

Consumption of terrestrial organic matter by estuarine molluscs determined by analysis of their stable isotopes and cellulase activity

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ABSTRACT

We examined the feeding niche of four species of molluscs by analysis of their stable isotope signatures and cellulase activities to determine if they could utilize terrestrial organic matter. The molluscs and potential food sources were collected from the upper, middle and lower estuary of the Yura River from spring 2007 to winter 2008. All species showed positive cellulase activity which highlighted their potential to digest terrestrial organic matter. Consumption and assimilation of terrestrial organic matter by estuarine molluscs however varied spatially and temporally, reflecting species-specific differences in feeding niche and in response to variations in food availability in the estuary. Thus, terrestrial primary production in the catchment area supports secondary production of molluscs in the Yura River estuary.

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1. Introduction

Estuarine molluscs constitute a diverse and abundant taxonomical group occupying a wide habitat range along river–estuary–coastal systems. They occupy variable feeding niches and provide an important link between organic matter and higher consumers in aquatic food webs. Most gastropods are considered to be deposit feeders of sedimentary materials (Kesler, 1983) while bivalves are filter feeders of suspended materials (Yamamuro and Koike, 1993). Organic matter in estuaries comes from various origins but the relative importance of these sources to estuarine molluscs is poorly resolved. Many studies have shown that primary production of marine phytoplankton and benthic microalgae are the main energy sources of estuarine (Herman et al., 2000; Moens et al., 2002; Kasai et al., 2004) and coastal (Kang et al., 2006; Yokoyama et al., 2005; Yokoyama and Ishihi, 2007) benthic consumers, while other studies emphasized the importance of littoral plants such as reeds and macrophytes (Tenore, 1983; Currin et al., 1995; Kang et al., 2007). However, terrestrial organic matter may be an important food source for estuarine consumers as it

accumulates rapidly on the bed and along flood plains of the river–estuary systems, supplying large amounts of allochthonous input. Few studies have shown the importance of terrestrial plants to aquatic consumers (Riera and Richard, 1996; Kasai and Nakata, 2005; Kanaya et al., 2008). This may be due to the fact that terrestrial detritus is typically composed of structural refractory materials such as cellulose and lignin that most macrobenthic detritus feeders are unable to digest and assimilate (Cividanes et al., 2002). Detritus from vascular plants is characterized by low caloric content, typically resistant to decay and becomes available to macroconsumers only after microbial breakdown (Tenore, 1983). However, animals possessing enzymes that have the ability to break glycosidic bonds of cellulose into oligosaccharides could take advantage of this energy source directly (Kesler, 1983). Gut extracts of freshwater molluscs and crustaceans were found to strongly hydrolyze cellulose (Monk, 2006). Endogenous cellulase genes have been cloned from clam *Corbicula japonica* (Sakamoto et al., 2007), abalone (Suzuki et al., 2003), snail (Wang et al., 2003), mussel (Xu et al., 2001) and crayfish (Byrne et al., 1999) proving direct digestion of cellulose by those aquatic invertebrates. Digestive enzymes could be used as a complementary tool for determining which dietary components are effectively metabolized (Johnston and Freeman, 2005) but it remains insufficient to determine to what extent these components contributed to the organism's diet.

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Stable isotope analysis is a powerful tool to investigate what food sources were assimilated by consumers. Animals typically possess more of the heavier isotopes than their food (Fry and Sherr, 1984) and stepwise enrichment occurs with each trophic level in the food chain (Peterson and Fry, 1987). Stable isotope ratios of carbon ($\delta^{13}\text{C}$) are used to analyze food sources because there is little fractionation from prey to predator (DeNiro and Epstein, 1978), while stable nitrogen ratios ($\delta^{15}\text{N}$) become enriched at successive trophic levels (Minagawa and Wada, 1984), thereby allowing estimates of consumer trophic position within food webs (Vander Zanden and Rasmussen, 1999).

Spatial and temporal variability in the diet of molluscs were previously documented (Yokoyama and Ishihi, 2003; Doi et al., 2005; Kang et al., 2006) and are considered mainly driven by food availability and hydrodynamics (Kasai et al., 2006; Yokoyama and Ishihi, 2007; Kanaya et al., 2008). In this study, we determined if molluscs could digest and assimilate terrestrial organic matter, based on their cellulase activity and stable isotopic ratios. We then assessed spatial and temporal variation in their feeding niche.

2. Materials and methods

2.1. Study area

The Yura River, with a total length of about 146 km, is one of the major rivers of Japan (Fig. 1). It has a total catchment area of 1880 km² and a mean annual discharge of 41 m³ s⁻¹ flowing into the western part of Wakasa Bay (Ministry of Land, Infrastructure, Transportation and Tourism, 2007). Natural forests remain in the headwaters, while secondary forests, natural levee and riparian vegetation are found along the terrace of the river's main channel. The bank of the estuary is characterized by narrow littoral zone and steep slope.

