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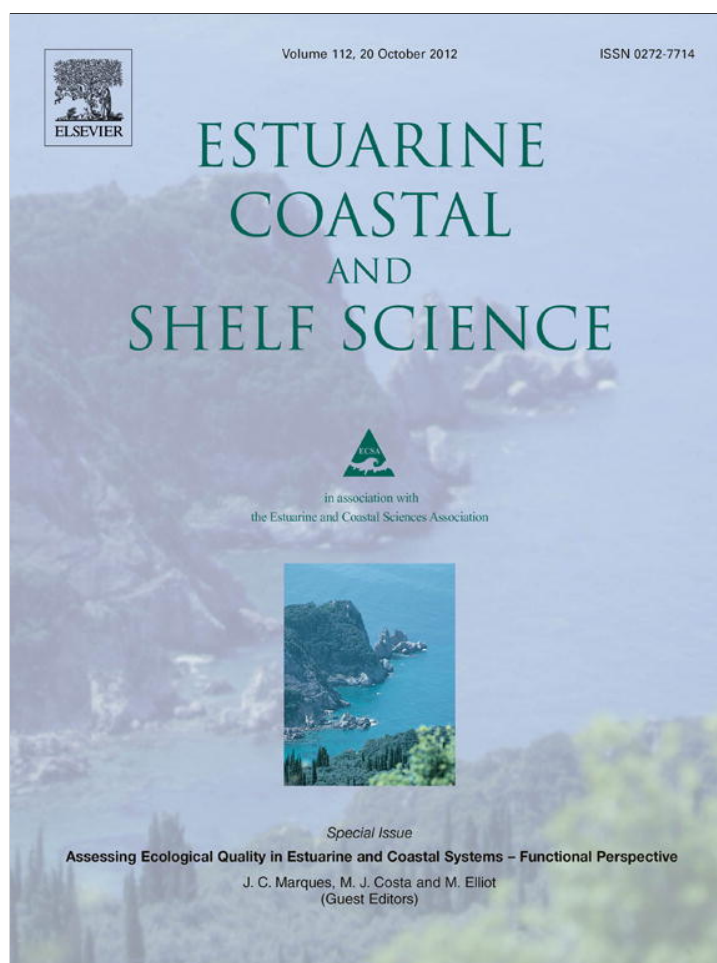216 PUBLICATIONS   4,254 CITATIONS

Spatial-temporal feeding dynamics of benthic communities in an estuary-marine gradient

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ABSTRACT

We investigated the fluctuations of carbon and nitrogen stable isotope ratios in benthic consumers and their potential food sources to determine the spatial and temporal variations in the utilization of available organic matter, indicating the origin and pathways of energy from Yura Estuary to Tango Sea, Japan. Field samplings were conducted from the upper estuary to offshore with sampling frequency of twice per season from April (spring) 2006 to February (winter) 2007. The $\delta^{13}C$ signatures of the upper and lower estuary benthos showed depleted and in wide range ($-28.9‰$ to $-13.5‰$) compared to the enriched and within narrow range signatures of marine benthos ($-20.6‰$ to $-14.0‰$) in all seasons. On the contrary, the $\delta^{15}N$ signatures of benthic communities showed decreasing trend seaward and summer values were different from the other seasons. Using the dual isotope and multisource mixing models, we estimated the relative contributions of potential food sources to the benthos diet. River POM played an important source of energy for the estuarine benthos, especially in winter when river discharge was high. Marine POM served as an important alternative food for the estuarine benthos from spring to autumn when seawater intruded the bottom estuary. Benthic microalgae were the major food source at the shallow coast throughout the year, while marine POM fueled the deep coast and offshore benthic food webs. Spatial and temporal feeding variations in estuarine benthic communities were driven by the hydrology of the estuary, whereas primary production and transport of food source dictated diet variations of marine benthic communities. The elucidation of the dynamic energy subsidy among aquatic systems highlights the importance of the land–sea transition zones that is crucial for benthic secondary productions.

1. Introduction

Horizontal gradients of organic matter flow from land to sea is coupled by temporal variations that vary from daily fluctuations of river flow to seasonal or annual climatic patterns. The combined effect of the landward movement of saltwater and the seaward flow of freshwater results to the conservation of nutrients within the estuary and coastal ecosystems (Connoly et al., 2009; Goñi et al., 2009; Kasai et al., 2010), that subsequently results to high productivity and biodiversity (Hughes et al., 2000; Hoffman and Bronk, 2006). The complex interactions among these habitats results to interdependence that is important for the healthy function of each ecosystem (Fisher et al., 2001; Zeug and Winemiller,

2008). Understanding how organisms utilize resources is important to trace energy flow and resource distribution across ecosystems from terrestrial to estuary (Kobayashi et al., 2011), and then to coastal and offshore areas (Antonio et al., 2010a).

Benthic organisms are classically used to characterize trophic relationships (Post, 2002; Nadon and Himmelman, 2010) and act as indicators of energy transfer and health of a system (Mincks et al., 2008). However, most of these studies were focused on certain population, conducted within a single habitat or within limited period, which did not always incorporate the complexity inherent to systems expose to variable energy sources and broad array of abiotic factors. The estuary and coastal food webs are diverse and complicated by physical mixing of materials from various sources (Hughes et al., 2000), therefore, it is inappropriate to analyze diet that represent static interactions or neglect fluctuations in space and time (Cividanes et al., 2002).

There is limited knowledge on the role of benthic communities in linking continuous aquatic systems across different environments.

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Primary producers such as terrestrial vegetation (e.g. Kasai and Nakata, 2005), marine phytoplankton (e.g. Yokoyama et al., 2005), benthic microalgae (e.g. Kang et al., 2003) and littoral plants (e.g. Currin et al., 1995) are known to fuel different benthic communities. However, only few studies describe the spatial (e.g. Deegan and Garritt, 1997; Yokoyama and Ishihi, 2007) and temporal (Fisher et al., 2001; Molina et al., 2011) variability in the utilization of various food sources by benthos. Determining the variability of energy pathways among benthic communities in the land–sea transition zone will contribute to our understanding of the factors that affect the function of these ecosystems and predictions can be made on how these ecosystems respond to environmental change.

Studies on energy flow and food web structures in aquatic ecosystems are increasingly recognizing the potential of stable isotope ratios as natural tracers. The $\delta^{13}C$ ratios in tissues of consumers are typically stable and do not change much (near 1.0‰) with increasing trophic level, thus become good indicators of origins of organic materials that are actually assimilated over a long period of time (DeNiro and Epstein, 1978). However, determination of food sources using solely carbon isotope composition is often ambiguous (Currin et al., 1995), while a dual isotope approach, like carbon and nitrogen, may be easier to interpret (Post, 2002). On the contrary, abundance of $\delta^{15}N$ in consumers is enriched by about 3.0‰ relative to their prey (DeNiro and Epstein, 1981), therefore may predict the trophic positions of organisms (Post, 2002). Minagawa and Wada (1984) found an average ^{15}N enrichment of $3.4 \pm 1.1‰$ per trophic level, independent of habitat. As of the moment, trophic enrichment of 1.0‰ for $\delta^{13}C$ and 3.4‰ for $\delta^{15}N$ are the generally acceptable enrichment values for food web studies, although few studies showed variations from these (e.g. Vanderklift and Ponsard, 2003).

In this study we evaluate the spatial and temporal variations in the carbon and nitrogen isotopic signatures of benthic communities and their potential food sources from the upper estuary of Yura River to coastal and offshore areas of Tango Sea, Japan in four seasons. Feeding variations were correlated to physical and chemical factors influencing the communities. We hypothesized that energy pathway in the estuary is more variable than in the marine environment. Spatial variation in summer has been published in the previous paper (Antonio et al., 2010a) and is included in the present study to analyze the seasonal changes.

