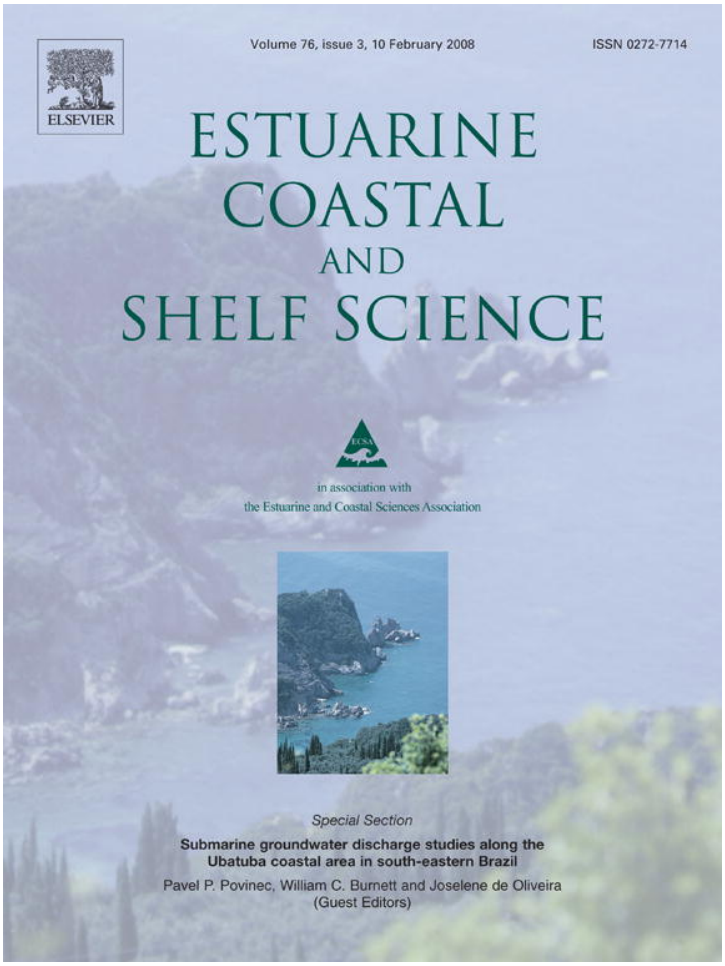

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Food sources of the pearl oyster in coastal ecosystems of Japan: Evidence from diet and stable isotope analysis

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Abstract

We estimated the composition of two food sources for the cultured pearl oyster *Pinctada fucata martensii* using stable isotopes and stomach content analysis in the coastal areas of the Uwa Sea, Japan. The $\delta^{13}C$ values of oysters (−17.5 to −16.8‰) were intermediate between that of particulate organic matter (POM, −20.2 to −19.1‰) and attached microalgae on pearl cages (−13.0‰). An isotope mixing model suggested that oysters were consuming 78% POM (mainly phytoplankton) and 22% attached microalgae. The attached microalgal composition of the stomach content showed a strong resemblance to the composition of that estimated through the isotope mixing model, suggesting preferential utilization of specific components is unlikely in this species. These results indicate that *P. fucata martensii* feed on a mixture of phytoplankton and attached microalgae, and that the attached microalgae on pearl cages can serve as an important additional food source.

Keywords: isotope mixing model; stable isotope analysis; phytoplankton; attached microalgae; benthic microalgae; *Pinctada fucata martensii*; marine coastal ecosystem

1. Introduction

Marine coastal ecosystems are commonly used for the cultivation of bivalves such as oysters, mussels and scallops. The food sources for these suspension-feeding bivalves often depend on particulate organic matter transported by tidal currents. Recent studies have suggested that in addition to phytoplankton, benthic microalgae are important food sources for suspension-feeding bivalves in shallow coastal areas, such as estuaries and lagoons (e.g., De Jonge and Van Beusekom, 1992; Kang et al., 1999; Sauriau and Kang, 2000; Yokoyama and Ishihi, 2003). This is probably because bivalves feed directly on the resuspended benthic microalgae from the sediment. However, information on the food sources for cultured bivalves has been limited (Hsieh et al., 2000). Knowledge of diet composition for cultured bivalves is crucial for understanding the coastal food web and ecosystem based management, particularly with regard to how culture systems may alter or compete with native fauna.

