

# Spatial shifts in food sources for macrozoobenthos in an estuarine ecosystem: Carbon and nitrogen stable isotope analyses

Hideyuki Doi <sup>a,\*</sup>, Masatoshi Matsumasa <sup>b</sup>, Terumasa Toya <sup>c</sup>, Nobuya Satoh <sup>d</sup>,  
Chitoshi Mizota <sup>d</sup>, Yonosuke Maki <sup>e</sup>, Eisuke Kikuchi <sup>f</sup>

<sup>a</sup> Graduate School of Life Sciences, Tohoku University, 2-1-1, Katahira, Aoba-ku, Sendai 980-8577, Japan

<sup>b</sup> Department of Biology, School of Liberal Arts and Sciences, Iwate Medical University, Honcho-dori 3-16-1, Morioka 020-0015, Japan

<sup>c</sup> Biological Institute, Graduate School of Sciences, Tohoku University, Aoba, Aoba-ku, Sendai 980-8576, Japan

<sup>d</sup> Department of Agro-biosciences, Faculty of Agriculture, Iwate University, Ueda 3-18-8, Morioka 020-8550, Japan

<sup>e</sup> Laboratory of Biology, Faculty of Humanities and Social Sciences, Iwate University, Ueda 3-18-34, Morioka 020-8550, Japan

<sup>f</sup> Center for Northeast Asian Studies, Tohoku University, Kawauchi, Aoba-ku, Sendai 980-8576, Japan

Received 1 November 2004; accepted 25 February 2005

Available online 19 April 2005

## Abstract

Carbon and nitrogen stable isotope ( $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$ , respectively) analyses were made on estuarine macrozoobenthos in order to examine the relationships between their feeding habits (feeding mode and food selectivity) and the spatial shifts in food sources from upstream to downstream in an estuary. The  $\delta^{13}\text{C}$  values of two ocy podid crabs were similar to those of benthic diatoms, indicating that they use their specialized mouth parts to selectively feed on benthic diatoms. The  $\delta^{13}\text{C}$  values of a gastropod and another ocy podid crab at the site furthest downstream were higher than values at an upstream site, suggesting that these unselective deposit feeders shift from feeding mainly on benthic diatoms downstream to feeding on sediment organic matter (SOM) upstream. The  $\delta^{13}\text{C}$  values of deposit feeding polychaetes were not significantly different among sampling sites, indicating that they feed mainly on SOM at all sites. These results show that species- and site-specific feeding habits must be considered when evaluating the roles of macrozoobenthos in regulating estuarine material flows.

© 2005 Elsevier Ltd. All rights reserved.

**Keywords:** feeding habits; deposit feeders; ocy podid crabs; gastropod; polychaetes; benthic microalgae; sediment organic matter

## 1. Introduction

Organic matter in an estuary is a mixture of allochthonous (i.e. imported) material from the river and sea, and autochthonous material that is produced within the estuary. The contribution of each component to the total organic budget changes spatially from the mouth to the head of an estuary (Little, 2000; Wetzel, 2001). Recently, stable isotope analyses have revealed

that the macrozoobenthos, which are important primary consumers in most estuaries, shift their main food source from one component to another as the composition of organic matter changes (e.g., Coach, 1989; Cifuentes et al., 1996). Thus, the roles of some macrozoobenthos in an estuarine food web are likely to change spatially, although few studies have examined this (but Wainright et al., 2000). Such a study requires clarifying the relationship between macrozoobenthos' feeding habits and food sources, because the role of benthic species depends on both their feeding mode (e.g., suspension, deposit or predatory feeding), and food selectivity (selective and unselective feeding that

\* Corresponding author.

E-mail address: [h-doi@cneas.tohoku.ac.jp](mailto:h-doi@cneas.tohoku.ac.jp) (H. Doi).

correspond with specialists and generalists, respectively). Other ecological variables, such as co-occurring macrozoobenthos and food source density and distribution, are also important. In addition, the contribution of allochthonous and autochthonous organic material differs between watersheds, depending on the geology, freshwater input, and tidal range of each estuary (Little, 2000; Mann, 2000). Therefore, empirical studies on various estuary types are needed.

The majority of macrozoobenthos in estuaries are suspension and deposit feeders. Their feeding activity forms an important link between the sediment and suspended material, and this affects nutrient cycling in estuarine ecosystems (see Little, 2000; Mann, 2000 for reviews). However, it is often difficult to evaluate macrozoobenthos' food sources. For example, the sediment organic matter (SOM) that benthic deposit feeders eat is a composite of microalgae, bacteria, and aquatic and terrestrial plants. Some deposit feeders selectively use only one part of the SOM (e.g., only the microalgae), while others feed unselectively on all SOM. Therefore, carbon and nitrogen stable isotope analyses, which are now widely used as a means of tracing material flow through food webs (e.g., Kurata et al., 2001), are recommended for estimating actual food sources for the benthic animals of the same feeding mode but having the different food selectivity.

