AMS ^{14}C MEASUREMENTS OF DISSOLVED INORGANIC CARBON IN PORE WATERS FROM A DEEP-SEA "COLD SEEP" GIANT CLAM COMMUNITY OFF HATSUSHIMA ISLAND, SAGAMI BAY, JAPAN

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ABSTRACT. We collected pore waters using an *in situ* pore water-squeezer for a submersible *Shinkai 2000* at six depths beneath the sediment surface within a deep-sea "cold seep" giant clam community off Hatsushima Island, Sagami Bay, Japan. A box core sample was also collected *ca.* 4.5 km east of the community and pore waters were separated. Dissolved inorganic carbon (DIC) was extracted and purified in a vacuum line and ^{14}C concentration was determined with a Tandetron accelerator mass spectrometer at Nagoya University after conversion to graphite targets using a batch Fe-catalytic hydrogen reduction method. Δ^{14}C values decreased with increasing depth to −938‰ at the sulfate concentration minimum. This indicates that methane used for the active reduction of sulfate and formation of hydrogen sulfide, which is used by symbiotic chemoautotrophic bacteria in gills of the giant clams, is almost dead and is likely supplied from the deep. Δ^{14}C values of DIC vary linearly with δ^{13}C values along a mixing line between that in the bottom water and that produced by the oxidation of dead methane. The δ^{13}C value of DIC oxidized from dead methane is estimated to be *ca.* −45‰.

INTRODUCTION

In the last 10 years, deep-sea cold seep biological communities have been found in tectonically active subduction zones off Oregon, Peru, in the Nankai Trough and the Japan Trench (*e.g.*, Suess *et al.* 1985; Ohta and Laubier 1987), and in tectonically passive areas off Florida, Louisiana and northern California (*e.g.*, Paull *et al.* 1984; Kennicutt *et al.* 1985). Dense deep-sea biological communities dominated by the giant clam, *Calyptogena soyoae,* were also found off Hatsushima Island, western Sagami Bay, Central Japan in 1984 by the submersible *Shinkai 2000* of the Japan Marine Science and Technology Center (Okutani and Egawa 1985; Fig. 1). The clam communities stretch for 7 km along the foot of a steep escarpment off Izu Peninsula at water depths of 900–1200 m (Hashimoto *et al.* 1989). The area is located near the convergence front of the northern tip of the Philippine Sea Plate, which is subducting beneath the Eurasian and North American Plates.

These cold seep communities are believed to be supported by microbial chemosynthesis that oxidizes reduced compounds in a manner similar to that occurring in deep-sea hydrothermal biological communities on mid-oceanic ridges (Jannasch and Mottl 1985). Issues in understanding "cold seep" biological communities are the origin of material and energy sources for these dense communities and the relations between the biological and tectonic activities.

Since 1986, *Shinkai 2000* has been conducting geochemical, biological and geological surveys in the largest community of the Hatsushima site (*e.g.*, Sakai *et al.* 1987). Masuzawa *et al.* (1992) found that microbial sulfate reduction using methane actively occurs just beneath the living giant clams. Our data on pore waters extracted from a 16-cm-long, weight-driven core showed that hydrogen sulfide produced by this process supports the giant clam community. We have developed an *in-situ* pore-water squeezer for a submersible (ISPS-S) to collect pore waters at several depths under the giant clams (Masuzawa *et al.* 1990; Masuzawa, Kitagawa and Handa 1991).