The suspension-feeding oyster, *Pinctada fucata martensii*, is commonly cultured in coastal areas of the Uwa Sea. This species is one of the most important commercial bivalves in Japan and is mostly used for the production of pearls. They are usually cultured in pocket nets that provide ample surface structure for attachment and growth of microalgae which also

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harbor a diverse invertebrate community. Doi et al. (in press) observed that attached microalgae on cage structures are important food sources for zooplankton in the surrounding waters. Attached microalgae may therefore be a significant source of food to the Japanese pearl oyster. From this, we hypothesized that the attached microalgae on cage structures are an available food source for cultured bivalves. To our knowledge, there is no evidence that the attached microalgae are an important source of food for cultured bivalves.

There are two methods to estimate the food source of an organism, including the pearl oyster. First, stomach content analysis provides a direct measure of what has been ingested by an individual, and usually represents an animal's diet over the last few hours. This analysis alone may require many samples and sometimes entails problems with the identification of prey items (Bradstreet, 1980; Gaston and Nettleship, 1981). Second, stable isotope analysis has been used as a complementary tool for animal diet analysis in many food web studies (France, 1994, 1996; Michener and Schell, 1994). In general, carbon isotope values of organisms reflect those of primary producers (DeNiro and Epstein, 1978; Fry and Sherr, 1984) and the nitrogen isotope values are an indicator of trophic levels (Minagawa and Wada, 1984). Stable isotope values in animal tissues are based on actual assimilation and reflect their average diet over the previous week to months (Hobson, 1999). The combination of stomach content analysis and the measurement of stable isotopes can thus provide insight in both short- and long-term dietary information.

The purpose of this study was to estimate the contribution of attached microalgae for *Pinctada fucata martensii* using carbon and nitrogen stable isotope analysis and stomach contents analysis. We evaluated the contribution of phytoplankton and attached microalgae to oysters using a two-source mixing model, and tested whether relative contribution of each food source was related to their stomach contents.

2. Materials and methods

2.1. Sample collection

Six individuals of *Pinctada fucata martensii* were collected at Miura and Uchiumi in the Uwa Sea, Shikoku Island, Japan, in 15 July and 25 August 2005 (Fig. 1). This area is entirely marine and there is no inflowing river or stream influencing the station. Its trophic status is variable due to water intrusions, but is normally oligotrophic (Kawabata and Satake, 1992; Koizumi and Kohno, 1994; Koizumi et al., 1997). Water temperature peaked in August (25–27 °C) and decreased to the minimum in February or March (ca. 16 °C). Salinity ranged ca. 33–34.5‰, and was relatively lower during July to October (<34‰). Chlorophyll *a* concentration fluctuated between 0.5 and 4.4 μg L^{-1} (Tomaru et al., 2002). A pearl farm is simply a structure that allows the oysters to be maintained in the water column at a depth of 2–3 m. All samples were examined for stable isotope analysis and stomach contents analysis ($N = 24$).

