

# Stable Isotope Analyses of the Trophic Structure of Macrobenthos on an Artificial Tidal Flat Developed Using Sediments Dredged from Pearl Oyster Farms in Ago Bay

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**Abstract** In a study organized by the Mie Prefectural Government, an artificial tidal flat was created on a sandy shore in Ago Bay using enriched sediments that had been dredged from pearl oyster farms. The aim of the artificial tidal flat (ATF) was to remove enriched sediments from pearl oyster farms and to re-mineralize the organic matter in the sediment through the activity by the biota. We investigated the trophic structure of the macrobenthos on the intertidal flat before (November, 2003) and after (November, 2004 and 2005) the ATF was constructed using the stable carbon and nitrogen isotope technique in order to evaluate the effect of the ATF. Before the ATF, the consumers were classified into four groups: group A, which was composed of suspension feeders and characterized by depleted  $\delta^{13}\text{C}$  values (-19.5 to -18.0‰), group B, in which members had intermediate  $\delta^{13}\text{C}$  values (-14.8 to -12.1‰), group C, in which members had enriched  $\delta^{13}\text{C}$  values (-11.5 to -10.7‰) and group D, in which members had enriched  $\delta^{15}\text{N}$  values (11.9 to 13.7‰). The members of groups A, B, and C are primary consumers and the food sources of each group are considered to have been a mixture of coastal phytoplankton and benthic microalgae, benthic microalgae, and a mixture of benthic microalgae and eelgrass or other unknown primary producers that have enriched  $\delta^{13}\text{C}$  values, respectively. The members of group D are secondary consumers. Benthic animals collected after ATF construction were also divided into four groups, however the number of species in groups C and D decreased due to the change of sediment composition from sand to sandy mud and to the short duration (8 months) after the completion of the ATF. After the ATF, the suspension feeding bivalves were enriched in  $^{13}\text{C}$  by 0.6 to 1.4‰ and were depleted in  $^{15}\text{N}$  by 0.5 to 1.3‰, suggesting the increased dependency on benthic microalgae that had increased their biomass by utilizing the nutrients from the pearl-oyster farm sediments.

**Key words:** intertidal flat, stable isotopes, benthic microalgae, phytoplankton, suspension feeders

## Introduction

Continuous culture of pearl oysters over extended periods within a confined farm area generates a large amount of biodeposition, oxygen deficient bottom water, and free hydrogen sulfide from the enriched sediments. Those conditions often result in a decline in pearl oyster productivity (Sawada and Taniguchi 1965). Some measures taken to improve farm environments

have been based on engineering approaches, such as digging trenches to create water routes along the seabed, widening the mouths of bays, aeration or vertical stirring of the water, and dredging enriched sediments from the seabed (Yokoyama *et al.* 2006). Among those measures, dredging enriched sediments leads to an immediate effect, however finding disposal sites for the enriched sediments has been a major problem.

In a study organized by the Mie Prefectural Government, an artificial tidal flat was created on

a sandy shore in Ago Bay using enriched sediments that were dredged from pearl oyster farms. The aim of the artificial tidal flat (ATF) was to remove enriched sediments from the pearl oyster farms and to re-mineralize sediment organic matter through the activities of the biota at the oxygen-rich sediment-water and sediment-air interfaces.

The stable isotope technique has been used successfully to follow the flows of organic matter and to point out linkages from primary producers to higher trophic levels. Stable carbon isotope ratios ( $\delta^{13}\text{C}$ ) have been extensively used to elucidate the sources of nutrition of consumers based on the close relationship between the  $\delta^{13}\text{C}$  of the food items and that of the consumer (DeNiro and Epstein 1978). On the other hand, stable nitrogen ratios ( $\delta^{15}\text{N}$ ) have been applied to investigations of the trophic levels because of the large and consistent  $^{15}\text{N}$  enrichment with increasing trophic level (DeNiro and Epstein 1981, Minagawa and Wada 1984). The coupling of  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values therefore results in a clear bi-dimensional separation of the different potential food sources for estuarine consumers. In this paper, we report on the application of the stable carbon and nitrogen technique to determine the food sources and trophic levels of intertidal animals before and after ATF construction in Ago Bay.

## Materials and Methods

### Study Area

Ago Bay has a ria style coastline with an area of 27 km<sup>2</sup> and a mean depth of 10 m (Figure 1). Pearl oyster farming began in the bay in the 1900s. In 2004, 6,600 rafts for pearl oyster farming covered 620,000 m<sup>2</sup> of the bay, and 2,000 kg of pearls were produced (Tokai Regional Agricultural Administration Office 2006).

The ATF was conducted at 34°18.0'N, 136°50.7'E in Ago Bay during the period from December 2003 to March 2005 using enriched sediments (COD<sub>sed</sub>, 12mg/g dry, mud content, 91.9%) that had been deposited on the seabed beneath rafts of pearl-oyster culture in Ago Bay (Katakura *et al.* 2005, Kokubu *et al.* 2005). The enriched sediments were

spread over the shore and were mixed with the existing sandy sediment to a depth of 1.0 m under the sediment surface, resulting in the emergence of a tidal flat with an area of 5,000 m<sup>2</sup>. After the ATF was constructed, the mud content of the sediments changed from 12.1 to 29.8%, COD<sub>sed</sub> changed from 0.46 to 4.8 mg/g-dry, and the chlorophyll a concentration on the sediment surface increased from 0.9 to 1.9 mg/kg (Kokubu 2005).