The present study conducted carbon and nitrogen stable isotope analyses for macrozoobenthos at three sites from the mouth to the head of the Kitakami estuary, in northeastern Japan. The macrozoobenthos species were compared among different feeding modes and/or food selectivity to determine if these feeding habits conferred different stable isotope ratios. The macrozoobenthos were categorized according to their feeding habits and potential food sources, and the stable isotope

data were used to identify spatial shifts in the use of organic material as its composition varies in the estuary.

## 2. Materials and methods

### 2.1. Study area

The Kitakami River estuary is located in the northeast of Honshu Island, Japan ( $38^{\circ}30'–34'N$ ,  $141^{\circ}18'–28'E$ ). Three sampling stations, Sts. 1, 2, and 3 were set at approximately 2, 8, and 16.5 km, respectively, upstream from the river mouth (Fig. 1). All the stations were on intertidal flats with salt marshes that consisted mainly of *Phragmites australis*. The salt marsh at St. 1 was smaller and had developed relatively large, bare sand flat, as compared with the larger marshes at Sts. 2 and 3, where bare areas were restricted to the marsh edges. At St. 1, the benthic microalgae were only readily available to collect samples in the sparse marsh, while at Sts. 2 and 3, the benthic microalgae were not available, probably due to light limitation by salt marsh. Water salinity varied considerably with tidal movements and the mass of river flow (e.g., during 1999, the range of salinity in the surface water at St. 1 was ca. 0–30, Matsumasa, personal communication). The macrozoobenthic species composition changes with the salinity gradient and the spatial arrangement of habitable substrate (see below Table 1; Matsumasa and Kikuchi, 1993; Matsumasa, 1994).

### 2.2. Sample collection and preparation for isotopic analyses

On 10 August 2001 the following dominant adult macrozoobenthos were collected with three individuals for the replicate sample at Sts. 1, 2, and 3: a bivalve

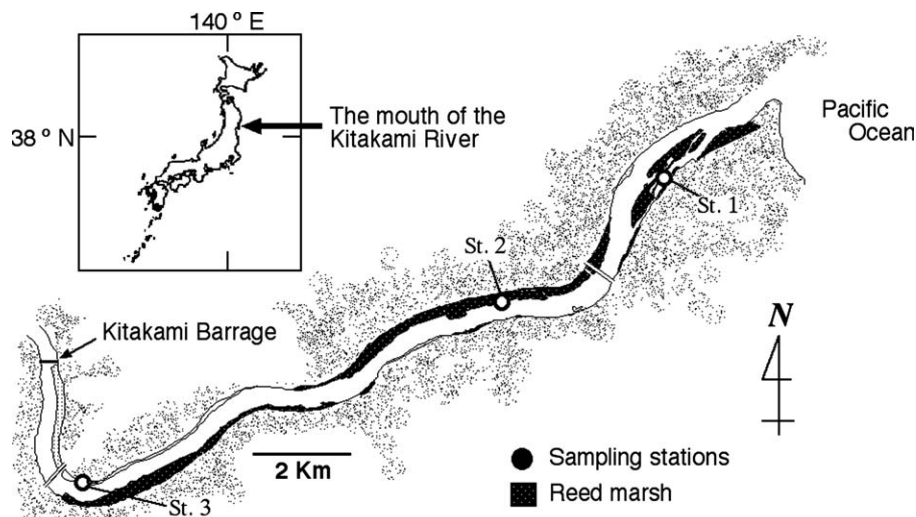


Fig. 1. Map of the Kitakami River estuary.

Table 1  
Presence and feeding habits (feeding mode and food selectivity) of the macrozoobenthos in the Kitakami River estuary

Species	St. 1	St. 2	St. 3	Feeding mode	Food selectivity	References
<i>Corbicula japonica</i> (B)	+	+	+	Filter feeding	Unselective	Yamamuro and Koike, 1994
<i>Assiminea japonica</i> (G)	+	+	+	Deposit feeding	Unselective	Abbott, 1958
<i>Deiratonotus cristatus</i> (C)	+	+		Deposit feeding	Unselective	Ono, 1967
<i>Ilyoplax pusilla</i> (C)	+			Deposit feeding	Selective	Wada, 1982
<i>Scopimera globosa</i> (C)	+	+		Deposit feeding	Very selective	Wada, 1982
<i>Chiromantes dehaani</i> (C)	+			Deposit feeding	Unselective	Ono, 1967
<i>Helice tridens tridens</i> (C)	+			Predatory feeding	Unselective	Takeda et al., 1988
<i>Hediste</i> spp. (P)	+	+	+	Deposit feeding	Unselective	Kikuchi and Wada, 1996
<i>Notomastus</i> sp. (P)	+	+	+	Deposit feeding	Unselective	Kikuchi and Wada, 1996
<i>Tylorrhynchus heterochaetus</i> (P)			+	Deposit feeding	Unknown	

The letters B, G, C, and P indicate bivalve, gastropod, crab, and polychaete, respectively. + indicates species presence.