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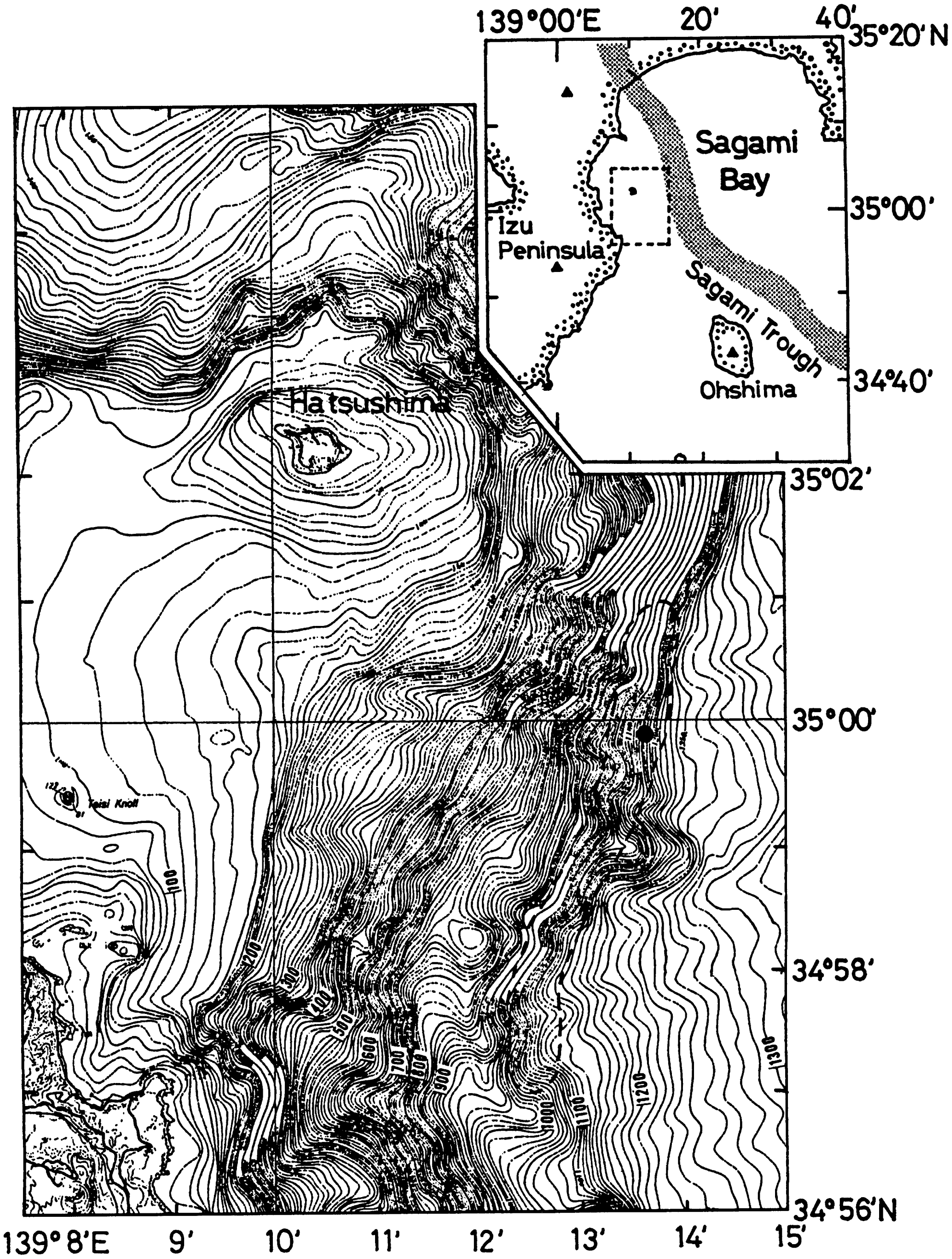

Fig. 1. Topographic map of the Hatsushima site, western Sagami Bay, Central Japan. A broken line encloses the area where deep-sea *Calyptogena* communities distribute. ● = location of the largest *Calyptogena* community at the Hatsushima site where pore water samples were collected with ISPS-S during Dives 521, 593 and 720 of the submersible *Shinkai 2000*.

We report here the collection of pore water samples from the giant clam community, and the measurements of $\Delta^{14}C$ and $\delta^{13}C$ of dissolved inorganic carbon (DIC) of pore waters. We clarify the nature of oxidized methane used for sulfate reduction in these systems. The results revealed extremely low $\Delta^{14}C$ values at the minimum of pore-water sulfate, indicating that the oxidized methane was almost dead and was supplied from the deep.

METHODS

The largest community dominated by a giant clam, *Calyptogena soyoae,* at the Hatsushima site (34°59.9′ N, 139°13.6′ E; 1146 m deep; Fig. 1) stretches for *ca.* 200 m from east to west and for *ca.* 50 m from south to north, including many dense *Calyptogena* patches of a few to 10 meters in diameter. We have collected pore water samples at a *Calyptogena* patch near the western end of the largest *Calyptogena* community since Dive 380 on 4 November 1988. By accident, we left a funnel with a float to collect emanating materials (Gamo and Sakai 1989) and a weight-driven corer during Dive 380, which made it possible to reach the same *Calyptogena* patch in the largest community at the Hatsushima site. We collected pore water samples with ISPS-S by setting it on the living *Calyptogena* patch for two hours during Dives 521 (14 November 1990), 593 (2 December 1991) and 720 (26 November 1993) of the submersible *Shinkai 2000* (Fig. 2). ISPS-S's squeezing ports with an opening diameter of 22 mm were at 0, 9, 18, 27, 36 and 45 cm below the sediment-water interface. We recovered pore water samples from ISPS-S and refrigerated them after appropriate chemical treatments for each chemical species (Masuzawa *et al.* 1992) on board the R/V *Natsushima* following each dive.

