

Carbon sources for demersal fish in the western Seto Inland Sea, Japan, examined by $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ analyses

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Abstract

The relative importance of benthic and pelagic primary production for demersal fish and some invertebrates in the western Seto Inland Sea of Japan was examined using carbon and nitrogen stable isotope analyses. A few fishes, such as juvenile black rockfish *Sebastes inermis* and large Japanese anchovies *Engraulis japonicus*, had isotopic carbon signatures similar to pelagic particulate organic matter ($-20.1 \pm 1.7\text{‰}$), which indicates that their food was derived from production in the water column. However, 92% of the 401 demersal fish that were analyzed had $\delta^{13}\text{C}$ signatures (-17.0 to -13.0‰) similar to those of benthic crustaceans, epilithic microphytobenthos, and macroalgae and unlike the signature of pelagic particulate organic matter or zooplankton. These results suggest that in the Seto Inland Sea there is not a tight coupling between pelagic primary production and the food web of demersal fishes, but rather that these fishes are dependent on carbon from benthic primary production.

Petersen and Curtis (1980) suggested that pelagic–benthic coupling in the marine ecosystem should be tighter at higher latitudes than at lower latitudes. It appears that this latitudinal difference can be seen in the coastal shallow waters along the North Pacific Ocean between the northernmost Arctic region and the western temperate region. In the highly productive Chukchi and northern Bering Seas where primary production ranges from 250 to 300 $\text{gC m}^{-2} \text{yr}^{-1}$ (Walsh et al. 1989), a large portion of the organic matters being produced in the water column falls ungrazed to the bottom (Grebmeier and Barry 1991). The downward organic carbon flux was reported to be 253–654 $\text{mgC m}^{-2} \text{d}^{-1}$ in the northern Bering Sea (Fukuchi et al. 1993), and low surface sediment C:N ratios (wt.:wt.) of 6–8 were found under the Bering Shelf–Anadyr Water, which suggests that a high quality, nitrogen-rich organic material was being supplied to the benthic animals (Grebmeier et al. 1988). On the other hand, in the shallow, productive Seto Inland Sea of Japan, surface sediment C:N ratios are relatively high (8–11) (Shinohara 1997; Mishima et al. 1999). This suggests that the quality of the organic matter supplied to the benthos may be lower than that in the Bering Sea, although annual primary production (280–460 gC m^{-2}) and the downward organic carbon flux (140–440 $\text{mgC m}^{-2} \text{d}^{-1}$) reported for the western

area of the Seto Inland Sea are similar to values in the Chukchi and northern Bering Seas (Seiki et al. 1985). In addition, macrobenthic biomass is only 56 $\text{g wet weight m}^{-2}$ in the Seto Inland Sea (Hashimoto et al. 1997), in contrast to levels of 118–2,377 $\text{g wet weight m}^{-2}$ in the benthos of the Bering Shelf–Anadyr Water (Grebmeier et al. 1988).

Here we address the relative importance of benthic primary production for the shallow coastal waters of Japan, where pelagic–benthic coupling is weak. In the Seto Inland Sea and arctic seas, benthic animals have been assumed to be primarily dependent on water column primary production (Tatara 1981), but recent studies showing the importance of benthic primary production challenge this assumption. Cahoon and Cooke (1992) demonstrated that the benthic primary production is comparable to the water column primary production not only in macroalgal or seagrass beds but also in so-called unvegetated habitats devoid of macrophytes. In the South Atlantic Bight, benthic microalgae contribute to approximately 40% of the total primary production at depths of 14–40 m (Jahnke et al. 2000). We hypothesized that benthic primary production might supply substantial organic carbon to the demersal fish in the Seto Inland Sea.

To test this hypothesis we analyzed the $\delta^{13}\text{C}$ of the demersal fish in the western Seto Inland Sea to determine their carbon source. Since the $\delta^{13}\text{C}$ of the animals increases only about 1‰ through a prey–predator trophic link (DeNiro and Epstein 1978), the $\delta^{13}\text{C}$ of the animals reflects the $\delta^{13}\text{C}$ of primary producers at the base of the food web. The reported average $\delta^{13}\text{C}$ value for marine phytoplankton is -22‰ , lower than the -17‰ signature for marine benthic algae (France 1995), thus allowing the relative contribution of water column and benthic primary production to be estimated. In the Chukchi Sea, the $\delta^{13}\text{C}$ values of the demersal fish were re-

Acknowledgments

We thank E. Wada for his critical reading of this manuscript. We are grateful to M. Ueno for his useful comments and advice and H. Yamaguchi for cooperation in the species identification of small crustaceans. We are also grateful to J. J. Middelburg and two anonymous reviewers for their valuable comments on the manuscript and to W. Wurtsbaugh for English revision of the manuscript. This study was supported by a grant from Japan Science and Technology Corporation.

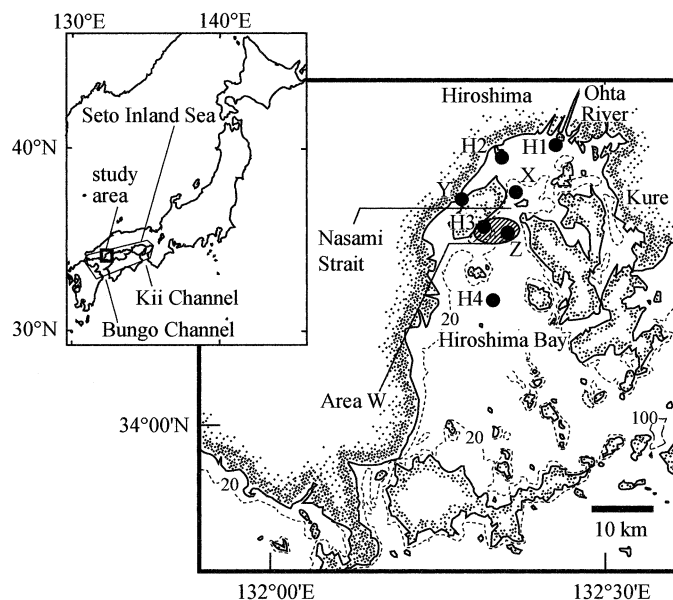


Fig. 1. Sampling locations in Hiroshima Bay. The isobaths are shown with dotted lines for 20 m and broken lines for 100 m.

ported to be very similar to the values of zooplankton, clearly reflecting the tight pelagic–benthic coupling there (Dunton et al. 1989; Schell et al. 1998). If water column primary production contributed significantly to the demersal fish in the Seto Inland Sea, the area's demersal fish would show depleted $\delta^{13}\text{C}$ values like the fish in the Chukchi Sea.

In this study, we also analyzed the nitrogen stable isotope ratio of the demersal fish as a secondary tracer. $\delta^{15}\text{N}$ levels in both invertebrates and vertebrates show an increase of about 3–4‰ per trophic level (Minagawa and Wada 1984), and thus the isotopic nitrogen signatures can be used to measure trophic position. Consequently, schematic food web structures depicted by $\delta^{13}\text{C}$ – $\delta^{15}\text{N}$ maps provide useful information about the transport pathways of organic matter from primary producers to top predators.

Study area

The Seto Inland Sea is the broad shallow area located to the southwest of the mainland of Japan (Fig. 1). It is 450 km long, with an area of 220,000 km² and a mean depth of 37 m. The sea consists of several shallower semienclosed areas and deeper narrow channels, leading to the open sea at three openings, the Bungo Channel, the Kii Channel, and the Kanmon Strait. The latter strait leads to the Japan Sea at its westernmost end, and is too narrow to affect the total water exchange of the sea. The former two straits lead to the Pacific Ocean at the southwestern (the Bungo Channel) and the southeastern (the Kii Channel) end of the sea. The mass of water exchanged through the Bungo Channel is twice that through the Kii Channel (Fujiwara 1983), and the residence time of the total water in the sea is assumed to be less than several years (Takeoka 1984).

Hiroshima Bay is located to the north of the Bungo Channel and has an area of ca. 1,000 km² and a mean depth of

24 m (Fig. 1). The bay is dotted with many islands in its central area, and thus its topography is very complicated. In particular, the Nasami Strait divides the bay into two semi-enclosed areas, northern and central Hiroshima Bay. The northern bay is one of the most eutrophic areas in the Seto Inland Sea and the polluted Ota River flows into this area from the northern shore. The chlorophyll *a* (Chl *a*) concentration increases and the dissolved oxygen becomes supersaturated in the surface layer of the northern bay in summer, in contrast to the small seasonal change in the central bay (Hashimoto et al. 1994).

