson & Howarth 1987, Currin et al. 1995, Heip et al. 1995). Therefore, benthic animals act as important links as primary consumers between organic materials and carnivorous fish, and consequently, between benthic and pelagic food webs.

In estuarine coastal areas the majority of benthic consumers are detritivores such as suspension and deposit feeders (Heip et al. 1995). In general, suspension feeders ingest particulate organic matter (POM) in the water column, whereas deposit feeders ingest sediment organic matter (SOM) (Fauchald & Jumars 1979, Tsuchiya & Kurihara 1980, Kanaya et al. 2005). However, it is difficult to determine the assimilated diets of detritivores, since POM and SOM are generally mixtures of organic compounds from a variety sources (Wada et al. 1987, de Jonge & van Beusekom 1992, Thornton & McManus 1994, Chanton & Lewis 2002). Consequently, stable isotope ratios have recently been used as tracers to assess the dietary components of

benthic consumers in aquatic environments (e.g. Incze et al. 1982, Sullivan & Moncreiff 1990, Riera & Richard 1996, Kang et al. 2003, Yokoyama et al. 2005). Since the stable isotope ratios of an animal reflect those of the assimilated diets over time with empirical trophic enrichments (δ^{13}C: 0 to +1‰ and δ^{15}N: +3 to 4‰ per trophic level, Fry & Sherr 1984, Minagawa & Wada 1984), we can estimate the food source and trophic level of a consumer from the isotopic signatures.

In estuarine and shallow coastal areas phytoplankton, benthic microalgae or both have been reported as the major dietary components of benthic detritivores (Riera & Richard 1996, Page 1997, Kang et al. 2003, Page & Lastra 2003, Yokoyama et al. 2005). In tidal flats resuspended microphytobenthos often exceed the phytoplankton biomass in the water column (e.g. de Jonge & van Beusekom 1992). Consequently microphytobenthos become an important diet of benthic suspension feeders inhabiting tidal flats or tidal channels (e.g. Riera & Richard 1996, Page 1997, Kang et al. 2003, Page & Lastra 2003). In contrast, marsh plant detritus sometimes plays an important role in reed dominated marsh systems (e.g. Peterson & Howarth 1987, Currin et al. 1995). These findings suggest that major dietary components of benthic detritivores differ from system to system in relation to the supply of potential food sources.

In estuarine environments environmental conditions change drastically in space and time (Kikuchi et al. 1992, Aikins & Kikuchi 2001, Chanton & Lewis 2002, Kanaya & Kikuchi 2004). Hence, it is expected that the food sources of a benthic consumer change spatially or temporally in relation to the environmental changes. However, few studies have reported the spatial or temporal dietary shift of benthic consumers within an estuarine system. Page & Lastra (2003) reported that the dietary importance of benthic microalgae changed over time and corresponded to changes of phytoplanktonic biomass in the water column. Spatial changes of salinity (indicative of the amount of freshwater inflow) often affect the dietary importance of riverine terrestrial materials for benthic animals (e.g. Incze et al. 1982, Riera & Richard 1996, Doi et al. 2005). Also, shading by marsh plants reduces the biomass of microphytobenthos on sediments and suppresses the availability of microphytobenthos for benthic consumers (Wainright et al. 2000).

In this study a series of field surveys was conducted in 2 neighboring locations (3 sites) within a semi-enclosed brackish lagoon, Gamo Lagoon, located on the eastern coast of Japan. The inner lagoon has stagnant water with ample autochthonous phytoplanktonic biomass (up to 200 µg l^{-1}, Kikuchi et al. 1992, Kanaya & Kikuchi 2004). Tidal current velocity is much higher near the lagoon mouth (>40 cm s^{-1}) than in the inner lagoon (~10 cm s^{-1}, Aikins & Kikuchi 2001). Therefore, we inferred that phytoplankton is the major dietary component in the inner lagoon, whereas microphytobenthos (including resuspended microphytobenthos), allochthonous sources (marine or riverine inputs) or both are more important near the lagoon mouth. We measured the stable carbon and nitrogen isotope ratios of consumers and potential food sources to: (1) assess the relative dietary importance of potential food sources in the benthic food web, and (2) demonstrate a spatial dietary shift of consumers between the locations in relation to the local environmental changes.

MATERIALS AND METHODS

Study sites. Gamo Lagoon is located at the north side of the Nanakita River estuary, Sendai Bay, eastern coast of Japan (Fig. 1). The lagoon is separated from the estuary by a stone levee with 3 water gates. Tides are semi-diurnal with a range of 0.8 m (spring tide), and salinity fluctuates tidally from near 0 to > 30 psu (diurnal average, 21 to 25 psu, Kurihara et al. 2000). Inner lagoon water maintains higher magnitudes of autochthonous phytoplanktonic biomass (up to 200 µg l^{-1}) than adjacent waters (Kikuchi et al. 1992). The phytoplanktonic community is mostly dominated by centric and pennate diatoms throughout the year (E. Nobata unpubl. data). A portion of lagoon water is diluted by inflowing water during high tides and discharged into the sea during ebb tides (Kikuchi et al. 1992, Kanaya & Kikuchi 2004). The edge of the estuary is vegetated with the marsh plant *Phragmites australis* (Fig. 1). Sediment of the inner lagoon is partly covered with a thick layer of the red alga *Gracilaria vermiculophylla* throughout the season, and the green alga *Enteromorpha prolifera* is also found during summer. Seagrass does not occur in the estuary. The microphytobenthic community is dominated by pennate diatoms (Kanaya et al. 2005, E. Nobata unpubl. data). Rich epiphytic microalgae are found on *G. vermiculophylla*, which are composed mostly of pennate diatoms (Aikins & Kikuchi 2002, E. Nobata unpubl. data).