### Sampling and Isotope Analysis

Surveys were conducted before and after the ATF was constructed. Before the ATF, we sampled the reeds (*Phragmites australis*) in August 2003; particulate organic matter in surface

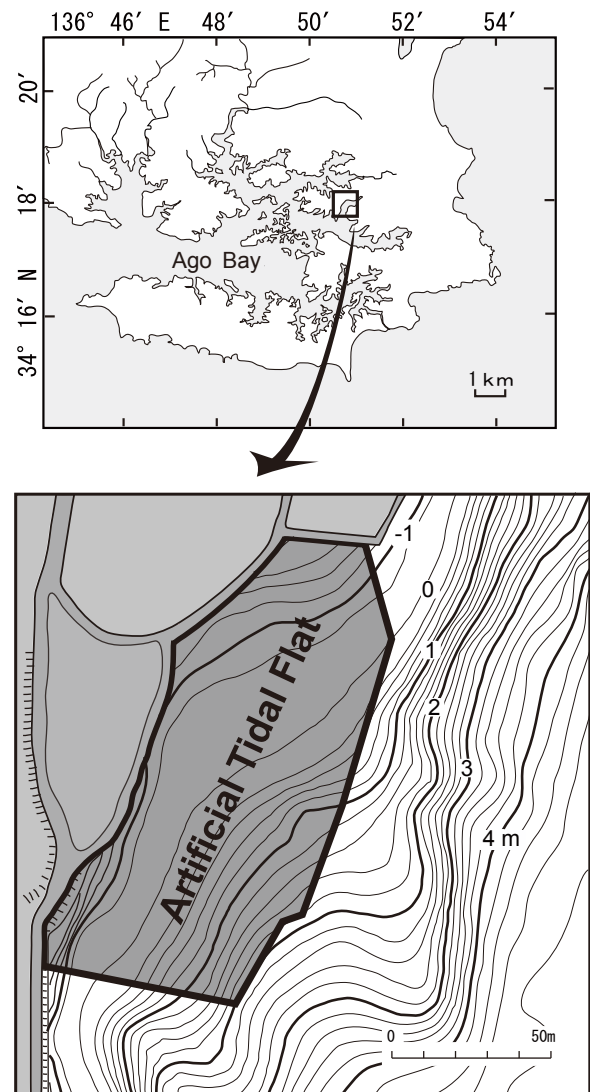


Fig. 1. Map of Ago Bay showing the artificial tidal flat with depth contours.

seawater (coastal POM) and benthic microalgae in May, July, and November 2003; seaweed in July, August, and November 2003; eelgrass (*Zostera marina*) in July and November 2003; and intertidal macrobenthos in November 2003 on and around the sandy shore in Ago Bay (Table 1). After the ATF, we also sampled coastal POM in February and May 2004; July and November 2005, and February 2006; seaweed in November 2005; benthic microalgae in January, July, and November 2005; eelgrass in February and May 2004; and intertidal macrobenthos in November 2004 and 2005 at the ATF. Benthic microalgae were extracted from the surface sediments following the procedure of Couch (1989) as modified by Riera and Richard (1996). The seawater for coastal POM was filtered on pre-combusted Whatman GF/F glass fiber filters, washed with 1.2N HCl, rinsed with distilled water, and dried at 60°C.

Collected animals were kept frozen until analysis. For the molluscs, the shell was removed and the soft tissues were used as the sample. The other animals were analyzed whole. The animal tissues were soaked in 1.2N HCl for a few minutes to remove traces of carbonates. The animal and plant samples were freeze-dried and ground to a fine powder.

The  $^{15}\text{N}$  and  $^{13}\text{C}$  compositions of the samples were determined using a mass spectrometer (MAT 252, Finnigan MAT) coupled online via a Finnigan ConFlo II interface with an elemental analyzer (EA 1110, ThermoQuest). Results are expressed in the standard  $\delta$  unit notation as  $\delta X = [(R_{\text{samples}} / R_{\text{reference}}) - 1] \times 10^3$ , where  $X$  is  $^{13}\text{C}$  or  $^{15}\text{N}$  and  $R = ^{13}\text{C} / ^{12}\text{C}$  for carbon and  $^{15}\text{N} / ^{14}\text{N}$  for nitrogen. The values are reported relative to the Vienna Pee Dee Belemnite standard (PDB) for carbon and to air  $\text{N}_2$  for nitrogen.