2. Materials and methods

2.1. Study area

Yura River has a total length of about 146 km (National Astronomical Observatory of Japan, 2005) that flows into Tango Sea, the western part of Wakasa Bay and opens to Japan Sea (35°N, 135°E) (Fig. 1). The downstream riverbed is nearly flat and the average depth is ∼3 m with a shallow sill at the river mouth. Typical normal tide range in the estuary is less than 0.5 m, and there is negligible effect of tidal currents on the physical and biological conditions of the estuary (Kasai et al., 2010). The seawater easily intrudes into the river, since the riverbed (until 20 km upstream from the river mouth) is lower than the sea surface. Salty bottom water is typically observed at downstream of Yura River from the middle of spring to autumn (Fig. 2).

2.2. Field sampling

There were 3 river stations (R1–R3) and 6 seaward stations (M1–M6) grouped as upper estuary (R1 and R2), lower estuary which was near the river mouth (R3), shallow coast 5–10 m in depth (M1 and M2), deep coast 30–60 m in depth (M3 and M4), and offshore 100–150 m in depth (M5 and M6) (Fig. 1). Samplings

were conducted twice a season: April 18–19 and May 24–25, 2006 for spring; June 20–22 and August 28–30, 2006 for summer; October 18–19 and November 20–21, 2006 for autumn; January 17–18 and February 8–9, 2007 for winter.

Particulate organic matter (POM) was collected by Van Dorn water sampler from the surface, middle and bottom layers of the water column at each station. The top 1 cm sediment surface for epipelic benthic microalgae (BMA) analysis was collected using the tube corer, while epilithic microalgae were brushed from the submerged stones of the estuary stations and included in the BMA samples. The water and sediment were collected at the same time we collected the animals. Benthic animals were collected by a 5 min tow of beam trawl net (2 m width, 20 cm height, 2 mm mesh size) at the seaward stations, while a smaller beam trawl (1.5 m width, 30 cm height, 2 mm mesh size), hand net, basket and pipe traps were used at the estuary stations where water is shallower and most benthic consumers were found near the river bank. Different sampling gears were used in the marine and estuary stations to make sure that important benthic consumers were sampled and represented from different environment. However, our data could not be used for the analysis of benthic community structure such as comparison of biomass and diversity.

2.3. Laboratory analyses of samples

Water samples for POM analysis were filtered onto glass-fiber filters (Whatman GF/F), treated with 1.2 N HCl vapor overnight to remove carbonates, neutralized with NaOH vapor and oven dried at 60 °C. The collected surface sediment was incubated with pre-treated (acid-washed and burned) sand for 15 h under artificial light to allow movement of epipelic benthic microalgae towards the pre-treated sand. BMA was separated by washing with distilled water and filtration though 63 μm fiber sieve, re-filtered onto GF/F and succeeding treatments similar to POM were employed (as per Antonio et al., 2010a).

Only abundant benthos were selected from the collected samples as representative species of each benthic communities. Benthic organisms were sorted, washed with distilled water, identified to the lowest possible taxa and stored at −30 °C. The dorsal trunk muscle behind the pectoral fin of fishes, the mantle of molluscs, the cheliped muscles of the crabs, the trunk muscles of shrimps, the tube feet of sea stars and the body discs of brittle stars were used for isotope analysis. Pooled whole body of amphipods, isopods, mysids and polychaetes in 3–20 individuals per sample of the same species (depending in size) were processed. The animal samples were oven dried and homogenized into fine powder. Acid treatment was known to influence nitrogen isotope ratios of organic materials, thus, was not used to remove other inorganic carbonates (Carabel et al., 2006; Mateo et al., 2008). Lipids were not extracted because we used mostly animal muscles that were of low lipid content (Bodin et al., 2007).

2.4. Stable isotope analyses

The $\delta^{13}C$ and $\delta^{15}N$ 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 with Pee Dee Belemnite for carbon and atmospheric N_2 for nitrogen as standards expressed in the following equation:

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

where X represents ^{13}C or ^{15}N and R is $^{13}C/^{12}C$ or $^{15}N/^{14}N$ ratio, respectively. The mean isotopic value from three or more samples
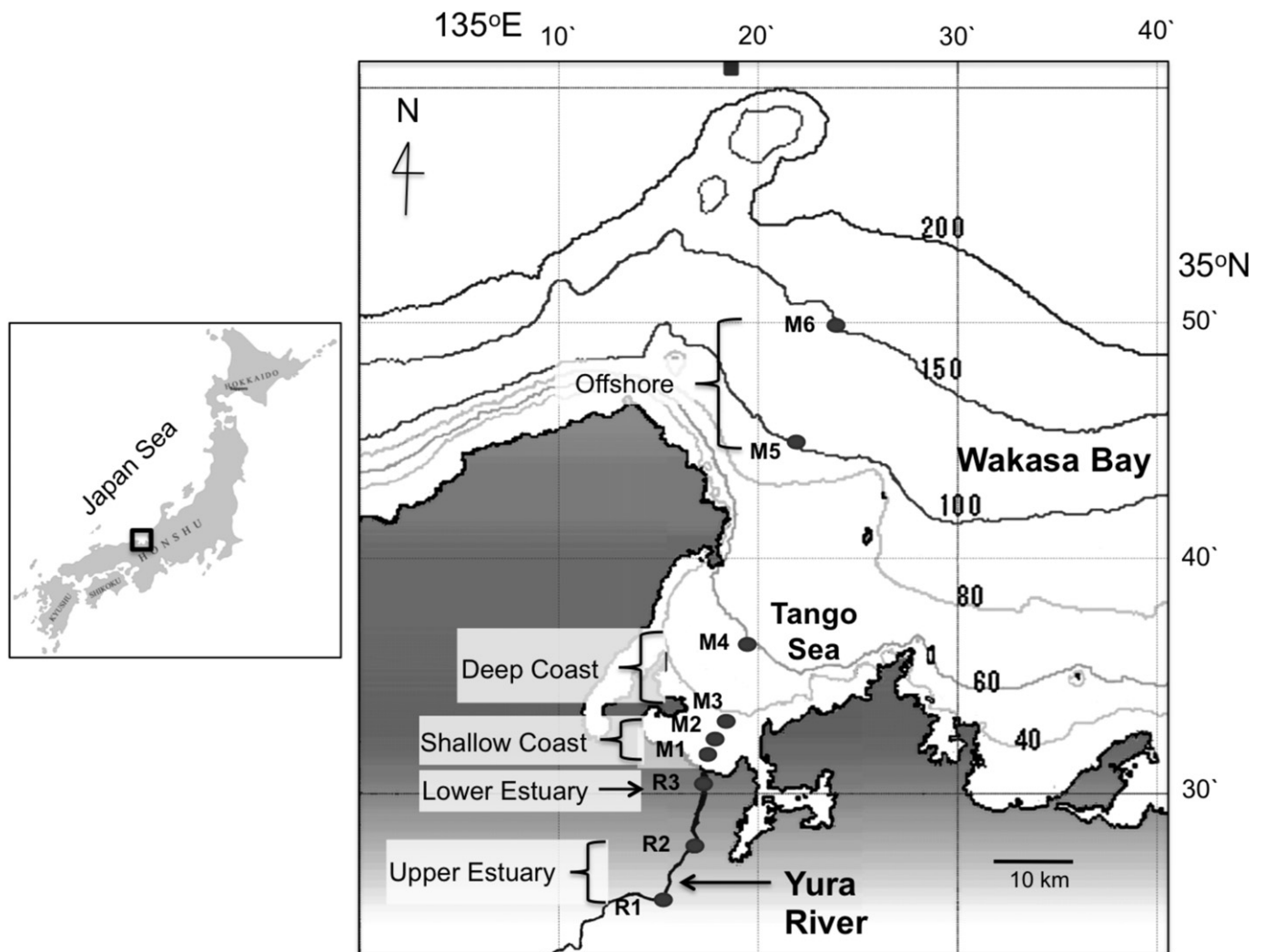
Fig. 1. The study area and location of the sampling stations: R1, R2 – upper estuary; R3 – lower estuary; M1, M2 – shallow coast; M3, M4 – deep coast; M5, M6 – offshore.

of the same species represents the species-specific isotopic signature in the carbon-nitrogen isotope graph (Fig. 3).