*Corbicula japonica* Prime, the gastropod *Assiminea japonica* (Martens), polychaetes including *Notomastus* sp., *Tylorrhynchus heterochaetus* (Quatrefages) and *Hediste* spp., and crabs including *Chiromantes dehaani*, (H. Milne Edwards), *Helice tridens tridens* (De Haan), *Deiratonotus cristatus* (De Man), *Ilyoplax pusilla* (De Haan) and *Scopimera globosa* (De Haan). The presence at each station and feeding habits of these macrozoobenthos are shown in Table 1. The whole body samples of these macrozoobenthos were freeze-dried and preserved in a freezer at  $-20^{\circ}\text{C}$  prior to analysis of stable isotopes.

Sediment samples containing sediment organic matter (SOM) were collected from depths of 0–0.5 cm (SOM<sub>surface</sub>) and 7–10 cm (SOM<sub>deep</sub>) at Sts. 1–3 with three replicates on 10 August 2001. Surface water samples for suspended particulate organic matter (POM) were collected at Sts. 1–3 on 10 August 2001. The salinity was measured using a salinometer (HORIBA U-21), and then the water was filtered through Whatman GF/F glass-fiber filters (precombusted at  $500^{\circ}\text{C}$  for 2 h) to collect the POM. SOM and POM samples were acidified with  $1\text{ mol l}^{-1}$  HCl to remove carbonates before the isotope analysis. The leaves of the marsh plants *Phragmites australis* and *Typha latifolia* were collected at Sts. 1–3 and at St. 2, respectively.

Benthic diatoms were collected from the sediment at Sts. 1, 2, and 3 making use of their phototactic movement with three replicates (see Coach, 1989; Doi et al., 2003a,b). Unfortunately, benthic diatoms were sufficiently collected only from the sediment at St. 1 by the extracting method, probably because of low availability of benthic diatoms at Sts. 2 and 3 due to light limitation by salt marsh. To do this, sediment samples from a depth of 1 cm were collected on 27 September 2002, and spread in a 1-cm-thin layer on petri dishes. For each sample, a nylon net (75- $\mu\text{m}$  mesh) was placed on the sediment surface and a 2-mm layer of 25- to 65- $\mu\text{m}$  silica powder (precombusted at  $500^{\circ}\text{C}$  for 2 h) was put on the net. The petri dishes were illuminated for 24 h with continuous spraying of filtered river water to retain moisture in the silica powder. After 24 h the powder was scraped off and mixed with filtered

deionized water to suspend the benthic diatoms. They were then decanted into glass vials and freeze-dried.

### 2.3. Stable isotope ratio analysis

The carbon and nitrogen isotope ratios of the samples were measured with a mass spectrometer (DELTA plus, Finnigan Mat) directly connected to an elemental analyzer (NA-2500, CE Instruments). All the isotopic data are reported in the conventional  $\delta$  notation as follows:

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

where  $R$  is the  $^{13}\text{C}/^{12}\text{C}$  or  $^{15}\text{N}/^{14}\text{N}$  ratio for  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$ , respectively. Pee Dee Belemnite (PDB) was used as the  $\delta^{13}\text{C}$  standard and nitrogen gas as the  $\delta^{15}\text{N}$  standard. The overall analytical error was within  $\pm 0.2\text{‰}$  for both  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$ .

The methodology for analyses of trophic relationships using stable isotope ratios is based on the premise that the enrichment of  $\delta^{13}\text{C}$  during trophic transfer is slight ( $+0.8 \pm 1.1\text{‰}$ ), whereas the enrichment of  $\delta^{15}\text{N}$  is fairly large ( $+3.4 \pm 1.1\text{‰}$ ) (DeNiro and Epstein, 1978; Minagawa and Wada, 1984).

## 3. Results

### 3.1. Isotopic signatures of the food sources

In this study area, the vascular plants (*Phragmites australis* and *Typha latifolia*,  $\text{C}_3$  plants) had similar  $\delta^{13}\text{C}$  values from  $-27.0$  to  $-25.2\text{‰}$  throughout all stations. The  $\delta^{15}\text{N}$  values of *P. australis* at St. 1 ( $10.5 \pm 0.4\text{‰}$ , mean  $\pm 1$  SD,  $n = 3$ ) were significantly higher than those at Sts. 2 and 3 ( $6.3$ – $7.5\text{‰}$ ) (multiple comparison Holm test,  $p < 0.05$ ,  $n = 3$ ). The  $\delta^{15}\text{N}$  value for *T. latifolia* at St. 3 was  $8.6 \pm 0.3\text{‰}$ .

The mean  $\delta^{13}\text{C}$  value of the benthic diatoms ( $-18.8 \pm 0.4\text{‰}$ ) were significantly higher than those of the higher plants, SOM and POM (Holm test,  $p < 0.05$ ,

$n = 3$ ), indicating that the benthic diatoms, collected only at St. 1, were enriched in  $\delta^{13}\text{C}$  relative to all other potential food sources.

The mean  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values of the  $\text{SOM}_{\text{surface}}$  (depth: 0–0.5 cm) ranged from  $-25.7$  to  $-24.1\text{‰}$  and from  $5.0$  to  $5.9\text{‰}$ , respectively, and those of the  $\text{SOM}_{\text{deep}}$  (depth: 7–10 cm) ranged from  $-25.7$  to  $-23.8\text{‰}$  and from  $6.0$  to  $6.4\text{‰}$ , respectively (Figs. 2–4). The  $\delta^{13}\text{C}$  values of SOM were matched with those of in situ vascular plants, however  $\delta^{15}\text{N}$  values of SOM were generally depleted in SOM relative to vascular plants.