## Results and Discussion

### Primary Producers

In the study area, there are various potential food sources for benthic animals, which include coastal phytoplankton (coastal POM), benthic microalgae, eelgrass, and reeds. Figure 2 is a dual isotope plot of the potential food sources. As the

Table 1. Isotopic compositions of primary producers. Mean values are indicated. Numbers in parentheses indicate sample size.

|                           | 2003      |           |           | 2004      |           |            | 2005      |           |           | 2006      |  | Total<br>mean $\pm$ SD |
|---------------------------|-----------|-----------|-----------|-----------|-----------|------------|-----------|-----------|-----------|-----------|--|------------------------|
|                           | May       | Jul       | Aug       | Nov       | Feb       | May        | Jan       | Jul       | Nov       | Feb       |  |                        |
| $\delta^{13}\text{C}$ (‰) |           |           |           |           |           |            |           |           |           |           |  |                        |
| reed                      |           |           | -26.7 (5) |           |           |            |           |           |           |           |  | -26.7 $\pm$ 0.5 (5)    |
| phytoplankton             | -20.4 (4) | -21.1 (4) |           | -21.9 (4) | -23.1 (4) | -21.5 (16) |           | -22.7 (3) | -21.4 (3) | -23.1 (2) |  | -21.7 $\pm$ 1.2 (40)   |
| seaweed                   |           | -16.3 (5) | -10.6 (2) | -17.1 (3) |           |            |           |           | -16.9 (5) |           |  | -16.7 $\pm$ 3.4 (15)   |
| benthic microalgae        | -15.7 (4) | -15.6 (3) |           | -18.0 (3) |           |            | -14.3 (5) | -14.6 (1) | -15.6 (3) |           |  | -15.8 $\pm$ 1.8 (19)   |
| eelgrass                  |           | -11.6 (5) |           | -10.4 (4) | -7.2 (5)  | -8.9 (24)  |           |           |           |           |  | -9.2 $\pm$ 1.6 (38)    |
| $\delta^{15}\text{N}$ (‰) |           |           |           |           |           |            |           |           |           |           |  |                        |
| reed                      |           |           | 3.4 (5)   |           |           |            |           |           |           |           |  | 3.4 $\pm$ 0.7 (5)      |
| phytoplankton             | 8.5 (4)   | 7.4 (4)   |           | 7.2 (4)   | 7.1 (4)   | 5.0 (16)   |           | 5.5 (3)   | 4.9 (3)   | 6.0 (2)   |  | 6.1 $\pm$ 1.6 (40)     |
| seaweed                   |           | 8.3 (5)   | 8.7 (2)   | 8.0 (3)   |           |            |           |           | 8.1 (5)   |           |  | 8.1 $\pm$ 0.5 (16)     |
| benthic microalgae        | 6.5 (4)   | 6.5 (3)   |           | 5.9 (3)   |           |            | 3.9 (6)   | 5.8 (1)   | 5.5 (3)   |           |  | 5.6 $\pm$ 1.4 (20)     |
| eelgrass                  |           | 5.6 (5)   |           | 4.2 (4)   | 4.2 (5)   | 4.6 (24)   |           |           |           |           |  | 4.6 $\pm$ 0.7 (38)     |

isotopic compositions of the primary producers showed large temporal variations and we could not find any seasonal pattern (Table 1), a mean ( $\pm$ SD) value for each producer was used for the identification of food sources. Among the primary producers, reeds had the most depleted  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values,  $-26.7 \pm 0.5\%$  and  $3.4 \pm 0.7\%$ . The eelgrass had enriched  $\delta^{13}\text{C}$  ( $-9.2 \pm 1.6\%$ ) and comparatively low  $\delta^{15}\text{N}$  ( $4.6 \pm 0.7\%$ ) values. The  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values for phytoplankton were  $-21.7 \pm 1.2\%$  and  $6.1 \pm 1.6\%$ . The values are within the range of those previously reported for marine organic matter in temperate regions (e.g. Fry and Sherr 1984, Owens 1987). The  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values of benthic microalgae were  $-15.8 \pm 1.8\%$  and  $5.6 \pm 1.4\%$ . The  $\delta^{13}\text{C}$  value was significantly different from that of phytoplankton (Mann-Whitney *U*-test,  $p < 0.05$ ). The  $\delta^{13}\text{C}$  of seaweeds ( $-16.7 \pm 3.4\%$ ) was close to the  $\delta^{13}\text{C}$  of benthic microalgae, whereas the  $\delta^{15}\text{N}$  of seaweeds ( $8.1 \pm 0.5\%$ ) was significantly more enriched than the  $\delta^{15}\text{N}$  of benthic microalgae (Mann-Whitney *U*-test,  $p < 0.01$ ). Dual isotope plots of  $\delta^{13}\text{C}$  vs.  $\delta^{15}\text{N}$  for consumers and their potential food sources at the sandy shore before the artificial tidal flat are shown in Figure 3.

#### Food Sources of Animals Before the ATF

Intertidal animals collected from the sandy shore before the ATF were composed of 41 species, including two actinians (sea anemone), two chitons (Polyplacophora), nine gastropods, 10 bivalves, five polychaetes, 12 decapods, and one holothurian (sea cucumber, Table 2). The  $\delta^{13}\text{C}$  of the animals

ranged from  $-19.5$  to  $-6.5\%$ , while the  $\delta^{15}\text{N}$  ranged from  $6.1$  to  $13.7\%$ . The animals were roughly divided into four groups: A, B, C, and D, based on the  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values.