2.5. Mixing models

The percent contribution (PC) of the food sources to the estimated diet of consumers was calculated using the conservative dual isotope model of Yokoyama and Ishihi (2007) when there were only two feasible food sources:

$$PC = 100(S \times C_C - S \times C_{E2} - N_C + N_{E2})/(S \times C_{E1} - S \times C_{E2} - N_{E1} + N_{E2})$$

where C_C and N_C are the $\delta^{13}C$ and $\delta^{15}N$ values of the consumer, C_{E1} and N_{E1} are the $\delta^{13}C$ and $\delta^{15}N$ of one of the end members, and C_{E2} and N_{E2} are the $\delta^{13}C$ and $\delta^{15}N$ of the other end member, and slope (S) of the trophic-enrichment as $S = 3.4$ (Minagawa and Wada, 1984). Conventional $\delta^{13}C$ (1.0‰) and $\delta^{15}N$ (3.4‰) fractionation values were utilized since species-specific fractionation values are unknown. The difference between the $\delta^{15}N$ of a consumer and its estimated food mixture was calculated by a formula (Yokoyama and Ishihi, 2007):

$$\Delta\delta^{15}N = \{S \times C_C (N_{E1} - N_{E2}) + S \times N_C (C_{E2} - C_{E1}) + S(N_{E2} \times C_{E1} - N_{E1} \times C_{E2})\}/\{S(C_{E2} - C_{E1}) - N_{E2} + N_{E1}\}$$

On the other hand, when many possible food sources may be utilized by benthos, the multisource mixing model (Phillips and Gregg, 2003) in software IsoSource version 1.3.1 was used to estimate PC. This study assumes that the $\delta^{13}C$ and $\delta^{15}N$ of a consumer is in isotopic equilibrium with its diet. Estimates of the percent contribution of potential food sources to the diet of a consumer are typically presented in a range, however, in this study we used the mean values of the model output for each species since we are interested on the percent contribution of a food source at the community level which could be an exemption in using the multiple mixing model (Phillips and Gregg, 2003). The relative contribution of each dietary source to each benthic communities was estimated by calculating the average PC from all the consumers in a community.

2.6. Analyses of data

Multiple Analysis of Variance was used to test for differences in the means of carbon and nitrogen isotopic ratios of consumers across communities and seasons. Tukey HSD test was employed to detect the magnitude of spatial and temporal differences as well as to test whether the difference in isotopic ratios of common species present in two or more stations was significant or not.
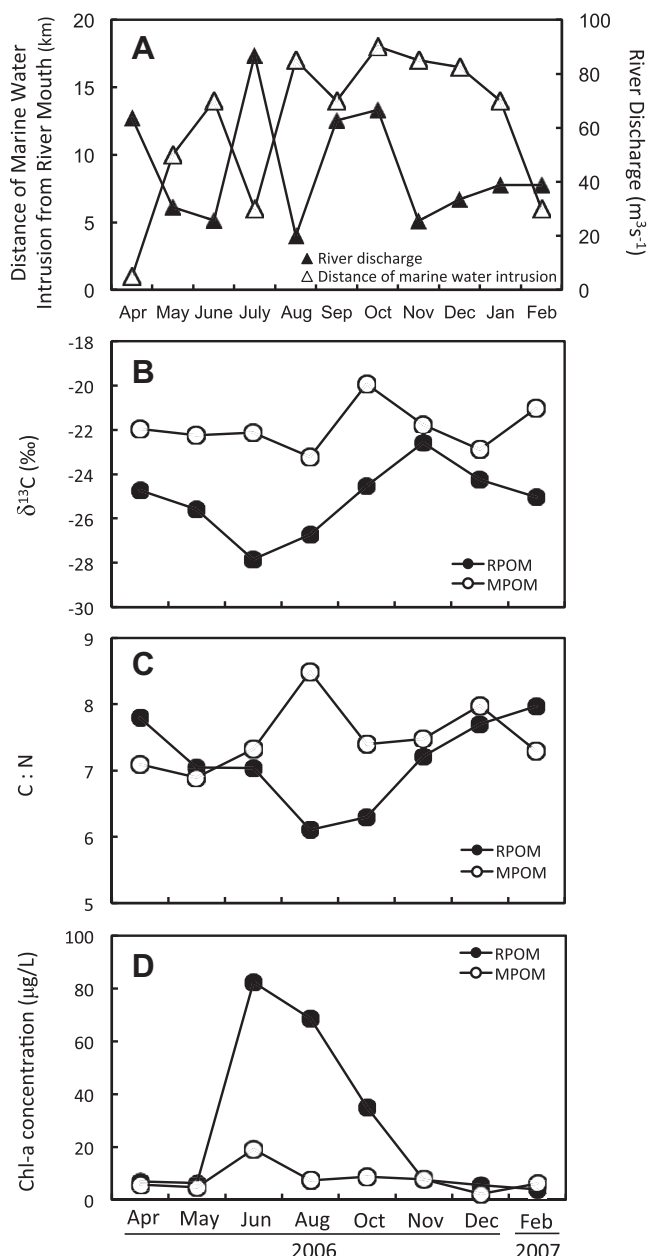
Fig. 2. Time series (April 2006 to February 2007) of (A) Yura River discharge (solid triangle) versus distance of marine water intrusion from the mouth (open triangle), (B) mean δ^{13}C, (C) mean C:N ratio, and (D) mean chlorophyll a concentrations of RPOM (solid circle) and MPOM (open circle).

3. Results

3.1. Potential primary food sources

Five potential food sources were identified and utilized as end members in this study: terrestrial leaf litter, river POM (RPOM), marine POM (MPOM), benthic microalgae (BMA), and macroalgae. Salt wedge develop from spring (April) in Yura Estuary, with maximum marine water intrusion of up to 18 km upstream in summer (August) and autumn (October), and disappears in winter (January) when the estuary becomes a river and consists mainly of freshwater (Fig. 2). In a stratified state, the surface POM showed depleted δ^{13}C while the saline bottom POM was relatively enriched from the estuary (R1, R2, R3) to shallow coast (M1, M2). POM with

salinity lower than 5.0 was regarded as RPOM, while POM with salinity higher than 25.0 was regarded as MPOM. RPOM showed depleted annual range of δ^{13}C (-31.1 to $-22.9‰$), and an annual mean of $-25.7‰ \pm 2.3$ (Fig. 3). MPOM in the estuary and shallow coast showed relatively depleted range of δ^{13}C (-24.8 to $-20.1‰$) compared to the deep coast (M3, M4) and offshore stations (M5, M6) (-23.3 to $-19.9‰$) and with an annual mean of $-21.8‰ \pm 1.2$. On the other hand, RPOM showed annual mean of $5.7‰ \pm 1.1$ for δ^{15}N that was almost similar to MPOM with an annual mean of $5.5‰ \pm 0.9$. The partially degraded leaf litter exhibited more depleted δ^{13}C and δ^{15}N signatures than RPOM with mean δ^{13}C of $-28.3‰ \pm 0.1$ and $3.8‰ \pm 0.4$ for δ^{15}N.

Generally, benthic microalgae (BMA) showed enriched and stable δ^{13}C signatures in all stations and seasons (-22.3 to $-13.2‰$) (Fig. 3). BMA showed a decreasing trend in the annual mean of δ^{15}N of BMA from the upper estuary ($7.6‰ \pm 0.8$) to offshore ($4.1‰ \pm 0.3$). On the other hand, the macroalgae collected from shallow coast and river mouth showed a wide range of δ^{13}C (-20.8 to $-13.7‰$) and δ^{15}N (3.2 to 8.2‰). The pooled isotopic values of abundant macroalgae species showed an annual mean of $-15.8‰ \pm 1.7$ for δ^{13}C and $5.6‰ \pm 1.4$ for δ^{15}N. The isotopic signatures of macroalgae were more enriched than RPOM and MPOM but relatively similar to BMA. Although the δ^{13}C signatures of macroalgae and BMA were comparably similar, the δ^{15}N of BMA is more enriched than macroalgae that significantly separates the two food sources.

3.2. Benthic species composition

A total of 135 species of benthic animals were selected from the trawled samples that consisted of 46 species of crustaceans, 41 species of fish, 32 species of molluscs, 8 species of echinoderms, 7 species of polychaetes and 1 species of ribbon worm (Table 1). Crustaceans were diverse at the lower estuary and deep coast while fish, mollusc and echinoderm species were most diverse at the shallow coast and offshore stations.