We collected a bottom water sample with a CTD-rosette sampler at a few meters above the bottom surface at the largest community (14 July 1989), and a box core (KT89-11, BX-10; 34°59.8′N, 139°16.4′E; 1368 m deep; 29 cm long; 17 July 1989) at *ca.* 4.5 km east of the Hatsushima site as a reference during cruise KT89-11 of the R/V *Tansei Maru* of the University of Tokyo. Pore waters were separated from the sediment samples by centrifugation at *in-situ* temperature.

Samples for ^{14}C and ^{13}C measurements were stored in syringes or in glass bottles with septum stoppers after poisoning with saturated $HgCl_2$ solution. DIC of these sample solutions was extracted by a gas-evolution technique modified from that of Hassan (1982). Each sample was introduced *via* a septum into a reaction vessel containing 3M $HClO_4$ and DIC was extracted by flushing with CO_2-free nitrogen gas, trapped with a Horibe trap dipped in liquid nitrogen. The CO_2 gas was introduced into a connected vacuum line, purified with a n-pentane cold trap and liquid nitrogen cold traps, quantified manometrically and finally sealed into two Pyrex™ glass tubes for ^{14}C and ^{13}C measurements. The recovery of DIC using known amounts of Na_2CO_3 solutions was quantitative (>99%). Particulate organic carbon (POC) in dried sediment samples of BX-10 was converted to CO_2 by the combustion method (Minagawa, Winter and Kaplan 1984) and sealed into two Pyrex™ glass tubes. DIC and POC concentrations were calculated from the resultant amounts of CO_2 and the amounts of pore water and sediment, respectively.

^{14}C concentrations in these CO_2 gases were measured relative to NBS SRM 4990-C (HOxII) with a Tandetron accelerator mass spectrometer (AMS) at Nagoya University (Nakamura, Nakai and Ohishi 1987) after converting graphite targets using the batch Fe-catalytic hydrogen reduction method (Kitagawa *et al.* 1993). $^{13}C/^{12}C$ ratios of these CO_2 gases were measured with a Finnigan MAT 252 gas ion-source mass spectrometer relative to the PDB standard within an accuracy of $\pm 0.1‰$. Measured ^{14}C concentrations are shown as $\Delta^{14}C$ values after Stuiver and Polach (1977). Cl, SO_4 and H_2S in pore waters were determined by methods reported previously (Masuzawa *et al.* 1992).