Group A was constituted of 10 bivalves and one polychaete. Animals in this group were all suspension feeders. The  $\delta^{13}\text{C}$  values for that group (range  $-19.5$  to  $-18.0\%$ ), was the most depleted among the four animal groups, and were intermediate between those of phytoplankton and benthic microalgae. The  $\delta^{15}\text{N}$  values ( $8.7$  to  $9.9\%$ ) for the group A were approximately 3-4% enriched relative to the phytoplankton and benthic microalgae, suggesting that the members of Group A incorporated a mixture of these two primary producers.

Group B was comprised of a variety of taxa, including eight gastropods (Nos. 5-7 and 9-13), two polychaetes (Nos. 30 and 33), six decapods (Nos. 39, 41 and 46-49) and one holothurian (No. 51). The  $\delta^{13}\text{C}$  values of that group ranged from  $-14.8$  to  $-12.1\%$ , while the  $\delta^{15}\text{N}$  showed a large range ( $6.7$  to  $10.7\%$ ). Taking into account the small difference in the  $\delta^{13}\text{C}$  between group B and benthic microalgae which are considered to be a major food source of the members of group B. In group B, four gastropods (Nos. 7, 9, 10 and 12) had relatively depleted  $\delta^{15}\text{N}$  values ( $6.7$  to  $8.4\%$ ), indicating that those animals are primary consumers. Animals showing the  $\delta^{15}\text{N}$  values of  $>10\%$  ( $> 4.3\%$  enriched relative to benthic microalgae) are considered to include omnivores and/or carnivores. The mean  $\delta^{15}\text{N}$  for seaweeds ( $8.3\%$ ) was the same level as, or more enriched than those of the

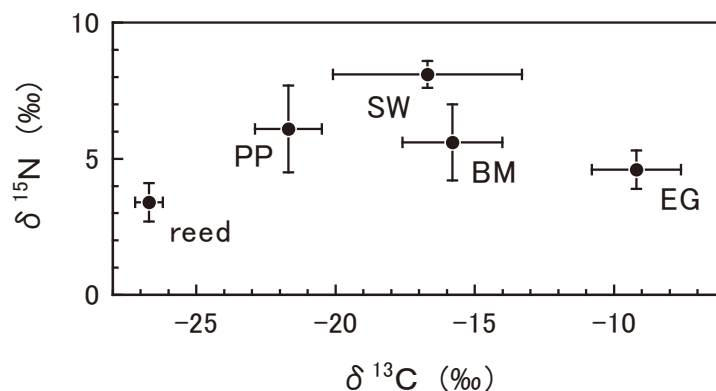


Fig. 2. Dual isotope plot of  $\delta^{13}\text{C}$  vs.  $\delta^{15}\text{N}$  for potential food sources for consumers. Error bars are SD. PP: coastal phytoplankton. BM: benthic microalgae. SW: seaweeds. EG: eelgrass.

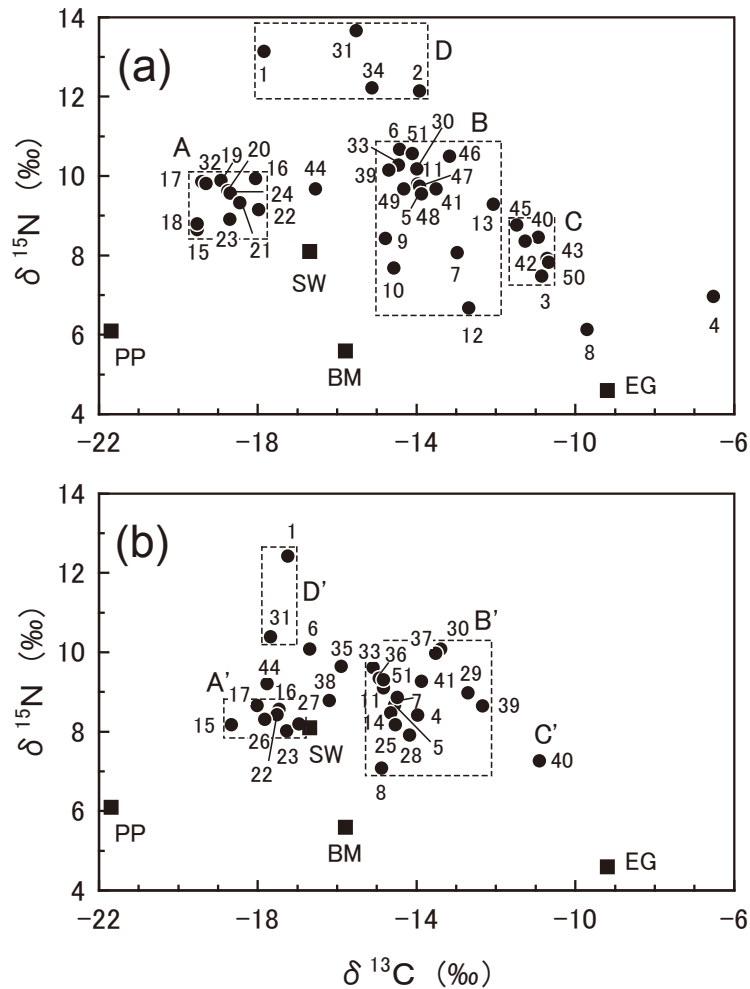


Fig. 3. Dual isotope plots of  $\delta^{13}\text{C}$  vs.  $\delta^{15}\text{N}$  for consumers (●) and their potential food sources (■) at the sandy shore before the artificial tidal flat making (a) and at the artificial tidal flat (b). See Table 1 for species identification numbers (1-51). Error bars are SD for the primary producers. PP: coastal phytoplankton. BM: benthic microalgae. SW: seaweeds. EG: eelgrass.