2.2. Field sampling

Molluscs and their possible food sources were collected in three stations; upper estuary (13–15 km from the river mouth RM), middle estuary (8–10 km from the RM) and lower estuary (0–2 km from the RM) (Fig. 1). Sampling was conducted on April 10, August 10, and October 18, 2007 and on January 30, 2008 to represent spring, summer, autumn and winter samples respectively. The gastropods were collected by hand, washed, identified and frozen ($-30\text{ }^{\circ}\text{C}$) until processed for stable isotope analysis. Together with these molluscs, we also collected water, sediment and stones with epilithic microalgae to quantify potential primary food sources. Leaf litter of broad leaf trees and silver grass were collected from the middle estuary.

Particulate organic matter (POM) as an indicator of phytoplankton density was collected from the surface and bottom layers of the water column at each station using a Van Dorn water sampler. Surface fresh water (salinity < 3) was considered as allochthonous riverine POM (RPOM). Marine water (salinity > 33) collected from the subtidal area which was not influenced by river discharge but could intrude into the bottom of the estuary and become a source of phytoplankton was considered as marine POM (MPOM) (Fig. 1). Epilithic organic matter (EOM) was randomly collected from submerged stones of the river bank of each station, brushed and washed with filtered river water. The EOM samples were initially filtered onto 100 μm mesh sieve to separate debris and small animals from epilithic microalgae. The top 1 cm surface of sedimentary organic matter (SOM) was collected by hand from each station (1 m depth).

2.3. Stable isotope analyses

The mantle of the molluscs was oven dried and homogenized into fine powder by mortar and pestle. Samples were not acid

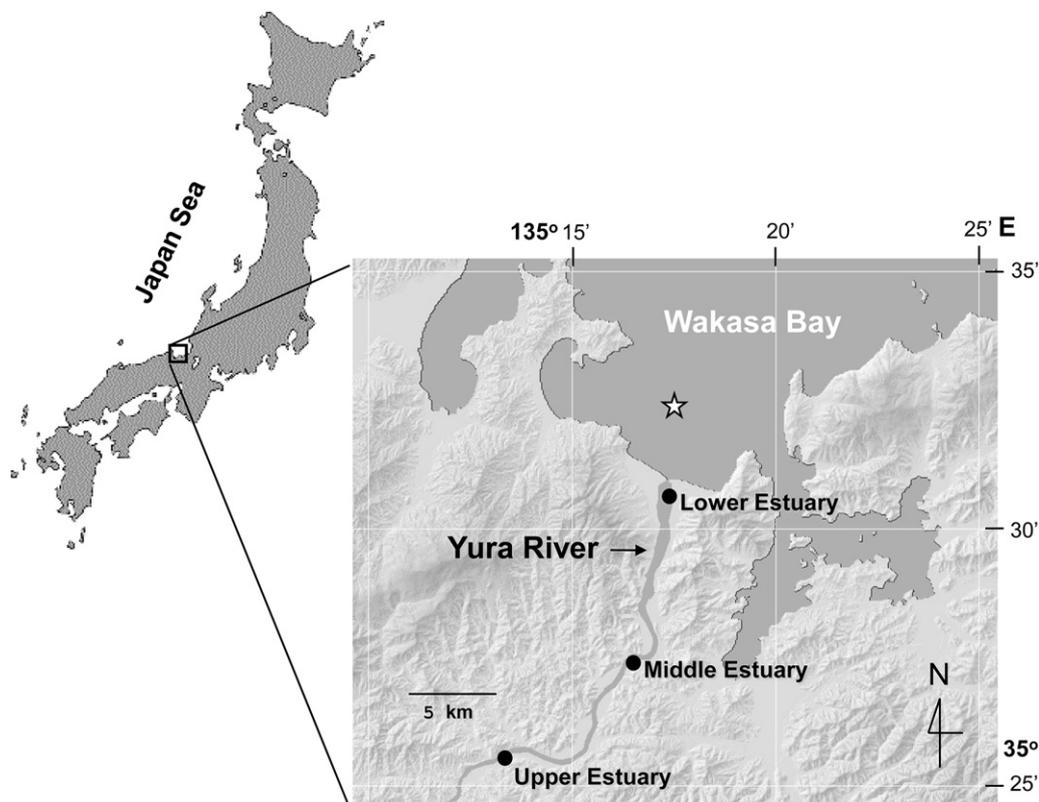


Fig. 1. Location of sampling stations: upper, middle and lower estuary of the Yura River, Kyoto, Japan. ☆Location of marine particulate organic matter (MPOM) sampling site.