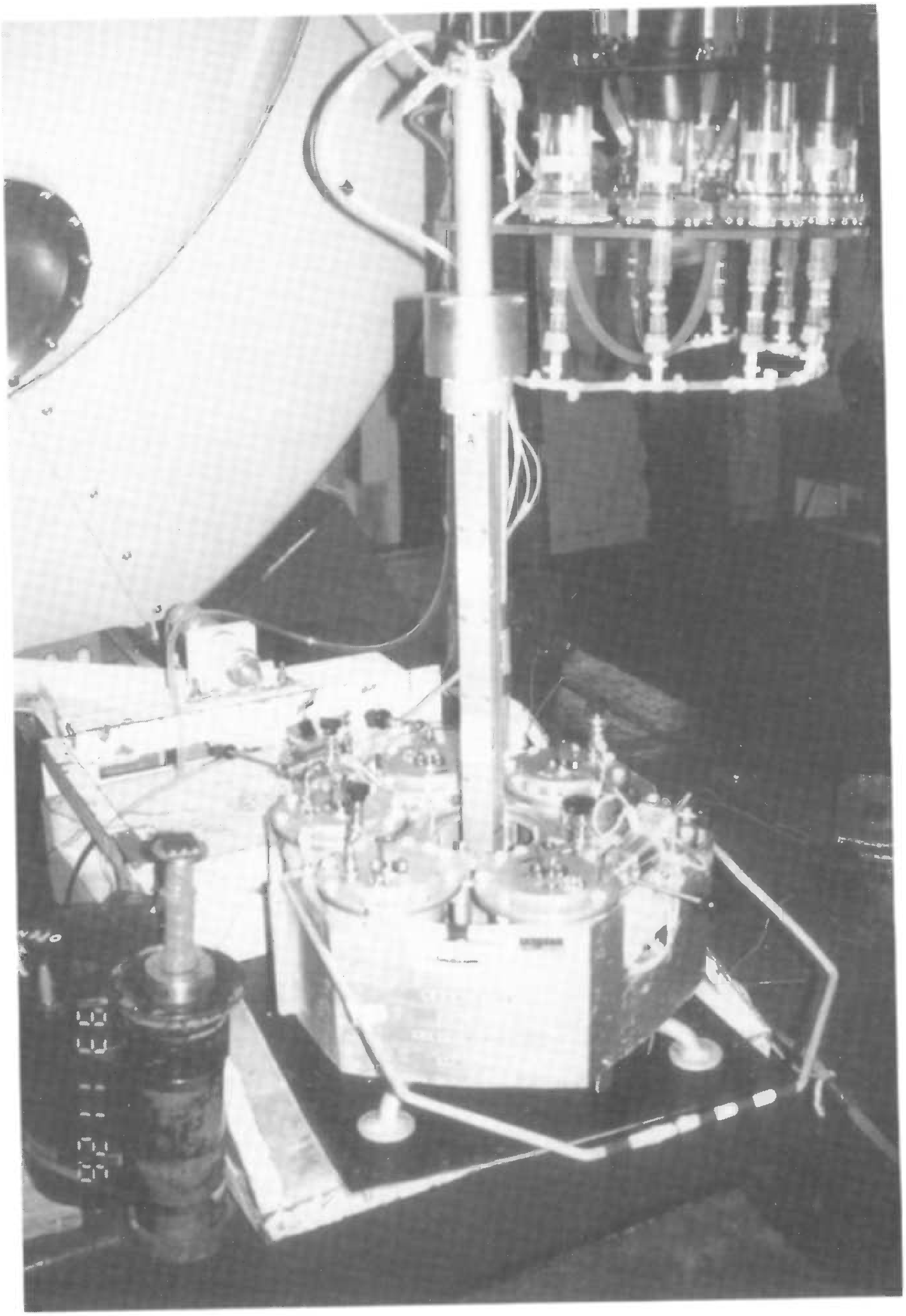

Fig. 2. A view of ISPS-S on the platform of *Shinkai 2000*

RESULTS AND DISCUSSION

Table 1 lists the analytical results of pore waters for BX-10 and the *Calyptogena* patch. Values of POC and $\delta^{13}C$ and $\Delta^{14}C$ of POC of Core BX-10 are shown in Table 2; vertical profiles are shown in Figure 3 (Core BX-10) and Figure 4 (the *Calyptogena* patch). During Dive 521, the lower three ports of ISPS-S did not work. For Dive 593, we could not get enough pore water for DIC extraction at the lower three ports because the amounts of sampled pore waters decreased with increasing depth. The chemical composition of pore water at 27 cm of Dive 720 showed a contamination from a shallower depth, and we did not accept it. We were not able to prepare good graphite targets to give enough carbon ion beams for the samples at 9 cm of Dive 593 and at 45 cm of Dive 720, probably because of interference from sulfur species. The $\Delta^{14}C$ value of the bottom water collected at *ca.* 1140 m depth at the Hatsushima site was −166 ± 14‰ (Table 1) and comparable to the values obtained of −161.3, and −210.6‰ at 997 and 1345 m depth, respectively, of GEOSECS Stn. 224 (34°15′N, 141°58′E; Ostlund and Stuiver 1980).

TABLE 1. Chemical Compositions, DIC and $\delta^{13}C$ and $\Delta^{14}C$ of DIC in Pore Waters from the Western Sagami Bay (KT89-11, BX-10) and from a Deep-Sea Giant Clam Community off Hatsushima Island in Western Sagami Bay, Japan