The mean  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values of POM from the water (salinity: 2.5) were  $-25.8 \pm 0.2$  and  $3.8 \pm 1.0\text{‰}$  (mean  $\pm 1$  SD,  $n = 3$ ), respectively, and those from the water (salinity: 0) were  $-24.7 \pm 0.2$  and  $4.8 \pm 1.0\text{‰}$ , respectively (Figs. 3 and 4). The mean  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values of POM from the water (salinity: 30) were  $-22.1 \pm 0.5$  and  $7.9 \pm 0.1\text{‰}$ , respectively, and were significantly higher than the values of POM from either the water (salinity: 2.5 or 0) (Holm test,  $p < 0.05$ ,  $n = 3$ ). The POM in the water column varied along the estuarine gradient and was more enriched in  $\delta^{13}\text{C}$  at the marine end (St. 1).

### 3.2. Isotopic signatures of macrozoobenthos

*Corbicula japonica* and *Assiminea japonica* largely tracked the gradient of  $\delta^{13}\text{C}$  values found in food sources, corresponding with their generalist (i.e.

unselective) food habits (Table 1). The mean  $\delta^{13}\text{C}$  values of *C. japonica* gradually decreased from St. 1 to St. 3 (Figs. 2–5). The  $\delta^{13}\text{C}$  values of *A. japonica* ranged from  $-21.8\text{‰}$  (St. 2) to  $-18.1\text{‰}$  (St. 1). The values for both species were significantly higher at St. 1 than at the other two sites (Holm test,  $p < 0.05$ ,  $n = 3$ ).

*Ilyoplax pusilla* maintained similar  $\delta^{13}\text{C}$  values across the estuarine gradient. The mean  $\delta^{13}\text{C}$  values of *I. pusilla* were  $-14.8 \pm 1.0\text{‰}$  (mean  $\pm 1$  SD,  $n = 3$ ) at St. 1 and  $-16.2 \pm 1.1\text{‰}$  at St. 2 (Figs. 2–5), were significantly different between Sts. 1 and 2 ( $t$ -test,  $p > 0.05$ ,  $n = 3$ ). In contrast, *Deiratonotus cristatus* changed  $\delta^{13}\text{C}$  values across the estuarine gradient. The mean  $\delta^{13}\text{C}$  value of *D. cristatus* was significantly higher at St. 1 ( $-17.6 \pm 1.2\text{‰}$ ) than at St. 2 ( $-22.3 \pm 0.4\text{‰}$ ) ( $t$ -test,  $p < 0.01$ ,  $n = 3$ ). The mean  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values of the grapsid crab *Chiromantes dehaani* were  $-22.8 \pm 0.0\text{‰}$  and  $11.0 \pm 0.8\text{‰}$ , respectively, and the mean  $\delta^{13}\text{C}$  values of an ocypodid crab, *Scopimera globosa* were  $-14.5 \pm 0.3\text{‰}$  and were the highest of all macrozoobenthos species examined in this study. The  $\delta^{15}\text{N}$  values of *Helice tridens tridens* ( $13.5 \pm 0.7\text{‰}$ ) were significantly higher than those of all other species (Holm test,  $p < 0.05$ ,  $n = 3$ ), indicating a slightly higher trophic level and some predatory food habits.

The isotope values of polychaete did not vary among stations. However, the  $\delta^{13}\text{C}$  values of *Notomastus* sp. at Sts. 2 and 3 were significantly higher than those of *Hediste* spp. in situ (Holm test,  $p < 0.05$ ,  $n = 3$ ). The