treated (Mateo et al., 2008) and lipids were not extracted because mantle has low lipid content (Bodin et al., 2007). Water and EOM samples were filtered onto precombusted (450 °C, 2 h) and pre-weighed glass-fiber filters (Whatman GF/F, 0.7 µm), treated with 1.2 N HCl vapor overnight to remove carbonates, neutralized with NaOH vapor for 24 h and oven dried at 60 °C. Leaf litters were washed with distilled water, dried and powdered. The sediment samples were filtered through 500 µm sieve, oven dried, homogenized, acid treated, oven dried again and finally re-homogenized and used as SOM for stable isotope analysis. About 0.7 mg of homogenized animal samples, 3 mg of leaf litter, POM and EOM filter samples were put into tin capsules, ready for combustion. The ^{13}C and ^{15}N composition of the samples were determined using a mass spectrometer (MAT 252, Finnigan MAT) with an elemental analyzer (EA 1110, ThermoQuest Italia). Stable isotope ratios were expressed in δ unit notation using the following equation:

$$\delta X = \left[\left(\frac{R_{\text{sample}}}{R_{\text{standard}}} \right) - 1 \right] \times 1000$$

where X represents ^{13}C or ^{15}N and R is the $^{13}\text{C}/^{12}\text{C}$ or $^{15}\text{N}/^{14}\text{N}$ ratio, respectively. Pee Dee Belemnite and atmospheric N_2 were used as carbon and nitrogen standards, respectively.

2.4. Cellulase CMC plate assay

Molluscs were separated from their shell. Samples of whole body and selected body parts were homogenized separately in 100 mM sodium acetate buffer (pH 5.5). The homogenates were utilized as enzyme samples and placed on a carboxymethyl cellulose (CMC) plate [0.5% (w/v) CMC, 1% (w/v) agarose, 100 mM sodium acetate buffer (pH 5.5)]. The plate was stained with 0.1% aqueous Congo Red solution after incubation at 37 °C for 15 h. Subsequently, the plate was washed with 1 M NaCl for 1 day. Cellulase activity was detected by the presence of a white halo zone and its strength was determined by the size of its diameter and area around the tissues. Replicates were made for each tissue analyzed. *Corbicula japonica* was previously known to have cellulase activity (Sakamoto et al., 2007), thus, was not measured in this study.

2.5. Data analysis

Two-way ANOVA was used to test for differences in carbon and nitrogen isotopic ratios of molluscs among stations and among seasons in the estuary. Linear correlation was used to determine relationship between fluctuations in the isotopic ratios of molluscs and possible food items. All tests were analyzed using the Kyplot statistical software.

3. Results

3.1. Isotopic trend of possible food sources

Five potential food sources were identified; terrestrial organic matter represented by plant detritus, riverine POM (RPOM) and sedimentary organic matter (SOM); phytoplankton estimated from marine POM (MPOM); and benthic microalgae represented by epilithic organic matter (EOM). Leaf litter of silver grass *Miscanthus sacchariflorus* and broad leaved trees (not identified) showed depleted $\delta^{13}\text{C}$ values (−27.5 and −29.9‰, respectively). Annual $\delta^{13}\text{C}$ mean of RPOM was almost similar in all stations (Table 1 and Fig. 2). However, seasonal $\delta^{13}\text{C}$ variations of RPOM were relatively large, with the most depleted value in summer. MPOM had relatively stable $\delta^{13}\text{C}$, fluctuating within only −22.4‰ in winter to −21.6‰ in spring with an annual mean of −21.9‰. The $\delta^{13}\text{C}$ of EOM varied over a large interval, with the highest variability observed in the

Table 1
 $\delta^{13}\text{C}$ (‰) and $\delta^{15}\text{N}$ (‰) of possible food items of molluscs.

Station and season	$\delta^{13}\text{C}$ (‰)			$\delta^{15}\text{N}$ (‰)		
	RPOM	EOM	SOM	RPOM	EOM	SOM
<i>Upper estuary</i>						
Spring	−24.0	−16.3	−24.9	6.3	6.3	1.6
Summer	−28.7	−17.1	−26.3	7.6	10.7	4.2
Autumn	−24.7	−18.4	−24.8	6.7	7.1	3.1
Winter	−24.8	−19.7	−27.4	4.2	6.4	3.8
Annual mean	−25.6	−17.9	−25.9	6.2	7.6	3.2
<i>Middle estuary</i>						
Spring	−24.0	−17.7	−26.4	6.7	6.6	3.0
Summer	−29.6	−17.2	−27.9	6.2	8.0	3.3
Autumn	−25.0	−16.4	−27.5	6.0	9.0	1.9
Winter	−24.7	−21.6	−24.2	4.1	6.2	5.9
Annual mean	−25.8	−18.2	−26.5	5.8	7.4	3.5
<i>Lower estuary</i>						
Spring	−25.0	−20.1	−24.9	5.7	7.7	3.9
Summer	−29.6	−16.4	−23.1	6.2	10.8	5.0
Autumn	−25.0	−18.8	−24.0	5.3	10.6	3.5
Winter	−24.7	−13.3	−25.1	4.2	7.2	1.8
Annual mean	−26.1	−17.1	−24.3	5.4	9.1	3.6