3.3. Spatial variation among benthic communities

The δ^{13}C values of benthic animals ranged from -28.9 to $-15.1‰$ in the upper estuary, -26.0 to $-13.5‰$ in the lower estuary, -20.0 to $-15.4‰$ in the shallow coast, -19.6 to $-15.1‰$ in the deep coast and -20.6 to $-14.0‰$ in offshore stations (Fig. 3). The means of δ^{13}C signatures of the upper and lower estuary benthic consumers were similar (Tukey HSD, $p > 0.05$) but were significantly different (MANOVA, $p < 0.01$) from the other marine communities. The estuarine stations showed depleted and wide range of δ^{13}C from about -29 to $-13‰$, a difference of 16‰. On the contrary, marine benthic consumers at the shallow coast, deep coast and offshore stations showed enriched and narrower range of δ^{13}C from around -21 to $-14‰$, a difference of only 7‰. Unlike δ^{13}C, there was no significant spatial difference in the means of δ^{15}N of benthic communities (MANOVA, $p > 0.05$).

3.4. Temporal variation among benthic communities

The means of δ^{13}C of benthic consumers in summer at the upper and lower estuary were significantly different from the other seasons (MANOVA, $p < 0.05$) (Fig. 3). Our results showed that δ^{13}C of benthic consumers in spring and summer was relatively similar (Tukey HSD, $p > 0.05$), so as autumn and winter (Tukey HSD, $p > 0.05$); whereas summer and winter were the most different seasons (Tukey HSD, $p < 0.05$), especially at the estuary. On the other hand, the means of δ^{15}N of benthos in spring at the lower estuary and shallow coast were significantly different from the other seasons of the year (MANOVA, $p < 0.05$).

3.5. Variation in $\delta^{13}C$ and $\delta^{15}N$ of common species

There was an observed general increasing trend of $\delta^{13}C$ and a decreasing trend of $\delta^{15}N$ for common species collected from the estuary to offshore stations (Fig. 4). Many species showed significant increase in $\delta^{13}C$ from upper estuary to lower estuary in all seasons (Tukey $p < 0.05$). This includes the bivalve *Corbicula japonica* (5) and *Mytilus trossulus* (14); the gastropod *Clithon retropictus* (24); the amphipod *Melita hoshinoi* (39); the mysid *Neomysis awatchensis* (53) and *Neomysis japonica* (54); the crab *Hemigrypsus takanoi* (78); as well as the goby fish *Favonigobius gymnauchen* (111) and *Tridentiger trigonocephalus* (133). Only the amphipod *Sternomoera* sp. (46) showed a significant increase in $\delta^{13}C$ from lower estuary to shallow coast. The sand shrimp *Crangon uritai* (65) and the seastar *Luidia quinaria* (92) showed significant increase in $\delta^{13}C$ from lower estuary to deep coast, while sole fish *Aseraggodes kobensis* (100) showed significant decrease from shallow coast to deep coast. There were no significant difference in $\delta^{13}C$ of common species in the deep coast and offshore stations (Tukey $p > 0.05$).

Some common species of the upper and lower estuary showed a significant decrease in $\delta^{15}N$ in all seasons (Tukey $p < 0.05$), particularly in winter (Fig. 4). The amphipod *Sternomoera* sp. (46) and mysid *Orientomysis japonica* (55) showed significant decrease in $\delta^{15}N$ from lower estuary to shallow coast. The shrimp *Crangon affinis* (61) and the fish *Amblychaeturichthys hexanema* (97) showed significant decrease in $\delta^{15}N$ from the lower estuary to deep coast. The fish *Apogon lineatus* (99) showed significant decrease in $\delta^{15}N$ from the deep coast to offshore, but the flounder *Paralichthys olivaceus* (117) showed a significant increase.

3.6. Percent composition

Spatial and temporal variations in the proportion of potential primary food sources were observed in the diet of benthic consumers. The $\delta^{13}C$ signatures of benthos in the upper estuary showed that they were depending on four sources of organic matter: leaf litter, RPOM, MPOM and BMA. River POM showed the highest contribution of organic matter utilized in the upper estuary, highest in winter ($\sim 65\%$) and lowest in spring ($\sim 32\%$) (Fig. 5). BMA is the second important energy source in the upper estuary throughout the year (10—30%), followed by MPOM with highest contribution in spring ($\sim 22\%$) and none (0%) in winter. In the lower estuary, the benthic consumers utilized organic matter from many sources: leaf litter, RPOM, MPOM, BMA and macroalgae. MPOM was important in the diet of lower estuary benthos in spring ($\sim 40\%$) and summer ($\sim 36\%$), RPOM was important in autumn ($\sim 29\%$) and winter ($\sim 37\%$), while BMA and macroalgae were alternative food throughout the year. Similarly, the shallow coast benthic consumers also utilized a mixture of organic matter from many sources, but BMA served as the main energy source (>30%) in all seasons. On the other hand, the deep coast and offshore stations were fueled by only two primary food sources: MPOM and BMA. The proportion of MPOM and BMA was almost the same in all seasons in the deep coast, except in summer when MPOM contributed $\sim 70\%$ in the benthos diet. The offshore benthos community relied most of their energy (70—90%) from the MPOM (phytodetritus), and less but significant amount of organic matter (10—30%) from BMA.

4. Discussion

4.1. Estuary and marine gradient

This study shows the spatial and temporal variations in the isotopic signatures of benthic communities across estuary-marine gradient, revealing the difference in energy sources utilized and energy subsidy among communities. In the upper estuary, estimation of the percent contribution to the benthos diet at the community level showed that RPOM and leaf litter provided the highest contribution especially in winter when there was high river discharge, while BMA and MPOM served as alternative food source. In the lower estuary, marine phytoplankton (MPOM) was an important energy source, especially in spring and summer during high phytoplankton production in the estuary. Macroalgae showed similar $\delta^{13}C$ signatures with small species of crustaceans suggesting contribution of macroalgal detritus to estuarine and shallow coast food web. Generally, feeding niche of lower estuary benthos can be described as seasonally shifting from more of MPOM-based in spring and summer to more of RPOM in autumn and winter, similar to other studies (e.g. Hughes et al., 2000). In all seasons the upper and lower estuary benthos exhibited wide range of $\delta^{13}C$ values that is the opposite to the narrow-ranged $\delta^{13}C$ signatures of marine benthos. The wide range of $\delta^{13}C$ of consumers at the upper and lower estuary demonstrates the variable food sources utilized in the system. On the other hand, narrow and less fluctuating $\delta^{13}C$ signatures of marine benthos emphasized the limited variety of energy source and a more stable system.

For instance, the sessile brackish bivalve *Corbicula japonica* (5) exhibited wide spatial and temporal variation in $\delta^{13}C$ and $\delta^{15}N$ signatures. This species showed depleted $\delta^{13}C$ signatures in the upper estuary ($-29\permil$ to $-26\permil$) compared to relatively enriched values in the lower estuary ($-26\permil$ to $-22\permil$). Terrestrial leaf litter (probably in small particle size suspended in water) and RPOM (a mixture of autochthonous and allochthonous organic matter) were the main energy source for this species in the upper estuary, however, at the lower estuary a mix diet of leaf litter, RPOM, MPOM and BMA were apparent. In summer, this bivalve may have selectively filtered MPOM at the lower estuary as reflected by its enriched $\delta^{13}C$ signature ($-23\permil$) similar to MPOM (mean value of $-22\permil$). *C. japonica* is a known opportunistic filter feeder, capable of switching diet from more of terrestrial-based organic matter (leaf litter and RPOM) during high river flow to MPOM-based during high phytoplankton production (Kasai and Nakata, 2005; Antonio et al., 2010b). Diet of a marine benthos however is less variable than an estuarine benthos. For instance the strict marine bivalve *Acila divaricata* (1) exhibited very little spatial and temporal variations of $\delta^{13}C$ at the deep coast ($-16\permil$) and offshore ($-17\permil$ to $-16\permil$), consistently reflecting dependence on enriched BMA-based energy, while higher marine trophic consumers such as the echinoderm *Luidia quinaria* (92) and the fishes *Apogon lineatus* (99) and *Paralichthys olivaceus* (117) consistently exhibited a mix diet of MPOM and BMA. On the other hand, when a benthic consumer is capable of moving between the marine and estuary habitats, there is an observed significant shift in the $\delta^{13}C$ signatures. The amphipod *Sternomoera* sp. (46) exhibited a depletion of $\delta^{13}C$ from $-18.7\permil$ at the shallow coast to $-23.9\permil$ at the lower estuary in winter, while the sand shrimp *Crangon uritai* (65) demonstrated a relatively enriched and stable $\delta^{13}C$ signatures in the shallow coast and deep coast stations ($-17\permil$ to $-16\permil$) but more depleted $\delta^{13}C$ were observed at the lower estuary ($-19\permil$ to $-17\permil$). A spatial decrease in $\delta^{13}C$ signature as the consumer moves from the sea into the estuary suggests the incorporation of a more depleted organic matter into its diet such as RPOM and terrestrial matter.