primary consumers. Assuming a 3-4‰ shift in N assimilation per trophic level, seaweeds were probably not important in the diet of the consumers.

The depleted  $\delta^{15}\text{N}$  values (7.5 to 8.8‰) for animals in group C, which was composed of a chiton (No. 3) and five crabs (Nos. 40, 42, 43, 45 and 50), indicate that those animals are primary consumers. Among the primary consumers, the members of the group showed the most enriched  $\delta^{13}\text{C}$ , ranging from -11.5 to -10.7‰. A possible explanation for the observed  $\delta^{13}\text{C}$  is that group C feed on a mixture of benthic microalgae and eelgrass. However, the chiton in group C, *Liolophura japonica*, and the other chiton species, *Acanthochitona* sp. (No. 4), showed a more enriched  $\delta^{13}\text{C}$  value (7.0‰) than those of group C overall,

can feed on crustose algae growing on stones and rocks (Steneck and Watling 1982). Thus, we conclude that their enriched  $\delta^{13}\text{C}$  values were not due to the assimilation of eelgrass. It may be that crustose algae had more enriched  $\delta^{13}\text{C}$  values than benthic microalgae, however we did not measure the isotopic compositions of crustose algae at that time.

Group D was comprised of four species: two actinians (Nos. 1 and 2) and two polychaetes (Nos. 31 and 34). The range of the  $\delta^{15}\text{N}$  for the animals in the group was from 11.9 to 13.7‰, which was 4.2 to 8.0‰ enriched relative to phytoplankton or microalgae, indicating that the animals in group D were secondary consumers. The actinian *Haliplanella luciae* showed a more depleted  $\delta^{13}\text{C}$  value (-17.8‰) than the other animals (-15.5 to

Table 2. Isotopic compositions of consumers before and after the artificial tidal flat making. . The mean  $\pm$  SD ( $n \geq 3$ ) or the range ( $n=2$ ) of the  $\delta^{13}\text{C}$   $\delta^{15}\text{N}$  values. Numbers in parentheses refer to the species identification number.

