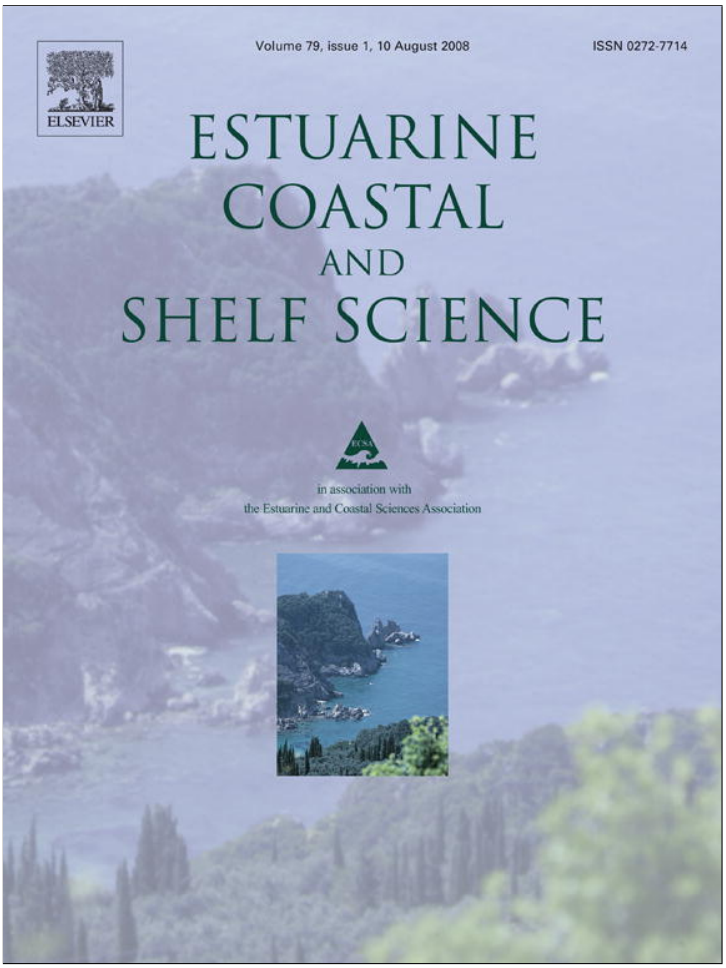

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Bivalve tissue as a carbon and nitrogen isotope baseline indicator in coastal ecosystems

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ABSTRACT

Pinctada fucata martensii mantle tissue and gut contents were examined as baseline indicators of carbon and nitrogen isotope composition at six stations in the Uwa Sea, Japan. Substantial variations in $\delta^{13}C$ and $\delta^{15}N$ values of oysters among stations were observed, with $\delta^{13}C$ being consistently lower at Hiburi Island (−18.1‰) than at other stations (−17.2‰). Oysters from fish farm sites were enriched in $\delta^{15}N$ (8.1‰) relative to those from unaffected sites (6.8‰), suggesting that fish farming tends to increase baseline $\delta^{15}N$ values. The mean $\Delta\delta^{13}C$ (0.8‰) was consistent over space and time, whereas the average $\Delta\delta^{15}N$ slightly increased in summer. The relatively low $\delta^{15}N$ enrichment compared to the theoretical isotope fractionation factor (3.4‰) may be due to oyster-specific physiological attributes. Carbon and nitrogen isotope turnover rates were roughly similar within a tissue, and mantle tissue turnover rate was estimated to be 120–180 days. These results indicated that oysters are long-term integrators of $\delta^{13}C$ and $\delta^{15}N$ from their diet and that $\delta^{13}C$ of oysters is a more accurate bioindicator of isotopic baselines than $\delta^{15}N$ for marine ecological studies.

1. Introduction

Stable carbon and nitrogen isotope values ($\delta^{13}C$ and $\delta^{15}N$) have found increasing use in providing time-integrated information of feeding relationships and energy flow through food webs (Peterson and Fry, 1987; Kling et al., 1992; Cabana and Rasmussen, 1994). Isotope values of animals are affected by isotope values of nutrients and organic compounds forming the base of their food web. For example, Hsieh et al. (2000) reported that $\delta^{13}C$ values of water column particulate organic matter (POM) in Chiku Lagoon varied spatially from −21.7 to −28.2‰. Jennings and Warr (2003) reported that $\delta^{15}N$ values of queen scallops in the northeastern Atlantic (Irish Sea, English Channel, North Sea) varied spatially from 4.2 to 11.0‰.