Collection and sample preparation of benthic consumers. We collected 14 consumer species including polychaetes, bivalves, decapods and amphipods in April 2004 at 2 sites in the lagoon (Sites A and B, Fig. 1), and 5 of the 14 animal species in August 2005 to check seasonal trends. Site A was located in a bare sandy flat near the lagoon mouth. Site B was located in a muddy sand flat (200 m from Site A) partly covered with *Gracilaria vermiculophylla*. Tidal current was much weaker at Site B (nearly 10 cm s^{-1}) than at Site A (10 to >40 cm s^{-1}; Aikins & Kikuchi 2001). Large animals were picked from the sediment by hand and
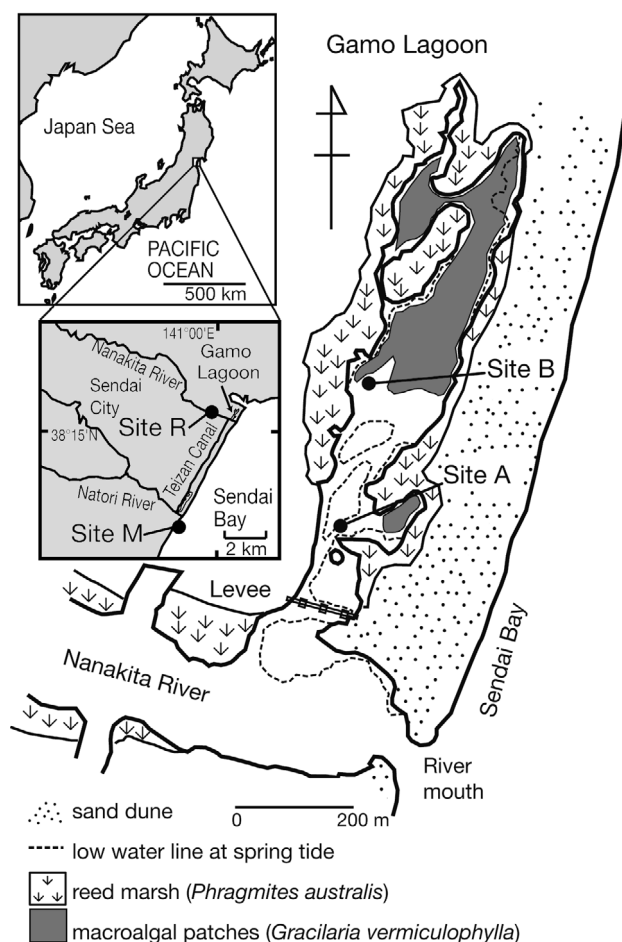
Fig. 1. Study sites in the Gamo Lagoon, Japan. Sites A and B are the sampling locations for food sources and benthic consumers. Sites R and M are the locations for riverine and marine POM sampling, respectively. Areas covered by the marsh plant *Phragmites australis* and the macroalga *Gracilaria vermiculophylla* are shown along with the low water line at spring tide and sand dune locations

small animals were collected using a 1 mm mesh sieve. At Site B, algae associating animals were sampled in the dense algal patch (Site B-algae, 100% coverage), whereas the others were sampled in the adjacent bare flat (Site B-bare). At Site B-algae, *G. vermiculophylla* was picked from the sediment and washed several times through a sieve to capture associating amphipods. Subsequently, the polychaete *Capitella* sp. was collected from the sediment below the algal patch. Animals were placed in filtered lagoon water kept at 4°C and taken to the laboratory. Muscle tissue was analyzed for bivalves and decapods. For the others, specimens were left for 12 h to evacuate the gut contents and the whole body was subsequently analyzed. The

samples were freeze-dried for 24 h, ground into powder and treated with a chloroform–methanol solution (2:1 by volume) for 24 h to remove lipids.

Collection and sample preparation of potential food sources. Potential food sources were sampled during the period from 2003 to 2005 (for dates and sample numbers see Table 2). To obtain particulate organic matter (POM), surface water was sampled at Sites A and B during ebb tide (only at Site A in September 2003 and March 2004). Additional samples were taken at Sites R and M (Fig. 1) in July and August 2005 to obtain riverine and marine POM, respectively. Salinity of each sample was determined using a hand-held water quality meter (U-22, Horiba). In July and August 2005, chlorophyll *a* (chl *a*) concentration of each sample was determined using a fluorometer (10-AU, Turner Designs) after an extraction with N, N-dimethylformamide. Water samples in September 2003 and March 2004 were filtered through 250 and 25 µm mesh screens, and particles on the 25 µm mesh were concentrated onto a precombusted glass fiber filter (GFF; 500°C, 2 h). Water samples in July and August 2005 were prefiltered through a 250 µm screen and particles in the filtrate were concentrated onto a precombusted GFF (500°C, 2 h). No systematic difference was found between the samples collected by the 2 methods (Table 2).

To obtain sediment organic matters (SOM), surface sediment (0 to 5 mm deep) was sampled at Sites A and B-bare. Benthic diatoms were extracted from surface sediments at Sites A and B-bare (0 to 5 mm deep) by making use of the diatoms' phototactic movement (Couch 1989). Samples were inspected with a microscope to confirm a composition of pennate motile diatoms such as *Navicula* spp., *Nitzschia* spp. and *Pleurosigma* spp. with little contamination of detrital matter. Epiphytic diatoms on *Gracilaria vermiculophylla* (Site B-algae) were sampled by brushing the algae into deionized water. These samples were free from algal debris and composed primarily of pennate diatoms such as *Achnanthes* spp., *Navicula* spp. and

Table 1. Effect of acidic treatment (1 M HCl) on the nitrogen isotope ratio of organic matter sampled on 9 March 2007 (mean ± SD, n = 4). A *t*-test was used to detect differences between the acidified and untreated samples. ns = not significant (p > 0.05)

Organic matter		$\delta^{15}N$ (‰)		*t*-test (df = 6)
	Site	Not acidified	Acidified	
Benthic diatoms	A	7.7 ± 0.4	7.7 ± 1.4	*t* = −0.00 ns
Epiphytic diatoms (on *Gracilaria*)	B	8.0 ± 0.2	8.2 ± 0.9	*t* = −0.49 ns
SOM	B	6.2 ± 0.3	6.3 ± 0.1	*t* = −0.26 ns
POM	B	6.7 ± 0.3	6.5 ± 0.8	*t* = 0.41 ns

Table 2. Carbon and nitrogen stable isotope ratios of primary producers and organic matter. SOM: sediment organic matter (0–5 mm deep); POM: particulate organic matter; Sep-03: sampling date 27 September 2003; Oct-03: 8 October 2003; Mar-04: 24 or 29 March 2004; Apr-04: 3 April 2004; Jul-04: 9 July 2004; Jul-05: 22 July 2005; Aug-05: 22 August 2005