| Species   | Before                       |                              |   | After                        |                              |    |
|---|------------------------------|------------------------------|---|------------------------------|------------------------------|----|
|   | $\delta^{13}\text{C}$<br>(‰) | $\delta^{15}\text{N}$<br>(‰) | n | $\delta^{13}\text{C}$<br>(‰) | $\delta^{15}\text{N}$<br>(‰) | n  |
| Actinia   |                              |                              |   |                              |                              |    |
| (1) <i>Haliplanella luciae</i>                        | -17.8 $\pm$ 0.5              | 13.1 $\pm$ 0.3               | 4 | -17.2 $\pm$ 0.4              | 12.4 $\pm$ 0.3               | 4  |
| (2) <i>Edwardsia</i> sp.                              | -14.4 to -13.5               | 11.9 to 12.4                 | 2 |                              |                              |    |
| Polyplocophora  |                              |                              |   |                              |                              |    |
| (3) <i>Liolophura japonica</i>                        | -10.8 $\pm$ 0.1              | 7.5 $\pm$ 0.1                | 3 |                              |                              |    |
| (4) <i>Acanthochitona</i> sp.                         | -6.5                         | 7.0                          | 1 | -15.0 to -13.0               | 7.9 to 8.9                   | 2  |
| Gastropoda  |                              |                              |   |                              |                              |    |
| (5) <i>Batillaria cumingii</i>                        | -13.9 $\pm$ 0.3              | 9.6 $\pm$ 0.1                | 5 | -14.6 $\pm$ 0.9              | 8.7 $\pm$ 0.6                | 9  |
| (6) <i>Reticunassa festiva</i>                        | -14.4 $\pm$ 0.8              | 10.7 $\pm$ 0.4               | 5 | -16.7 $\pm$ 0.8              | 10.1 $\pm$ 0.4               | 9  |
| (7) <i>Lunella coronata coreensis</i>                 | -13.0 $\pm$ 0.5              | 8.1 $\pm$ 0.2                | 5 | -14.5 $\pm$ 0.5              | 8.9 $\pm$ 0.3                | 10 |
| (8) <i>Patelloida pygmaea</i>                         | -9.7 $\pm$ 1.5               | 6.1 $\pm$ 1.1                | 5 | -14.9 $\pm$ 2.9              | 7.1 $\pm$ 1.6                | 10 |
| (9) <i>Monodonta labio confusa</i>                    | -14.8 $\pm$ 0.5              | 8.4 $\pm$ 0.2                | 5 |                              |                              |    |
| (10) <i>Notoacmea radula</i>                          | -14.6 $\pm$ 0.8              | 7.7 $\pm$ 0.3                | 5 |                              |                              |    |
| (11) <i>Batillaria multiformis</i>                    | -14.0 $\pm$ 0.4              | 9.8 $\pm$ 0.3                | 3 | -14.8                        | 9.1                          | 1  |
| (12) <i>Littorina brevicula</i>                       | -12.7 $\pm$ 0.5              | 6.7 $\pm$ 0.3                | 3 |                              |                              |    |
| (13) <i>Cerithideopsisilla cingulata</i>              | -12.2 to -12.0               | 9.2 to 9.4                   | 2 |                              |                              |    |
| (14) <i>Clypeomorus coralia</i>                       |                              |                              |   | -14.7 $\pm$ 0.7              | 8.5 $\pm$ 0.4                | 5  |
| Bivalvia  |                              |                              |   |                              |                              |    |
| (15) <i>Vignadula atrata</i>                          | -19.5 $\pm$ 0.2              | 8.7 $\pm$ 0.2                | 5 | -18.7 $\pm$ 0.5              | 8.2 $\pm$ 0.3                | 4  |
| (16) <i>Anomalocardia squamosa</i>                    | -18.1 $\pm$ 0.1              | 9.9 $\pm$ 0.2                | 5 | -17.5 $\pm$ 0.3              | 8.6 $\pm$ 0.6                | 4  |
| (17) <i>Crassostrea gigas</i>                         | -19.4 $\pm$ 0.4              | 9.9 $\pm$ 0.4                | 5 | -18.0 $\pm$ 0.3              | 8.7 $\pm$ 0.1                | 10 |
| (18) <i>Hormomya mutabilis</i>                        | -19.5 $\pm$ 0.3              | 8.8 $\pm$ 0.1                | 5 |                              |                              |    |
| (19) <i>Claudiconcha japonica</i>                     | -18.9 $\pm$ 0.4              | 9.9 $\pm$ 0.6                | 5 |                              |                              |    |
| (20) <i>Barbatia virescens</i>                        | -18.8 $\pm$ 0.3              | 9.6 $\pm$ 0.2                | 5 |                              |                              |    |
| (21) <i>Cyclina sinensis</i>                          | -18.7 $\pm$ 0.2              | 9.6 $\pm$ 0.4                | 4 |                              |                              |    |
| (22) <i>Ruditapes philippinarum</i>                   | -18.1 to -17.8               | 8.9 to 9.4                   | 2 | -17.5 $\pm$ 0.2              | 8.4 $\pm$ 0.2                | 5  |
| (23) <i>Laternula limicola</i>                        | -18.7                        | 8.9                          | 1 | -17.3 $\pm$ 0.2              | 8.0 $\pm$ 0.2                | 3  |
| (24) <i>Gafrarium divaricatum</i>                     | -18.5                        | 9.3                          | 1 |                              |                              |    |
| (25) <i>Macoma incongrua</i>                          |                              |                              |   | -14.5                        | 8.2                          | 1  |
| (26) <i>Pitar sulfreum</i>                            |                              |                              |   | -18.0 to -17.7               | 8.3 to 8.3                   | 2  |
| (27) <i>Musculus senhousia</i>                        |                              |                              |   | -17.0 $\pm$ 0.3              | 8.2 $\pm$ 0.1                | 5  |
| (28) <i>Moerella rutila</i>                           |                              |                              |   | -14.2 $\pm$ 0.6              | 7.9 $\pm$ 0.4                | 8  |
| Sipunculida   |                              |                              |   |                              |                              |    |
| (29) <i>Siphonosoma cumanense</i>                     |                              |                              |   | -12.7                        | 9.0                          | 1  |
| Polychaeta  |                              |                              |   |                              |                              |    |
| (30) <i>Notomastus</i> sp.                            | -14.0 $\pm$ 0.6              | 10.2 $\pm$ 0.9               | 6 | -13.4                        | 10.1                         | 1  |
| (31) <i>Lumbrineris nipponica</i>                     | -15.5 $\pm$ 1.1              | 13.7 $\pm$ 0.4               | 4 | -17.7                        | 10.4                         | 1  |
| (32) <i>Chaetopterus cautus</i>                       | -19.4 to -19.3               | 9.6 to 10.1                  | 2 |                              |                              |    |
| (33) <i>Cirriiformia tentaculata</i>                  | -14.5                        | 10.3                         | 1 | -15.1 $\pm$ 0.5              | 9.6 $\pm$ 0.5                | 10 |
| (34) <i>Marphysa sanguinea</i>                        | -15.1                        | 12.2                         | 1 |                              |                              |    |
| (35) <i>Perinereis nuntia</i> var. <i>brevicirris</i> |                              |                              |   | -15.9 $\pm$ 0.4              | 9.6 $\pm$ 0.9                | 3  |
| (36) <i>Perinereis nuntia</i> var. <i>vallata</i>     |                              |                              |   | -14.9 $\pm$ 1.8              | 9.4 $\pm$ 1.2                | 5  |
| (37) <i>Ceratonereis erythraeensis</i>                |                              |                              |   | -13.5 $\pm$ 0.3              | 10.0 $\pm$ 0.8               | 10 |
| Crustacea   |                              |                              |   |                              |                              |    |
| (38) <i>Sphaeroma sieboldii</i>                       |                              |                              |   | -16.2 $\pm$ 2.3              | 8.8 $\pm$ 1.0                | 5  |
| (39) <i>Hemigrapsus penicillatus</i>                  | -14.7 $\pm$ 0.2              | 10.1 $\pm$ 0.7               | 5 | -13.5 to -11.2               | 7.3 to 10.0                  | 2  |
| (40) <i>Ilyoplax pusillus</i>                         | -10.9 $\pm$ 0.3              | 8.5 $\pm$ 0.1                | 5 | -10.9 $\pm$ 0.9              | 7.3 $\pm$ 0.5                | 5  |
| (41) <i>Pagurus dubius</i>                            | -13.5 $\pm$ 0.6              | 9.7 $\pm$ 0.3                | 5 | -13.9 $\pm$ 1.2              | 9.3 $\pm$ 0.6                | 9  |
| (42) <i>Macrophthalmus japonicus</i>                  | -11.3 $\pm$ 0.5              | 8.4 $\pm$ 0.3                | 5 |                              |                              |    |
| (43) <i>Scopimera globosa</i>                         | -10.7 $\pm$ 0.4              | 7.9 $\pm$ 0.7                | 5 |                              |                              |    |
| (44) <i>Diogenes spinifrons</i>                       | -16.6 $\pm$ 0.9              | 9.7 $\pm$ 0.5                | 4 | -17.8 $\pm$ 0.9              | 9.2 $\pm$ 0.2                | 3  |
| (45) <i>Macrophthalmus dilatatus</i>                  | -11.5 $\pm$ 0.4              | 8.8 $\pm$ 0.3                | 3 |                              |                              |    |
| (46) <i>Charybdis japonica</i>                        | -13.3 to -13.1               | 10.5 to 10.5                 | 2 |                              |                              |    |
| (47) <i>Nihonotrypaea</i> sp.                         | -14.4 to -13.5               | 9.7 to 9.8                   | 2 |                              |                              |    |
| (48) <i>Alpheus brevicristatus</i>                    | -13.9                        | 9.5                          | 1 |                              |                              |    |
| (49) <i>Laomedea astacina</i>                         | -14.3                        | 9.7                          | 1 |                              |                              |    |
| (50) <i>Macrophthalmus banzai</i>                     | -10.7                        | 7.8                          | 1 |                              |                              |    |
| Holothuroidea   |                              |                              |   |                              |                              |    |
| (51) <i>Patinapta ooplax</i>                          | -14.4 to -13.8               | 10.5 to 10.7                 | 2 | -14.8                        | 9.3                          | 1  |