This spatial variability highlights the importance of understanding the control of carbon and nitrogen isotope baselines in food web studies (Cabana and Rasmussen, 1996; Vander Zanden and Rasmussen, 1999). The fact that $\delta^{13}C$ and $\delta^{15}N$ values of primary producers are highly variable depending on their surrounding physicochemical environments has hindered the accurate determination of isotope values (Post, 2002). To assess the spatial variation of $\delta^{13}C$ and $\delta^{15}N$ values of primary producers, suspension-feeding bivalves have been used to determine isotope values at the base of the food web in studies of freshwater systems, because they live long, have a low metabolic rate, and integrate highly variable isotope values among primary producers (Cabana and Rasmussen, 1996; McKinney et al., 2001; Post, 2002). In addition, since bivalves are sedentary suspension feeders, their tissue isotope values well reflect the spatial differentiation of their food sources compared to other mobile consumers. Although bivalves have been used in a variety of studies to estimate isotope baselines in freshwater habitats (Cabana and Rasmussen, 1996; Raikow and Hamilton, 2001; Howard et al., 2005; Gustafson et al., 2007), they are rarely used to estimate the baseline in marine ecosystems (Jennings and Warr, 2003). In marine ecosystems, food web analyses using stable isotopes are increasing (e.g., Moncreiff and Sullivan, 2001; Takai et al., 2002; Fredriksen, 2003). Moreover, compared to freshwater systems, large fishes and mammals having long life spans are common in marine ecosystems. To estimate the food source of

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these long-living species, we considered the utility of the isotope baseline using suspension-feeding bivalves in marine ecosystems.

Pinctada fucata martensii is a suspension-feeding bivalve that is commonly cultured in the coastal areas of the Uwa Sea. Fukumori et al. (2008) showed that *P. fucata martensii* feeds relatively non-selectively on fine particles. This indicates that the stable isotope values of oyster gut contents are nearly equivalent to those of particulate organic matter in the water column, and that oysters would be a good indicator of the source of carbon and nitrogen in an ecosystem.

The present study examined the spatial and temporal variations of δ^{13}C and δ^{15}N in adult *P. fucata martensii* mantle tissue and gut contents. We predicted that the baseline isotopic signatures would vary over space and time and that the oysters would reflect the carbon and nitrogen isotope values of primary producers at each station. We estimated oyster isotope turnover rates from a time lag between isotopic change of primary producers and oysters. From this information, we assessed the role of oysters as isotopic baselines in marine coastal ecosystems.

2. Methods

2.1. Sample collection

Six *P. fucata martensii* individuals were collected monthly at Hiburi Island, Yusu, Miura, Shitaba, Shimonada, and Uchiumi in the Uwa Sea, Shikoku Island, Japan from May to November 2005, except August (Fig. 1). In August, only four stations of oysters (Hiburi Island, Miura, Shimonada, and Uchiumi) were available. All oysters were 2 years old and the oysters collected from each station were approximately the same size (mean shell height, 62.2 ± 16.2 mm; total wet weight, 34.6 ± 8.6 g; one-way ANOVA, all, $P > 0.05$). The samples were used for stable isotope analysis ($N = 234$).

The Uwa Sea is entirely marine and there is only limited terrestrial runoff to any of the stations. The trophic status of the Uwa Sea varies due to oceanic intrusion, but it is normally oligotrophic (Kawabata and Satake, 1992; Koizumi and Kohno, 1994; Koizumi et al., 1997). The pearl farm is structured simply, allowing oysters to be maintained in the water column at a depth of 2–3 m. The pearl farms at Yusu, Miura, and Shitaba are located near fish farms.

2.2. Stable isotope analysis

Mantle tissue and gut contents of *P. fucata martensii* were dried at 60 °C for at least 24 h prior to use in stable isotope analysis. Our previous study suggested that preferential utilization of algal-specific components is unlikely in this species (Fukumori et al., 2008). Thus, we regarded the isotope values of oyster gut contents as those of primary producers in the water column. The oysters were individually ground to fine powder and immersed in chloroform/methanol (2:1) solution for 24 h to remove lipids.