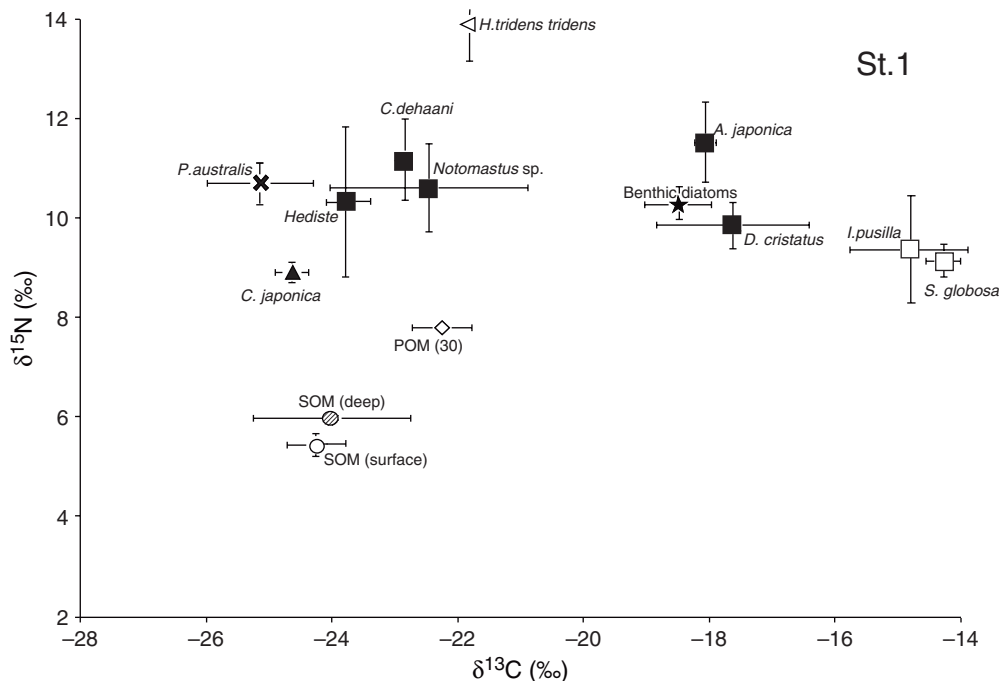


Fig. 2. Carbon and nitrogen stable isotope plots of macrozoobenthos and their food sources at St. 1. Each symbol shows a mean  $\pm 1$  SD ( $n = 3$ ). POM (30) indicates particulate organic matter (salinity: 30), respectively. SOM (surface and deep) indicates sediment organic matter at 0–0.5 and 7–10 cm depth, respectively. Black squares, white squares, black triangle and white triangle showed unselective deposit feeders, selective deposit feeders, filter feeder and predator, respectively.

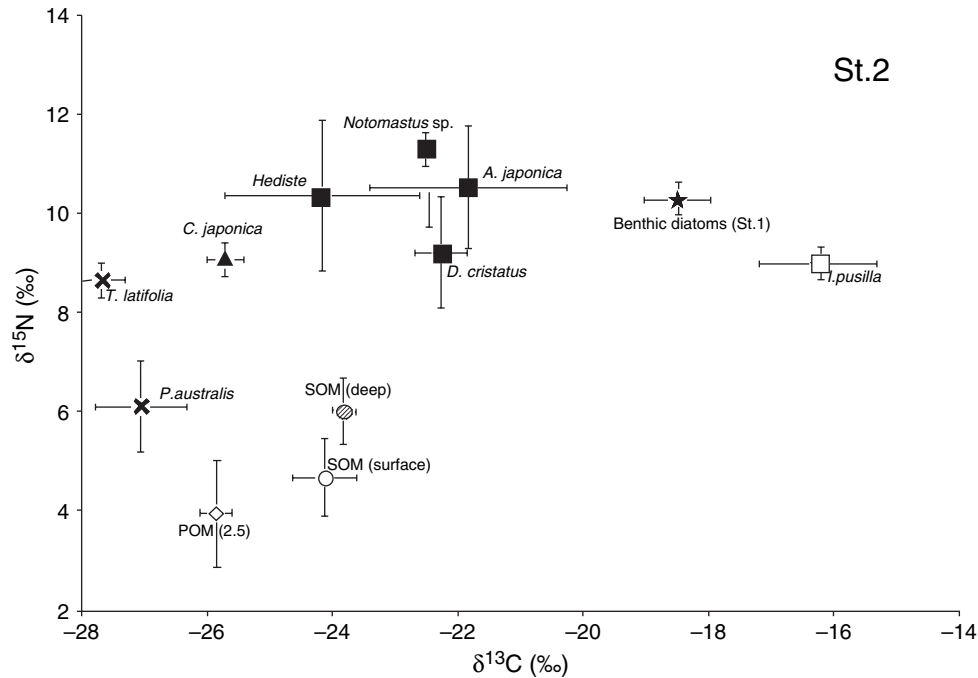


Fig. 3. Carbon and nitrogen stable isotope plots of macrozoobenthos and their food sources at St. 2. Each symbol shows a mean  $\pm 1$  SD ( $n = 3$ ). POM (2.5) indicates particulate organic matter (salinity: 2.5), respectively. SOM (surface and deep) indicates sediment organic matter at 0–0.5 and 7–10 cm depth, respectively. Black squares, white squares and black triangle showed unselective deposit feeders, selective deposit feeders and filter feeder, respectively.

$\delta^{13}\text{C}$  values of *Tylorrhynchus heterochaetus* ( $-22.0 \pm 0.9\text{‰}$ , mean  $\pm 1$  SD,  $n = 3$ ) were significantly higher than those of *Hediste* spp. (Holm test,  $p < 0.01$ ,  $n = 3$ ) and were close to those of *Notomastus* sp. The  $\delta^{15}\text{N}$  values of these three polychaetes were similar to each other (range: 9.2–11.1‰).

#### 4. Discussion

The  $\delta^{13}\text{C}$  value of the suspension feeding bivalve *Corbicula japonica* significantly decreased from downstream to upstream (Fig. 5). This correlates with the changing contributions of marine and terrestrial organic material to the POM and the decreasing water salinity from the mouth to the head of the estuary. The  $\delta^{13}\text{C}$  values of the POM differed at high and low salinities (30 at St. 1 vs. 2.5 and 0 at Sts. 2 and 3, respectively), and therefore, *C. japonica* was using a POM food source that differed in composition along the estuary.