The feeding variations of the benthos consumers discussed above reflect the spatial and temporal energy dynamics at the community level. Fig. 4 shows a trend of enrichment in $\delta^{13}C$ and the opposite depletion in $\delta^{15}N$ signatures of common species from the upper estuary to the lower estuary. The spatial enrichment in $\delta^{13}C$ is explained by the general shift of benthos diet from more of RPOM-based at the upper estuary to more of a mix diet at the lower
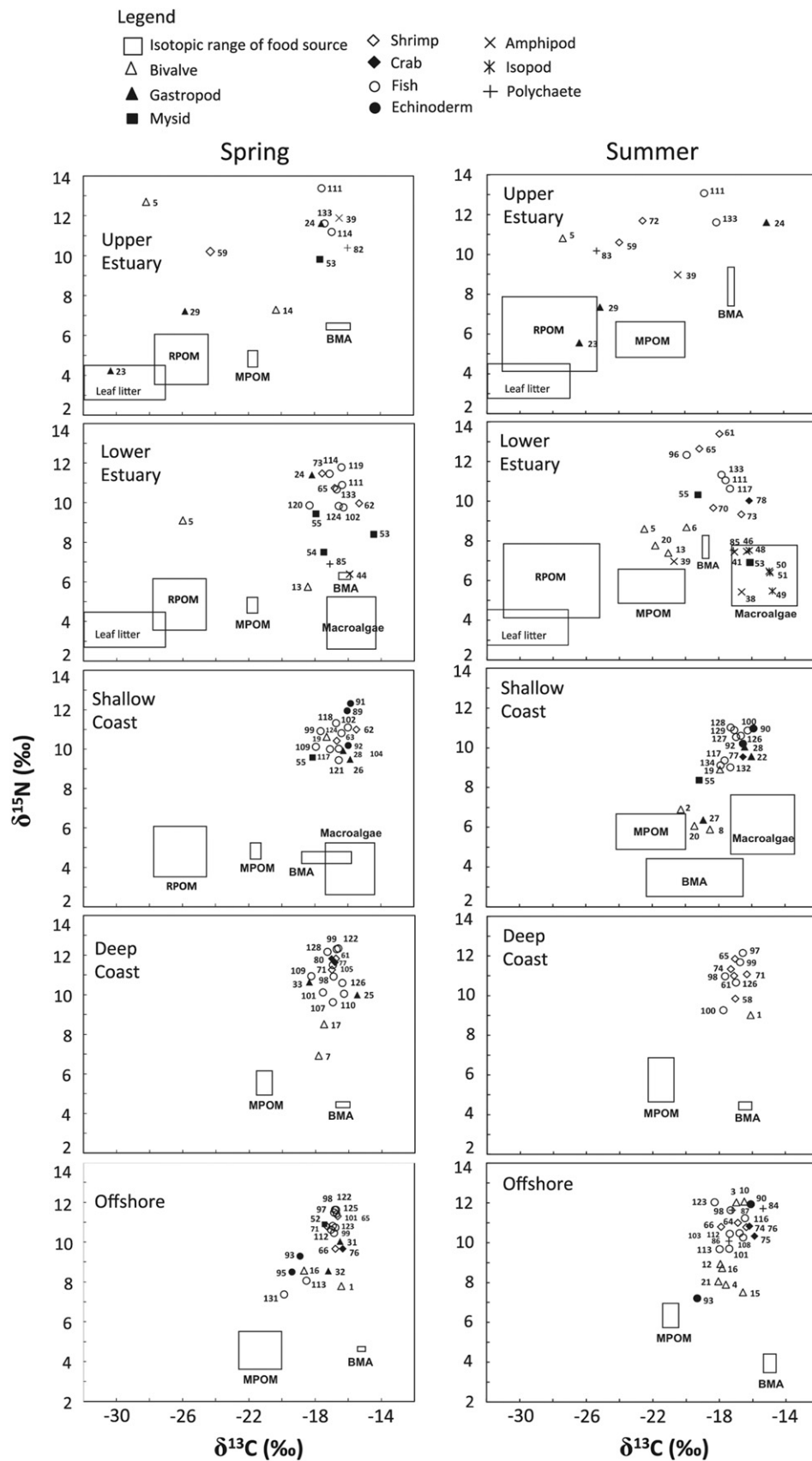
Fig. 3. Carbon and nitrogen isotope plot of benthic communities in four seasons. Refer to Table 1 for the species name of the corresponding species number.
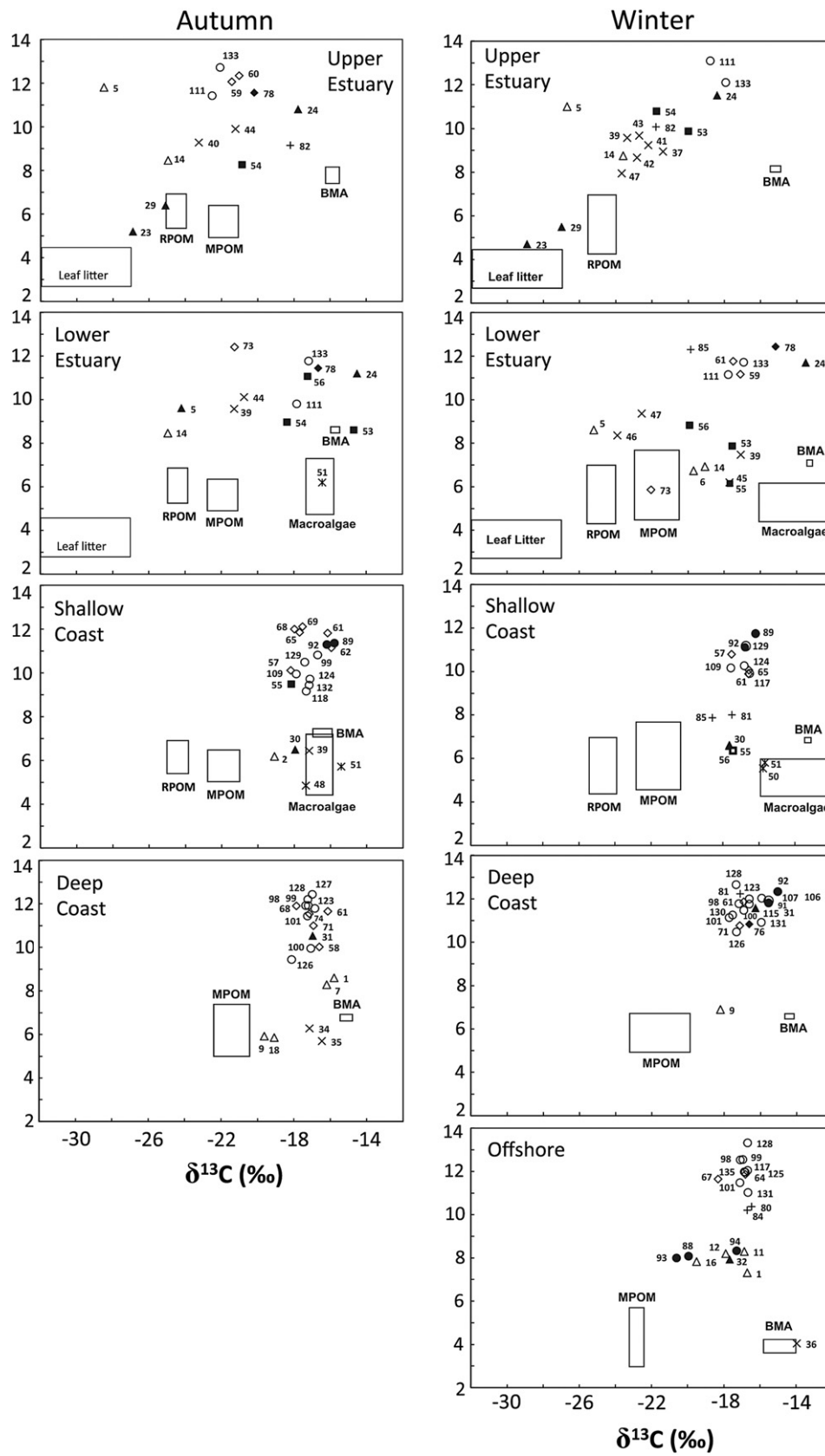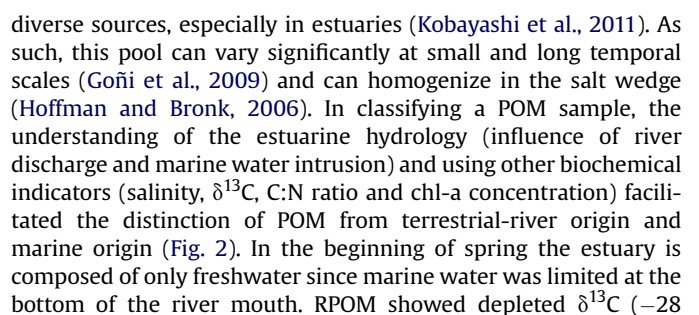
Fig. 3. (*continued*).