Stable carbon and nitrogen isotopes were measured with an ANCA-SL mass spectrometer (PDZ Europa Ltd.). Carbon (δ^{13}C) and nitrogen (δ^{15}N) isotope values are expressed as per mil (‰) deviation from the standard with the following equation:

$$\delta^{13}\mathrm{C} \text{ or } \delta^{15}\mathrm{N} = \left(\frac{R_{\mathrm{sample}}}{R_{\mathrm{standard}}} - 1\right) \times 1000$$

where $R = {}^{13}\mathrm{C}/{}^{12}\mathrm{C}$ or ${}^{15}\mathrm{N}/{}^{14}\mathrm{N}$ for δ^{13}C or δ^{15}N, respectively. Standards for δ^{13}C and δ^{15}N were referenced to Pee Dee belemnite (PDB) limestone carbonate and atmospheric N_2, respectively.

We calculated the isotope fractionation values ($\Delta\delta^{13}$C and $\Delta\delta^{15}$N) of the oyster tissues from the isotope values of their gut contents. $\Delta\delta^{13}$C and $\Delta\delta^{15}$N values are defined as

$$\Delta\delta^{13}\mathrm{C} \text{ or } \Delta\delta^{15}\mathrm{N}(‰) = \delta X_{\mathrm{consumer}} - \delta X_{\mathrm{gut\ content}}$$

with $X = {}^{13}\mathrm{C}$ or ${}^{15}\mathrm{N}$.

2.3. Statistical analysis

We used one-way analyses of variance (ANOVA) to test for seasonal changes in the δ^{13}C and δ^{15}N values of oyster mantle tissue and gut contents. We also used ANOVA to compare the δ^{13}C and δ^{15}N values of oysters among stations. To compare δ^{15}N between oysters at the fish farm sites and those at unaffected sites, we used unpaired *t*-tests. To monitor changes in δ^{13}C and δ^{15}N of oyster mantle tissue in response to changes in isotope composition of oyster gut contents, we used a generalized linear model (GLM). We applied δ^{13}C and δ^{15}N values of oyster mantle tissue and gut contents to the GLM, and the model was selected on the basis of Akaike's Information Criterion (AIC; Akaike, 1974) to determine the best model for the relationships (lower values indicate relatively better fit to data). GLM is (δ^{13}C or δ^{15}N of oysters) $= \beta_1\mathrm{month}_1 + \beta_2\mathrm{month}_2 + \beta_3\mathrm{month}_3 + \alpha$, where β and month are coefficients of δ^{13}C or δ^{15}N of gut content, and α is a constant (e.g., Gratton and Forbes, 2006). The model is based on the assumption that isotope fractionation is persistent over the season. Variables of gut contents were added retroactively to the first sampling month (May) from the last sampling month (November, October, and September, see

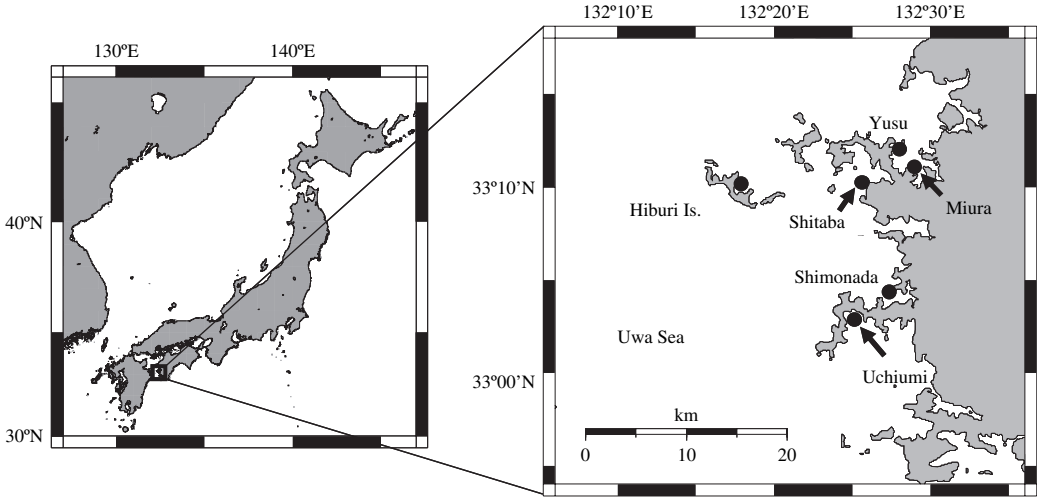

Fig. 1. Location of sample stations in Uwa Sea, Japan.

the models in Table 1). The estimation of oyster turnover rate was discontinued in September because of the relatively long turnover rates (see Section 3). We performed statistical analyses using R version 2.5.1 software (R Development Core Team, 2007). For descriptive purposes, means ± 1 SD are given. All *P* values were based on the two-tailed test.