Producers / organic matter	Site	Date	$\delta^{13}C$		$\delta^{15}N$	
Microphytobenthos						
Benthic diatoms	A	Mar-04	−17.3 ± 0.8	(3)	7.6 ± 0.1	(3)
	A	Jul-05	−17.7	(2)	9.9 ± 0.2	(3)
	B	Mar-04	−11.8 ± 1.8	(3)	7.3 ± 0.2	(3)
	B	Jul-05	−19.8 ± 0.5	(3)	8.8 ± 0.3	(3)
		Mean	**−16.5 ± 3.4**	**(11)**	**8.4 ± 1.1**	**(12)**
Epiphytic diatoms	B	Oct-03	−16.7 ± 0.4	(3)	7.5 ± 0.3	(3)
(on *Gracilaria*)	B	Apr-04	−13.3 ± 0.2	(3)	8.1 ± 0.2	(3)
		Mean	**−15.0 ± 1.9**	**(6)**	**7.8 ± 0.3**	**(6)**
Macroalgae and marsh plants						
Gracilaria vermi-	B	Jul-04	−17.8 ± 0.2	(3)	9.3 ± 0.3	(3)
culophylla	B[a]	Aug-05	−21.7 ± 1.0	(6)	9.6 ± 2.1	(6)
(red alga)		**Mean**	**−20.4 ± 2.1**	**(9)**	**9.5 ± 1.7**	**(9)**
Enteromorpha	B	Jul-04	−11.8 ± 0.6	(3)	10.7 ± 1.2	(3)
prolifera	B[a]	Aug-05	−13.8 ± 1.9	(6)	10.5 ± 2.3	(6)
(green alga)		**Mean**	**−13.1 ± 1.9**	**(9)**	**10.5 ± 1.9**	**(9)**
Phragmites australis (reed)						
dead foliage	B	Jul-04	−26.1 ± 0.2	(3)	4.2 ± 0.6	(3)
fresh foliage	A, B[a]	Aug-05	−25.5 ± 0.7	(9)	9.3 ± 1.5	(9)
		Mean	**−25.6 ± 0.7**	**(12)**	**8.0 ± 2.7**	**(12)**
Pinus spp. (pine)[b]						
dead foliage		Aug-05	−28.9 ± 0.7	(3)	−3.1 ± 0.6	(3)
fresh foliage		Aug-05	−27.4 ± 1.5	(3)	−0.2 ± 2.3	(3)
		Mean	**−28.1 ± 1.3**	**(6)**	**−1.6 ± 2.2**	**(6)**
Organic matter						
SOM	A	Mar-04	−21.2 ± 0.4	(3)	7.9 ± 0.2	(3)
	A	Jul-05	−22.2 ± 0.9	(3)	7.5	(2)
		Mean	**−21.7 ± 0.8**	**(6)**	**7.7 ± 0.3**	**(5)**
	B	Mar-04	−21.8 ± 0.1	(3)	7.6 ± 0.6	(3)
	B	Jul-05	−24.0 ± 0.7	(3)	6.5 ± 0.4	(3)
		Mean	**−22.9 ± 1.3**	**(6)**	**7.1 ± 0.7**	**(6)**
POM	A	Sep-03	−23.6 ± 0.1	(3)	7.2 ± 0.8	(3)
	A	Mar-04	−24.1 ± 0.2	(3)	6.6 ± 0.2	(3)
	A	Jul-05	−23.2 ± 0.1	(3)	5.1 ± 0.8	(3)
	A	Aug-05	−21.6 ± 0.2	(3)	5.0 ± 0.3	(3)
		Mean	**−23.1 ± 1.0**	**(12)**	**6.0 ± 1.1**	**(12)**
Autochthonous	B[c]	Jul-05	−23.7 ± 0.2	(3)	7.4 ± 0.9	(3)
POM	B[c]	Aug-05	−23.6 ± 0.1	(3)	8.3 ± 0.2	(3)
		Mean	**−23.6 ± 0.2**	**(6)**	**7.9 ± 0.8**	**(6)**
Riverine POM	R	Jul-05	−26.0 ± 0.2	(3)	4.7 ± 0.8	(3)
	R	Aug-05	−28.9	(2)	7.8 ± 0.4	(3)
		Mean	**−27.2 ± 1.4**	**(5)**	**6.2 ± 1.0**	**(6)**
Marine POM	M	Jul-05	−21.3 ± 0.4	(3)	5.5 ± 0.4	(3)
	M	Aug-05	−18.4 ± 0.4	(3)	6.8	(2)
		Mean	**−19.9 ± 1.6**	**(6)**	**6.0 ± 0.8**	**(5)**

[a]Three additional samples were collected at the innermost part of the lagoon; [b]Sampled in the adjacent pine forest; [c]Composed mostly of autochthonous phytoplankton (see Table 3)

Nitzschia spp. Diatoms were concentrated onto a precombusted GFF (500°C, 2 h).

All POM, SOM and diatom samples were freeze-dried for 24 h after treatment with 1 M HCl to remove carbonates. Acidic treatment did not significantly change the $\delta^{15}N$ values of POM, SOM or diatom samples (*t*-test, p > 0.05, Table 1). The macroalgae *Gracilaria vermiculophylla* and *Enteromorpha prolifera* at Site B-algae and the inner lagoon, the marsh plant *Phragmites australis* near Site B-algae and the inner lagoon, and the terrestrial plant *Pinus* spp. in the adjacent pine forest were also sampled by hand, washed in deionized water, freeze-dried for 24 h and ground into powder.

Stable isotope analyses. Carbon and nitrogen stable isotope ratios were determined using a mass spectrometer (Finnigan MAT Delta plus). Isotope ratios are represented in delta notation:

$$\delta^{13}C \text{ or } \delta^{15}N \text{ (‰)} = (R_{sample} / R_{standard} - 1) \times 1000$$

where R is the $^{13}C/^{12}C$ or $^{15}N/^{14}N$ ratio for $\delta^{13}C$ or $\delta^{15}N$, respectively. Pee Dee Belemnite (PDB) and atmospheric N_2 were used as references for $\delta^{13}C$ and $\delta^{15}N$, respectively. Analysis errors were within ± 0.2‰ for both $\delta^{13}C$ and $\delta^{15}N$ (±SD of 10 repeated measurements for the laboratory standard, L-histidine).

Statistics. Data are given as means with SD. The mean $\delta^{13}C$ values of all consumers sampled in each habitat were compared among the habitats using a 1-way ANOVA and the Tukey–Kramer *a posteriori* test. Spatial and temporal changes in $\delta^{13}C$ and $\delta^{15}N$ values of 4 consumer species collected at both Sites A and B-bare on the 2 sampling occasions (*Pseudopolydora kempi japonica*, *Ruditapes philippinarum*, *Nuttallia olivacea* and *Macoma contabulata*) were tested by a 2-way ANOVA. Homogeneity of the data was tested in advance using Bartlett's test. Temporal changes in $\delta^{13}C$ and $\delta^{15}N$ values of *Upogebia yokoyai* were analyzed with a Student's *t*-test.