An ocypodid crab, *Scopimera globosa* showed the highest  $\delta^{13}\text{C}$  values among the macrozoobenthos in this study. The crab scrapes organic material from the surface of sediment particles using specialized hairs on the inner and outer sides of its merus mouth parts (Ono, 1967). *Ilyopla pusilla* feeds in a similar way, although it is less selective than *S. globosa* (Wada, 1982). The high  $\delta^{13}\text{C}$  values were not significantly different between Sts. 1 and 2, probably reflecting selective feeding on

benthic diatoms. This contrasts with the  $\delta^{13}\text{C}$  values of the two unselective deposit feeders, *Deiratonotus cristatus* and *Assiminea japonica*, which showed significant site differences (Fig. 5).

Two unselective deposit feeders, the gastropod *Assiminea japonica* (Abbott, 1958) and the ocypodid crab *Deiratonotus cristatus* (Ono, 1967), also responded to spatial changes in the availability of food items. The  $\delta^{13}\text{C}$  values of *A. japonica* and *D. cristatus* at St. 1 were close to the values for benthic diatoms and *Ilyopla pusilla* (selective grazer) in situ, indicating that they fed mainly on benthic diatoms at the site furthest downstream. On the other hand, the  $\delta^{13}\text{C}$  values of *A. japonica* and *D. cristatus* at St. 2 were similar to the values for SOM in situ, suggesting a higher contribution of SOM to their diet. Neither of these benthic species feed directly on fresh terrestrial plants. A possible explanation of the food source shifts of *A. japonica* and *D. cristatus* is the availability of benthic microalgae.

In temperate estuarine tidal flats, large areas of the intertidal zone are often covered by dense stands of marsh plants (e.g., *Phragmites australis*). Under these densely vegetated conditions, benthic microalgae living on the sediment surface become less abundant because shading by the marsh plants reduces light intensity and thereby decreases algal productivity (Wainright et al., 2000). In the Kitakami River estuary, St. 1 had a relatively large, open area that was not shaded by marsh plants, while Sts. 2 and 3 were vegetated and had only

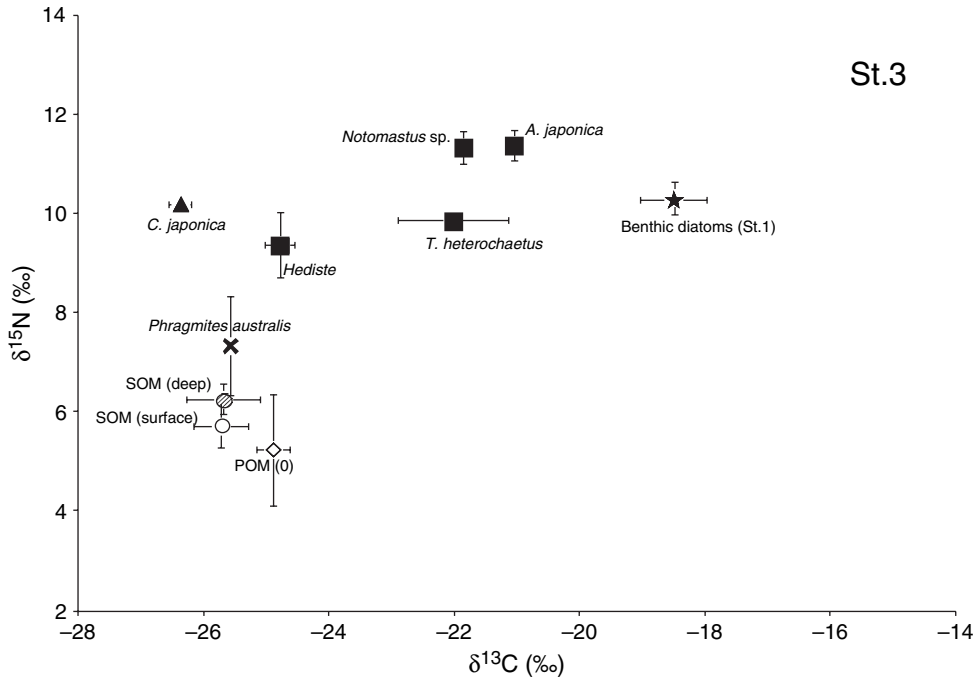


Fig. 4. Carbon and nitrogen stable isotope plots of macrozoobenthos and their food sources at St. 3. Each symbol shows a mean  $\pm$  1 SD ( $n = 3$ ). POM (0) indicates particulate organic matter (salinity: 0), respectively. SOM (surface and deep) indicates sediment organic matter at 0–0.5 and 7–10 cm depth, respectively. Black squares, white squares and white triangle showed unselective deposit feeders, selective deposit feeders and filter feeder, respectively.

narrow, bare areas at their edges. The light intensity on the bottom sediment of the salt marsh (mean during July–October, 2002 ca. 10,000 lx) was much lower than in the adjacent, open flats. In addition, the chlorophyll *a* concentration (mean ca. 1.0  $\mu\text{g cm}^{-2}$ ) was also much lower in the salt marsh than on the open mud flat (mean ca. 2.4  $\mu\text{g cm}^{-2}$ ) (Kudo et al., unpublished data). Thus, the availability of benthic diatoms as food sources was higher at St. 1 than at Sts. 2 and 3. Since *Assiminea japonica* and *Deiratonotus cristatus* feed unselectively on the sediment surface (Table 1), they may ingest more benthic diatoms at St. 1 and more SOM at Sts. 2 and 3.