RPOM, riverine particulate organic matter; EOM, epilithic organic matter; SOM, sedimentary organic matter. Annual mean per station is provided.

lower estuary and the least in the upper estuary. Sedimentary $\delta^{13}\text{C}$ showed a similar variability range with RPOM, with the middle estuary having the widest range and the lower estuary the least.

Leaf litter from silver grass and terrestrial trees had $\delta^{15}\text{N}$ of 5.4 and 6.3‰, respectively. The $\delta^{15}\text{N}$ of RPOM had the highest annual mean and widest range in the upper estuary, while the lower estuary was the opposite (Table 1 and Fig. 2). The $\delta^{15}\text{N}$ of MPOM fluctuated within 5.7–7.4‰ (annual mean of 6.6‰). Seasonal fluctuations in $\delta^{15}\text{N}$ of RPOM and MPOM were smaller compared to EOM and SOM which fluctuated greatly. The $\delta^{15}\text{N}$ of EOM showed the highest fluctuation in the upper estuary and the lowest in the middle estuary. In the case of $\delta^{15}\text{N}$ of SOM, the middle estuary showed the highest fluctuation, while the upper estuary showed the lowest.

3.2. Isotopic signatures of molluscs

Three species of estuarine gastropod were identified in the Yura River; *Cipangopaludina japonica*, *Clithon retropictus*, and *Semisulcospira libertina*, as well as one species of bivalve, *Corbicula japonica* (Table 2). *Cipangopaludina japonica* was collected only in the middle estuary (Table 2 and Fig. 2). There were significant differences in its $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ among seasons, with a relatively enriched $\delta^{13}\text{C}$ in summer and autumn compared to more depleted values in spring and winter, while its $\delta^{15}\text{N}$ fluctuated within a minimal range throughout the year (Table 3). The fluctuations in $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ of *Cipangopaludina japonica* showed a significant linear correlation with that of SOM (Table 4).

Clithon retropictus was collected from all stations and showed the most enriched $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ among the molluscs analyzed (Table 2 and Fig. 2). Its $\delta^{13}\text{C}$ showed highly significant differences both among stations and seasons (Table 3). Among the stations, its annual mean $\delta^{13}\text{C}$ was most enriched in the lower estuary compared to the upper and middle estuary. Its $\delta^{13}\text{C}$ showed the widest variation in the middle estuary, with highest values in spring and lowest in winter. The $\delta^{15}\text{N}$ of *C. retropictus* was relatively stable. Fluctuations in $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ of *C. retropictus* were correlated with those of EOM (Table 4).

Semisulcospira libertina was present in the upper and middle estuary in all seasons. This species showed a more depleted annual mean values of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ in the middle estuary than in the