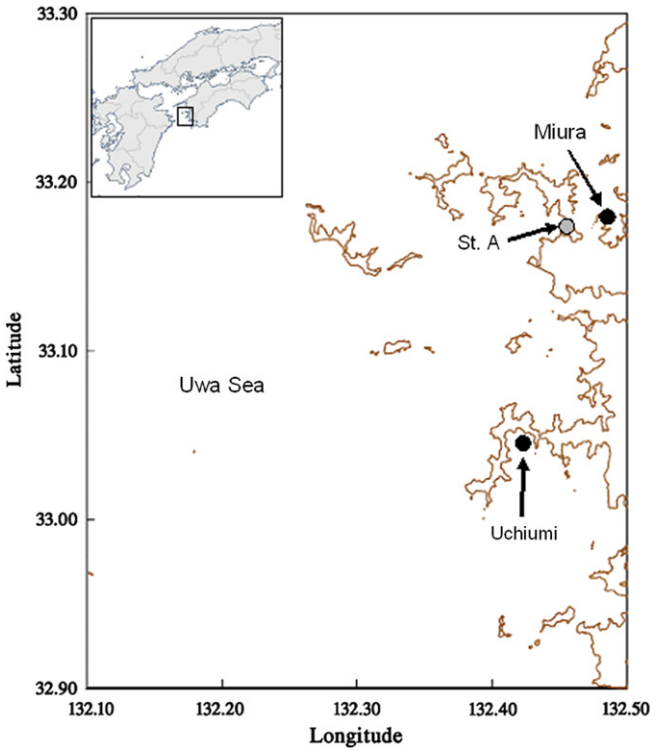

Fig. 1. Locations of sample stations in the Uwa Sea, Japan.

As the primary producers, phytoplankton, benthic microalgae and attached microalgae were collected. Particulate organic matter (POM, mainly phytoplankton) was collected at St. A and Uchiumi in July and August 2005 (Fig. 1). The surface waters were brought to the laboratory and filtered through Whatman GF/F glass fiber filters (precombusted at 550 °C for 3 h). Zooplankton remaining on the filter was removed. Benthic and attached microalgae were collected at Uchiumi in August and July 2005, respectively. Benthic microalgae were separated from sediments using phototactic movement, by in situ barrel-shaped clear acrylic chambers (a circular area of 280 cm^2, a volume of 5 L), which were placed on the sea floor of the station at a depth of 5 m for 24 h (unpublished methods). The bottom of chamber was made of a nylon net (75 μm mesh) and its inside was covered with a 3 mm layer of silica powder (0.1 mm in diameter, precombusted at 550 °C for 3 h). After 24 h incubation, the powder containing benthic microalgae was scraped off and mixed with filtered deionized water. Suspended benthic microalgae were poured into glass vials and freeze-dried. Our method is a modified procedure from Coach (1989) and Doi et al. (2003a,b). We randomly collected microalgae, which were attached on the cotton ropes of the farms, from 0–1 m depth by hand. Benthic microalgae and attached microalgae were filtered through Whatman GF/F glass fiber filters (precombusted at 550 °C for 3 h) to collect the isotope samples.

2.2. Stable isotope analysis

All samples for isotope analysis were dried at 60°C for 24 h. For *Pinctada fucata martensii*, mantle tissue was used

for stable isotope analysis. The mantle tissue was individually ground to a fine powder and immersed in chloroform/methanol (2:1) solution for 24 h to remove lipids. For primary producers (POM, benthic microalgae, and attached microalgae), ground samples were exposed to a vapor of 12 mol L^{-1} HCl for 24 h to remove carbonates prior to isotope measurements.

Carbon and nitrogen stable isotopes were measured using an ANCA-SL mass spectrometer (PDZ Europa Ltd.). Isotope ratios of carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) are expressed as per mil deviations from the standard by the following equation:

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

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

To determine which of the two food sources (POM and attached microalgae) for *Pinctada fucata martensii*, we calculated the relative contribution of each source to the biomass of the oysters using two-source mixing model (Phillips, 2001). Oysters were not expected to directly assimilate benthic microalgae (see Results). A carbon isotope fractionation for bivalves of 0.8‰ was used for the model (France and Peters, 1997). The model is defined as follows:

$$\delta^{13}C_O = f_P(\delta^{13}C_P + 0.8) + f_A(\delta^{13}C_A + 0.8),\ 1 = f_P + f_A,$$

where the subscripts O, P, and A refer to the oysters, POM, and attached microalgae, respectively; f_P and f_A are the fractional contributions of POM and attached microalgae to the oysters, respectively.