3. Results

3.1. $\delta^{13}C$ and $\delta^{15}N$ of oyster tissue and gut content

Seasonal changes in $\delta^{13}C$ values of *P. fucata martensii* mantle tissue and gut contents are shown in Fig. 2 for each sampling station. Significant differences in $\delta^{13}C$ values were noted among the stations: $\delta^{13}C$ of oyster tissue was lower at Hiburi Island ($-18.1 \pm 0.4‰$) than at the other stations ($-17.2 \pm 0.4‰$) throughout the sampling period (one-way ANOVA, $P < 0.0001$). Similarly, $\delta^{13}C$ of oyster gut contents at Hiburi Island ($-18.8 \pm 0.6‰$) was lower than that at the other stations ($-18.1 \pm 0.6‰$) throughout the sampling period (one-way ANOVA, $P < 0.001$).

The $\Delta\delta^{13}C$ values of oyster mantle tissue ranged from 0.1 to 1.7‰, with an overall mean of $0.8 \pm 0.4‰$ (Fig. 2). These values were similar among stations and seasons (one-way ANOVA, $P > 0.05$).

The $\delta^{15}N$ values of oyster mantle tissue were significantly different among stations (one-way ANOVA, $P < 0.0001$), as shown in Fig. 3. To examine the effect of fish farms on $\delta^{15}N$ of oyster mantle tissue, we compared $\delta^{15}N$ of oyster mantle tissue at fish farm sites (Yusu, Miura, and Shitaba) with those at the unaffected sites (Hiburi Island, Shimonada, and Uchiumi), and found that the mean $\delta^{15}N$ value of oyster mantle tissue at fish farm sites ($8.1 \pm 0.7‰$) was significantly higher than that at the unaffected sites ($6.8 \pm 0.6‰$) (unpaired *t*-test, $P < 0.0001$, Fig. 3). A similar tendency was found in the $\delta^{15}N$ values of oyster gut content: the mean $\delta^{15}N$ value of oyster gut content at fish farm sites ($7.3 \pm 0.4‰$) was significantly higher than that at the other sites ($6.5 \pm 0.4‰$) (unpaired *t*-test, $P < 0.0001$).

The $\Delta\delta^{15}N$ values ranged from −0.4 to 2.0‰, with an overall mean of $0.5 \pm 0.7‰$ (Fig. 3). The $\Delta\delta^{15}N$ values at Yusu, Miura, Shitaba, and Shimonada (0.7‰) were significantly higher than those at Hiburi Island and Uchiumi (0.3‰), especially in summer (unpaired *t*-test, $P < 0.0001$).

Table 1
Akaike's Information Criterion (AIC) of linear models of carbon isotope turnover rates of oysters, which is estimated from the change in carbon isotope composition of oyster tissue in response to the change in isotope composition of gut content. α was omitted from the model. The best-performing model for each group is indicated in bold

Model and variable group	AIC
$\delta^{13}C$ of oyster in November	
+0.6Nov	−5.4
+0.7Nov + 0.1Oct	−3.9
+0.4Nov + 0.04Oct + 0.5Sep	−20.6
+0.4Nov + 0.01Oct + 0.6Sep − 0.04Aug	−10.5
+0.4Nov − 0.1Oct + 0.3Sep − 0.1Aug + 0.2Jul	−12.1
+0.4Nov − 0.1Oct + 0.1Sep − 0.1Aug + 0.04 Jul + 0.2Jun	−19.3
+0.3Nov − 0.1Oct + 0.1Sep − 0.1Aug + 0.01Jul + 0.1Jun + 0.2May	**−28.7**
$\delta^{13}C$ of oyster in October	
+0.6Oct	−11.3
+0.5Oct − 0.2Sep	−13.3
+0.5Oct − 0.2Sep + 0.2Aug	−16.7
+0.4Oct − 0.3Sep + 0.2Aug + 0.1Jul	−15.3
+0.5Oct − 0.4Sep + 0.2Aug − 0.1Jul + 0.2Jun	**−20.7**
+0.5Oct − 0.4Sep + 0.2Aug − 0.1Jul + 0.2Jun + 0.01May	−18.8
$\delta^{13}C$ of oyster in September	
+0.8Sep	−5.7
+1.0Sep − 0.1Aug	−9.3
+0.9Sep − 0.1Aug + 0.1Jul	−8.1
+0.8Sep − 0.1Aug − 0.02Jul + 0.2Jun	−10.4
+0.7Sep − 0.1Aug − 0.03Jul + 0.1Jun + 0.2May	**−11.0**