Table 1
Species composition of dominant benthic consumers collected from the upper estuary of Yura River to offshore of Tango Sea, Japan.

Bivalve	Continuation of amphipod	Ribbon worm
1 *Acila divaricata*	46 *Sternomoera* sp.	87 Nemertinea
2 *Callista chinensis*	47 *Tesogammarus* sp.	
3 *Cardiomya kashimana*		**Echinoderm**
4 *Crenulilimopsis oblonga*	**Isopod**	88 *Amphiophiura penichira*
5 *Corbicula japonica*	48 *Cleantoides planicauda*	89 *Astropecten latespinosus*
6 *Crassostrea gigas*	49 *Gnorimosphaeroma* sp.	90 *Astropecten scoparius*
7 *Fulvia nutica*	50 *Sphaeroma seiboldii*	91 *Ctenopleura fisheri*
8 *Laternula anatina*	51 *Synidotea laevidorsalis*	92 *Luidia quinaria*
9 *Leinucula niponica*		93 *Ophiura kinbergi*
10 *Leptomya cuspidariaeformis*	**Mysid**	94 *Parastichopus nipponensis*
11 *Limopsis belcheri*	52 *Holmesiella affinis*	95 Unidentified Ophiuroidea
12 *Modiolus margaritaceus*	53 *Neomysis awatchensis*	
13 *Musculista senshousia*	54 *Neomysis japonica*	**Fish**
14 *Mytilus trossulus*	55 *Orientomysis japonica*	96 *Acanthogobius lactipes*
15 *Naculana acinacea*	56 *Rhopalophthalmus orientalis*	97 *Amblychaeturichthys hexanema*
16 *Pandorella otukai*		98 *Amblychaeturichthys sciistius*
17 *Philine argentata*	**Shrimp**	99 *Apogon lineatus*
18 *Ratellops pulchellus*	57 *Acetes japonicus*	100 *Aseraggodes kobensis*
19 *Solidicorbula erythrodon*	58 *Alpheus japonicus*	101 *Chamsodon snyderii*
20 *Veremolpa micra*	59 *Caridina leucosticta*	102 *Chelidonichthys spinosus*
21 *Yoldia similis*	60 *Caridina serratirostris*	103 *Cottiusculus schmidti*
	61 *Crangon affinis*	104 *Cynoglossus gracilis*
Gastropod	62 *Crangon amurensis*	105 *Cynoglossus interruptus*
22 *Balylonia japonica*	63 *Crangon cassiope*	106 *Dasyatis akajei*
23 *Cipangopaludina japonica*	64 *Crangon hakodatei*	107 *Dipturus kwangtungesis*
24 *Clithon retropictus*	65 *Crangon uritai*	108 *Dixistes rikuzenius*
25 *Fusinus ferrugineus*	66 *Eualus spathilostris*	109 *Engraulis japonicus*
26 *Glassaulax didyma*	67 *Holmosiella affinis*	110 *Eosetta grigojewi*
27 *Mitrella bicincta*	68 *Latreutes anoponya*	111 *Favonigobius gymnauchen*
28 *Niotha livescens*	69 *Latreutes planirostris*	112 *Glytocephalus stelleri*
29 *Semisulcospira libertina*	70 *Macrobracium nipponense*	113 *Hippoglossoides pinetorum*
30 *Umbonium costatum*	71 *Metapenaeopsis acclivis*	114 *Lateolabrax japonicus*
31 *Zeuxis cactus*	72 *Palaemon paucedens*	115 *Lepidotrigla microptera*
	73 *Palaemon serrifer*	116 *Lophius litulon*
Scaphopod	74 *Pandalus hypsinotus*	117 *Paralichthys olivaceus*
32 *Striodentalium rhabdotum*		118 *Paraplagusia japonica*
	Crab	119 *Platycephalus* sp.
Squid	75 *Carcinoplax surugensis*	120 *Plecoglossus altivelis altivelis*
33 *Sepiola* sp.	76 *Charybdis bimaculata*	121 *Pleuronectes yokohamae*
	77 *Diogenes spinitrons*	122 *Pseudorhombus cinnamoneus*
Amphipod	78 *Hemigrypsus takanoi*	123 *Pseudorhombus pentophthalmus*
34 *Ampelisca brevicornis*	79 *Portunus hastatoides*	124 *Repomucenus curvicornis*
35 *Amphitoe* sp.		125 *Repomucenus lunatus*
36 Unidentified Atylidae	**Polychaete**	126 *Repomucenus valenciennei*
37 *Grandidierella japonica*	80 *Ehlersileanira* sp.	127 *Saurida elongata*
38 *Hyale* sp.	81 *Glycinde* sp.	128 *Saurida undosquamis*
39 *Melita hoshinoi*	82 *Hediste* sp.	129 *Sillago japonica*
40 *Melita quadridentata*	83 *Neanthes japonica*	130 *Symphurus orientalis*
41 *Melita rylovae*	84 *Nyphtys californiensis*	131 *Tanakius kitaharai*
42 *Melita satiflagella*	85 *Platynereis dumerlii*	132 *Tarphops oligolepis*
43 *Melita shimizui*	86 Unidentified Sigaliodae	133 *Tridentiger trigonocephalus*
44 *Melita* sp.		134 *Upeneus japonicus*
45 *Photis* sp.		135 *Uranoscopus japonicus*

estuary (Fig. 5). The spatial enrichment in $\delta^{13}C$ was strongly observed among the common species in autumn and winter, while little variation was apparent in spring and summer. The relatively homogenous $\delta^{13}C$ signature of benthic communities in Yura Estuary during spring and summer may be caused by either a greater organism exchange among habitats or by underlying biochemical processes that tend to establish a more homogenous isotopic values across the system. Strong marine water intrusion into the estuary coupled by high phytoplankton production in spring and summer has resulted to benthos selective utilization of a more nutritious MPOM-based energy available in the estuary. Whereas, abundant supply of allochthonous terrestrial materials and freshwater autochthonous matter during high river discharge could have resulted to a benthos diet shift to RPOM-based energy in autumn when marine intrusion is weak and becomes a total freshwater system in winter.