2.3. Stomach contents analysis

To compare the food habits of oysters, we conducted stomach contents analysis. For respective samples from each station, stomach contents of oysters were extracted and preserved with 5% glutaraldehyde on a glass slide. Then microalgae were microscopically examined at 400× magnification and classified into phytoplankton and attached microalgae (see Kawamura and Hirano, 1992 for details). For each genus, the number of cells were counted and relative abundance of each genus was calculated as the percent number ($\%N$) to the total cell number.

2.4. Statistical analysis

We used Student's *t*-test and one-way analysis of variance (ANOVA) to test for significant differences in the $\delta^{13}C$ and $\delta^{15}N$ values of POM (mainly phytoplankton), benthic microalgae, and attached microalgae. We also used these analyses to compare the $\delta^{13}C$ and $\delta^{15}N$ values of oysters between stations. For descriptive purposes, the means ± 1 SD are given. All *p* values were based on a two tailed test.

3. Results

3.1. $\delta^{13}C$ and $\delta^{15}N$ of potential food sources and oysters

The $\delta^{13}C$ and $\delta^{15}N$ values of *Pinctada fucata martensii* and their potential diets are shown in Table 1. There were no significant differences in $\delta^{13}C$ values among the stations (Student's *t*-test, $t_{22} = 1.2$, $p = 0.3$), but the $\delta^{15}N$ values were significantly higher at Miura than at Uchiumi ($t_{22} = 11.2$, $p < 0.001$; Fig. 2). There were significant differences both in $\delta^{13}C$ (one-way ANOVA, $F_{3,12} = 23.3$, $p < 0.001$) and $\delta^{15}N$ values ($F_{3,12} = 11.2$, $p < 0.001$) among POM (St. A), POM (Uchiumi), benthic microalgae and attached microalgae.

3.2. Contribution of two food sources to oyster diet

The contributions of each food source to *Pinctada fucata martensii*, as estimated by the two-source mixing model, are shown in Fig. 3. The contribution of POM (mainly phytoplankton) was much higher than that of attached microalgae in all cases, ranging from 63.9 (Miura in August) to 86.9% (Uchiumi in July); the contribution of attached microalgae ranged from 13.1 (Uchiumi in July) to 36.1% (Miura in August).

3.3. Stomach contents of oysters

Food items found in stomachs of *Pinctada fucata martensii* are listed in Table 2. According to the classification of Kawamura and Hirano (1992), the stomach contents of oysters consisted of 59.6–78.8% phytoplankton and 11.5–30.1% attached microalgae. The attached microalgal composition of the stomach contents was similar to the attached microalgal contribution obtained by the two-source mixing model (Fig. 3).

4. Discussion

Our isotopic results indicate that *Pinctada fucata martensii* fed primarily on phytoplankton but also fed on attached microalgae in both stations. A similar attached microalgal composition (12–30%) was found in their diets by stomach contents

Table 1
$\delta^{13}C$ (‰) and $\delta^{15}N$ (‰) for *Pinctada fucata martensii* and their potential diets. Isotopic values indicate the means ± 1 SD. Number of samples in given in parentheses

Sample	$\delta^{13}C$ (‰)	$\delta^{15}N$ (‰)
Oysters		
P. fucata martensii (Miura)	−17.2 ± 0.4 (12)	8.4 ± 0.4 (12)
P. fucata martensii (Uchiumi)	−17.4 ± 0.2 (12)	7.0 ± 0.2 (12)
Diets		
POM (St. A)	−20.2 ± 0.6 (4)	6.0 ± 1.1 (4)
POM (Uchiumi)	−19.1 ± 0.2 (4)	4.2 ± 0.3 (4)
Benthic microalgae	−17.4 ± 0.6 (4)	2.1 ± 0.3 (4)
Attached algae	−13.0 ± 0.3 (4)	5.3 ± 0.5 (4)