Depth (cm)	Cl (mmol kg^{-1})	SO_4 (mmol kg^{-1})	H_2S (mmol kg^{-1})	DIC (mmol kg^{-1})	$\delta^{13}C_{DIC}$ (‰)	$\Delta^{14}C_{DIC}$ (‰)
Western Sagami Bay: KT89, BX-10 (34°59.8′N, 139°16.4′; 1368 m deep; 17 July 1989)						
0–5	528	27.3	--	5.47	−7.9	−66 ± 15
5–10	530	27.1	--	7.58	−9.7	−49 ± 12
10–15	534	26.9	--	3.35	−5.8	−128 ± 15
15–20	530	26.6	--	3.50	−6.7	−150 ± 0
20–25	532	25.4	--	3.67	−6.8	−178 ± 13
Giant clam community off Hatsushima Island in Western Sagami Bay (34°59.9′N, 139°13.6′E; 1146 m deep)						
BW*	532	27.5	--	2.37	−1.9	−166 ± 14
D521 (14 Nov. 1990)						
0	516	27.0	--	2.33	−1.0	−244 ± 37
9	527	26.7	--	2.05	−2.4	−152 ± 25
18	533	23.9	--	4.31	−23.3	−582 ± 11
D593 (2 Dec. 1991)						
0	512	26.6	--	2.34	−0.4	−244 ± 12
9	527	24.6	0.13	4.19	−16.9	--
18	549	8.0	6.80	11.98	−29.5	−938 ± 20
27	552	1.6	8.94	--	--	--
36	554	2.2	8.98	--	--	--
45	553	5.0	7.18	--	--	--
D720 (26 Nov. 1993)						
0	536	27.8	0.01	2.34	−2.7	−209 ± 15
9	538	27.0	0.03	3.34	−9.7	−367 ± 14
18	563	6.8	6.28	5.36	−16.1	−480 ± 8
36	558	1.7	13.84	11.45	−41.6	−938 ± 4
45	558	7.5	8.26	10.31	−40.8	--

*Bottom water collected at a few meters above the colony with a CTD-rosette sampler by R/V *Tansei Maru*, 14 July 1989

TABLE 2. POC and $\delta^{13}C$ and $\Delta^{14}C$ in Core KT89-11, BX10, Western Sagami Bay

Depth (cm)	POC (%)	$\delta^{13}C$ (‰)	$\Delta^{14}C$ (‰)
0–1	2.68	–20.7	–51 ± 11
4–5	2.72	–21.9	–130 ± 8
8–9	2.62	–21.0	–138 ± 11
12–13	2.43	–21.7	–161 ± 13
16–17	2.04	–21.3	–117 ± 10
24–25	1.77	–22.8	–121 ± 9

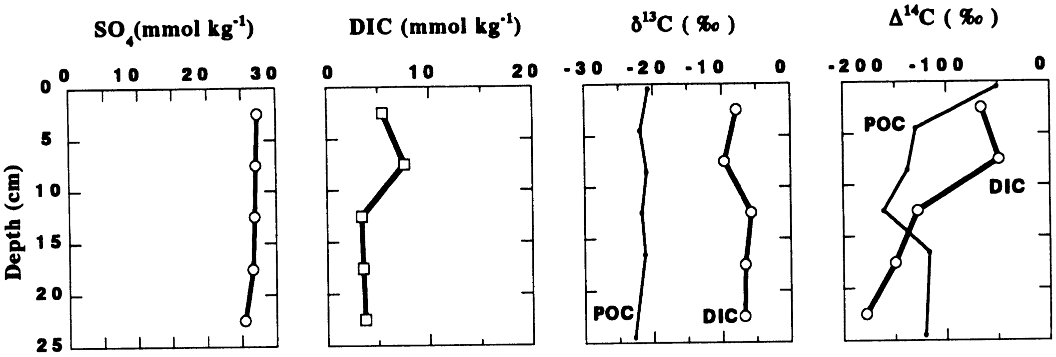

Fig. 3. Vertical profiles of SO_4, DIC, and $\delta^{13}C$ and $\Delta^{14}C$ of DIC in pore waters, as well as $\delta^{13}C$ and $\Delta^{14}C$ of POC of sediments in Core KT89-11, BX-10 from western Sagami Bay.