On the other hand, the enriched $\delta^{15}N$ signature of common benthos species at the upper estuary compared to the other stations may be influenced by the incorporation of ^{15}N-enriched dissolved inorganic nitrogen from anthropogenic activities of the catchment area (Goñi et al., 2009; Kobayashi et al., 2011). Previous study revealed higher Nitrate and Nitrite concentrations at the upstream of Yura River compared to the river mouth, and was especially high in winter when river discharge was high (Kasai et al., 2010). However, since there was no significant spatial and temporal variation in $\delta^{15}N$ among the overall estuarine benthic communities, this implies that anthropogenic effect on the nitrogen isotope signature of benthos was not strong enough to be conspicuous at the community level, or there may be other biochemical factors that have caused the elevated $\delta^{15}N$ in some species. Change in the trophic level during migration and the utilization of $\delta^{15}N$-enriched bacterial by-products may also cause
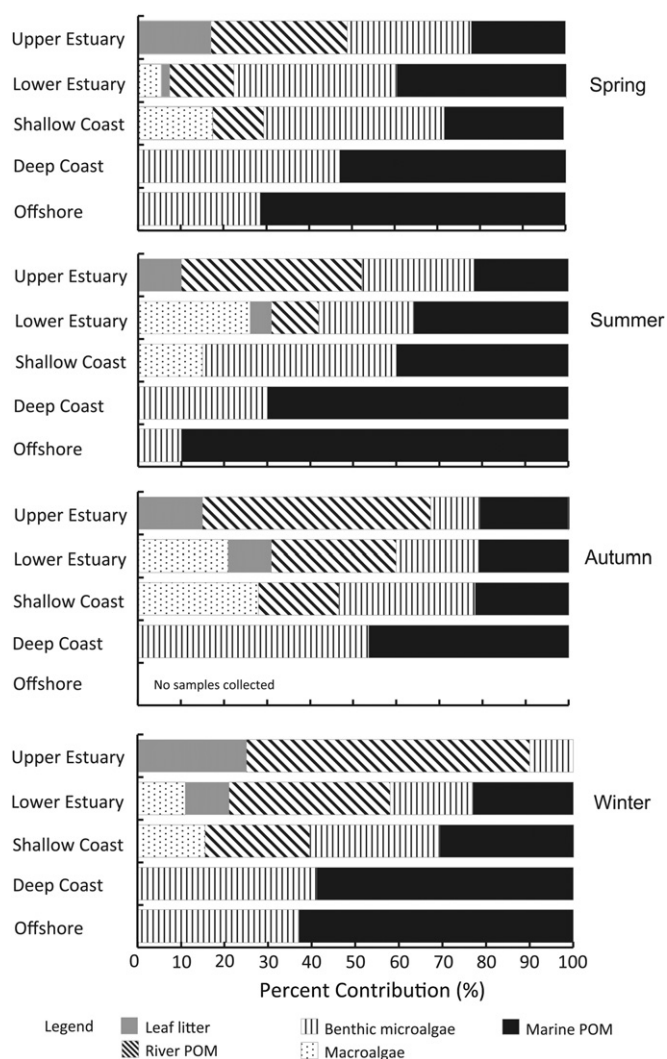
Fig. 4. Spatial and temporal variations in the δ^{13}C (left) and δ^{15}N (right) signatures of common benthic consumers. Refer to Table 1 for the species name of the corresponding species number.

spatial difference in δ^{15}N among the benthic consumers (Vanderklift and Ponsard, 2003; Zeug and Winemiller, 2008). On the other hand, spatial and temporal isotopic variation was not apparent among marine benthos in the deep and offshore stations that rely on phytoplankton (MPOM) and BMA primary productions, and not influenced by the estuary.

4.2. The ambiguous POM in the estuary

Utilizing POM as a food source is problematic since it is a dynamic pool of autochthonous and allochthonous matter from diverse sources, especially in estuaries (Kobayashi et al., 2011). As such, this pool can vary significantly at small and long temporal scales (Goñi et al., 2009) and can homogenize in the salt wedge (Hoffman and Bronk, 2006). In classifying a POM sample, the understanding of the estuarine hydrology (influence of river discharge and marine water intrusion) and using other biochemical indicators (salinity, δ^{13}C, C:N ratio and chl-a concentration) facilitated the distinction of POM from terrestrial-river origin and marine origin (Fig. 2). In the beginning of spring the estuary is composed of only freshwater since marine water was limited at the bottom of the river mouth. RPOM showed depleted δ^{13}C (-28

Fig. 5. Mean percent contribution (PC) of potential food sources to the diet of benthic communities in four seasons.

similar $\delta^{13}C$, C:N ratio and chl-a concentration of MPOM and RPOM suggested mixing in the water column. High precipitation in the form of heavy snow and rain in winter (typical along the coastal area of Japan Sea) pushed back marine water to the river mouth, and the estuary becomes a river again. The depleted $\delta^{13}C$ values (−26 to −24‰), high C:N ratio (7.7−8) and almost zero chl-a concentration of RPOM in winter implied the dominance of terrestrial detritus in the estuary. Thus, RPOM consisted mainly of terrestrial detritus in spring and winter, and containing more of freshwater phytoplankton in summer and autumn. This means that the allochthonous river subsidy to the lower estuary and shallow coast is a temporal shift between terrestrial detritus and freshwater phytoplankton, and the upper estuary may have a more endogenous food web than hypothesized. On the other hand, MPOM which was dominated by marine phytoplankton was a significant alternative food in the estuary, subsidizing estuarine food web from spring to autumn.

The present study provides supporting evidence that the estuarine to shallow coast benthic communities assimilated organic matter of terrestrial and river origin (leaf litter and RPOM). This is opposite to the results of studies dealing on estuary and coastal benthic food web that have shown very little or totally no dependence on terrestrial matter (e.g. Deegan and Garritt, 1997). In Yura Estuary, the proportion of RPOM in benthos diet was highest in the upper estuary (30−80%), and decreased seaward in the lower estuary (10−40%) to the shallow coast (0−25%), while the deep coast and offshore communities did not show utilization of RPOM. Our results are parallel to the reports of Connoly et al. (2009) that showed an evidence of trophic subsidy of coastal benthic fisheries by river discharge plumes from a small estuary in Australia and Doi et al. (2005) that showed a mix diet of terrestrial organic matter, BMA and marine phytoplankton by estuarine benthos. Moreover, Schlacher and Wooldridge (1996) found that in saline estuaries, detrital-based energy sources were secondary to water primary production; while Fisher et al. (2001) concluded that allochthonous resources and detritus are important during periods of inundation.

Terrestrial matter is rich with refractory compounds such as cellulose and lignin that are difficult to digest and assimilate by most aquatic consumers (Cividanes et al., 2002). Only those animals that have digestive enzymes able to break glycosidic bonds of cellulose into oligosaccharides can utilize such sources of energy (Sakamoto et al., 2007). In our previous study, the Yura estuarine molluscs exhibited positive cellulase activities and depleted $\delta^{13}C$ similar to RPOM that clearly indicated their capability to digest cellulose and assimilate RPOM (Antonio et al., 2010b), thereby becoming important links of terrestrial primary production and estuarine secondary production. Moreover, feeding of microbially-mediated RPOM by benthos may have a significant impact on the utilization of decomposed terrestrial organic materials. Bacterial colonization and growth can improve the quality of POM even for terrestrial-derived detritus (Edwards, 1987) and has the potential to enhance terrestrial-derived material transfer in aquatic food webs (Zeug and Winemiller, 2008). Assimilation of terrestrial and river derived matter by estuarine molluscs has huge impact on the estimation of the community percent contribution of leaf litter and RPOM, thereby may cause over estimation of the allochthonous subsidy to lower estuary and shallow coast during high river flow. Moreover, some overlapping in the isotopic signatures among food sources may have resulted to uncertainties in the estimation of the contribution of an organic matter to the benthos diet. For instance, the range of $\delta^{13}C$ and $\delta^{15}N$ of RPOM and leaf litter overlapped in spring and summer, BMA and macroalgae in spring and autumn at the shallow coast, as well as RPOM and MPOM in summer and autumn when marine and freshwater were together in the estuary. Although the mean isotopic values of each food sources were used