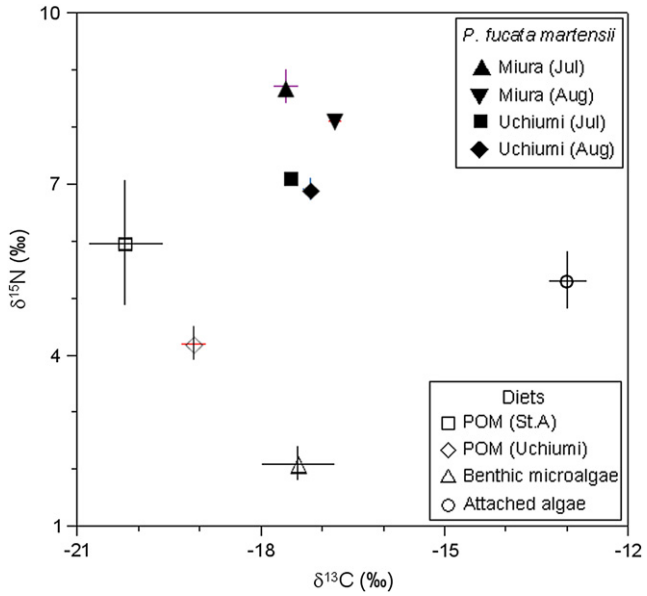

Fig. 2. Carbon and nitrogen stable isotope values of oysters and their potential diets. Error bars indicate standard deviations.

analysis. Although oysters are cultured on hanging cages in the water column, and their diets may be planktonically dominated (Hsieh et al., 2000), our results indicate that *P. fucata martensii* fed on a mixture of phytoplankton and attached microalgae, and that the attached microalgae on pearl cages can serve as an important food source in addition to phytoplankton.

There are two possibilities as to how cultured oysters feed on farm-attached microalgae. The first is that *Pinctada fucata martensii* feed on resuspended attached microalgae in the water column from pearl cages, which are peeled off by their pumping activity and through natural diffusion of water. In fact, the volume of water pumped by pearl oysters is the highest of any bivalve, with the pumping rate of the black lip pearl oyster, *Pinctada margaritifera*, reported at 25 L^{-1} h^{-1} g^{-1} dry wt. of oyster soft tissue (Pouvreau et al., 1999). In the coastal area of the Uwa Sea, 40–50 oysters are placed in a suspended pearl cage and may induce a large amount of filtration around the cage. Thus, attached microalgae can contribute to a portion of the oyster diet via their resuspension by oyster pumping. Another possible explanation is that oysters may have the opportunity to feed on attached microalgae because of their physical proximity. On the pearl-cultivated cage, large amounts of algae, sponges, barnacles, and invertebrates are attached. This fouling often reduces water flow through culture enclosures and ultimately the flux of food particles to the bivalves (Claereboudt et al., 1994; Lodeiros and Himmelman, 1996, 2000). Thus, under high fouling conditions cultured oysters are less likely to feed on phytoplankton that is supplied from outside of the pearl cage. The type and degree of fouling varies with locality (Claereboudt et al., 1994), cage type (Mendoza et al., 2003), and oyster age (Guenther et al., 2006), suggesting that the relative algal utilization by oysters depends on a number of factors. In our study area, oysters and their cages were regularly cleaned, supporting the possibility that oyster pumping may be the major cause of attached microalgal ingestion by cultured oysters.