Core KT89-11, BX-10 was taken as a reference showing the ordinary Sagami Bay sediment outside the *Calyptogena* community area. As shown in Figure 3, pore-water SO_4 decreased slightly at the bottom of the core, indicating that the core was under oxic and suboxic diagenesis. $\delta^{13}C$ values of DIC ranging from –9.7 to –5.8‰ were found among those of seawater (*ca.* 0‰) and POC (–22.8 to –20.7‰; Table 2). $\Delta^{14}C$ values of DIC change from –66 ± 15‰ at 0–5 cm depth to –178 ± 13‰ at 20–25 cm depth, and are higher in the upper layer and lower in the deeper layer than those of POC (Fig. 2). As suggested by the maximum value of DIC and minimum value of $\delta^{13}C$ of DIC at 5–10 cm depth, the higher values in the upper layer are due to oxic respiration of fresh organic matter with high $\Delta^{14}C$ values (Druffel and Williams 1990) accelerated by benthos activity. The lower values in the deeper part are due to upward diffusion of older DIC from the deep. $\Delta^{14}C$ values of both DIC and POC are not lower than *ca.* -200‰ in Core BX-10, a 27-cm long ordinary sediment core in Sagami Bay.

Masuzawa *et al.* (1992) showed that microbial sulfate reduction and hydrogen sulfide formation, using methane as the reductant, occurs under the living giant clams in the *Calyptogena* patch at the Hatsushima site as follows

$$CH_4 + SO_4^{2-} \rightarrow CO_3^{2-} + H_2S + H_2O\,. \qquad (1)$$

With the increase in carbonate alkalinity in Equation (1), calcium carbonate, probably with magnesium, deposits in the pore waters

$$Ca^{2+} + HCO_3^- \rightarrow CaCO_3 + H^+\,. \qquad (2)$$

Figure 4 shows vertical profiles of chemical and isotopic compositions of pore waters collected with ISPS-S from the *Calyptogena* patch during Dives 521 (14 November 1990), 593 (2 December 1991) and 720 (26 November 1993), as well as those extracted from a weight-driven core collected from the