-14.0‰), suggesting that *H. luciae* depended on phytoplankton and that the other animals incorporated benthic microalgae through primary consumers.

#### Food Sources of Animals After the ATF

Eight months after the completion of the ATF, we sampled animals from the artificial tidal flat. The 30 species collected were composed of one actinian, one chiton, six gastropods, nine bivalves, one sipunculid, six polychaetes, five decapods and one holothurian. Benthic animals collected after the ATF were also classified into 4 groups: group A', group B', group C', and group D', which corresponded to groups A, B, C and D before the ATF.

Group A' included seven bivalves representing five species (Nos. 15-17, 22 and 23) that had occurred before the ATF and two species (Nos. 26 and 27) that did not occur before the ATF. Five bivalves (Nos. 18-21 and 24) and one polychaete (No. 32) disappeared after the ATF. The  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values of group A' ranged from -18.7 to -17.0‰ and from 8.0 to 8.7‰, of which the range shifted considerably after the ATF. That is, the  $\delta^{13}\text{C}$  enriched, while the  $\delta^{15}\text{N}$  values decreased. Figure 4 shows the shift of the isotopic compositions of five bivalves after the ATF. For

all five species that occurred before and after the ATF, the  $\delta^{13}\text{C}$  values increased by 0.6‰ (*Ruditapes philippinarum*) to 1.4‰ (*Crassostrea gigas* and *Laternula limicola*), while  $\delta^{15}\text{N}$  values decreased by 0.5‰ (*Vignadula atrata*) to 1.3‰ (*Anomalocardia squamosa*) after the ATF. There were significant differences (Mann-Whitney U-test, all  $p < 0.05$ ) in the  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  for *Vignadula atrata*, *Crassostrea gigas*, and *Anomalocardia squamosa* before and after the ATF.