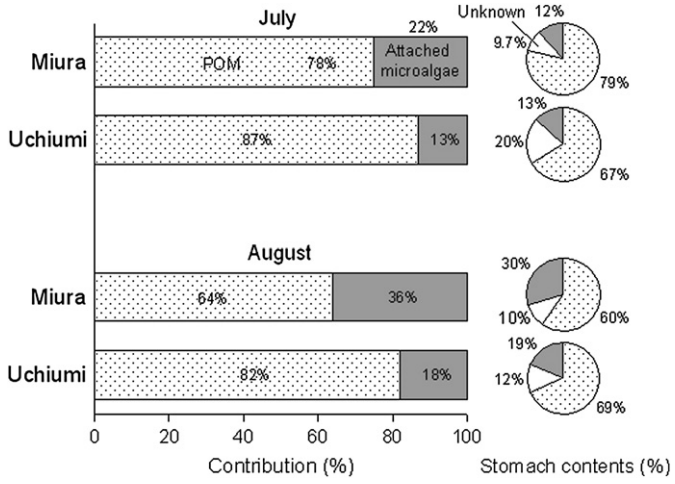

Fig. 3. Contribution (%) of two food sources (POM and attached algae) of *Pinctada fucata martensii* and the composition of their stomach contents at Miura and Uchiumi in July and August 2005. Dotted, gray, and white areas represent the contribution or proportion of phytoplankton, attached microalgae, and unknown microalgae, respectively.

Table 2
Mean stomach contents of *Pinctada fucata martensii* at Miura and Uchiumi in July and August 2005 ($N = 24$). Microalgae abundance is expressed as the percentage number (*%N*) to the total cell number

Order	July		August	
	Miura	Uchiumi	Miura	Uchiumi
Thalassiosira	57.5	27.0	26.8	22.2
Bacteriastrum	4.2	0.4	4.4	16.3
Fragilaria	2.2	4.6	0.0	0.0
Rhizosolenia	1.9	2.7	3.3	0.0
Skeletonema	0.0	4.9	0.0	11.8
Thalassionema	2.9	8.4	15.1	3.3
Thalassiothrix	3.9	7.2	5.2	6.5
Dinophysis	0.0	1.1	0.2	0.0
Prorocentrum	0.9	0.8	3.8	8.5
Distephanus	5.4	9.5	0.8	0.0
Phytoplankton	78.8	66.5	59.6	68.6
Diploneis	1.4	1.1	2.9	2.6
Gyrosigma	0.3	0.4	0.4	0.0
Navicula	3.4	1.9	9.4	5.2
Nitzschia	6.0	9.1	14.9	5.9
Licmophora	0.3	0.4	2.5	5.2
Attached microalgae	11.5	13.0	30.1	19.0
Ciliate	0.0	1.1	0.1	2.0
Unknown	9.7	19.4	10.0	10.5

The attached microalgal composition of the stomach contents strongly resembled the composition of contribution rate from the two-source mixing model, indicating that preferential utilization of specific components is unlikely in this species. The ability of bivalves to select for specific organic components within the POM pool was observed previously (Riera and Richard, 1996). For the pacific oyster, *Crassostrea gigas*,

preferential assimilation of selected algal components was observed under conditions of food limitation, which leads to the reduction of trophic competition between sympatric bivalve species (Decottignies et al., 2007). In the present study, it seems unlikely that *Pinctada fucata martensii* compete for food resources, because they were held in suspended culture on a surface layer, and fouling organisms were removed regularly from the pearl cages.

Recent isotopic studies suggested that benthic microalgae on sediments can contribute a significant portion of the diet for bivalves via their resuspension in shallow coastal areas (De Jonge and Van Beusekom, 1992; Riera and Richard, 1996; Takai et al., 2004). However, this is not the case in the pearl oyster. In the present study, benthic microalgae did not contribute to the diet of cultured oysters. Since the cultured oysters are water-column rather than benthic dwelling, they may have less opportunity to access the benthic microalgae that is resuspended from the bottom (Hsieh et al., 2000). In addition, our study areas are sufficiently deep (average water depth of 30 m in Miura and 500 m in Uchiumi) and that benthic microalgae in bottom sediments are less likely to be transported to the food source pool for the oysters.

In conclusion, the present study provides evidence of the importance of attached microalgae as well as POM (mainly phytoplankton) as a food source for the cultured oyster in marine coastal environments. The δ^{15}N values measured in the Uwa Sea suggest spatial isotopic variation for *Pinctada fucata martensii*. More research using an isotope approach is needed to more precisely identify the contribution of the attached microalgal sources and to consider the effects of localities.