Materials and methods

Sampling—Fish were captured with a throw net on the island shore (Sta. Z) from 15 June 1999 to 30 June 2000, with a boat seine at Sta. Y (<10 m in depth) on 21 October 1997 and 16 April 1998, and with a bottom trawl at Stas. H1–H4 (10–30 m in depth) on 14 May 1999 (Fig. 1). Standard lengths of the fish are reported here. Small benthic crustaceans including amphipods, an isopod, and decapods were collected from the surfaces of stones, macroalgae, and shells of bivalves at Sta. Z. Mysids (*Acanthomysis tenuicauda* and *Acanthomysis* spp.) and copepods that were extracted from the stomach contents of Japanese horse mackerel *Trachurus japonicus* captured at Sta. H4 were also analyzed for stable isotope ratios. As supplementary data, bivalves, cephalopods, large decapods, a starfish, holothurians, and an ascidian were also analyzed for stable isotope ratios. We collected the Japanese oyster *Crassostrea gigas* from several different kinds of habitat (the island shore [Sta. Z], the sea bottom [Sta. H3], and the oyster raft [Sta. X]) in order to examine the habitat-related differences in isotopic values. The oysters collected at Sta. X were hanging from a raft in the surface layer (0–6 m).

Particulate organic matter (POM) (0.7–125 μm) for isotopic analysis was collected from sea surface water at Stas. H1–H4 on 14 May 1999 and filtered onto precombusted (450°C, 2 h) glass-fiber filters (Whatman GF/F type) after being sieved through a 125- μm mesh sieve. The POM in the surface water of area W was collected and processed from 10 August 1999 to 10 November 2000. We also sampled larger particles in the layer <20 m on 14 May 1999 with a vertical haul of a 350- μm mesh plankton net. These were sieved through 350- μm and 125- μm mesh sieves, and the stable isotope ratios of POM in each fraction were analyzed.

The epilithic organic matter (EOM) as an indicator of epilithic algae was collected at Sta. Z from 10 August 1999 to 12 October 2000. Several submerged greenish stones were collected at the sublittoral fringe at low tides of spring tide, and the surfaces of the stones were brushed in the sea water that had been filtered through GF/F filters. The brushed material from the biofilm was passed through a 125- μm sieve and then filtered onto GF/F filters for the isotopic analyses of EOM of 0.7–125 μm . This EOM collected at the sublittoral fringe consisted mainly of diatoms and detritus (Takai unpubl. data). Using the same procedure, we also analyzed the green organic matter (0.7–125 μm) attached to the shells

Table 1. The carbon (upper) and nitrogen (lower) stable isotope ratios (‰) of POM, mysids (*Acanthomysis tenuicauda* and *Acanthomysis* spp.), copepods, and SOM collected on 14 May 1999. The seasonal means of POM in the surface layer of area W from 14 May 1999 to 10 Nov 2000 and EOM at the sublittoral fringe of Sta. Z from 10 Aug 1999 to 12 Oct 2000 are also shown.

Location	POM*			Mysids†	Copepods†	SOM	EOM 0.7–125 μm
	0.7–125 μm	125–350 μm	>350 μm				
14 May 1999							
H1‡	-22.3 9.5	-22.0 10.7	—			-22.2 7.2	
H2‡	-21.3 9.2	—	—			-20.7 7.7	
H3	-21.9 8.5	-22.6 10.6	-22.0 10.4			-20.6 7.4	
H4	-22.2 8.2	-22.6 9.3	-22.1 10.2	-21.0 8.4	-20.8 8.5	-20.2§ 6.9	
The seasonal mean (±SD)							
W	-20.1±1.7 8.3±1.3 (n = 10)						
Z							-15.4±1.8 8.4±1.4 (n = 7)

* The POMs of 0.7–125 μm were collected from the sea surface. The POMs of 125–350 μm and >350 μm were collected from the depth layer of 0–20 m with a plankton net.

† These were extracted from the stomach of Japanese horse mackerel *Trachurus japonicus* and were defatted.

‡ Dash indicates no data.

§ The SOM at Sta. H4 was collected on 27 Oct 1998.

of Japanese oysters that were collected from the sea bottom (Sta. H3) and the oyster raft (Sta. X).

The surface sedimentary organic matter (SOM) was collected with a bottom sampler at Stas. H1–H3 on 14 May 1999. At Sta. H4, additional sediment was collected on 27 October 1998 and analyzed for isotope ratios, since the sample collection at this station at the trawling date was unsuccessful.

Stable isotope analyses—The samples were stored at -20°C. We analyzed the muscle tissues of fish, bivalves, cephalopods, large decapods, a starfish, holothurians, and an ascidian. The muscles were excised from the trunk behind a pectoral fin in fish, from the adductor muscle in the bivalves, from the mantle in the cephalopods, and from the tube-foots in the starfish. The isotopic ratios of small crustaceans were analyzed for mixed individuals except *Melita rylovae*, *Cleantiella isopus*, and *Heptacarpus futirostris*. The animal tissues were dried at 60°C and ground to a fine powder and lipids were removed with a chloroform:methanol (2:1) solution. The POM and EOM samples were exposed to the vapor of concentrated HCl for a day in order to eliminate carbonates and then were dried in a vacuum desiccator. The SOM samples were saturated with 1 M HCl solution for a day in order to eliminate carbonates and then were dried on a hot plate. We analyzed one sample from each sampling date for POM, EOM, SOM, and the shell-attached organic matter.

Stable isotope ratios of carbon and nitrogen were measured with a MAT 252 mass spectrometer (Finnigan MAT) coupled with an element analyzer (Carlo Erba). Isotope ra-

tios, $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$, are expressed as per mil deviations from the standard as defined by the following equation:

$$\delta^{13}\text{C}, \delta^{15}\text{N} = [R_{\text{sample}}/R_{\text{standard}} - 1] \times 1,000 \quad (\text{‰})$$

where $R = {}^{13}\text{C}/{}^{12}\text{C}$ or ${}^{15}\text{N}/{}^{14}\text{N}$. Belemnite (PDB) and atmospheric nitrogen were used as the isotope standards of carbon and nitrogen, respectively. The analytical precision for the isotopic analyses was $\leq 0.28\text{‰}$ for both $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$.

Results

Stable isotopic distribution of EOM, POM, and SOM—The $\delta^{13}\text{C}$ of EOM (0.7–125 μm) at the sublittoral fringe of the island shore (Sta. Z) averaged $-15.4 \pm 1.8\text{‰}$ (±SD) from August 1999 to October 2000 ($n = 7$) (Table 1, Fig. 2). Likewise the $\delta^{13}\text{C}$ of the organic matter (0.7–125 μm) attached to the shells of the Japanese oysters from the sea bottom (10–30 m in depth) had enriched $\delta^{13}\text{C}$ values of -13.9‰ and those from the oyster raft in the surface layer had enrichments of -15.6‰ (Fig. 2). These values were clearly more enriched relative to the average $\delta^{13}\text{C}$ of POM (0.7–125 μm) collected in the surface layer of the central bay (area W) from May 1999 to November 2000 ($n = 10$, $-20.1 \pm 1.7\text{‰}$; Table 1, Fig. 2). This $\delta^{13}\text{C}$ difference between attached organic matter and POM was consistent with the general characteristics of the benthic-planktonic difference in isotopic ratios compiled by France (1995).

