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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

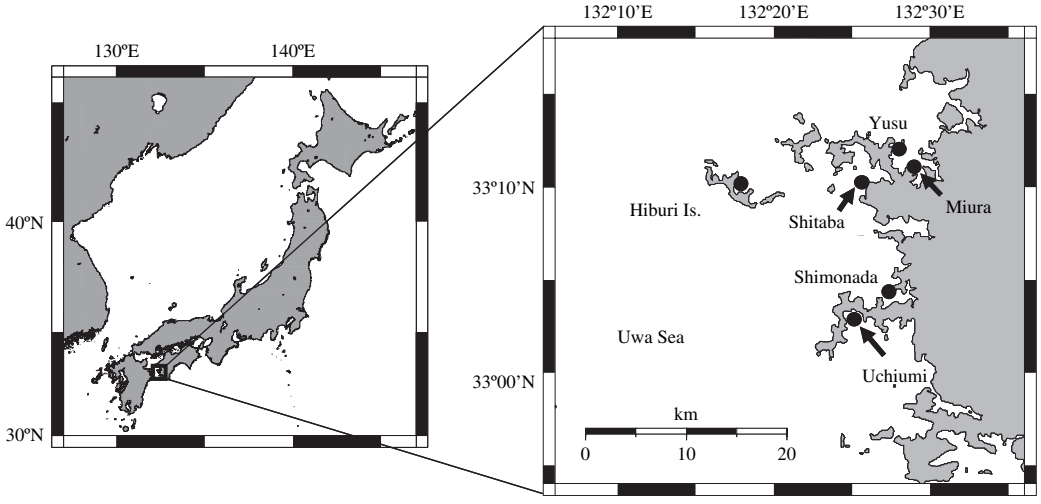

Fig. 1. Location of sample stations in Uwa Sea, Japan.