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References

Bradstreet, M.S.W., 1980. Thick-billed murres and black guillemots in the Barrow Strait area, N.W.T., during spring: diets and food availability along ice edges. Canadian Journal of Zoology 58, 2120–2140.

Claereboudt, M.R., Bureau, D., Côté, J., Himmelman, J.H., 1994. Fouling development and its effect on the growth of juvenile giant scallops (*Placopecten magellanicus*) in suspended culture. Aquaculture 121, 327–342.

Coach, C.A., 1989. Carbon and nitrogen stable isotopes of meiobenthos and their food resources. Estuarine, Coastal and Shelf Science 28, 433–441.

Decottignies, P., Beninger, P.G., Rincé, Y., Riera, P., 2007. Trophic interactions between two introduced suspension-feeders, *Crepidula fornicata* and *Crassostrea gigas*, are influenced by seasonal effects and qualitative selection capacity. Journal of Experimental Marine Biology and Ecology 342, 231–241.

DeNiro, M.J., Epstein, S., 1978. Influence of diet on the distribution of carbon isotopes in animals. Geochimica et Cosmochimica Acta 42, 495–506.

De Jonge, V.N., Van Beusekom, J.E.E., 1992. Contribution of resuspended microphytobenthos to total phytoplankton in the Ems estuary and its possible role for grazers. Netherlands Journal of Sea Research 30, 91–105.

Doi, H., Chang, K.-H., Obayashi, Y., Yoshihara, M., Shime, M., Yamamoto, T., Nishibe, Y., Nakano, S. Attached microalgae contribute to planktonic food webs in bays with fish and pearl oyster farms. Marine Ecology Progress Series, in press.

Doi, H., Kikuchi, E., Hino, S., Itoh, T., Takagi, S., Shikano, S., 2003a. Isotopic (δ^{13}C) evidence for autochthonous origin of sediment organic matter in small, acidic Lake Katanuma, Japan. Marine and Freshwater Research 54, 253–257.

Doi, H., Kikuchi, E., Hino, S., Itoh, T., Takagi, S., Shikano, S., 2003b. Seasonal dynamics of carbon stable isotope ratios of particulate organic matter and benthic diatoms in strongly acidic Lake Katanuma. Aquatic Microbial Ecology 33, 87–94.

France, R., 1994. Nitrogen isotopic composition of marine and freshwater invertebrates. Marine Ecology Progress Series 115, 205–207.

France, R., 1996. Ontogenetic shift in crayfish δ^{13}C as a measure of land-water ecotonal coupling. Oecologia 107, 239–242.

France, R.L., Peters, R.H., 1997. Ecosystem differences in the trophic enrichment of ^{13}C in aquatic food webs. Canadian Journal of Fisheries and Aquatic Sciences 54, 1255–1258.

Fry, B., Sherr, E.B., 1984. δ^{13}C measurements as indicators of carbon flow in marine and freshwater ecosystems. Marine Science 27, 13–47.

Gaston, A.J., Nettleship, D.N., 1981. The Thick-Billed Murres of Prince Leopold Island. Canadian Wildlife Service Monograph No. 6, 350 pp.

Guenther, J., Southgate, P.C., de Nys, R., 2006. The effect of age and shell size on accumulation of fouling organisms on the Akoya pearl oyster *Pinctada fucata* (Gould). Aquaculture 253, 366–373.

Hsieh, H.L., Kao, W.Y., Chen, C.P., Liu, P.J., 2000. Detrital flows through the feeding pathway of the oyster (*Crassostrea gigas*) in a tropical shallow lagoon: δ^{13}C signals. Marine Biology 136, 677–684.

Hobson, K.A., 1999. Tracing origins and migration of wildlife using stable isotopes: a review. Oecologia 120, 314–326.

Kang, C.K., Sauriau, P.-G., Richard, P., Blanchard, G.F., 1999. Food sources of the infaunal suspension-feeding bivalve *Cerastoderma edule* in a muddy sandflat of Marennes-Oléron Bay, as determined by analyses of carbon and nitrogen stable isotopes. Marine Ecology Progress Series 187, 147–158.