At St. 1, the  $\delta^{13}\text{C}$  values of the grapsid crab *Chiromantes dehaani*, a deposit feeder, were approximately 2‰ higher than for the in situ SOM. The enrichment factor of *C. dehaani* was slightly higher than the general carbon-isotopic enrichment factor ( $0.8 \pm 1.1\text{‰}$ , DeNiro and Epstein, 1978), indicating *C. dehaani* might assimilate the parts of SOM, which have higher isotope ratios. The  $\delta^{13}\text{C}$  values of another grapsid, *Helice tridens tridens*, were close to those of *C. dehaani* and the polychaetes *Notomastus sp.* and *Tylorrhynchus heterochaetus*. The  $\delta^{15}\text{N}$  values of *H. tridens tridens* were 2.4–4.2‰ higher than for other macrozoobenthos (Fig. 2). This suggests that *H. tridens tridens* is carnivorous and feeds on other macrozoobenthos, such as crabs and polychaetes, as well as on the SOM (Kurihara et al., 1988; Takeda et al., 1988).

The polychaete *Notomastus sp.* is a head-down deposit feeder and feeds on deeper layers of SOM

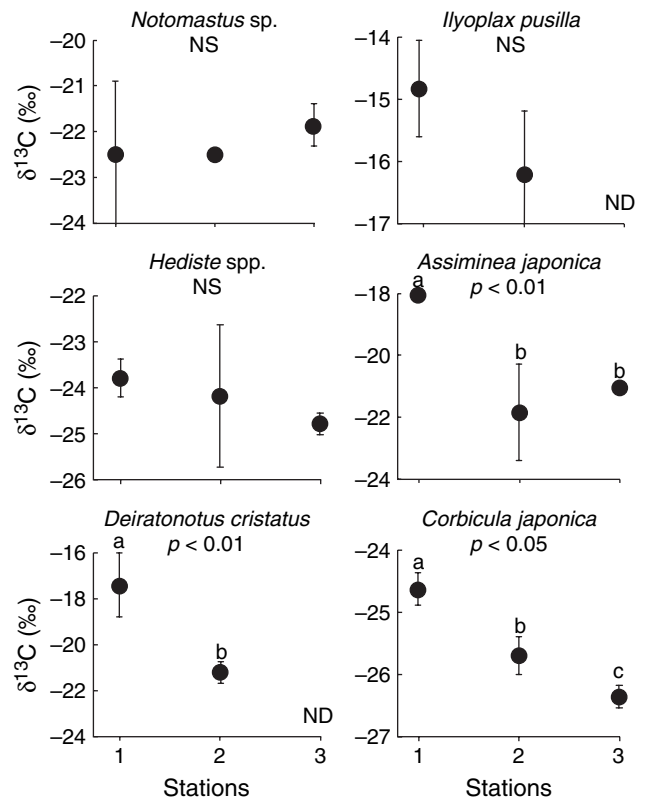


Fig. 5. Spatial differences in the carbon isotopes of macrozoobenthos. Each symbol shows a mean  $\pm$  1 SD ( $n = 3$ ). The *p*-values are indicated for Holm and *t*-tests. The letters a, b, and c indicate significant differences among carbon isotope values.

(Kikuchi and Wada, 1996). The  $\delta^{13}\text{C}$  values of *Notomastus* sp. were close to those of the SOM, confirming that this polychaete feeds on SOM. Since the  $\delta^{13}\text{C}$  values of *Tylorrhynchus heterochaetus* were close to those of *Notomastus* sp., *T. heterochaetus* probably also feeds mainly on SOM, although details of its feeding habits are not known. The  $\delta^{13}\text{C}$  values of *Hediste* spp., another polychaete, were significantly lower than those of *Notomastus* sp. and *T. heterochaetus*. This indicates that *Hediste* spp. feeds on organic material in both POM and SOM, presumably reflecting the fact that this polychaete feeds on freshly deposited organic matter (Kikuchi and Wada, 1996).

In the Kitakami River estuary, the food sources of a suspension feeder and two unselective surface deposit feeders (“grazers”) changed from the head to the mouth of the estuary, while the food sources of a selective grazer and two unselective deposit feeders did not change. Our results clearly show that the feeding habits of macrozoobenthos affect the spatial use of food sources. Therefore, spatial changes in the composition of organic matter from the head to the mouth of an estuary and the feeding habits (i.e. feeding mode and food selectivity) of macrozoobenthos are essential components of the estuarine food web. Our stable isotopes results showed that unselective feeders of a filter feeder and deposit feeders tracked the in situ composition of food sources, and selective deposit feeders (i.e. grazers) tracked their particular food sources. Species-specific and site-specific feeding outcomes along the estuarine gradient should be considered in the study of the roles played by macrozoobenthos in the regulation of estuarine material flows.