The $\delta^{15}\text{N}$ of EOM (0.7–125 μm) at Sta. Z averaged $8.4 \pm 1.4\text{‰}$ ($n = 7$), which is very similar to the average $\delta^{15}\text{N}$ of $8.3 \pm 1.3\text{‰}$ ($n = 10$) in offshore POM (0.7–125 μm) in area W (Table 1; Fig. 2). The organic matter attached to

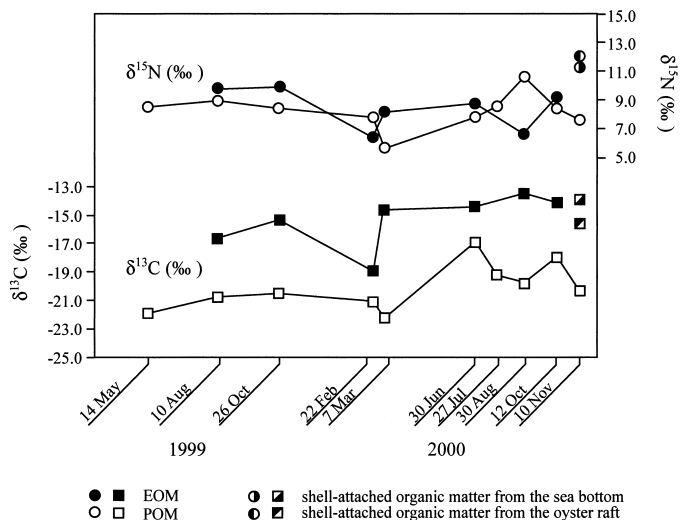


Fig. 2. The carbon (square) and nitrogen (circle) stable isotope ratios (‰) of POM (0.7–125 μm) in the surface layer in area W and of EOM (0.7–125 μm) at the sublittoral fringe of Sta. Z. The values of shell-attached organic matter are also shown for Japanese oysters *Crassostrea gigas* from the sea bottom of Sta. H3 and the oyster raft of Sta. X on 10 November 2000.

shells (0.7–125 μm) was slightly enriched in $\delta^{15}\text{N}$ with 11.3–12.0‰ relative to the EOM and POM (Fig. 2).

On 14 May 1999, the local differences and the particle-size-related differences in the POM isotopic values were examined (Table 1). Irrespective of the sampling station and particle size, the POMs had depleted $\delta^{13}\text{C}$ ranging from -22.6 to -21.3‰. These $\delta^{13}\text{C}$ values were very similar to the $\delta^{13}\text{C}$ of -21.0 to -20.8‰ in the mysids (*Acanthomysis tenuicauda* and *Acanthomysis* spp.) and the copepods extracted from the stomach of the Japanese horse mackerel at Sta. H4. On the other hand, the $\delta^{15}\text{N}$ of POM clearly showed both local and particle-size-related differences (Table 1). The $\delta^{15}\text{N}$ of POM >125 μm was more enriched (9.3–10.7‰) relative to the POMs of 0.7–125 μm (8.2–9.5‰), and the $\delta^{15}\text{N}$ of POM increased northward from 8.2‰ (Sta. H4) to 9.5‰ (Sta. H1) in 0.7–125- μm particles and from 9.3‰ (Sta. H4) to 10.7‰ (Sta. H1) in 125–350- μm particles. The $\delta^{15}\text{N}$ values of 8.4–8.5‰ in the mysids and copepods were similar to the $\delta^{15}\text{N}$ of 0.7–125 μm POM.

The $\delta^{13}\text{C}$ of the SOM ranged from -22.2 to -20.2‰ and was similar to the values of the POM (Table 1). The SOM from the mouth of the Ohta River was slightly depleted in $\delta^{13}\text{C}$ (-22.2‰, Sta. H1). The $\delta^{15}\text{N}$ variation in the SOM was small, ranging from 6.9 to 7.7‰.

Stable isotope ratios of fish in the island shore—The $\delta^{13}\text{C}$ of fish in the island shore (Sta. Z) ranged from -18.6‰ in black rockfish *Sebastes inermis* to -13.8‰ in motleystripe rainbowfish *Halichoeres tenuispinnis* (Table 2). The fish $\delta^{13}\text{C}$ was distributed from -17.0 to -13.0‰ in 166 (88%) of the 189 samples and was similar to the $\delta^{13}\text{C}$ values of -17.3 to -12.5‰ in the small benthic crustaceans that are thought to be the principal prey for these fish (Table 3). The $\delta^{13}\text{C}$ values derived from benthic primary production from EOM and the shell-attached organic matter overlapped those of most of

the demersal fishes, whereas the depleted signatures of offshore POM did not (Table 1; Fig. 2).

The $\delta^{15}\text{N}$ of the fish averaged 14.6 ± 0.5 ‰, ranging from 13.1‰ in black rockfish to 15.8‰ in spottilybelly greenling *Hexagrammos agrammus* (Table 2). These values were much more enriched than those of the EOM (6.5–9.9‰) and of the small benthic crustacean (6.7–11.8‰; Tables 1, 3).

The young-of-the-year black rockfish sampled in June showed clearly depleted isotopic values for both $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ (-18.6 to -17.0‰ in $\delta^{13}\text{C}$ and 13.1 to 14.4‰ in $\delta^{15}\text{N}$; Fig. 3). The $\delta^{15}\text{N}$ of the rockfish increased linearly with length, consistent with size-related $\delta^{15}\text{N}$ changes reported for carnivorous fish species (Takai and Sakamoto 1999), while the $\delta^{13}\text{C}$ increased in a stepwise manner for fish between 62 and 66 mm. There were significant correlations between the fish length and the stable isotope ratios ($n = 52$; $\delta^{13}\text{C}$, $r = 0.74$, $P < 0.0001$; $\delta^{15}\text{N}$, $r = 0.85$, $P < 0.0001$).

Stable isotope ratios of fish in the deeper area—The $\delta^{13}\text{C}$ of fish in the deeper area (Stas. Y, H1, H2, H3, and H4) ranged from -19.5‰ in Japanese anchovy *Engraulis japonicus* (Sta. Y) to -12.6‰ in surf fish *Ditrema temminckii* (Sta. Y) (Table 4). The fish $\delta^{13}\text{C}$ was distributed from -17.0 to -13.0‰ in 203 (96%) of the 212 samples and was very similar to the $\delta^{13}\text{C}$ distribution of the fish at Sta. Z (Table 2). Likewise, benthic invertebrates in the deeper area mostly had enriched $\delta^{13}\text{C}$ values of -17.0 to -13.0‰ (Table 5). In particular, the Japanese oysters showed enriched $\delta^{13}\text{C}$ values of -15.7 to -14.9‰, in contrast to the $\delta^{13}\text{C}$ distribution of other filter-feeding benthos, the egg cockles *Fulvia mutica*, and the ascidian *Styela plicata*.

The $\delta^{15}\text{N}$ of fish in the deeper area ranged from 7.4‰ in Japanese anchovy (Sta. Y) to 17.5‰ in cutlassfish *Trichiurus japonicus* (Sta. H4) (Table 4), showing much more variability relative to the fish in the littoral areas (Sta. Z) (Table 2). The $\delta^{15}\text{N}$ distribution of benthic invertebrates in the deeper area also had highly variable $\delta^{15}\text{N}$ distributions, with values ranging from 9.5‰ in Japanese trepang *Apostichopus japonicus* (Sta. H2) to 17.9‰ in Japanese squid *Loliolus japonica* (Sta. H2) (Table 5).

In two fish species, cardinal fish *Apogon lineatus* and young-of-the-year gurnard *Lepidotrigla microptera* (48–76 mm), clear local variations in $\delta^{15}\text{N}$ were found (Fig. 4). The $\delta^{15}\text{N}$ of these fishes was significantly more enriched in the northern bay than in the central bay. The $\delta^{15}\text{N}$ of the cardinal fish was 16.7 ± 0.4 ‰ ($n = 4$) at Sta. H1 and 15.0 ± 0.3 ‰ ($n = 41$) at Stas. H3 and H4 (Mann-Whitney's U -test; $P = 0.001$). The $\delta^{15}\text{N}$ of the young gurnard was 15.7 ± 0.3 ‰ ($n = 17$) at Sta. H1 and 14.6 ± 0.4 ‰ ($n = 9$) at Sta. H4 (Mann-Whitney's U -test; $P < 0.0001$).

Four large Japanese anchovies ≥ 125 mm showed peculiarly depleted isotopic values in both $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ (Fig. 5). These anchovies ranged from -19.5 to -18.1‰ for $\delta^{13}\text{C}$ and from 7.4 to 9.4‰ for $\delta^{15}\text{N}$ and were in contrast to the enrichments of anchovies ≤ 120 mm with -17.2 to -13.1‰ for $\delta^{13}\text{C}$ and 12.7 to 16.6‰ for $\delta^{15}\text{N}$.