Group B' consisted of 16 species, including one chiton (No. 4), six gastropods (Nos. 5-8, 11 and 14), two bivalves (Nos. 25 and 28), four polychaetes (Nos. 30, 33, 36 and 37), two decapods (Nos. 39 and 41), and one holothurian (No. 51). Among the members of group B', nine species, including four gastropods (Nos. 5-7 and 11), two polychaetes (Nos. 30 and 33), two decapods (Nos. 39 and 41), and one holothurian (No. 51) were collected at both before and after the ATF. After the ATF, one gastropod (No. 14), two bivalves (Nos. 25 and 28), two polychaetes (Nos. 36 and 37) and one isopod (No. 38) were new to the area, while five gastropods (Nos. 9-13) and four decapods (Nos. 46-49) disappeared. The change of species composition may have been due to the change in the sediment composition from sand to sandy mud. We excluded the gastropod *Reticunassa festiva* (No. 6), which

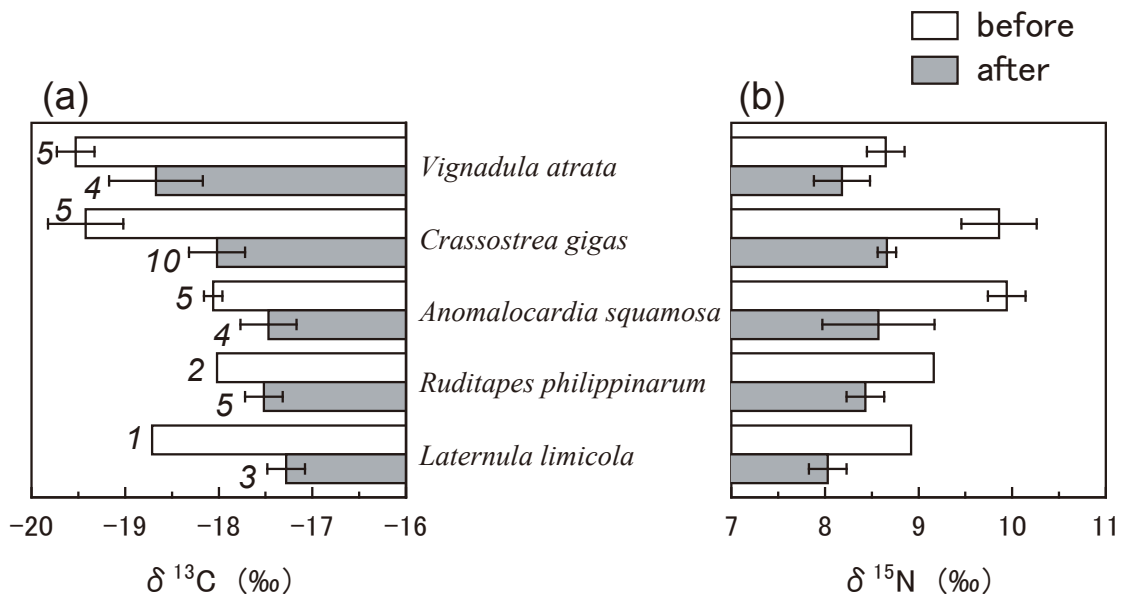


Fig. 4. (a)  $\delta^{13}\text{C}$  and (b)  $\delta^{15}\text{N}$  values for 5 bivalve species of suspension feeders collected from the sandy shore before the artificial tidal flat making (□) and at the artificial tidal flat (■). Error bars are SD. Numerals denoted in italics indicate sample size (n).

was a member of group B before the ATF, from group B', because the species showed a markedly depleted  $\delta^{13}\text{C}$  after the ATF. On the other hand, the  $\delta^{13}\text{C}$  values of the chiton *Acanthochitona* sp. (No. 4) and the gastropod *Patelloida pygmaea* (No. 8) shifted in the range of group B'. The range of the  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  of group B' was from -15.1 to -12.4‰ and from 7.1 to 10.1‰, which was close to the range of group B before the ATF, suggesting a close similarity in the food item benthic microalgae between the members of groups B and B'.

Group C' consisted of a single species, the decapod *Ilyoplax pusillus* (No. 40) that showed a  $\delta^{13}\text{C}$  of -10.9‰ and a  $\delta^{15}\text{N}$  of 7.3‰. The other five species in group C disappeared after the ATF, probably due to the change of the sediment composition.

Group D' contained two species, the actinian *Haliplanella luciae* and the polychaete *Lumbrineris nipponica* and had  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values of -17.2‰ and 12.4‰ for the actinian and -17.7‰ and 10.4‰ for the polychaete. Two members in group C, the actinian *Edwardsia* sp. and the polychaete *Marphysa sanguinea* were not collected after the ATF.

The most characteristic findings in the present study are that the dependency of suspension feeders on benthic microalgae increased after the ATF. It has been suggested that ATF resulted in the enrichment of the sediments and increased the biomasses of benthic microalgae and macrobenthos (Kokubu *et al.* 2005). The present study suggests that macrobenthic animals assimilated benthic microalgae that increased their biomass using nutrients that were contained in the pearl oyster farm sediments. Thus, the ATF may be effective from the viewpoint of giving the disposal site of enriched sediments that are produced from mariculture and of mineralizing the accumulated organic matter in the sediment. On the other hand, we must note that the ATF may exert an influence on the local biota as shown by the fact that the macrofauna changed from that characteristic of a sandy shore to one characteristic of a mudflat after the ATF.

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