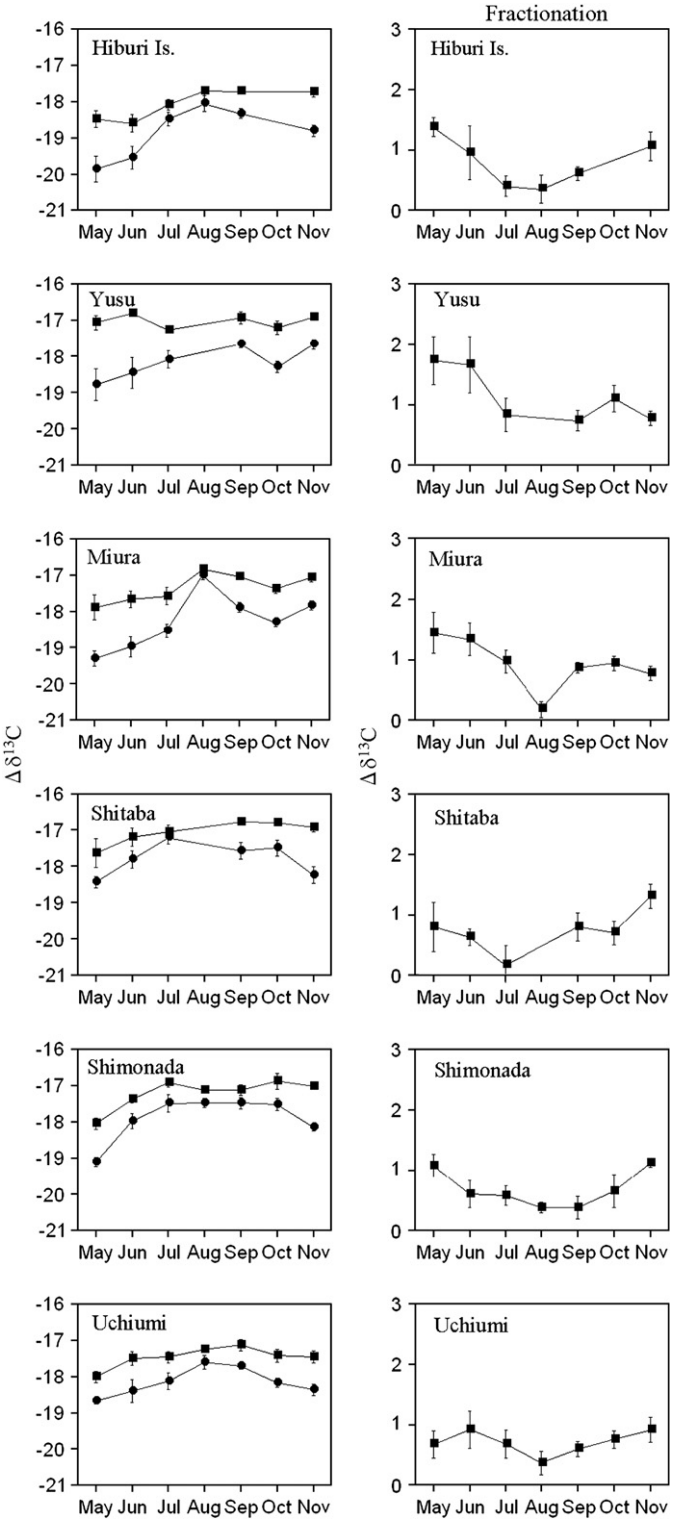

Fig. 2. Seasonal changes in $\delta^{13}C$ of oyster mantle tissue and gut content, and carbon fractionation ($\Delta\delta^{13}C$) values. Oyster mantle tissue is represented by squares and gut content by circles. Error bars indicate standard deviations.