The $\delta^{13}\text{C}$ - $\delta^{15}\text{N}$ diagram for the Hiroshima Bay food web—The fish analyzed in this study were divided into three distinct groups on the $\delta^{13}\text{C}$ - $\delta^{15}\text{N}$ diagram (Fig. 6). One group

Table 2. The carbon (upper) and nitrogen (lower) stable isotope ratios (‰; mean \pm SD) of fish captured with a throw net in the island shore (Sta. Z) in Hiroshima Bay. The sample number (left) and standard length (mm; right) are shown in parentheses.

Species	15 Jun 99	10 Aug 99	26 Oct 99	22 Feb 00	7 Mar 00	30 Jun 00
Perciformes						
Surf fish						
<i>Ditrema temmincki</i>	-14.9 15.0 (1; 52)				-14.1 15.2 (1; 123)	-17.3 \pm 0.1 13.8 \pm 0.2 (5; 62-69)
Richardson's dragonet						
<i>Repomucenus richardsonii</i>	-15.5 \pm 0.3 13.9 \pm 0.4 (3; 62-80)					
Motleystripe rainbowfish						
<i>Halichoeres tenuispinnis</i>	-14.7 \pm 0.5 14.8 \pm 0.3 (20; 50-90)	-14.8 \pm 0.4 14.3 \pm 0.2 (10; 59-102)	-15.5 \pm 0.6 14.2 \pm 0.3 (8; 54-94)			-15.3 \pm 0.3 14.3 \pm 0.2 (10; 75-84)
Pudding wife						
<i>Halichoeres poecilopterus</i>	-14.9 \pm 0.4 15.0 \pm 0.3 (13; 54-112)	-15.5 \pm 0.3 14.5 \pm 0.3 (4; 58-99)	-14.1 14.2 (1; 83)			-15.6 14.6 (1; 120)
Scorpaeniformes						
Spottybelly greenling						
<i>Hexagrammos agrammus</i>	-15.0 \pm 1.1 15.1 \pm 0.7 (3; 66-146)	-15.5 \pm 0.2 14.5 \pm 0.2 (6; 81-102)	-15.9 \pm 0.3 14.6 \pm 0.2 (3; 90-141)	-14.5 \pm 0.1 14.7 \pm 0.6 (6; 43-135)	-14.5 \pm 0.1 14.9 \pm 0.9 (3; 52-136)	
Tiny stinger						
<i>Hypodytes rubripinnis</i>	-15.2 \pm 0.7 14.4 \pm 0.5 (8; 35-70)		-14.8 \pm 0.2 14.7 \pm 0.4 (10; 50-59)	-14.0 15.3 (1; 63)	-14.4 14.6 (1; 52)	-14.8 \pm 0.2 14.8 \pm 0.2 (7; 44-66)
Black rockfish						
<i>Sebastes inermis</i> (62 mm \geq SL)	-17.7 \pm 0.7 14.1 \pm 0.2 (6; 45-53)		-17.1, -16.8 13.8, 14.1 (2; 51, 55)			-18.0 \pm 0.3 13.7 \pm 0.4 (11; 41-62)
Black rockfish						
<i>Sebastes inermis</i> (66 mm \leq SL)			-15.6 \pm 0.3 15.2 \pm 0.2 (10; 107-127)	-15.2 \pm 0.3 14.8 \pm 0.3 (14; 66-115)	-14.9 \pm 0.2 15.0 \pm 0.2 (5; 78-118)	-15.9, -15.2 14.9, 15.2 (2; 91, 122)
Tetraodontiformes						
Net-work filefish						
<i>Rudarius ercodes</i>	-16.0 \pm 0.9 14.7 \pm 0.5 (4; 33-39)	-15.9 \pm 0.3 14.6 \pm 0.3 (10; 38-44)				

(A) consisted of only young-of-the-year black rockfish at Sta. Z in June. These fish were distributed in the range from -18.6 to -17.0‰ in $\delta^{13}\text{C}$ and from 13.1 to 14.4‰ in $\delta^{15}\text{N}$. Group B consisted only of the large Japanese anchovies ≥ 125 mm. Both $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ in these fish were strikingly depleted, with -19.5 to -18.1‰ in $\delta^{13}\text{C}$ and from 7.4 to 9.4‰ in $\delta^{15}\text{N}$. Group C includes all the other fish collected in both the island shore and deeper area. The isotopic ratios of these fish were mostly distributed from -17.0 to -13.0‰ in $\delta^{13}\text{C}$ and from 13.0 to 18.0‰ in $\delta^{15}\text{N}$.

Discussion

Carbon source and trophic position of fish dependent on water column primary production—All the young-of-the-year black rockfish at Sta. Z in June showed peculiarly de-

pleted $\delta^{13}\text{C}$ values ranging from -18.6 to -17.0‰ in both 1999 and 2000, in contrast to enriched values of -16.0 to -14.6‰ in the larger rockfish ≥ 66 mm (Table 2, Fig. 3). Stomach content analysis has shown that the rockfish feed mainly on copepods at the planktonic larval stage in winter and on benthic crustaceans after the fish settle in spring (Harada 1962). This indicates that the ^{13}C depletion of planktonic rockfish does reflect their diet of zooplankton and the $\delta^{13}\text{C}$ isotopic ratios of -20.1 \pm 1.7‰ of water column primary producers. We assumed that the rockfish would change their carbon source from water column primary production to benthic primary production with their life history change in feeding habit. However, the two young-of-the-year black rockfish collected at Sta. H1 on 14 May 1999 showed enriched isotopic values of -16.4 to -15.3‰ in $\delta^{13}\text{C}$ and 14.4 to 14.7‰ in $\delta^{15}\text{N}$ (Table 4, Fig. 3). These young inhabiting

Table 3. The carbon (left) and nitrogen (right) stable isotope ratios (‰) of crustaceans in the island shore (Sta. Z) in Hiroshima Bay. A mixture of some samples were analyzed for the crustaceans. The mixed sample number (left) and body length (mm; right) are shown in parentheses.

Species	10 Aug 99	26 Oct 99	22 Feb 00	7 Mar 00	30 Jun 00	30 Aug 00	12 Oct 00
Amphipoda							
Gammaridea							
<i>Ampithoe lacertosa</i>	-15.7; 6.7 (8; 5.5–13.2)				-17.3; 7.4 (10; 11.6–18.8)	-13.5; 7.5 (10; 11.4–14.0)	
<i>Pontogeneia rostrata</i>					-16.7; 7.9 (20; 3.2–5.1)		
<i>Melita koreana</i>		-13.1; 9.2 (20; 5.8–8.2)				-14.2; 8.1 (10; 4.5–7.0)	-12.5; 8.1 (10; 5.0–7.5)
<i>Melita rylovae</i>						-14.6; 7.7 (1; 9.0)	
<i>Hyale</i> sp. 1			-14.2; 8.6 (10; 7.8–11.4)	-14.0; 8.7 (10; 10.8–12.0)			
<i>Hyale</i> sp. 2			-15.0; 8.5 (7; 6.2–9.5)	-14.7; 8.3 (10; 8.0–9.7)			
<i>Podocerus</i> sp.					-16.8; 8.8 (19; 2.0–6.5)		
Caprellidea							
<i>Caprella penantis</i>				-15.2; 9.8 (10; 5.9–9.0)			
Isopoda							
<i>Cleantiella isopus</i>							-13.5; 9.5 (1; 16.9)
Decapoda							
<i>Heptacarpus futilirostris</i>						-15.8; 11.8 (1; 12.4)	
<i>Eualus sinensis</i>					-16.9; 11.5 (2; 6.0, 10.0)	-15.7; 11.4 (2; 8.4, 10.0)	

the deeper area might have settled the bottom and changed their feeding habit much earlier relative to the young in the shore.

Depleted $\delta^{13}\text{C}$ values of -19.0 to -17.0 ‰ were also found for two kinds of filter-feeding benthos, egg cockles (Sta. H4) and an ascidian (Sta. H1), ranging from -18.2 to -17.7 ‰ (Table 5). As expected, these benthic filter feeders appear to depend on water column primary production.