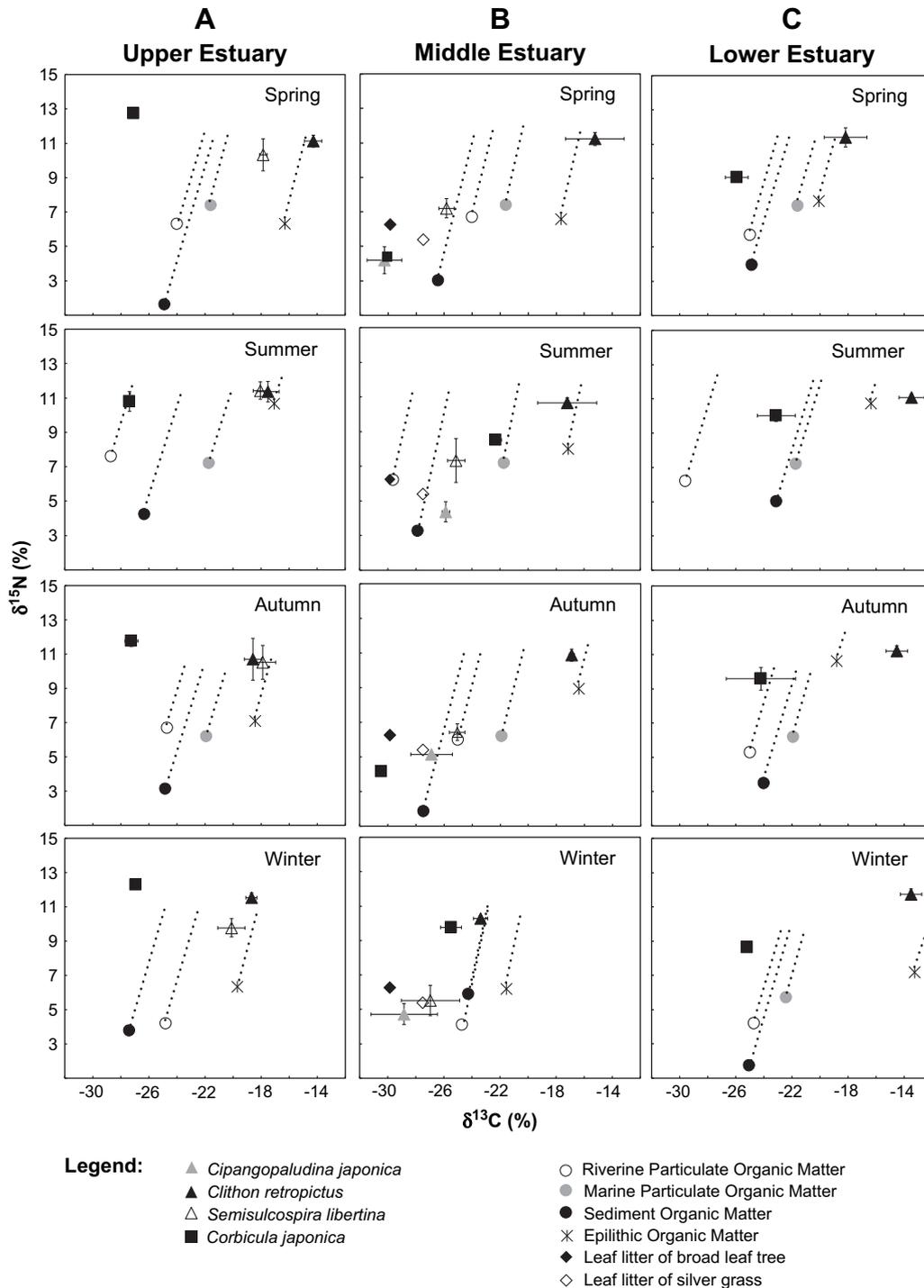


Fig. 2. $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ of molluscs and their potential diet. Error bars indicate standard deviations. Dotted lines are the expected trophic enrichment of each potential diet, increase of 1.0 for $\delta^{13}\text{C}$ (deNiro and Epstein, 1978) and 3.4 for $\delta^{15}\text{N}$ (Minagawa and Wada, 1984).

upper estuary (Table 2 and Fig. 2). There were significant differences in its $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ among stations but not among seasons (Table 3). Its stable isotope signatures were not correlated with any of the possible food sources (Table 4).

Corbicula japonica was collected from all stations in the estuary. Its isotopic signature in the upper estuary was stable compared to the middle and lower estuary. In the middle and lower estuary this bivalve showed depleted $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ in spring and autumn but enriched values in summer and autumn (Table 2 and Fig. 2). No significant difference was observed in its $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ among

seasons, although its $\delta^{15}\text{N}$ had significant difference among stations (Table 3). Its stable isotope signatures were not correlated with any of the potential food sources (Table 4).

3.3. Cellulase activity

The three gastropod species (*Cipangopaludina japonica*, *Clithon retropictus* and *Semisulcospira libertina*) showed positive cellulase activities detected by the white halo zones in the CMC plate assay (Table 5). The whole body and midgut glands of *Cipangopaludina*

Table 2
 $\delta^{13}\text{C}$ (‰) and $\delta^{15}\text{N}$ (‰) of four species of molluscs.