3.2. Tissue turnover

Oyster isotope turnover rates were estimated from stepwise GLM with AIC. The best models for oyster carbon isotope turnover

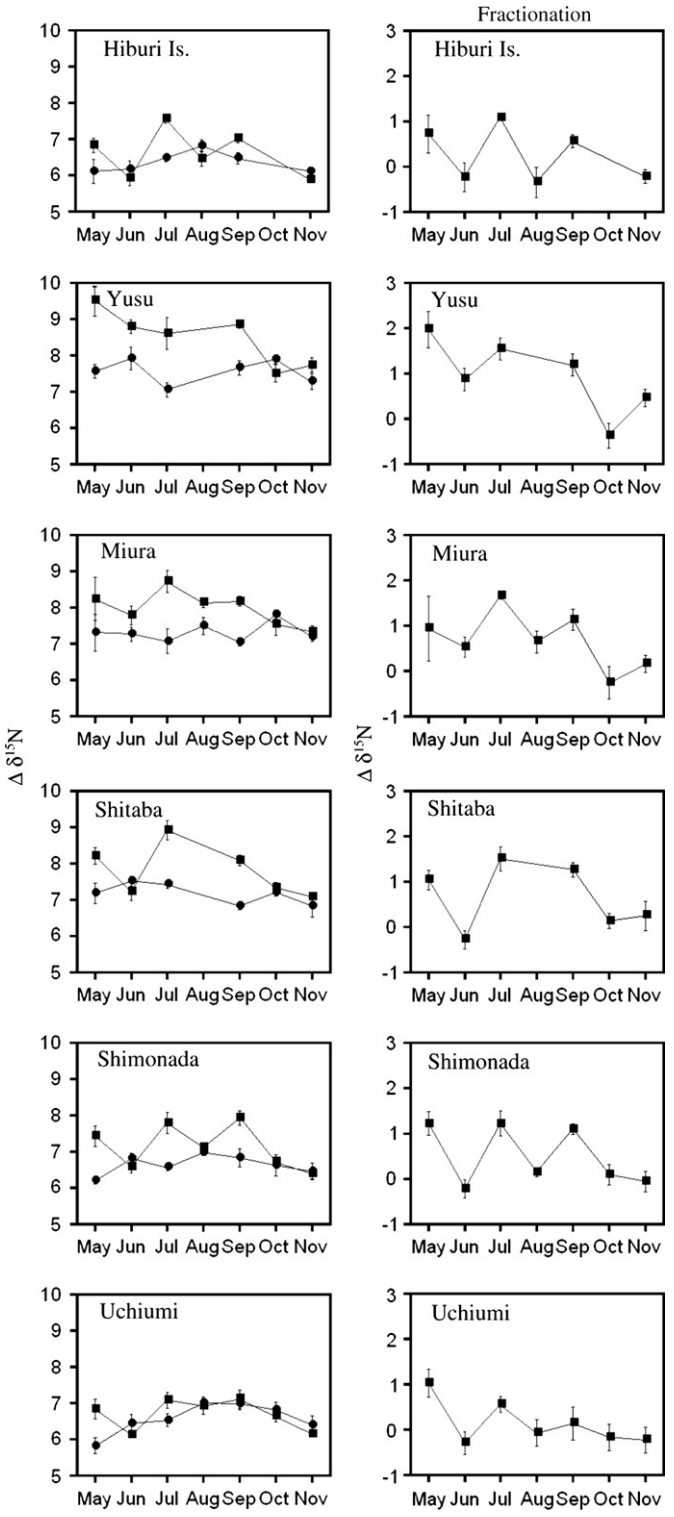

Fig. 3. Seasonal changes in δ^{15}N of oyster mantle tissue and gut content, and nitrogen fractionation ($\Delta\delta^{15}$N) values. Oyster mantle tissue is represented by squares and gut content by circles. Error bars indicate standard deviations.

rates in November, October, and September were 6, 4, and 4 months (November, AIC = −28.7; September, AIC = −20.7; October, AIC = −11.0, Table 1). Similarly, the best models for oyster nitrogen isotope turnover rates in November, October, and September were 6, 5, and 4 months (November, AIC = −5.7; September, AIC = 2.9; October, AIC = −2.2, Table 2). Since oyster turnover rate was relatively longer than the sampling period, the estimation of turnover rate was discontinued in September. Thus, oyster mantle tissue turnover rate was 120–180 days. The coefficients of the last month were consistently higher than those of previous months, suggesting that δ^{13}C and δ^{15}N values of oysters were strongly affected by the isotope composition of gut contents in the sampled month rather than that of the previous months.