The $\delta^{15}\text{N}$ of the young-of-the-year black rockfish in June averaged 13.8 ± 0.4 ‰ (range: 13.1 to 14.4 ‰; $n = 17$; Table 2, Fig. 3). This average value was 3 – 4 ‰ more enriched than the POMs of >350 and 125 – 350 μm , and 5 – 6 ‰ more enriched than the POMs of 0.7 – 125 μm , mysids, or copepods from the central bay (Stas. H3 and H4). Considering that the $\delta^{15}\text{N}$ increase per trophic level in the shallow water near Japan was reported to be 3.4 ± 1.1 ‰ (East China Sea; Minagawa and Wada 1984), the ^{15}N -depleted POMs (0.7 – 125 μm), mysids, and copepods are too depleted in $\delta^{15}\text{N}$ to be the main food source for the young rockfish. It is likely that some kinds of zooplankton composing the POMs >125 μm would be the main food source for the young rockfish.

The $\delta^{15}\text{N}$ of 13.4 ‰ in the ascidian collected at Sta. H1 was very similar to the values of the young rockfish, and thus it appears as if their trophic positions were similar (Table 5). However, the ascidian likely feeds on slightly smaller components in POMs than the young rockfish, since the $\delta^{15}\text{N}$

differences between the ascidian and POMs at Sta. H1 were smaller: 3.9 ‰ for 0.7 – 125 - μm particles and 2.7 ‰ for 125 – 350 - μm particles relative to the differences in the young rockfish. The ascidian may feed not only on zooplankton, but also on phytoplankton.

The egg cockles at Sta. H4 showed peculiarly depleted $\delta^{15}\text{N}$ values of 10.0 ± 0.1 ‰ (range: 9.8 to 10.1 ‰; $n = 4$) in contrast to the young rockfish and the ascidian (Table 5). These $\delta^{15}\text{N}$ values were as depleted as the value (10.2 ‰) of POM (>350 μm) at Sta. H4 (Table 1). Consequently, they likely feed on ^{15}N -depleted components of POMs, such as phytoplankton.

Carbon source and trophic position of migrators from the open sea—Japanese anchovies ≥ 125 mm showed a peculiar isotopic distribution (Table 4, Fig. 5). The 20 anchovies ≤ 120 mm had mean $\delta^{13}\text{C}$ signatures of -15.5 ± 1.2 ‰ and mean $\delta^{15}\text{N}$ signatures of 14.4 ± 1.0 ‰, similar to the adult anchovy in the Sagami Bay (Pacific side of the central Japan) in October 1993 (-17 to -15 ‰ for $\delta^{13}\text{C}$ and 11 to 14 ‰ for $\delta^{15}\text{N}$; Lindsay et al. 1998), while the four anchovies ≥ 125 mm had averages of -18.8 ± 0.6 ‰ in $\delta^{13}\text{C}$ and 8.5 ± 0.8 ‰ in $\delta^{15}\text{N}$. The latter were similar to the anchovies captured in the western North Pacific Ocean off Japan (-20 to -18 ‰ in $\delta^{13}\text{C}$ and 7 to 10 ‰ in $\delta^{15}\text{N}$; Mitani 2000). Considering that most populations of the anchovy are supposed

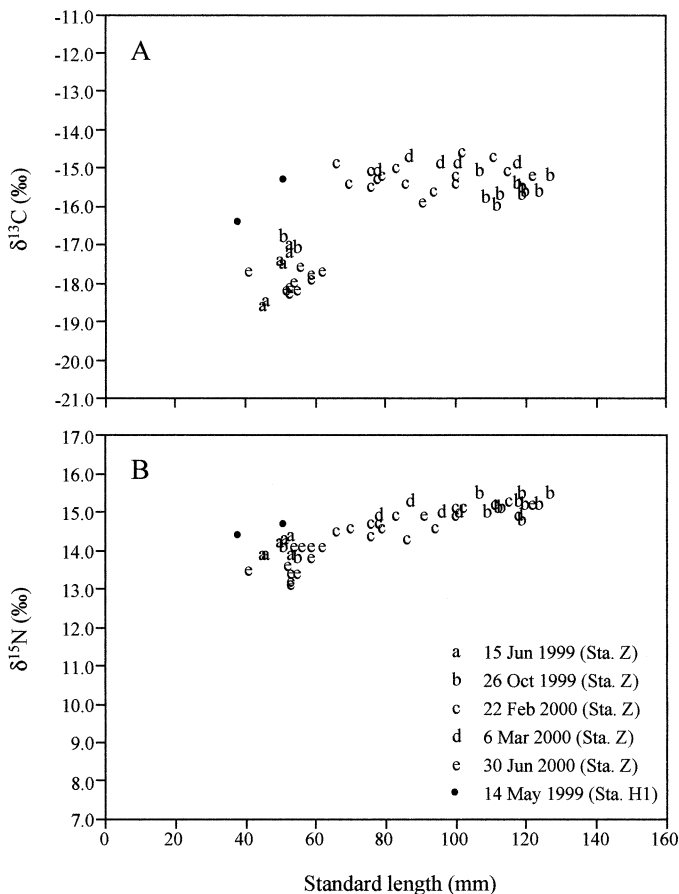


Fig. 3. The relationship between standard length and stable isotope ratios in black rockfish *Sebastes inermis* collected at Stas. Z and H1 ($n = 52$). (A) $\delta^{13}\text{C}$. (B) $\delta^{15}\text{N}$.

to migrate into the Seto Inland Sea from the open sea in spring and leave there in winter (Takao 1985), the extremely depleted $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of the anchovies in April are interpreted as feeding records of the pelagic diets in the open sea during the winter. This means that the isotopic values of these ^{13}C -depleted anchovies would reflect an oceanic water column carbon supply.

Japanese anchovies have generally been assumed to feed primarily on copepods, larvae, and diatoms (Kondo 1971), but the stomach content analysis of anchovies captured in tidelands of the western Seto Inland Sea at flood tide showed that they feed not only on plankton but also on large numbers of benthic animals including decapods, mysids, amphipods, and polychaetes (Zinnouchi 1977). It is thus likely that diverse feeding of the anchovies during their stay in the Seto Inland Sea increased both their $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ signatures.

Carbon source and trophic position of fish depending on the benthic primary production—The $\delta^{13}\text{C}$ signatures of most fish and small crustaceans collected from both the shore and the deeper area were distributed from -17.0 to -13.0 ‰ (Tables 2, 3, 4, Fig. 6). Although small benthic crustaceans were not collected in the deeper area in Hiroshima Bay, Yamaguchi (2000) found similarly enriched $\delta^{13}\text{C}$ values of amphipods, isopods, and decapods in samples col-

lected from temperate coastal waters <10 m of the Japan Sea. Accordingly, the enriched $\delta^{13}\text{C}$ values of the small benthic crustaceans were inferred to be ubiquitous not only at the sublittoral fringe but also in the deeper area (up to at least 10 m in depth).

These ^{13}C -enriched fish and small benthic crustaceans overlapped the $\delta^{13}\text{C}$ signatures of epilithic and shell-attached organic matter but not the depleted signatures of planktonic POM (Table 1, Fig. 2). Table 6 shows reference data of the isotopic ratios of macroalgae (nondefatted; Takai et al. 2001) and seagrasses *Zostera marina* (nondefatted; Takai unpubl. data). The $\delta^{13}\text{C}$ signatures of the seagrasses were clearly more enriched than the values of the animals, while the $\delta^{13}\text{C}$ distribution of the macroalgae overlapped with that of the ^{13}C -enriched animals.

Based on the overlapping $\delta^{13}\text{C}$ distributions of the macroalgae, epilithic organic matter, and the ^{13}C -enriched animals, it appears that the organisms receive a substantial carbon supply from the benthic primary producers. Quantitatively important carbon transport from microphytobenthos to macrobenthic animals was recently confirmed in a tidal flat ecosystem with a stable isotope tracer-addition experiment and by analyzing natural isotope abundances (Herman et al. 2000; Middelburg et al. 2000). The results in our study indicate that such benthic carbon transport would be important not only in the shore ecosystem but also in the 10–30 m depth strata.