Kawabata, Z., Satake, M., 1992. Changes in water movement, nutrient concentrations and phytoplankton biomass caused by the *kyucho* in a bay. Bulletin on Coastal Oceanography 30, 27–36 (in Japanese).

Kawamura, T., Hirano, R., 1992. Seasonal changes in benthic diatom communities colonizing glass slides in Aburatsubo bay, Japan. Diatom Research 7, 227–239.

Koizumi, Y., Kohno, Y., 1994. An influence of the *kyucho* on a mechanism of diatom growth in Shitaba Bay in summer. Bulletin on Coastal Oceanography 32, 81–89 (in Japanese).

Koizumi, Y., Nishikawa, S., Yakushiji, F., Uchida, T., 1997. Germination of resting stage cells and growth of vegetative cells in diatoms caused by Kyucho events. Bulletin of the Japanese Society of Fisheries Oceanography 61, 275–287 (in Japanese).

Lodeiros, C.J., Himmelman, J.H., 1996. Influence of fouling on the growth and survival of the tropical scallop, *Euvola (Pecten) ziczac* (L. 1758) in suspended culture. Aquaculture Research 27, 749–756.

Lodeiros, C., Himmelman, J.H., 2000. Identification of environmental factors affecting growth and survival of the tropical scallop *Euvola (Pecten) ziczac* in suspended culture in the Golfo de Cariaco, Venezuela. Aquaculture 182, 91–114.

Mendoza, Y., Freites, L., Lodeiros, C.J., López, J.A., Himmelman, J.H., 2003. Evaluation of biological and economical aspects of the culture of the scallop Lyropecten (Nodipecten) nodosus in suspended and bottom culture. Aquaculture 221, 207–219.

Michener, R.H., Schell, D.M., 1994. Stable isotope ratios as tracers in marine aquatic food webs. In: Lajtha, K., Michener, R.H. (Eds.), Stable Isotopes in Ecology and Environmental Science. Blackwell Scientific Publications, Oxford, pp. 138–157.

Minagawa, M., Wada, E., 1984. Stepwise enrichment of ^{15}N along food chains: further evidence and the relation between $\delta^{15}N$ and animal age. Geochimica et Cosmochimica Acta 48, 1135–1140.

Phillips, D.L., 2001. Mixing models in analyses of diet using multiple stable isotopes: A critique. Oecologia 130, 114–125.

Pouvreau, S., Jonquières, G., Buestel, D., 1999. Filtration by the pearl oyster, *Pinctada margaritifera*, under conditions of low seston load and small particle size in a tropical lagoon habitat. Aquaculture 176, 295–314.

Riera, P., Richard, P., 1996. Isotopic determination of food sources of *Crassostria gigas* along a trophic gradient in the estuarine bay of Marennes-Oléron. Estuarine, Coastal and Shelf Science 42, 347–360.

Sauriau, P.G., Kang, C.K., 2000. Stable isotope evidence of benthic microalgae-based growth and secondary production in the suspension feeder *Cerastoderma edule* (Mollusca, Bivalvia) in the Marennes-Oléron Bay. Hydrobiologia 440, 317–329.

Takai, N., Yorozu, A., Tanimoto, T., Hoshika, A., Yoshihara, K., 2004. Transport pathways of microphytobenthos-originating organic carbon in the food web of an exposed hard bottom shore in the Seto Inland Sea, Japan. Marine Ecology Progress Series 284, 97–108.

Tomaru, Y., Udaka, N., Kawabata, Z., Nakano, S., 2002. Seasonal change of seston size distribution and phytoplankton composition in bivalve pearl oyster *Pinctada fucata martensii* culture farm. Hydrobiologia 481, 181–185.

Yokoyama, H., Ishihi, Y., 2003. Feeding of the bivalve *Theora lubrica* on benthic microalgae: isotopic evidence. Marine Ecology Progress Series 255, 303–309.