Station and season	<i>Cipangopaludina japonica</i>		<i>Clithon retropictus</i>		<i>Semisulcospira libertina</i>		<i>Corbicula japonica</i>	
	$\delta^{13}\text{C}$ (‰)	$\delta^{15}\text{N}$ (‰)	$\delta^{13}\text{C}$ (‰)	$\delta^{15}\text{N}$ (‰)	$\delta^{13}\text{C}$ (‰)	$\delta^{15}\text{N}$ (‰)	$\delta^{13}\text{C}$ (‰)	$\delta^{15}\text{N}$ (‰)
<i>Upper estuary</i>								
Spring	nd	nd	-14.3 ± 0.6	11.1 ± 0.4 (3)	-17.9 ± 0.3	10.3 ± 0.9 (4)	-27.1 ± 0.2	12.7 ± 0.0 (5)
Summer	nd	nd	-17.5 ± 0.8	11.4 ± 0.6 (3)	-18.1	11.4 (2)	-27.4 ± 0.3	10.8 ± 0.6 (3)
Autumn	nd	nd	-18.6 ± 0.6	10.7 ± 1.2 (3)	-17.9 ± 0.9	10.5 ± 1.0 (3)	-27.3 ± 0.5	11.8 ± 0.3 (3)
Winter	nd	nd	-18.7 ± 0.4	11.5 ± 0.3 (3)	-20.1 ± 1.0	9.8 ± 0.5 (3)	-27.0 ± 0.4	12.3 ± 0.2 (3)
Annual mean			-17.3 ± 0.6	11.2 ± 0.6	-18.5 ± 0.7	10.5 ± 0.8	-27.2 ± 0.3	11.9 ± 0.3
<i>Middle estuary</i>								
Spring	-30.3 ± 1.2	4.2 ± 0.8 (8)	-15.3 ± 2.1	11.2 ± 0.4 (5)	-25.9 ± 0.5	7.2 ± 0.6 (3)	-30.1	4.4 (2)
Summer	-25.9 ± 0.3	4.4 ± 0.6 (3)	-17.2 ± 2.1	10.7 ± 0.3 (3)	-25.1	7.4 (2)	-22.3 ± 0.4	8.5 ± 0.1 (3)
Autumn	-26.9 ± 1.5	5.2 ± 0.1 (3)	-16.9 ± 0.2	10.9 ± 0.4 (3)	-25.1 ± 0.6	6.4 ± 0.5 (3)	-30.5	4.2 (2)
Winter	-28.9 ± 2.4	4.7 ± 0.6 (3)	-23.4 ± 0.5	10.3 ± 0.2 (3)	-27.0 ± 2.1	5.5 ± 0.9 (3)	-25.5 ± 0.7	9.78 ± 0.3 (3)
Annual mean	-28.0 ± 1.3	4.6 ± 0.5	-18.2 ± 1.2	10.8 ± 0.3	-25.8 ± 1.1	6.6 ± 0.6	-27.1 ± 0.6	6.7 ± 0.2
<i>Lower estuary</i>								
Spring	nd	nd	-18.2 ± 1.5	11.4 ± 0.6 (11)	nd	nd	-26.0 ± 0.8	9.1 ± 0.1 (3)
Summer	nd	nd	-13.5 ± 0.9	11.1 ± 0.2 (3)	nd	nd	-23.1	10.0 (2)
Autumn	nd	nd	-14.5 ± 0.8	11.2 ± 0.3 (5)	nd	nd	-24.2	9.6 (2)
Winter	nd	nd	-13.5 ± 0.8	11.7 ± 0.3 (3)	nd	nd	-25.2 ± 0.3	8.6 ± 0.1 (3)
Annual mean			-14.9 ± 1.0	11.4 ± 0.3			-24.6 ± 0.5	9.3 ± 0.1

Isotopic values indicate mean \pm SD. Number of samples in given parentheses. nd, no data.

japonica and *C. retropictus* showed moderate cellulase activities, while the midgut gland and crystalline style of *S. libertina* had strong cellulase activities. On the contrary, gonad of the gastropods showed weak cellulase activity.

4. Discussion

4.1. Variations in feeding niche of molluscs

Cipangopaludina japonica showed consistently depleted $\delta^{13}\text{C}$ in all seasons (annual mean 28.0‰) similar to $\delta^{13}\text{C}$ of leaf litter of broad leaved trees (-29.9‰), silver grass *Miscanthus sacchariflorus* (-27.5‰), and SOM (annual mean -26.5‰) and thus could be sources of organic carbon. On the other hand, assuming that the diet–tissue fractionation of $\delta^{15}\text{N}$ is 3.4‰ (Minagawa and Wada, 1984; Post, 2002), its estimated food source will have $\delta^{15}\text{N}$ values of 0.8‰ in spring, 1.0‰ in summer, 1.8‰ in autumn and 1.3‰ in winter. These values are reduced by $>2.2\text{‰}$ relative to those of SOM except in autumn and by $>3.6\text{‰}$ relative to leaf litters of silver grass and terrestrial tree. Fry (1991) described that typical terrestrial C_3 plants in temperate region have $\delta^{15}\text{N}$ values ranging from -5 to 2‰ . The $\delta^{15}\text{N}$ values of the estimated diet of *Cipangopaludina japonica* were within this range, suggesting that the gastropod obtained its nutrition mainly from terrestrial C_3 plants. The enriched $\delta^{15}\text{N}$ of leaf litters and SOM could be due to seasonal variability in isotopic signatures of terrestrial plants and microbial mineralization and nitrification/denitrification that produced ^{15}N -enriched inorganic nitrogen in aquatic sediments (Cloern et al., 2002). The temporal variation in $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ of this gastropod and its significant

correlation with SOM may imply that it could have selectively utilized different terrestrial organic matter from the sediment depending on the season. Surface SOM comprises recently-settled organic matter (Kikuchi and Wada, 1996). SOM could be from various origins and terrestrial plants could be one of the main sources. Therefore, *Cipangopaludina japonica* could select terrestrial plant organic material from SOM, and take the typical feeding niche of a terrestrial plant deposit feeder.