Similarly enriched $\delta^{13}\text{C}$ values were also found for most benthic invertebrates in the deeper area of Hiroshima Bay, which suggests substantial carbon supply from the benthic primary production (Table 5, Fig. 6). In particular, all the Japanese oysters showed enriched $\delta^{13}\text{C}$ values of -15.7 to -14.9 ‰, in contrast to the $\delta^{13}\text{C}$ distribution of the other filter-feeding benthos, the egg cockles, and the ascidian. According to Riera and Richard (1996), oysters in an estuarine bay in France were also enriched in $\delta^{13}\text{C}$, which suggests that they were feeding on microphytobenthos from an adjacent wide mudflat with signatures of about -16 ‰. It is likely that the enriched $\delta^{13}\text{C}$ values of the oysters in Hiroshima Bay and the estuarine oysters in France indicate that they are feeding on microphytobenthos. Here we need to pay attention to the enriched $\delta^{13}\text{C}$ values of -15.2 ± 0.3 ‰ in the oysters from the raft at Sta. X (Table 5). Since the water depth at Sta. X was about 30 m, it is unlikely that the oysters hanging in the upper 6 m of the water column would feed on the microalgae inhabiting the sea bottom. We suggest that the enriched $\delta^{13}\text{C}$ values of those oysters might be derived from the shell-attached organic matter, which was similarly enriched (-15.6 ‰) (Table 5).

The average $\delta^{15}\text{N}$ (11.6 ± 0.2 ‰) of the small decapods at Sta. Z was 3.2‰ more enriched than the average of the EOM (8.4 ± 1.4 ‰), 3.0‰ more enriched than that of macroalgal Chlorophyceae (8.6 ± 0.5 ‰; Takai et al. 2001), and 3.1‰ more enriched than that of Phaeophyceae (8.5 ± 1.0 ‰; Takai et al. 2001) (Table 3, Fig. 6). These differences were consistent with the $\delta^{15}\text{N}$ increase per trophic level in the East China Sea (3.4 ± 1.1 ‰; Minagawa and Wada 1984), and accordingly the small decapods were estimated to be typical primary consumers dependent on the organic matter originating from the benthic primary producers.

Table 4. The carbon (upper) and nitrogen (lower) stable isotope ratios (‰; mean \pm SD) of fish captured with a boat seine (Sta. Y) and a bottom trawl (Stas. H1–H4) in Hiroshima Bay. The sample number (left) and standard length (mm; right) are shown in parentheses.

Species	21 Oct 1997	16 Apr 1998	14 May 1999			
	Y	Y	H1	H2	H3	H4
Clupeiformes						
Gizzard shad <i>Konosirus punctatus</i>		–15.1 14.7 (1; 230)	–14.5 \pm 0.2 15.3 \pm 0.5 (7; 152–228)	–14.6 \pm 0.1 14.6 \pm 0.7 (3; 220–230)	–14.7 \pm 0.8 14.3 \pm 0.5 (11; 207–232)	–14.5 \pm 0.3 14.8 \pm 0.7 (20; 203–238)
Japanese anchovy <i>Engraulis japonicus</i> (120 mm \geq SL)	–14.3 \pm 0.9 14.4 \pm 0.7 (8; 110–120)	–16.4, –15.3 12.7, 15.0 (2; 110, 115)	–16.3 \pm 0.5 14.8 \pm 1.1 (6; 69–91)	–16.4 \pm 0.7 14.0 \pm 1.2 (4; 74–114)		
Japanese anchovy <i>Engraulis japonicus</i> (125 mm \leq SL)		–18.8 \pm 0.6 8.5 \pm 0.8 (4; 125–140)				
Perciformes						
Cardinal fish <i>Apogon lineatus</i>			–14.8 \pm 0.1 16.7 \pm 0.4 (4; 43–53)		–15.1 15.2 (1; 46)	–15.8 \pm 0.3 15.0 \pm 0.3 (40; 32–56)
Japanese horse mackerel <i>Trachurus japonicus</i>	–15.3, –14.4 16.0, 16.0 (2; 160, 180)					–15.3 \pm 0.1 15.1 \pm 0.0 (3; 150–155)
Ponyfish <i>Leiognathus nuchalis</i>			–15.4 \pm 0.3 15.8 \pm 0.4 (20; 78–117)	–15.2 \pm 0.4 16.1 \pm 0.6 (20; 78–115)		
Surf fish <i>Ditrema temmincki</i>		–12.6 16.7 (1; 200)	–14.7 \pm 0.1 14.6 \pm 0.1 (3; 152–180)			
Cutlassfish <i>Trichiurus japonicus</i>						–14.6 17.5 (1; 700)*
Scorpaeniformes						
Black rockfish <i>Sebastes inermis</i>			–16.4, –15.3 14.4, 14.7 (2; 38, 51)			
Gurnard <i>Lepidotrigla microptera</i> (76 mm \geq SL)			–15.0 \pm 0.1 15.7 \pm 0.3 (17; 56–76)			–16.6 \pm 0.4 –14.6 \pm 0.4 (9; 48–68)
Gurnard <i>Lepidotrigla microptera</i> (141 mm \leq SL)			–14.7, –14.3 16.6, 16.8 (2; 152, 164)	–14.2 \pm 0.4 16.3 \pm 1.1 (4; 141–198)	–14.3, –14.0 15.8, 17.3 (2; 142, 157)	–14.3 15.6 (1; 190)
Tetraodontiformes						
Net-work filefish <i>Rudarius ercodes</i>			–15.0 \pm 0.5 15.1 \pm 0.6 (6; 31–48)			
Finepatterned puffer <i>Takifugu poecilonotus</i>			–15.0 \pm 0.9 14.4 \pm 0.3 (7; 59–85)			–14.3 15.8 (1; 84)

* The total length was measured for *Trichiurus japonicus*.

Table 5. The carbon and nitrogen stable isotope ratios (‰; mean \pm SD) of benthic invertebrates in Hiroshima Bay. The isotopic values of shell-attached organic matter (0.7–125 μ m) from Japanese oysters are also shown in parentheses.

Species	Sampling date	Station	Depth (m)	n	$\delta^{13}\text{C}$	$\delta^{15}\text{N}$
Mollusca						
Bivalvia						
Japanese oyster <i>Crassostrea gigas</i>						
Shore	22 Feb 00	Z	—	13	-15.4 ± 0.2	11.0 ± 0.3
Sea bottom	10 Nov 00	H3	10–30	2	$-15.6, -15.4$ [-13.9]	12.6, 12.9 [11.3]
Oyster raft	10 Nov 00	X	0–6	5	-15.2 ± 0.3 [-15.6]	12.9 ± 0.2 [12.0]
Egg cockle <i>Fulvia mutica</i>	14 May 99	H4	10–30	4	-17.9 ± 0.2	10.0 ± 0.1
Cephalopoda						
Kobi cuttlefish <i>Sepia kobeensis</i>	14 May 99	H1	10–30	1	-13.7	14.3
Golden cuttlefish <i>Sepia esculenta</i>	14 May 99	H4	10–30	1	-13.6	14.8
Japanese squid <i>Loliolus japonica</i>	14 May 99	H2	10–30	2	$-14.3, -14.1$	16.8, 17.9
Long-armed octopus <i>Octopus minor</i>	14 May 99	H4	10–30	1	-14.4	15.2
Arthropoda						
Crustacea (Decapoda)						
Mantis shrimp <i>Oratosquilla oratoria</i>	14 May 99	H1	10–30	1	-14.7	15.8
<i>Myra fugax</i> (Leucosiidae)	14 May 99	H1	10–30	1	-14.2	16.0
Echinodermata						
Asteroidea						
Starfish <i>Asterias amurensis</i>	14 May 99	H2	10–30	1	-14.0	14.1
Holothuroidea						
Japanese trepang <i>Apostichopus japonicus</i>	14 May 99	H2	10–30	5	-15.7 ± 0.4	10.2 ± 0.5
	14 May 99	H4	10–30	1	-14.9	11.7
Chordata						
Ascidiacea						
<i>Styela plicata</i> (Styelidae)	14 May 99	H1	10–30	1	-18.2	13.4

By contrast, the average $\delta^{15}\text{N}$ ($8.2 \pm 0.8\text{‰}$) of the amphipods was 3.4‰ more depleted than the $\delta^{15}\text{N}$ of the small decapods, being as depleted as the $\delta^{15}\text{N}$ of primary producers such as macroalgae and EOM (0.7–125 μ m) (Table 3, Fig. 6). This difference of $\delta^{15}\text{N}$ between small decapods and amphipods may be related to the $\delta^{15}\text{N}$ variation in microalgae inhabiting the shore. The $\delta^{15}\text{N}$ of EOM collected at Sta. Z

on 22 December 2000 was 9.8‰ in the size fraction of 15–32 μ m at the sublittoral fringe where Bacillariophyceae was predominant, while the $\delta^{15}\text{N}$ was 3.8‰ in the fraction of 63–125 μ m at the littoral fringe (high shore) where Cyanophyceae was predominant (Takai unpubl. data). This suggests that the amphipods may use the ^{15}N -depleted organic matter produced at the littoral fringe.