to −24‰), relatively high C:N ratio (∼8), and almost zero chl-a concentration that suggested RPOM may consist mainly of allochthonous terrestrial detritus. As marine water intruded into the estuary from late spring to autumn, the water became stratified. Marine water was moving up and down the bottom of the river (depending on rainfall), while freshwater was on the surface moving seaward. High chl-a concentration (maximum at 82 µg/L in June) and low C:N ratio (6−7, similar to Redfield ratio of 6.6) indicated high phytoplankton production in the upper and lower estuary in summer. However, depleted $\delta^{13}C$ (−31 to −25‰) of RPOM further suggested that the abundant phytoplankton was of river origin since freshwater phytoplankton are known to have depleted $\delta^{13}C$ around −28‰ (Cloern et al., 2002) and varies considerably depending on locality (Kendall et al., 2001), while marine phytoplankton have enriched $\delta^{13}C$ around −21‰ (Yokoyama et al., 2005). At the same time, MPOM intruding the bottom of the estuary in summer also showed an increased in chl-a concentration (although lower than RPOM), while the enriched $\delta^{13}C$ (−23 to −20‰) and high C:N ratio (max 8.5 in August) of MPOM suggested a mixture of marine phytoplankton and terrestrial detritus that may be deposited on the bottom after rainy season in summer. From November to January marine water slowly retreated back to the sea, stratification weakened, and the relatively

in the model, similarities in the isotopic values of food sources could have caused some degree of uncertain error in attributing percent contribution to the diet of each species, and may be reflected at the community level.

4.3. Locally produced versus imported energy source for the marine benthos

One of our important results is the successful determination of the variations of stable isotope signature of epilithic microalgae (BMA). The $\delta^{13}C$ of BMA showed significantly enriched values ($-22.3‰$ to $-14.0‰$) compared to MPOM ($-24.8‰$ to $-19.9‰$), about 4–6‰ difference throughout the year from the shallow coast to offshore stations, thereby a good tracer of the bottom-produced BMA in each benthic communities. On the contrary, the $\delta^{15}N$ of BMA ($2.4‰–7.4‰$) was relatively depleted compared to $\delta^{15}N$ of MPOM ($3.0‰–7.8‰$), especially at the offshore stations. Thus, carbon and nitrogen isotopes of BMA and MPOM are isotopically distinct and could distinguish benthic primary production from the pelagic phytoplankton primary production in the analysis of benthic food web.

Spatial shift in the dominance between benthic and pelagic energy source was apparent, from more of BMA-based at the shallow coast to more of MPOM-based at the offshore that agrees to previous studies (Yokoyama and Ishihi, 2007; Nadon and Himmelman, 2010). The significant contribution of BMA (30–45%) to the benthos diet highlights the importance of BMA in shallow coastal areas (MacIntyre et al., 1996; Kang et al., 2003). High irradiance and unstable sandy floor moved by waves and tidal currents in the shallow coast could have resulted to high benthic primary production and resuspension of BMA that became available to primary consumers like the filter feeder bivalves (8, 19), grazer gastropods (20, 22, 25, 26, 27, 28, 30) and mysid (55) (Pinckney and Lee, 2008). Meanwhile, both phytoplankton (MPOM) and BMA fueled the benthic communities in the deep coast, contributing almost similar proportion (50:50) to the benthos diet, except in summer when MPOM was more dominant (70%). At the offshore stations, phytoplankton (MPOM) was the main source of energy (70–90%) throughout the year, while BMA contributed less but still significant proportion to the benthos diet (10–30%). Species of bivalves (1, 4, 7, 9, 11, 12, 15, 16, 17, 18, 21), gastropod (31), scaphopod (32) and amphipods (34, 35, 36) in the deep coast and offshore benthic communities assimilated partly BMA-based organic matter. Assimilation of BMA by primary consumers effectively transferred BMA-based energy to higher trophic consumers and emphasized the importance of BMA to diet of benthic communities (MacIntyre et al., 1996; Kang et al., 2003; Nadon and Himmelman, 2010).

Similar to previous studies on coastal benthic food web (Kang et al., 2003; Yokoyama and Ishihi, 2007), our results show that benthic primary production by BMA was an important energy source for benthos, especially at the shallow coast. However, it was surprising that BMA still provided substantial amount of organic matter to offshore stations at 100–150 m depths where light is very limited or none at all, and benthic community was hypothesized as fueled solely by phytoplankton rain (MPOM) from the surface. Our results showed that the $\delta^{13}C$ signature of offshore BMA was enriched by $\sim 1.0‰$ while their $\delta^{15}N$ signature was depleted by $\sim 1.5‰$ relative to the isotopic signature of the shallow and deep coast BMA in all seasons, suggesting the possibility that the offshore BMA was of different origin or was locally produced. Distribution of BMA was found to be mainly regulated by water column light penetration, nutrient supply and grazing (MacIntyre et al., 1996; Sarker et al., 2009). Primary production in the sediment was not measured in this study, but

other studies in the nearby bay suggested between 4.0 and 74.0 mg C m^{-2} d^{-1} (Sarker et al., 2009). Thus, we can only speculate that BMA associated with the sediment at the offshore stations could be either natural flora of the bottom (Loassachan et al., 2009; Grippo et al., 2010) or was transported from the shallower area by resuspension and strong tidal currents (Kang et al., 2003; Pinckney and Lee, 2008).

The $\delta^{13}C$ of MPOM collected from the surface, mid-water and bottom water of the deep coast (-23.3 to $-19.1‰$) and offshore (-23.2 to $-20.0‰$) stations did not vary significantly over the seasons which reflected the stable signature of the marine phytoplankton far from the influences of the terrestrial environment (Yokoyama et al., 2005). This means that the conservative $\delta^{13}C$ signature of the fallen phytodetritus was not changed by decomposition through depth and time, thus, becomes a good tracer of imported energy source from the surface for the benthic communities (Mincks et al., 2008). However, the $\delta^{15}N$ of the MPOM at the deep coast and offshore stations vary from enriched values in summer (6‰) to depleted values in winter (4‰). This trend is similar to other studies that reported $\delta^{15}N$ enrichment of MPOM during high phytoplankton production in summer and the opposite during low production in winter (e.g. Doi et al., 2005; Yokoyama et al., 2005; Mincks et al., 2008). Furthermore, it is expected that MPOM that reaches the deeper regions are more refractory in its biochemical composition because higher proportion of the labile material has been metabolized in the water column (Darrow et al., 2003). Giles et al. (2007) estimated about 13% (at 150 m depth) to 34% (at < 30 m depth) remineralization of MPOM in the sediment, and that sediment oxygen fluxes are affected by primary production of BMA. Remineralized nutrients from the declining phytoplankton bloom are taken up by heterotrophic bacteria in the water column and by benthic microalgae in the sediments (Darrow et al., 2003), revealing a tight benthic-pelagic coupling between BMA and MPOM.

5. Conclusion

This study attempts a large-scale spatial and temporal analysis of isotopic composition of benthic consumers, and the contribution of the potential primary food sources to the benthos diet at the community level. Our results showed a wide range and seasonally shifting isotope signatures at the estuary and shallow coastal benthic communities that reflected the various and seasonally fluctuating energy sources in the Yura Estuary. However, the benthic communities at the deep coast and offshore of Tango Sea reflected the conservative isotopic signature of phytodetritus, but at the same time assimilated benthic microalgae as an important alternative energy source. The intensity of river discharge and marine water intrusion moving in opposite direction has driven the dynamic energy subsidy between estuarine and marine environments (Hoffman and Bronk, 2006; Connoly et al., 2009; Goñi et al., 2009), highlighting the instability and vulnerability of these benthic communities to changes in the environment and anthropogenic disturbance. However, diet of deep coast and offshore benthos were relatively stable and has integrated the mixture of MPOM-based and BMA-based energy, with some seasonal fluctuations correlated to primary production and transport of food sources (Mincks et al., 2008). This study highlights the importance of the estuary in linking terrestrial and marine productions (Hughes et al., 2000). The isotopic depiction of energy pathways at the community level and relating it to the physical and chemical fluctuations in the estuary-marine gradient can facilitate comprehension and predictions of energy flow, elucidating ecosystem response to environmental change.

Acknowledgments

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