RESULTS

Isotopic signatures of potential food sources

The isotopic signatures ranged from -28.9 to $-11.8‰$ for $\delta^{13}C$ values and from -3.1 to $10.7‰$ for $\delta^{15}N$ values and clearly differed among the primary producers (Table 2). The microphytobenthos (benthic and epiphytic diatoms) and the macroalgae exhibited more ^{13}C-enriched isotope values (benthic diatoms, $-16.5 \pm 3.4‰$; epiphytic diatoms, $-15.0 \pm 1.9‰$; *Gracilaria vermiculophylla*, $-20.4 \pm 2.1‰$; *Enteromorpha prolifera*, $-13.1 \pm 1.9‰$) than those of the terrestrial C_3 plants (*Phragmites australis*, $-25.6 \pm 0.7‰$; *Pinus* spp., $-28.1 \pm 1.3‰$). Benthic and epiphytic diatoms exhibited similar $\delta^{15}N$ values (8.4 ± 1.1 and $7.8 \pm 0.3‰$, respectively), whereas those of the macroalgae were somewhat higher (*G. vermiculophylla*, $9.5 \pm 1.7‰$; *E. prolifera*, $10.5 \pm 1.9‰$). *Pinus* spp. and dead leaves of *P. australis* exhibited low mean $\delta^{15}N$ values (-1.6 ± 2.2 and $4.2 \pm 0.6‰$, respectively) compared with those of micro- and macroalgae (7.3 to $10.7‰$).

The $\delta^{13}C$ values of POM differed markedly among the sites. This value was lowest at the upper estuarine site (Site R, $-27.2 \pm 1.4‰$), intermediate at the lagoon sites (Site A, $-23.1 \pm 1.0‰$, Site B, $-23.6 \pm 0.2‰$) and highest at the marine site (Site M, $-19.9 \pm 1.6‰$), which was the most ^{13}C-enriched. Chl *a* content of the water sample was 1 order of magnitude higher at the inner lagoon site (Site B, 110 to 113 µg l^{-1}) than at the lagoon mouth, adjacent sea and upper estuary (Sites A, M and R, 3.3 to 17.9 µg l^{-1}; Table 3). Salinity of the water sample also differed among the sites and was lowest at Site R (1.4 to 3.7 psu) and highest at Site M (24.6 to 31.0 psu). In Gamo Lagoon salinity was lower in the

inner part (Site B, 8.3 to 15.6 psu) than at Site A near the lagoon mouth (17.4 to 30.0 psu). The $\delta^{13}C$ value of POM increased correspondingly with the increasing salinity of the samples. In contrast $\delta^{15}N$ values of POM showed little spatial difference (Site R, $6.2 \pm 1.0‰$; Site A, $6.0 \pm 1.1‰$; Site B, $7.9 \pm 0.8‰$; Site M, $6.0 \pm 0.8‰$). Isotopic signatures of SOM ($\delta^{13}C$, -24 to $-21.2‰$; $\delta^{15}N$, 6.5 to 7.9‰) were similar to those of POM in the lagoon ($\delta^{13}C$, -24.1 to $-21.6‰$; $\delta^{15}N$, 5 to 8.3‰).

Isotope signatures of the consumers

The 14 benthic consumer species collected from the 3 habitats (Sites A, B-bare and B-algae) were classified into 5 feeding groups based on the literature (Table 4). The deposit feeder *Macoma contabulata* at Site A exhibited the highest mean $\delta^{13}C$ value ($-13.5 \pm 0.8‰$) and the bivalve *Nuttallia olivacea* at Site B-bare had the lowest value ($-21.5 \pm 0.7‰$). The mean $\delta^{13}C$ value of consumer species (-21.5 to $-13.5‰$) was much more ^{13}C-enriched than those of C_3-plants and riverine POM (-28.9 to $-25.5‰$; Table 2), but fell within the range of values in the marine POM ($-19.9‰$), lagoon POM (-23.6 to $-23.1‰$), microphytobenthos (-19.8 to $-11.8‰$) and macroalgae (-21.7 to $-11.8‰$). The mean $\delta^{13}C$ value of all consumers sampled in each habitat differed significantly among sites (Sites A, B-bare and B-algae: 1-way ANOVA, df = 2, 74, $F = 47.8$, p < 0.001) and a pairwise comparison indicated a significant difference between all pairs (Tukey–Kramer's multiple comparison, p < 0.05). The value was highest at Site B-algae ($-15.7 \pm 1.3‰$), lowest at Site B-bare ($-20.2 \pm 1.3‰$) and intermediate at Site A ($-17.1 \pm 1.7‰$).

Stable nitrogen isotope ratios, which are indicators of trophic level, ranged from $6.8 \pm 0.8‰$ for the amphipod *Eogammarus possjeticus* at Site B-algae to $16.6 \pm 1.1‰$ for the polychaete *Armandia lanceolata* at Site A. The mean $\delta^{15}N$ value of the consumers in each habitat ranged from 9.1 to 16.6‰ at Site A, from 9.5 to 11.3‰ at Site B-bare, and from 6.8 to 10.5‰ at Site B-algae. At Site A the suspension feeders *Ruditapes philippinarum*, *Upogebia yokoyai* and *Nuttallia olivacea* exhibited lower mean $\delta^{15}N$ values (9.1 to 10.5‰) than the other consumer species at this site (10.8 to 16.6‰). The deposit feeder *A. lanceolata* (this species is regarded as a predator or scavenger, see 'Discussion'), *Heteromastus* sp. and *Macoma contabulata* at Site A exhibited ^{15}N-enriched isotopic values (>13‰). In contrast, the amphipods sampled at Site B-algae exhibited unexpectedly low mean $\delta^{15}N$ values (6.8 to 8.1‰) compared with those of the other consumers (>9.1‰) collected in this lagoon.

The $\delta^{13}C$ values of the 4 consumer species (*Pseudopolydora kempi japonica*, *Macoma contabulata*, *Nut-*

Table 3. Salinity (psu) and chl *a* content (µg l^{-1}) of the surface water for POM samples at the 4 stations. nd: not determined; Sep-03: sampling date 27 September 2003; Mar-04: 24 March 2004; Jul-05: 22 July 2005; Aug-05: 22 August 2005

Site	Date	Salinity (psu)	Chl *a* (µg l^{-1})
Gamo Lagoon (ebb tide)			
Site A	Sep-03	21.6 (1)	nd
	Mar-04	25.2 (1)	nd
	Jul-05	17.4 (1)	5.4 ± 0.8 (3)
	Aug-05	30.0 (1)	6.1 ± 0.2 (3)
Site B	Jul-05	8.3 (1)	110 ± 9.4 (3)
	Aug-05	15.6 (1)	113 ± 3.9 (3)
River (ebb tide)			
Site R	Jul-05	1.4 (1)	7.9 ± 0.2 (3)
	Aug-05	3.7 (1)	17.9 ± 0.8 (3)
Sea (high tide)			
Site M	Jul-05	24.6 (1)	3.3 ± 1.0 (3)
	Aug-05	31.0 (1)	9.1 ± 3.4 (3)