The $\delta^{15}\text{N}$ of the fish at Sta. Z (except the young black rockfish) ranged from 13.4 to 15.8‰ and was 1.8–4.2‰ more enriched than the average $\delta^{15}\text{N}$ of $11.6 \pm 0.2\text{‰}$ in the decapods at Sta. Z (Fig. 6). This difference was consistent with the $\delta^{15}\text{N}$ increase of $3.4 \pm 1.1\text{‰}$ per trophic level (Minagawa and Wada 1984), thus suggesting that a large portion of the fish collected at Sta. Z would be secondary consumers in a food chain based on benthic primary producers. Likewise the $\delta^{15}\text{N}$ of 93% of the 89 fish collected in the deeper area of the central bay (Stas. H3 and H4) was distributed from 14.0 to 16.0‰. This suggests that the fish in the deeper area would also be mostly secondary consumers dependent on the benthic primary producers. Here it is necessary to pay attention to potential mixed feeding on prey from different trophic positions. It appears that such complexity in feeding links varied the $\delta^{15}\text{N}$ values of the fish and as a consequence distributed the $\delta^{15}\text{N}$ continuously in the range from 14.0 to 16.0‰ (Tables 2, 4).

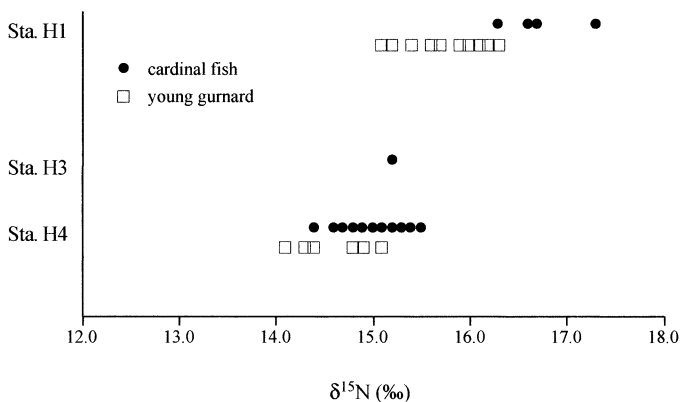


Fig. 4. The local differences of $\delta^{15}\text{N}$ in cardinal fish *Apogon lineatus* ($n = 45$) and young-of-the-year gurnard *Lepidotrigla microptera* (48–76 mm; $n = 26$).

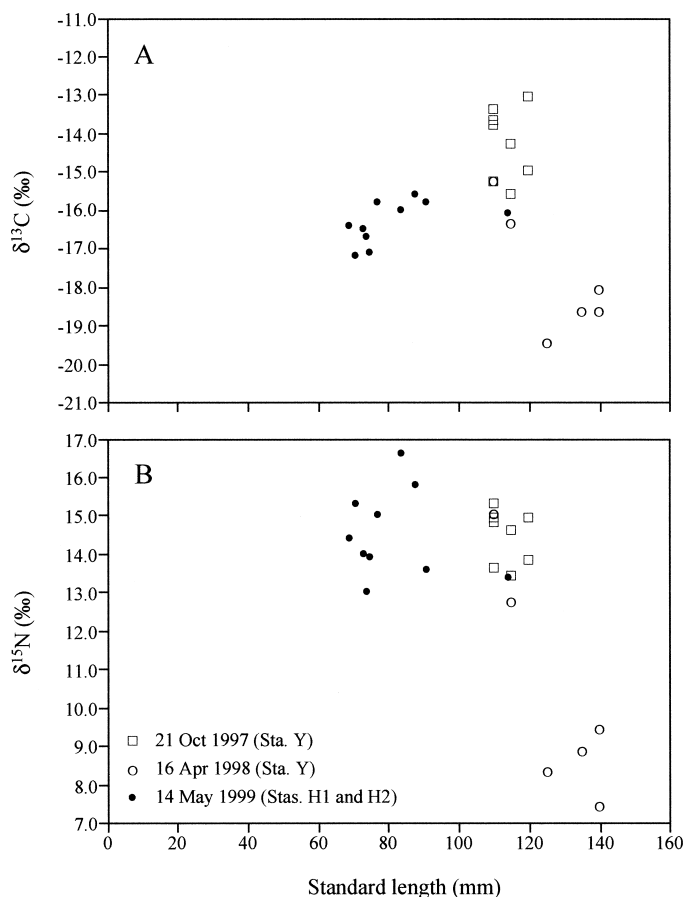


Fig. 5. The relationship between standard length and stable isotope ratios in Japanese anchovy *Engraulis japonicus* collected at Stas. Y, H1, and H2 ($n = 24$). (A) $\delta^{13}\text{C}$. (B) $\delta^{15}\text{N}$.

The maximum $\delta^{15}\text{N}$ value among the fish was found in the cutlassfish of 17.5‰ at Sta. H4 (Table 4). Since this value was 5.9‰ more enriched than that of the decapods at Sta. Z ($11.6 \pm 0.2\text{‰}$), it is evident that this carnivorous fish would be the typical tertiary consumer in the bay.

The $\delta^{15}\text{N}$ values of the fish collected in the deeper area of the northern bay (Stas. H1 and H2) were slightly more enriched than those of fish at Stas. H3 and H4, being distributed from 14.0 to 16.0‰ in 66% and over 16.0‰ in 30% of the fish (Table 4). Although this might suggest that fish in the north had higher trophic positions, it is more likely that the higher $\delta^{15}\text{N}$ values were simply a reflection of baseline enrichments of POM (Table 1) and macroalgae (Takai et al. 2001) that were higher in the north than in the central bay. This baseline variation was clearly reflected in the $\delta^{15}\text{N}$ values for the cardinal fish and the young-of-the-year gurnard (Fig. 4). In aquatic ecosystems, ^{15}N -enriched nitrogen from wastewater increases $\delta^{15}\text{N}$ of the aquatic life (McClelland et al. 1997). Since 82% of total nitrogen load from rivers that feed into Hiroshima Bay flows into the northern area (Lee and Hoshika 2000), the $\delta^{15}\text{N}$ increases in the primary producers and fish were considered to reflect the enriched ^{15}N signature of this wastewater.

We note that the fish analyzed in this study were mostly adults. The clearly depleted $\delta^{13}\text{C}$ values in the young-of-the-