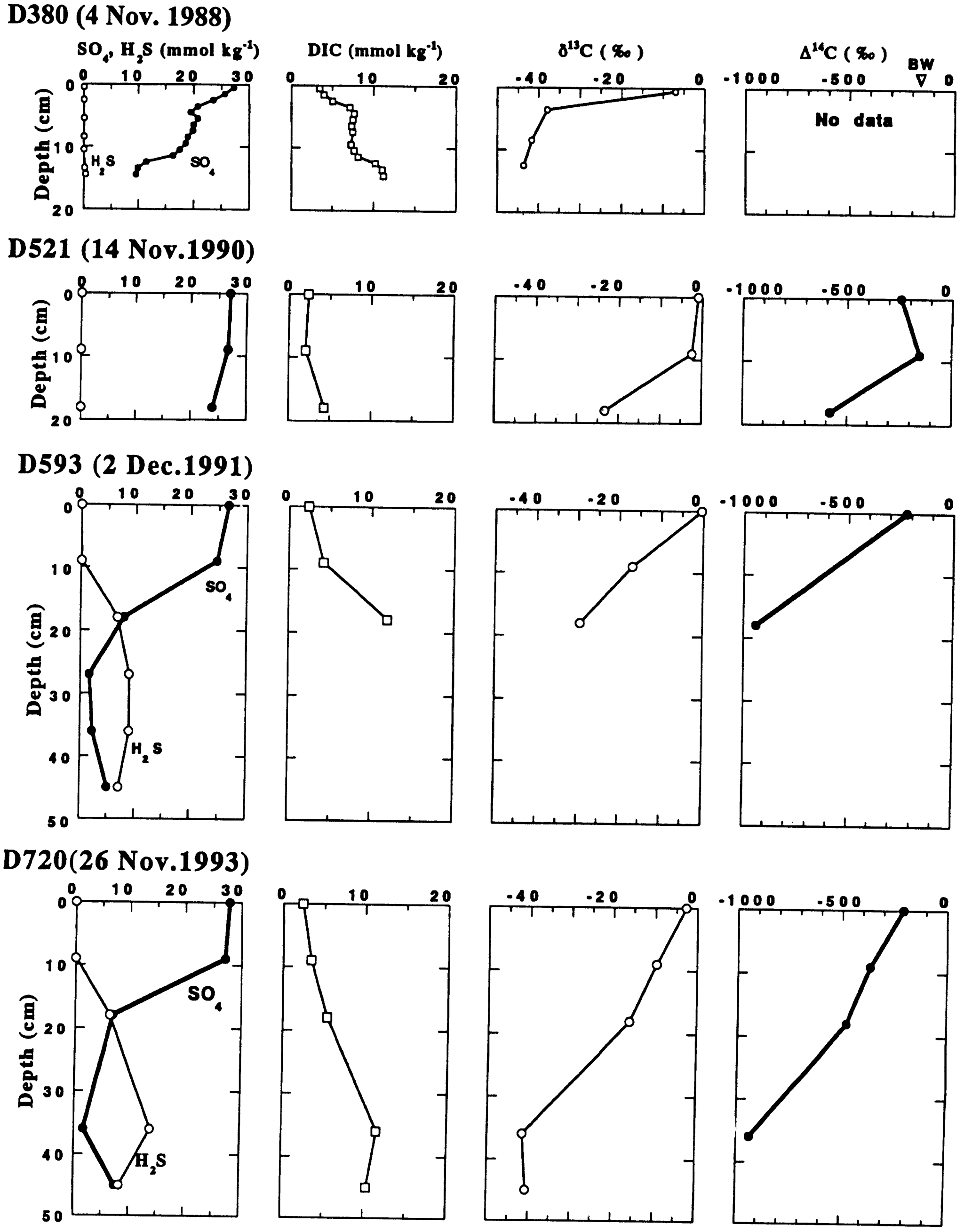

Fig. 4. Vertical profiles of SO_4 and H_2S, DIC, and $\delta^{13}C$ and $\Delta^{14}C$ of DIC in pore waters collected with an *in-situ* pore water squeezer for a submersible (ISPS-S) from a *Calyptogena* patch in the largest *Calyptogena* community at the Hatsushima site during Dives 521 (14 Nov. 1990), 593 (2 Dec. 1991) and 720 (26 Nov. 1993) of the submersible *Shinkai 2000*. The top row shows those for pore waters extracted from a weight driven core collected at the same *Calyptogena* patch during Dive 380 (4 Nov. 1988), from Masuzawa *et al.* (1992). A $\Delta^{14}C$ value of bottom water (BW) is also shown in the top row (▽).

same *Calyptogena* patch during Dive 380 (4 November 1988; Masuzawa *et al.* 1992). The vertical profiles of SO_4 for Dives 593 and 720 are very similar to each other, with the minimum at 27–36 cm depth; H_2S is maximum at 36 cm depth with very high concentrations such as 9.0 mmol kg^{-1} (Dive 593) and 13.8 mmol kg^{-1} (Dive 720). The SO_4 profile of Dive 380 is also similar to those of respective depths for Dives 593 and 720, which indicates that the active depth of microbial sulfate reduction is at *ca.* 20–40 cm under the living clams.

DIC increases inversely to SO_4 as the product of oxidation of organic carbon by SO_4, and $\delta^{13}C$ and $\Delta^{14}C$ values of DIC reflect those of organic carbon oxidized by sulfate reduction. $\delta^{13}C$ values of DIC decrease with increasing depth to as low as –41.6‰ at 36 cm depth of Dive 720, a value similar to those of Dive 380 (Fig. 3). These low $\delta^{13}C$ values indicate that methane is the most probable oxidized organic carbon through microbial sulfate reduction, as shown in Equation (1).

$\Delta^{14}C$ values of DIC decrease remarkably with depth and extremely low values were observed at the SO_4 minimum: –938 ± 20‰ at 18 cm of Dive 593 and –938 ± 4‰ at 36 cm of Dive 720 (Fig. 4; Table 1). Such extremely low $\Delta^{14}C$ values suggest that the oxidized methane was virtually dead and supplied probably from the deeper layer in the sediment. Figure 5 shows the relations among DIC, $\delta^{13}C$ and $\Delta^{14}C$ of DIC *vs.* SO_4 in the pore waters from the *Calyptogena* patch for Dives 521, 593 and 720. It is obvious that, with the decrease in sulfate, DIC increases, $\delta^{13}C$ decreases remarkably and $\Delta^{14}C$ decreases greatly from respective bottom water values, although the relations are divergent as to sampled date. The lowest $\delta^{13}C$ and $\Delta^{14}C$ values appear at the lowest SO_4 concentrations.

Figure 6 demonstrates the relation between $\Delta^{14}C$ and $\delta^{13}C$ of DIC in pore waters at the *Calyptogena* patch as well as those of DIC and POC of Core BX-10. Surface particulate organic carbon (SPOC) indicates fresh particulate organic carbon (phytoplankton) in the surface ocean with average $\delta^{13}C$ and $\Delta^{14}C$ values of –20‰ (Libes 1992) and +140‰ (Druffel and Williams 1990), respectively. DIC values of Core BX-10 are plotted along the mixing line between SPOC and bottom water (BW) with a slight shift in the lower layer from the mixing line. $\Delta^{14}C$ values of POC in Core BX-10 changed mostly by the decay of ^{14}C with time, with $\delta^{13}C$ values similar to the average marine phytoplankton (–20‰).