Table 2
Akaike's Information Criterion (AIC) of linear models of nitrogen isotope turnover rates of oysters, which is estimated from the change in nitrogen isotope composition of oyster tissue in response to the change in isotope composition of gut content. α was omitted from the model. Best-performing model for each group is indicated in bold

Model and variable group	AIC
δ^{15}N of oyster in November	
+1.3Nov	21.8
+1.2Nov + 0.1Oct	16.8
+0.9Nov − 0.1Oct + 0.6Sep	10.0
+0.8Nov − 0.2Oct + 0.8Sep − 0.1Aug	7.1
+0.6Nov + 0.02Oct + 0.7Sep − 0.1Aug + 0.4Jul	3.9
+0.5Nov + 0.2Oct + 0.2Sep − 0.006Aug + 0.2Jul + 0.4Jun	−2.5
+0.4Nov + 0.2Oct − 0.002Sep + 0.1Aug + 0.2Jul + 0.4Jun + 0.3May	**−5.7**
δ^{15}N of oyster in October	
+0.7Oct	9.9
+0.7Oct − 0.3Sep	8.4
+0.7Oct − 0.3Sep + 0.1Aug	5.7
+0.8Oct − 0.3Sep + 0.1Aug + 0.1Jul	7.2
+0.7Oct − 0.1Sep + 0.1Aug + 0.2Jul − 0.2Jun	8.2
+0.7Oct + 0.3Sep − 0.1Aug + 0.3Jul − 0.1Jun − 0.3May	**2.9**
δ^{15}N of oyster in September	
+1.2Sep	51.6
+1.3Sep + 0.1Aug	19.3
+1.1Sep + 0.1Aug + 0.6Jul	3.8
+0.7Sep + 0.2Aug + 0.3Jul + 0.3Jun	0.8
+0.5Sep + 0.4Aug + 0.3Jul + 0.3Jun + 0.3May	**−2.2**

4. Discussion

Our results show that the isotope values of oyster mantle tissue changed in response to changes in the isotope values of their gut contents as primary producers at each site, and that oyster mantle tissue turnover rate was 120–180 days. The tissue turnover rate was lower than the response time of whole-body tissue (30–60 days) described for the oyster *Crassostrea gigas* (Riera and Richard, 1997) and hemolymph (113 days) described for the freshwater mussel *Elliptio complanata* (Gustafson et al., 2007), but higher than that of whole-body tissue (333 days) described for the marine mussel *Mytilus edulis* (Hawkins, 1985). An appropriate baseline is needed to integrate the variation in isotope values at the base of food webs (Post, 2002). Long-living consumers, such as fish, have tissue turnover rates ranging from months to years (Hesslein et al., 1993), while short-living consumers, such as zooplankton, have high tissue turnover rates, similar to that of phytoplankton (Yoshioka and Wada, 1994). Sedentary oysters having a turnover rate of 4–6 months would therefore be useful indicators of isotopic baseline in the coastal ecosystem. The GLM also showed that δ^{13}C and δ^{15}N values of oysters were strongly affected by the isotope composition of gut contents in the last month rather than that in the previous months, suggesting that this analysis will be useful to determine at any time during the turnover rate.

Previous laboratory studies of trophic fractionation typically reported little change or slight enrichment of δ^{13}C from prey to predator (Fry and Sherr, 1984; Peterson and Fry, 1987). We reported the oyster overall mean $\Delta\delta^{13}$C value of 0.8‰, which was consistent over space and time. Bivalves have received attention for their potential role as time-averaged integrators of highly variable

isotopic values of short-lived producer organisms (Cabana and Rasmussen, 1996; Hsieh et al., 2000; Post, 2002). In the coastal areas of the Uwa Sea, *P. fucata martensii* well reflected $\delta^{13}C$ values at the base of the food web throughout the season and may therefore be a good indicator of carbon source in an ecosystem.