The enriched $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ of *Clithon retropictus* reflected its epilithic microalgal diet from upper to lower estuary and from spring to winter. One exception, however, was in the middle estuary during winter when it showed depleted $\delta^{13}\text{C}$ similar to SOM. During this time, almost no greenish mat was observed on rocks in the middle estuary which means EOM was scarce during winter in this particular area. Thus, this species could have assimilated organic matter from the sediment only when benthic microalgae were scarce in the environment. Spatial and temporal variations in the isotopic signature of this gastropod could be explained by the differences in the dominant benthic microalgal species composition along the Yura River (Negoro and Gotoh, 1983) and seasonal shifts in $\delta^{13}\text{C}$ of EOM (Currin et al., 1995; Kanget al., 2006). We therefore conclude that *C. retropictus* occupied a selective feeding niche – an epilithic microalgal grazer.

Our results showed that *Semisulcospira libertina* manifested a wider range of $\delta^{13}\text{C}$ (-27.0 to -17.9‰) and $\delta^{15}\text{N}$ (5.5 – 11.4‰) compared to the results of Doi et al. (2006) which showed a narrower range and more depleted $\delta^{13}\text{C}$ (-28.0 to -26.2‰) and $\delta^{15}\text{N}$ (2.0 – 3.0‰). The difference may be due to a greater number of stations and more frequency of sampling in our study compared to

Table 3

The results of ANOVA, indicating *P* values: <0.001 (highly significant difference); <0.01 (moderately significant difference); <0.05 (significant difference); and >0.05 (no significant difference).

Factors	<i>Cipangopaludina japonica</i>		<i>Clithon retropictus</i>		<i>Semisulcospira libertina</i>		<i>Corbicula japonica</i>	
	$\delta^{13}\text{C}$	$\delta^{15}\text{N}$	$\delta^{13}\text{C}$	$\delta^{15}\text{N}$	$\delta^{13}\text{C}$	$\delta^{15}\text{N}$	$\delta^{13}\text{C}$	$\delta^{15}\text{N}$
Among seasons (A)	<0.05	<0.05	<0.001	>0.05	>0.05	>0.05	>0.05	>0.05
Among stations (B)			<0.001	<0.01	<0.05	<0.001	>0.05	<0.05
A \times B			<0.001	>0.05	>0.05	>0.05	>0.05	>0.05

Table 4

Linear correlations of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ signatures of four species of molluscs against four possible food sources.

Species	RPOM	MPOM	EOM	SOM
<i>Cipangopaludina japonica</i>	NS	NS	NS	0.69**
<i>Clithon retropictus</i>	NS	NS	0.78**	NS
<i>Semisulcospira libertina</i>	NS	NS	NS	NS
<i>Corbicula japonica</i>	NS	NS	NS	NS

Numerical values are adjusted *R* values of correlation statistic. NS, no significant linear correlation; RPOM, riverine particulate organic matter; MPOM, marine particulate organic matter; EOM, epilithic organic matter; SOM, sedimentary organic matter.

** Significant linear correlation.

the latter study. Depleted values of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ were observed in *S. libertina* collected from the middle estuary in almost all seasons, suggesting its assimilation of SOM and RPOM. However, in summer its $\delta^{13}\text{C}$ was between SOM and MPOM, suggesting a mixed diet and consumption of MPOM whenever it was abundant. In the upper estuary, isotopic values were enriched similar to EOM throughout the year. Thus, this species could shift feeding niche depending on available organic matter in its vicinity. We therefore consider *S. libertina* as an opportunist deposit feeder.

The bivalve *Corbicula japonica* generally showed a depleted $\delta^{13}\text{C}$ signature, suggesting dependence on TOM, from upper to lower estuary throughout the year. However, close examination of the data revealed an exception in summer when $\delta^{13}\text{C}$ of this bivalve became enriched at a similar level as MPOM in the middle estuary, and at a level intermediate to MPOM and RPOM in the lower estuary. This suggests an opportunistic consumption of phytoplankton of marine origin during high abundance. The absence of significant correlation in the fluctuation of isotope signatures of *Corbicula japonica* with any of the potential food sources suggests that this bivalve is an opportunistic filter feeder. Our results are consistent with those of Kasai et al. (2006) who showed that this species utilized TOM in low primary production zones, while it relied on phytoplankton when primary production was high. Riera and Richard (1996) also observed similar spatial variability in the carbon sources used by the oyster *Crassostrea gigas* along the estuary gradient in the Bay of Marennes-Oleron, France. The relative contribution of terrestrial organic matter to the diet of *Corbicula japonica* was estimated to be over 90% (Kasai and Nakata, 2005) in the Kushida Estuary, suggesting significant importance of TOM to the diet of *Corbicula japonica*.