Table 4. Carbon and nitrogen stable isotope ratios of benthic consumers in Gamo Lagoon collected on 5 or 6 April 2004 (Apr-04) and 29 August 2005 (Aug-05). The reported and surmised feeding habits of the consumers determined from the literature and the present results are shown. Feeding habits: DDF, deep-deposit feeder; DF, deposit feeder; FSF, facultative suspension feeder; G, epiphyte grazer; OSF, obligatory suspension feeder; P, predator; P or S, predator or scavenger; SDF, surface-deposit feeder

Site and species	Date	Stable isotope ratio (‰)				Feeding habit	
		$\delta^{13}C$		$\delta^{15}N$		Reported	Surmised
Site A (bare flat)							
Polychaeta							
Armandia lanceolata	Apr-04	−17.1 ± 0.3	(3)	16.6 ± 1.1	(3)	DF[a]	P or S
Eteone longa	Apr-04	−16.8	(1)	12.5	(1)	P[a]	SDF
Heteromastus sp.	Apr-04	−18.2 ± 0.2	(3)	13.6 ± 0.2	(3)	DDF[a]	DDF
Pseudopolydora kempi	Apr-04	−17.7 ± 1.5	(3)	11.8 ± 1.7	(3)	FSF[a]	FSF
japonica	Aug-05	−17.2 ± 0.1	(3)	11.0 ± 0.2	(3)		
	Mean	−17.5 ± 1.0	(6)	11.4 ± 1.2	(6)		
Bivalvia							
Macoma contabulata	Apr-04	−14.0 ± 1.0	(3)	12.8 ± 0.5	(3)	SDF[c]	SDF
	Aug-05	−13.1 ± 0.1	(3)	13.7 ± 0.3	(3)		
	Mean	−13.5 ± 0.8	(6)	13.2 ± 0.6	(6)		
Nuttallia olivacea	Apr-04	−17.9 ± 0.2	(3)	9.9 ± 0.3	(3)	FSF[b, c]	OSF
	Aug-05	−18.0 ± 0.6	(3)	10.2 ± 0.4	(3)		
	Mean	−18.0 ± 0.4	(6)	10.0 ± 0.4	(6)		
Ruditapes philippinarum	Apr-04	−17.8 ± 0.0	(3)	9.2 ± 0.3	(3)	OSF[c]	OSF
	Aug-05	−17.0 ± 0.7	(3)	9.0 ± 0.1	(3)		
	Mean	−17.4 ± 0.7	(6)	9.1 ± 0.2	(6)		
Decapoda							
Nihonotrypaea japonica	Apr-04	−16.9	(1)	10.8	(1)	DF[d]	DF
Upogebia yokoyai	Apr-04	−18.4 ± 0.9	(3)	10.3 ± 0.7	(3)	OSF[e]	OSF
	Aug-05	−18.6 ± 0.7	(3)	10.7 ± 0.3	(3)		
	Mean	−18.5 ± 0.7	(6)	10.5 ± 0.5	(6)		
Mean of all consumers		−17.1 ± 1.7	(38)	11.6 ± 2.2	(38)		
Site B-bare (bare flat)							
Polychaeta							
Pseudopolydora kempi	Apr-04	−20.3 ± 0.1	(3)	12.1 ± 0.9	(3)	FSF[a]	FSF
japonica	Aug-05	−19.0 ± 0.1	(3)	10.6 ± 0.1	(3)		
	Mean	−19.7 ± 0.7	(6)	11.3 ± 1.0	(6)		
Bivalvia							
Macoma contabulata	Apr-04	−18.6 ± 1.6	(3)	10.1 ± 0.5	(3)	SDF[c]	SDF
	Aug-05	−19.0 ± 0.9	(3)	11.3 ± 1.1	(3)		
	Mean	−18.8 ± 1.2	(6)	10.7 ± 1.0	(6)		
Mya arenaria	Apr-04	−19.7 ± 0.1	(3)	10.2 ± 0.6	(3)	OSF[f]	SDF?
Nuttallia olivacea	Apr-04	−21.9 ± 0.8	(3)	9.9 ± 0.2	(3)	FSF[b, c]	OSF
	Aug-05	−21.2 ± 0.3	(3)	9.9 ± 0.3	(3)		
	Mean	−21.5 ± 0.7	(6)	9.9 ± 0.2	(6)		
Ruditapes philippinarum	Apr-04	−21.1 ± 0.7	(3)	9.6 ± 0.2	(3)	OSF[c]	OSF
	Aug-05	−20.7 ± 0.5	(3)	9.4 ± 0.3	(3)		
	Mean	−20.9 ± 0.6	(6)	9.5 ± 0.3	(6)		
Mean of all consumers		−20.2 ± 1.3	(27)	10.4 ± 0.9	(27)		
Site B-algae (a dense patch of the macroalga *Gracilaria vermiculophylla*)							
Polychaeta							
Capitella sp.	Apr-04	−14.4 ± 0.4	(3)	10.5 ± 0.8	(3)	SDF[a]	DF
Amphipoda							
Corophium uenoi	Apr-04	−17.1 ± 0.8	(3)	7.9 ± 0.5	(3)	G[g]	G
Eogammarus possjeticus	Apr-04	−16.4 ± 0.8	(3)	6.8 ± 0.8	(3)	G[g]	G
Grandidierella japonica	Apr-04	−14.9 ± 0.8	(3)	7.4 ± 0.1	(3)	G[g]	G
Mean of all consumers		−15.7 ± 1.3	(12)	8.1 ± 1.6	(12)		

References: [a]Fauchald & Jumars (1979), [b]Tsuchiya & Kurihara (1980), [c]Kanaya et al. (2005), [d]Tamaki & Ueno (1998), [e]Kinoshita et al. (2003) for *Upogebia major*, [f]Incze et al. (1982), [g]Aikins & Kikuchi (2002)

tallia olivacea and *Ruditapes philippinarum*) differed significantly between sites (Sites A and B-bare: 2-way ANOVA, p < 0.01; Table 5), but temporal changes were negligible (p > 0.05). The $\delta^{13}C$ values were 2.2 to 5.3‰ lower at Site B-bare (–21.5 to –18.8‰) than at Site A (–18.0 to –13.5‰). The $\delta^{15}N$ value of *M. contabulata* and *R. philippinarum* also significantly changed in space (2-way ANOVA, p < 0.05). At Site B-bare, the mean $\delta^{15}N$ value of *M. contabulata* was 2.5‰ lower, whereas that of *R. philippinarum* was 0.4‰ higher than at Site A. $\delta^{15}N$ values of *M. contabulata* also changed significantly between seasons (2-way ANOVA, p < 0.05), whereas those of *P. kempi japonica*, *N. olivacea* and *R. philippinarum* did not (p > 0.05). There were no significant changes in the $\delta^{13}C$ and $\delta^{15}N$ values of the mud shrimp *Upogebia yokoyai* between sampling occasions (*t*-test, df = 4, *t* = 0.26 for $\delta^{13}C$ and –0.86 for $\delta^{15}N$, p > 0.05).