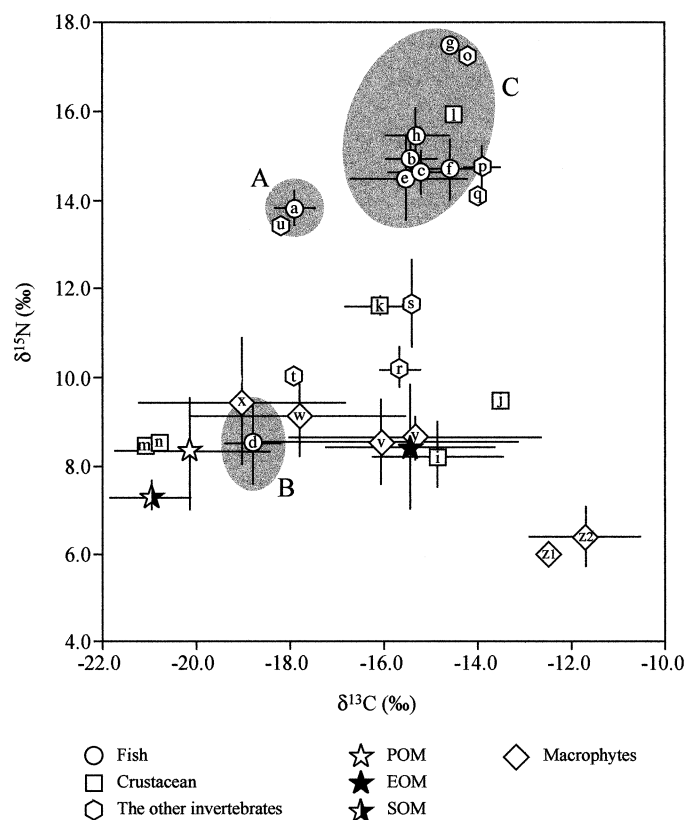


Fig. 6. The $\delta^{13}\text{C}$ - $\delta^{15}\text{N}$ food web diagram for Hiroshima Bay. The fish were divided into three distinct groups (shaded areas): (A) fish dependent upon water column primary production, (B) migrators from the open sea, and (C) fish dependent on benthic primary production. The seasonal means of POM (0.7–125 μm) in the surface layer of area W from 14 May 1999 to 10 November 2000 ($n = 10$) and EOM at the sublittoral fringe of Sta. Z from 10 August 1999 to 12 October 2000 ($n = 7$), and the mean of SOM in the bay ($n = 4$) are also shown. (a) The young-of-the-year black rockfish *Sebastes inermis* in June (Sta. Z; $n = 17$). (b) The other black rockfish at Sta. Z ($n = 33$). (c) All fish at Sta. Z except black rockfish ($n = 139$). (d) Japanese anchovy *Engraulis japonicus* ≥ 125 mm (Sta. Y; $n = 4$). (e) Japanese anchovy ≤ 120 mm (Stas. H1, H2, and Y; $n = 20$). (f) Gizzard shad *Konosirus punctatus* (Stas. H1–H4, Y; $n = 42$). (g) Cutlassfish *Trichiurus japonicus* (Sta. H4; $n = 1$). (h) All fish in the deeper zone except the anchovies, the shad, and the cutlassfish (Stas. H1, H2, H3, H4, and Y; $n = 145$). (i) Amphipods (Sta. Z; $n = 14$). (j) Isopod (Sta. Z; $n = 1$). (k) Small decapods (Sta. Z; $n = 3$). (l) Large decapods (Sta. H1; $n = 2$). (m) Mysids (*Acanthomysis tenuicauda* and *Acanthomysis* spp.; Sta. H4; $n = 1$). (n) Copepods (Sta. H4; $n = 1$). (o) Japanese squid *Loliolus japonica* (Sta. H2, $n = 2$). (p) The other cephalopod (Stas. H1 and H4, $n = 3$). (q) Starfish *Asterias amurensis* (Sta. H2, $n = 1$). (r) Japanese trepang *Apostichopus japonicus* (Stas. H2 and H4; $n = 6$). (s) Japanese oysters *Crassostrea gigas* (Stas. Z, H3, and X; $n = 20$). (t) Egg cockles *Fulvia mutica* (Sta. H4, $n = 4$). (u) Ascidian *Styela plicata* (Sta. H1; $n = 1$). Macroalgae (v–y) were collected at Sta. Z by Takai et al. (2001) and seagrasses *Zostera marina* (z_1 , z_2) were collected in the western Seto Inland Sea by Takai (unpubl. data). (v) Phaeophyceae ($n = 30$). (w) Rhodophyceae except samples with special $\delta^{13}\text{C}$ values of $< -30\text{‰}$ ($n = 20$). (x) Ulvophyceae ($n = 8$). (y) Chlorophyceae ($n = 3$). (z_1) A seagrass collected near Sta. H1 on 25 Oct 1999. (z_2) Seagrasses collected in the embayment of the sea from 19 July to 10 October in 2000 ($n = 7$). The values are shown as mean values \pm SD.

Table 6. The reference data of carbon and nitrogen stable isotope ratios (‰; mean \pm SD) of macroalgae collected at Sta. Z and seagrasses collected in the north Hiroshima Bay (near Sta. H1) and the embayment of the western Seto Inland Sea.

Species	<i>n</i>	Sampling date	$\delta^{13}\text{C}$	$\delta^{15}\text{N}$	Reference
Macroalgae					
Phaeophyceae	30	15 Jun 99–30 Jun 00	-16.0 ± 2.9	8.5 ± 1.0	Takai et al. (2001)
Rhodophyceae (except samples with special $\delta^{13}\text{C}$ values of $< -30\text{‰}$)	20	15 Jun 99–30 Jun 00	-17.8 ± 2.3	9.1 ± 0.9	Takai et al. (2001)
Ulvophyceae	8	15 Jun 99–30 Jun 00	-19.0 ± 2.2	9.4 ± 1.4	Takai et al. (2001)
Chlorophyceae	3	15 Jun 99–30 Jun 00	-15.3 ± 2.7	8.6 ± 0.5	Takai et al. (2001)
Seagrass					
<i>Zostera marina</i> (the north Hiroshima Bay)	1	25 Oct 99	-12.5	6.0	Takai (unpubl. data)
<i>Zostera marina</i> (the embayment)	7	19 Jul 00–10 Oct 00	-11.7 ± 1.2	6.4 ± 0.7	Takai (unpubl. data)

year black rockfish in June suggest that the early life history stages of juvenile fish that feed on plankton may be depleted in $\delta^{13}\text{C}$. Future studies should address changes in isotopic signatures that could occur with ontogenetic shifts in habitat and feeding and the consequent change in the source of carbon.

Apparent $\delta^{13}\text{C}$ discrepancy between muscle tissues and stomach contents in fish—In gizzard shad *Konosirus punctatus* and Japanese horse mackerel, striking differences were found for the $\delta^{13}\text{C}$ values between the fish muscle tissue and the stomach content. Although the stomach content of the shad consisted only of mud (Takai unpubl. data), the $\delta^{13}\text{C}$ of shad muscles was mostly distributed from -15.1 to -14.1‰ (Table 4), being much more enriched than the $\delta^{13}\text{C}$ of SOM, which ranged from -22.2 to -20.2‰ (Table 1). Riera et al. (1996) also reported a large isotopic difference between the diet and body tissue of intertidal nematodes that presumably consumed SOM. Their stable isotopic study suggested that the nematodes do not exploit all the components of the SOM, but rather use microphytobenthos as their main food source. Our data suggest that the Hiroshima Bay shad selectively use or assimilate ^{13}C -enriched components in the mud. This possibility is supported by the $\delta^{15}\text{N}$ values of 13.4 – 15.9‰ in the shad that were 1.8 – 4.3‰ more enriched than the small benthic decapods (primary consumers), which suggests that the shad are secondary consumers feeding on infaunal or epifaunal primary consumers inhabiting the surface sediments.

The $\delta^{13}\text{C}$ value (Sta. H4, -15.2‰) of the muscle tissue of a Japanese horse mackerel was also strikingly enriched relative to the values of the small crustaceans in its stomach; -21.0‰ in mysids (*Acanthomysis tenuicauda* and *Acanthomysis* spp.) and -20.8‰ in copepods from which lipids were removed. This discrepancy could be the result of the time-integrated isotopic signatures of the diet that are recorded in the tissue relative to the transient information of the diet items on the sampling date (Michner and Schell 1994). Young and adult horse mackerel generally feed not only on zooplankton but also on fish, cephalopods, shrimps, and polychaetes (Ochiai and Tanaka 1986). In Hiroshima Bay, fish, cephalopods, and shrimps had generally high $\delta^{13}\text{C}$ values of -17.0 to -13.0‰ (Tables 2–5), and polychaetes were also reported to be enriched in $\delta^{13}\text{C}$ in the easternmost Seto Inland Sea (-16.0 to -14.8‰ ; Yamada et al. 1996).

Consequently, the isotopic signature of the horse mackerel was likely a consequence of feeding on ^{13}C -enriched benthic animals rather than on only the depleted mysids and copepods encountered in the gut on the sampling date.

Importance of benthic primary production in the western Seto Inland Sea—Although the benthic animals inhabiting the Seto Inland Sea have been assumed to depend on organic carbon from the water column, most of the fish and the invertebrates in the Hiroshima Bay showed clearly enriched $\delta^{13}\text{C}$ values relative to the ^{13}C -depleted POM. In contrast, in the tight pelagic–benthic coupling system of the Chukchi Sea, isotopic signatures of all the demersal fish, crustaceans, bivalves, and ascidians were mostly distributed from -21 to -19‰ (Dunton et al. 1989), overlapping closely with ratios of zooplankton (Schell et al. 1998) (Note that their samples were not defatted in either study). Such sharp contrast in the $\delta^{13}\text{C}$ distributions between the Seto Inland Sea and the Chukchi Sea supports our conclusion that the demersal fish in the relatively loose pelagic–benthic coupling system of the Seto Inland Sea receive substantial carbon supply from benthic primary production.

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Received: 21 August 2001

Accepted: 17 January 2002

Amended: 24 January 2002