Slight differences in $\delta^{15}N$ values between oyster gut content and mantle tissue were observed compared to the averaged isotope fractionation factor (3.4‰; Minagawa and Wada, 1984). Several studies have reported low nitrogen isotopic fractionation for bivalves (Raikow and Hamilton, 2001; Post, 2002; Marín Leal et al., 2008), suggesting that the low $\delta^{15}N$ enrichment may be due to oyster-specific physiological attributes. Our results also indicated that oysters at some stations exhibited slightly higher nitrogen isotopic fractionation in summer. Nitrogen isotopic enrichment is generally attributed to fractionation during amino acid deamination and transamination, whereby ^{14}N amine groups are preferentially removed to produce isotopically light metabolites, leaving the remaining nitrogen pool enriched in ^{15}N (Macko et al., 1986; Gannes et al., 1997). Since the metabolic rates of organisms increase with an increase in water temperature, $\delta^{15}N$ enrichment of oysters in summer can be partially accounted for by the elevated metabolic rates. Although the $\delta^{15}N$ value of oysters reflected changes in the $\delta^{15}N$ values of primary producers, we should be careful using $\delta^{15}N$ of oysters as a baseline indicator, taking seasonality of isotope fractionation into consideration.

Our results also indicated that baseline $\delta^{13}C$ and $\delta^{15}N$ values varied among stations. Some studies have reported that the $\delta^{13}C$ and $\delta^{15}N$ values of mussels are highly variable among sites, reflecting baseline $\delta^{13}C$ and $\delta^{15}N$ values in coastal areas ($\delta^{13}C$, Hsieh et al., 2000; $\delta^{15}N$, McKinney et al., 2001; Jennings and Warr, 2003). In our study areas, the $\delta^{13}C$ values of oyster mantle tissue at Hiburi Island (approximately 35 km offshore) were consistently lower than those at the other stations, and a similar tendency was noted in the gut content. The $\delta^{13}C$ values of organisms in a marine trophic system are influenced by phytoplankton growth rate (Laws et al., 1995), the occurrence of phytoplankton blooms (Nakatsuka et al., 1992; Gervais and Riebesell, 2001), primary productivity (Laws et al., 1995; Schell, 2000), and CO_2 concentration (Burkhardt et al., 1999; Tortell et al., 2000). Although the factors influencing $\delta^{13}C$ have not yet been investigated in our study area, the variability may be due to geographical factors.

The $\delta^{15}N$ values of oyster mantle tissue at Yusu, Miura, and Shitaba were higher than those at the other stations throughout the season. McKinney et al. (2001) reported a large variation of $\delta^{15}N$ values of ribbed mussel in coastal salt marshes and suggested that $\delta^{15}N$ of the mussel is influenced by nitrogen derived from human activities in the adjoining marsh watershed. Although there is no inflowing river or stream that would influence our study areas, the oyster farms at Yusu, Miura, and Shitaba are located near fish farms, and a large amount of food is provided to the fish. Fish farms generally enrich surrounding waters and sediments with nutrients and organic matter, and this loading can cause a variety of environmental problems, such as algal blooms and sediment anoxia (e.g., Angel et al., 2002). In general, organisms from fish farms are enriched in $\delta^{15}N$ compared to those from unaffected offshore reference sites (Dolenec et al., 2006, 2007), suggesting that the relatively high $\delta^{15}N$ values of oysters at some stations may be partly due to the effects of fish farming.

In conclusion, the present study showed that cultured oysters are good indicators of isotopic baselines in coastal marine ecosystems. Although the variability of $\delta^{15}N$ of oysters was approximately similar to that of primary producers, it can be used as a tracer of eutrophic or organic enriched sites. In addition, $\delta^{13}C$ of oysters can serve as a more accurate indicator of isotopic baselines for ecological study. By using oysters as baseline indicators of carbon sources, it is possible to estimate food sources of long-living species, such as fish and mammals. In addition, the $\delta^{13}C$ values of oysters may be used as an indicator of primary productivity in coastal marine areas, since the $\delta^{13}C$ values of marine POM are constant compared to those of lake or freshwater POM (France, 1995). Further studies are necessary to determine if the isotope values of marine bivalves can be useful to coastal zone managers in assessing and monitoring coastal environments.

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