DISCUSSION

Isotopic signatures of the potential food sources

In Gamo Lagoon $\delta^{13}C$ and $\delta^{15}N$ values of benthic and epiphytic diatoms (Table 2) fell within the range of reported values (–18.8 to –12.1‰ for $\delta^{13}C$ and 3.8 to 11‰ for $\delta^{15}N$; Couch 1989, Moncreiff & Sullivan 2001, Kang et al. 2003, Doi et al. 2005). Ito (2002) cultured benthic diatoms in the Natori River estuary (6 km south of our study area) using a permeable tube and reported the mean $\delta^{13}C$ value of –14.8‰ (range, –17.9 to –11.1‰), which is similar to our data. Isotopic signatures of the macroalgae also fell within the ranges of reported values in other estuarine and coastal areas (Moncreiff & Sullivan 2001, Chanton & Lewis 2002, Yokoyama et al. 2005).

In general, riverine POM derived from terrestrial plant detritus exhibit more ^{13}C-depleted values (<–25‰) than those of marine or estuarine POM (–23.5 to –19‰; Fry & Sherr 1984, Meyers 1994, Kang et al. 2003, Doi et al. 2005). In our study site POM at the upper estuarine site (Site R, salinity < 3.7 psu; Table 3) and the C_3-plants (*Phragmites australis* and *Pinus* spp.) exhibited much more ^{13}C-depleted values (–28.1 to –25.6‰; Table 2) than POM at the marine site (salinity, 24.6 to 31 psu; $\delta^{13}C$, –19.9‰). We regarded the POM samples at Sites M and R as marine POM (i.e. phytoplankton) and riverine terrestrial materials, respectively. Chl *a* content in the water column was 1 order of magnitude higher in the inner lagoon (Site B, 110 to 113 µg l^{-1}) than in Site A (5.4 to 6.1 µg l^{-1}) and in the adjacent waters (Sites M and R, 3.3 to 17.9 µg l^{-1}; Table 3). This indicates that POM in the inner lagoon water was composed of autochthonous phytoplankton.

Role of macroalgae in the food web

Adin & Riera (2003) reported that stranded macroalgae comprised the major dietary component of amphipods in an open sandy shore; however, macroalgae and their derived detritus generally play only minor roles in estuarine and coastal food webs (e.g. Kang 2003, Page & Lastra 2003, Yokoyama et al. 2005).

At our study site the green alga *Enteromorpha prolifera* was found only during the summer and was not found outside the algal patches (G. Kanaya pers. obs.). The $\delta^{13}C$ value of *E. prolifera* (–13.1‰) was distinctively high compared with those of most consumers (Table 4). Thus, it seemed that the alga was of little dietary importance in the benthic food web. The red alga *Gracilaria vermiculophylla* was also rarely found outside the algal patches (G. Kanaya pers. obs.). How-

Table 5. Spatial and temporal changes in stable isotope signatures of 4 consumer species. A 2-way ANOVA for the $\delta^{13}C$ and $\delta^{15}N$ values as a function of site (Sites A and B-bare) and season (April 2004 and August 2005) of sampling is shown. Significant p-values are in bold. Δ = difference in the mean isotopic values between sites or seasons (‰)

Species	Site (df = 1)				Season (df = 1)			
	MS	*F*	p	Δ	MS	*F*	p	Δ
$\delta^{13}C$								
Pseudopolydora kempi japonica	14.5	24.2	**<0.01**	2.2	2.41	4.02	>0.05	0.9
Macoma contabulata	83.1	77.5	**<0.001**	5.3	0.23	0.21	>0.05	0.3
Nuttallia olivacea	37.4	125	**<0.001**	3.5	0.25	0.84	>0.05	0.3
Ruditapes philippinarum	37.0	114	**<0.001**	3.5	1.18	3.65	>0.05	0.6
$\delta^{15}N$								
Pseudopolydora kempi japonica	0.008	0.008	>0.05	0.1	4.00	4.20	>0.05	1.2
Macoma contabulata	18.9	41.9	**<0.001**	2.5	3.20	7.11	**<0.05**	1.0
Nuttallia olivacea	0.04	0.37	>0.05	0.1	0.11	1.21	>0.05	0.2
Ruditapes philippinarum	0.46	7.53	**<0.05**	0.4	0.13	2.15	>0.05	0.2

ever, the δ^{13}C value (−20.4‰) fell within the range of marine POM (−19.9 ± 1.6‰) and it is difficult to assume the dietary contribution of the algae judging from the δ^{13}C value alone. The δ^{34}S values of suspension and deposit feeding animals have been measured in this lagoon (the deposit feeding polychaete *Hediste* spp., and the bivalves *Macoma contabulata*, *Nuttallia olivacea* and *Ruditapes phillipinarum*) and were less than +12.1‰ at Site A and less than +10.1‰ at Site B-bare (Y. Maki, Iwate Univ., Japan, pers. comm.). The values were much more ^{35}S-depleted than that of *G. vermiculophylla* in this lagoon (19.3‰, G. Kanaya unpubl. data). Since the δ^{34}S value is nearly constant through trophic chains (McCutchan et al. 2003), *G. vermiculophylla* does not appear to be a major dietary component of the consumers in the benthic food web.

Diet of benthic consumers near the lagoon mouth (Site A)

The δ^{13}C values of consumers at Site A fell within the range of animals assimilating marine POM (−19.9‰) or benthic diatoms (−16.5‰; Fig. 2a), assuming trophic enrichment of 0 to +1‰ (Fry & Sherr 1984). Enriched δ^{13}C and δ^{15}N values of the deposit feeders imply a larger dietary contribution of benthic diatoms. In contrast, the suspension feeders *Upogebia yokoyai*, *Nuttallia olivacea* and *Ruditapes philippinarum*, which have lower δ^{13}C and/or δ^{15}N values, should assimilate marine POM or other isotopically depleted food sources. The low chl *a* concentration at this site (Table 3) implies a small supply of autochthonous phytoplankton to the area. Additionally, Site A is emergent during ebb tides when the inner lagoon water, which is rich with autochthonous POM, is discharged (G. Kanaya unpubl. data). Therefore, marine POM may be a more accessible diet for suspension feeders at this site. Riverine POM, the other ^{13}C-depleted potential food, is generally derived from terrestrial plant litter (Wada et al. 1987), which is indigestible by most marine invertebrates (Kristensen 1972). In Gamo Lagoon Kurata et al. (2001) experimentally demonstrated that marsh snails preferentially assimilated microalgae in their diet even though they could assimilate marsh plant litter. Consequently, the food web should be based on benthic diatoms and marine POM with little contribution from other ^{13}C-depleted food sources (e.g. autochthonous POM, riverine POM and C_3-plants).