4.2. Cellulase activity, life history and distribution

Cipangopaludina japonica had a very narrow distribution, only found in silty freshwater channels. This species was previously documented to feed primarily on bacterial biomass derived from algal detritus, sludge and compost of soft paddy soil (Kurihara and Kadowaki, 1988). It was argued that this gastropod mainly uses proteins and starch but not high molecular weight carbohydrates. However, our results show that this species digests cellulose and

Table 5

Cellulase activity of three gastropod species based on the presence (+) or absence (–) of the halo zone around the organ/body of the gastropod in the carboxymethyl cellulose (CMC) plate.

Organ/body	<i>Cipangopaludina japonica</i>	<i>Clithon retropictus</i>	<i>Semisulcospira libertina</i>
Whole body	+	+	+
Midgut gland	+	+	+
Gonad	+	+	–
Crystalline style	na	na	+

na, not applicable.

assimilated terrestrial plant detritus and SOM. Thus, further study on the metabolic characteristic of this gastropod is needed to elucidate the contribution of terrestrial matter and bacterial biomass to its diet.

Clithon retropictus is a diadromous gastropod that reproduces in freshwater, spends its veliger period in the sea, migrates upstream from the estuaries and spends most of its adult life in freshwater (Shigemiya and Kato, 2001). Along its estuary–freshwater migrations, epilithic microalgae may be a more stable source of food in terms of availability compared to phytoplankton or terrestrial matter. This gastropod has a well developed teeth-like radula that enables it to graze efficiently on epilithic microalgae (Kawamura et al., 2001). The positive cellulase activity of this species would also allow utilization of TOM as a substitute for epilithic microalgae when the latter became scarce in the environment. This kind of feeding niche would allow this species to exploit a wide habitat range, from brackish to freshwater environments.

Semisulcospira libertina was observed both on the rocks (like *Clithon retropictus*) and on the bottom sediment (like *Cipangopaludina japonica*). This gastropod showed positive cellulase activity and was found to have an opportunistic type of feeding niche, able to graze on EOM in the upper estuary, and selectively assimilate TOM in the middle estuary. During summer *S. libertina* assimilated marine phytoplankton as part of its diet when primary production was high in the estuary. Stomach content analysis of this gastropod revealed its polyphagous diet (Ohara and Tomiyama, 2000), thus allowing it to share the same feeding niche with *C. retropictus* in the upper estuary, and with *Cipangopaludina japonica* and *Corbicula japonica* in the middle estuary.

Corbicula japonica was previously known to favor high salinity waters (>10) of the estuary after juvenile stages (Sekiguchi et al., 2005). Unlike the gastropods, this bivalve cannot actively search for better feeding grounds, but can only wait and filter available organic matter from the water column by holding its inhalant siphon above the sediment surface (Yamamuro and Koike, 1993). This species possesses an endogenous cellulase gene (Sakamoto et al., 2007) allowing it to assimilate TOM (Kasai and Nakata, 2005; Kasai et al., 2006).

4.3. Diet shift among opportunists

The high primary production in the water column during summer resulted in an enriched isotopic signature for *Corbicula japonica* and *Semisulcospira libertina* in the middle estuary. These two species mainly consumed TOM, except in summer when they assimilated MPOM as part of their diet. However, they were not affected by marine water intrusion in the upper estuary where *Corbicula japonica* assimilated TOM while *S. libertina* grazed on EOM throughout the year. This spatial difference in diet could be attributed to a more river-dominated upper estuary relative to the marine-dominated middle and lower estuaries. Variations in the availability of food is concluded to generate marked effects on the net organic carbon fluxes (Jassby et al., 1993; Hoffman and Bronk, 2006) and consequently on biotic metabolism (Ibarrola et al., 1998; Fry and Allen, 2003), thereby influencing the reshaping of individual feeding history and niche (Doi et al., 2005; Shimoda et al., 2007).

5. Conclusions

All four species of estuarine molluscs were characterized by cellulase activity, showing their potential to digest terrestrial matter. However, assimilation varied highly in time and space, reflecting species-specific feeding niches and different responses to variations in availability of potential food sources. Terrestrial

organic matter was an important energy source for molluscs in the Yura estuary, serving either as a main source of nutrition or as periodic subsidy. By their consumption of terrestrial organic matter, estuarine molluscs could play an important role in transferring energy from terrestrial to aquatic ecosystems.

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