### Acknowledgements

We are grateful to Dr. K. Itoh, Faculty of Agriculture, Tohoku University for providing analytical facility for stable isotope analysis. We also thank Mr. G. Kanaya, Faculty of Sciences, Tohoku University, for his comments on the first version of this manuscript. This work is supported in part by the River Environmental Fund (REF) in charge of the Foundation of River and Watershed Environment Management (FOREM), Japan, and by a Grant-in-Aid for Scientific Research (B) from Japan Society for the Promotion of Science (No. 14380262).

### References

Abbott, R.T., 1958. The gastropod genus *Assimineia* in the Philippines. Proceedings of Academy of Natural Sciences of Philadelphia 110, 213–273.

- Cifuentes, L.A., Coffin, R.B., Solorzano, L., Cardenas, W., Espinoza, J., Twilley, R.R., 1996. Isotopic and elemental variations of carbon and nitrogen in a mangrove estuary. Estuarine, Coastal and Shelf Science 43, 781–800.
- Coach, C.A., 1989. Carbon and nitrogen stable isotopes of meiobenthos and their food resources. Estuarine, Coastal and Shelf Science 28, 433–441.
- DeNiro, M.J., Epstein, S., 1978. Influence of diet on the distribution of carbon isotopes in animals. Geochimica et Cosmochimica Acta 48, 1135–1140.
- Doi, H., Kikuchi, E., Hino, S., Itoh, T., Takagi, S., Shikano, S., 2003a. Isotopic ( $\delta^{13}\text{C}$ ) evidence for autochthonous origin of sediment organic matter in small, acidic Lake Katanuma, Japan. Marine and Freshwater Research 54, 253–257.
- Doi, H., Kikuchi, E., Hino, S., Itoh, T., Takagi, S., Shikano, S., 2003b. Seasonal dynamics of carbon stable isotope ratios of particulate organic matter and benthic diatoms in strongly acidic Lake Katanuma. Aquatic Microbial Ecology 33, 87–94.
- Kikuchi, E., Wada, E., 1996. Carbon and nitrogen stable isotope ratios of deposit-feeding polychaetes in the Nanakita River Estuary, Japan. Hydrobiologia 321, 69–75.
- Kurata, K., Minami, H., Kikuchi, E., 2001. Stable isotope analysis of food sources for salt marsh snails. Marine Ecology Progress Series 223, 167–177.
- Kurihara, Y., Sekimoto, K., Miyata, M., 1988. Wandering behaviour of the mud-crab *Helice tridens tridens* related to evasion of cannibalism. Marine Ecology Progress Series 49, 41–50.
- Little, C. (Ed.), 2000. The Biology of Soft Shores and Estuaries. Oxford University Press Inc., New York, pp. 176, 252.
- Mann, K.H. (Ed.), 2000. Ecology of Coastal Waters: With Implications for Management, second ed. Blackwell Scientific Inc., Malden, Massachusetts, pp. 406.
- Matsumasa, M., 1994. Effect of secondary substrate on associated small crustaceans in a brackish lagoon. Journal of Experimental Marine Biology and Ecology 176, 245–256.
- Matsumasa, M., Kikuchi, S., 1993. Blood osmoregulatory type and gill ultrastructure of an estuarine crab *Hemigrapsus penicillatus* (de Haan) (Crustacea: Brachyura). Annual Report of Iwate Medical University, School of Liberal Arts and Sciences 28, 37–45.
- Minagawa, M., Wada, E., 1984. Stepwise enrichment of  $^{15}\text{N}$  along food chains: further evidence and the relation between  $\delta^{15}\text{N}$  and animal age. Geochimica et Cosmochimica Acta 48, 1135–1140.
- Ono, Y., 1967. On the ecological distribution of ocyroid crabs in the estuary. Memoirs of the Faculty of Science, Kyushu University, Series E, Biology 4, 1–60.
- Takeda, S., Matsumasa, M., Kurihara, Y., 1988. Seasonal changes in the stomach contents of the burrowing mud-crab, *Helice tridens* (De Haan). The Bulletin of the Marine Biological Station of Asamushi, Tohoku University 18, 77–86.
- Wada, K., 1982. Substratum preference and feeding activity in *Scopimera globosa* De Haan and *Ilyoplax pusillus* (De Haan) (Crustacea: Ocypodidae). Benthos Research 23, 14–26 (in Japanese with English abstract).
- Wainright, S.C., Weinstein, M.P., Able, K.W., Currin, C.A., 2000. Relative importance of benthic microalgae, phytoplankton and the detritus of smooth cordgrass *Spartina alterniflora* and the common reed *Phragmites australis* to brackish-marsh food webs. Marine Ecology Progress Series 200, 77–91.
- Wetzel, R.G. (Ed.), 2001. Limnology Lake and River Ecosystems, third ed. Academic Press, San Diego, pp. 731–751.
- Yamamuro, M., Koike, I., 1994. Diel changes of nitrogen species in surface and overlying water of an estuarine lake in summer: evidence for benthic-pelagic coupling. Limnology and Oceanography 39, 1726–1733.