Trophic position of a consumer can be inferred from the δ^{15}N value because of a 3 to 4‰ increase per trophic level (Minagawa & Wada 1984). At this site, *Armandia lanceolata* (δ^{15}N 16.6‰) appeared to be a secondary consumer, whereas the others were primary
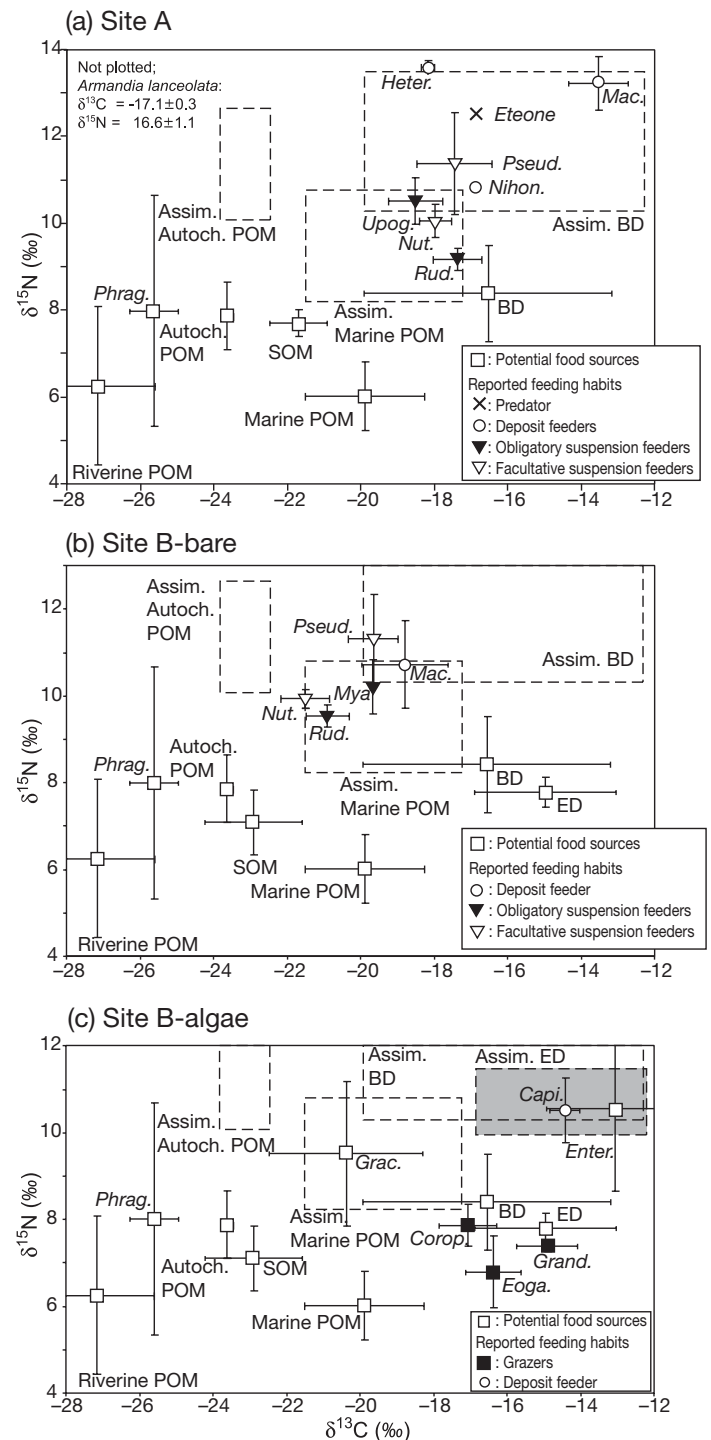

Fig. 2. δ^{13}C and δ^{15}N values of macrozoobenthos and their available food sources at (a) Site A, (b) Site B-bare and (c) Site B-algae. The first 3 to 6 characters of each genus name of the consumer, macroalgae, and plants are denoted in italics (see Tables 2 & 4 for full names). POM: particulate organic matter; SOM: sediment organic matter at each station; BD and ED: benthic and epiphytic diatoms (average of all samples). Broken lines indicate the expected isotope range of a consumer assimilating the dietary components. Bars indicate ± SD. See Tables 2 & 4 for sample sizes (n)

consumers based on marine POM or benthic diatoms (see Fig. 2a). *Eteone longa* is a common hunting predator (Fauchald & Jumars 1979); however, at this site, it appeared to be a deposit feeding primary consumer. It was also notable that the deposit feeders *Heteromastus* sp. and *Macoma contabulata* exhibited relatively high $\delta^{15}N$ values (>13‰). Based on Fauchald & Jumars (1979) and Kanaya et al. (2005), they are not considered predators or scavengers. Kikuchi & Wada (1996) reported that $\delta^{15}N$ values of the deposit feeding capitellid polychaete *Notomastus* sp. were >3.8‰ higher than the SOM and proposed a selective assimilation of ^{15}N-enriched fractions in its diet. In general, detritus is enriched in ^{15}N during decomposition by microbial transformation of nitrogen (Wada et al. 1987, Thornton & McManus 1994). Hence, the discrepancy in the $\delta^{15}N$ values between the deposit feeders and the diets may be due to bacterial trophic mediation.

The $\delta^{13}C$ values of the suspension feeders in this site (>1‰ higher than marine POM) indicate the assimilation of benthic diatoms together with marine POM or other isotopically depleted food sources. Results (Table 5) apparently showed that the surface deposit feeders fed mostly on the benthic diatoms (74 to 174%) and the suspension feeders assimilated a certain amount of benthic diatoms (27 to 59%). It is widely recognized that water movement resuspends microphytobenthos into the water column (e.g. de Jonge & van Beusekom 1992). At Site A, tidal current velocity reached over 40 cm s^{-1} during flood tides (Aikins & Kikuchi 2001). Therefore, the high tidal currents would resuspend the sediment surface and provide the suspension feeders with rich resuspended diatom cells.

Dietary changes of benthic consumers at Site B-bare

In Site B-bare, mean $\delta^{13}C$ value of all consumers (–20.2 ± 1.3‰, Table 4) was significantly lower than that at Site A (–17.1 ± 1.7‰) (Tukey-Kramer's multiple comparison, p < 0.05) and mean $\delta^{13}C$ values of each consumer (–21.5 to –18.8‰) fell within the range of autochthonous POM (–23.6‰), marine POM (–19.9‰) and benthic diatoms (–16.5‰) (see Fig. 2b). This implies the dietary importance of the 3 food sources for the consumers.

Chl *a* content in the water column increased dramatically from Site A to Site B (Table 3). This would increase the availability of phytoplankton for benthic suspension feeders in this site. In contrast, the availability of resuspended benthic diatoms may be reduced in Site B since tidal current is much lower in this site (nearly 10 cm s^{-1}) than at Site A (>40 cm s^{-1}; Aikins & Kikuchi 2001). The $\delta^{13}C$ values of the suspension feeders *Pseudopolydora kempi japonica*, *Nuttallia*

olivacea and *Ruditapes philippinarum* (–19.7, –21.5 and –20.2‰, respectively; Tables 4 & 5), were in fact significantly lower than those at Site A (–17.5, –18.0 and –17.8‰, respectively). This indicates the increasing dietary contribution of autochthonous phytoplankton or other isotopically depleted sources for the suspension feeders at this site. Kanaya et al. (2005) transplanted the suspension feeder *R. philippinarum* from Site B to enclosures set near Site A. After 3 mo, the $\delta^{13}C$ value of the transplanted *R. philippinarum* significantly increased from –20.5 to –18.9‰, which is in accordance with our results.

As well as the suspension feeders, the surface deposit feeder *Macoma contabulata* also showed a marked isotopic value shift between the 2 sites ($\Delta\delta^{13}C$ = 5.3‰, Table 5). The lower $\delta^{13}C$ value of *M. contabulata* at this site implies the assimilation of deposited autochthonous POM on the sediment surface. In Gamo Lagoon SOM in the inner area is derived mostly from settling phytoplankton (Kanaya & Kikuchi 2004). The isotopic signatures of SOM (see Fig. 2b), which resembled those of autochthonous POM, also indicate that deposited POM is the major source of SOM. It seems that phytoplankton becomes a significant food source for deposit feeders after it settles onto the sediment surface.

Our results clearly showed the spatial dietary shift of the infaunal suspension and deposit feeders within a brackish lagoon system. A spatial dietary shift of benthic consumers could occur even at small scales (~200 m) corresponding to the changes in potential food supply such as current-induced resuspension, phytoplanktonic biomass in the water column and/or deposited phytoplanktonic biomass on the sediment.

Diet of benthic consumers in the macroalgal patch (Site B-algae)

The $\delta^{13}C$ values of the algae associating amphipods *Corophium uenoi*, *Eogammarus possjeticus* and *Grandidierella japonica* suggested the trophic importance of epiphytic or benthic diatoms or both ($\delta^{13}C$, –16.5 to –15.0‰), whereas the other potential food sources were less important (see Fig. 2c). The $\delta^{13}C$ values of epiphytic and benthic diatoms were not distinctive and, thus, we could not assess the relative importance of the 2 diatom pools for the consumers' diet. However, we can infer relative dietary importance by considering feeding ecology and the supply of these 2 sources in the habitats. The amphipods maintain populations on the algal body and graze on diatoms attached to the algae (Aikins & Kikuchi 2002). Thus, it is likely that the amphipods assimilated mainly epiphytic diatoms in this site. The $\delta^{15}N$ values of the amphipods, however,

did not correspond to those of epiphytic diatoms (Fig. 2c). Macko et al. (1982) reported that the amphipod *Amphithoe valida* showed negative trophic enrichment (–0.7 to –0.1‰) when fed algal detritus. Similar inconspicuous trophic enrichment was also reported for another consumer taxon (McCutchan et al. 2003). The unexpectedly low δ^{15}N values of the amphipods in this site may, therefore, have been caused by a particular trophic fractionation or a selective assimilation of a ^{15}N-depleted fraction in their diets (e.g. N$_2$-fixing cyanobacteria; Currin et al. 1995). However, further detailed examination is needed to fully understand amphipod feeding ecology.

The δ^{13}C and δ^{15}N values of the deposit feeding polychaete, *Capitella* sp., also suggested the trophic importance of epiphytic or benthic diatoms or both (see Fig. 2c). *Capitella* sp. was sampled from the sediment below the dense algal patch (nearly 100% coverage), which extensively limits light availability for benthic diatoms on the sediment. Therefore, it is more likely that this worm assimilated detached epiphytes in the SOM pool. Capitellid polychaetes are known to ingest SOM with low or no selectivity (Fauchald & Jumars 1979). However, *Capitella* sp. at this site selectively assimilated the diatom cells in the SOM pool, since the δ^{13}C values were 8.5‰ higher than those of SOM.

Major dietary components in the lagoon food web

In estuarine and coastal food webs many ecologists have reported the trophic importance of microalgae (Riera & Richard 1996, Moncreiff & Sullivan 2001, Kang et al. 2003, Page & Lastra 2003, Yokoyama et al. 2005). In contrast, riverine materials became major dietary components in benthic food webs where riverine input is predominant (e.g. Incze et al. 1982, Riera & Richard 1996, Chanton & Lewis 2002) and marsh plant detritus sometimes plays a significant role in reed dominated marsh systems (Peterson & Howarth 1987, Currin et al. 1995, Wainright et al. 2000).

In our study site the benthic food web depended primarily on marine input and estuarine production (benthic and epiphytic diatoms and autochthonous phytoplankton); organic matter derived from the riverine terrestrial sources were rarely incorporated into the food web (Fig. 2). The microalgaebased food web is attributable, in part, to high primary productivity in the lagoon system. The lagoon shows no light limitations through the water column (Kikuchi et al. 1992). Enough nutrients released from the bottom sediments also support rapid growth of microalgae (Kikuchi et al. 1992). As a result, productivity in Gamo Lagoon (2.4 g C m^{-2} d^{-1}; Kikuchi et al. 1992) is comparable with that of the most productive estuaries in the world (Heip

et al. 1995). The rapid growth of microalgae would contribute to the benthic secondary production in the lagoon.

The microalgae-based food web would also be related to the high nutritive values of microalgae compared with that in riverine terrestrial organic material. In general, riverine POM is derived mainly from terrestrial plant litter (Wada et al. 1987), which is generally rich in cellulose and lignin that are indigestible for most marine invertebrates (Kristensen 1972). In contrast, microalgae with a low C/N ratio (<8; Meyers 1994, Kurata et al. 2001) are rich in proteins and carbohydrates, which are highly nutritive for benthic consumers (Kristensen 1972). Therefore, benthic consumers in Gamo Lagoon would preferentially assimilate more nutritive microalgae in their diet, whereas much more of the indigestible fractions, i.e. riverine terrestrial organic materials, would be excreted as feces or pseudofeces.

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