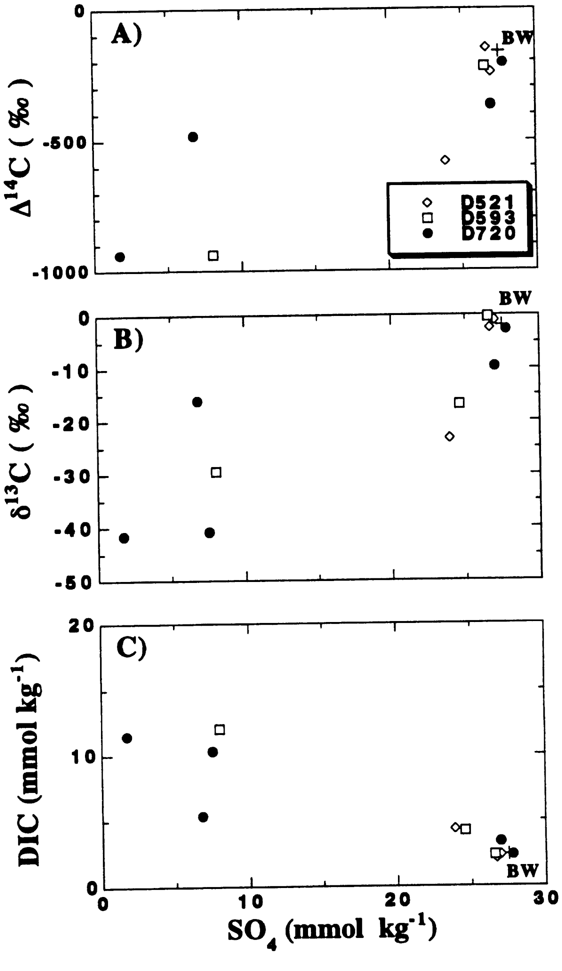

Fig. 5. Relation of $\Delta^{14}C$ (A), $\delta^{13}C$ (B) and DIC (C) *vs.* SO_4 in pore waters and those of BW collected at a *Calyptogena* patch at the Hatsushima site.

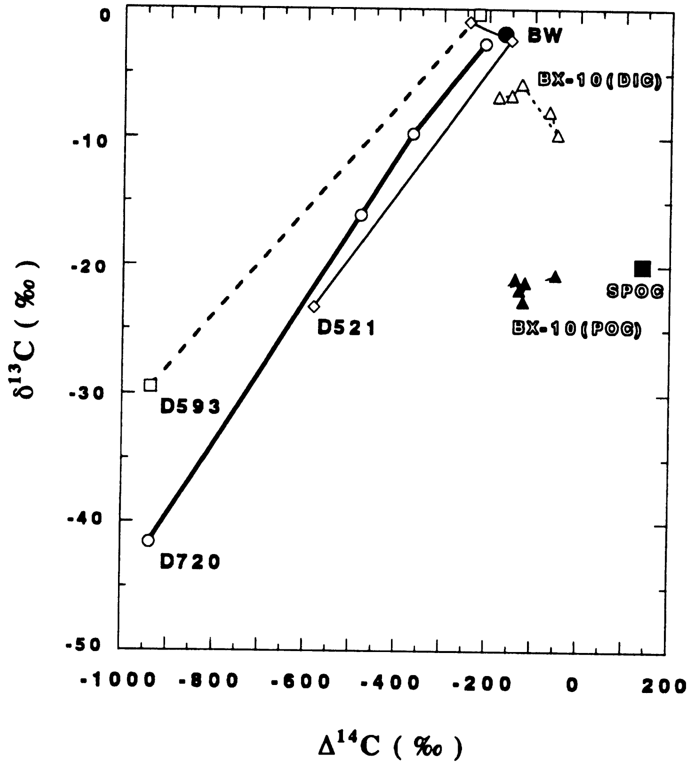

Fig. 6. Relation between $\Delta^{14}C$ and $\delta^{13}C$ of DIC in pore waters and those of BW collected at a *Calyptogena* patch at the Hatsushima site, as well as those of DIC and POC of Core KT89-11, BX-10 from western Sagami Bay.

The relation between $\Delta^{14}C$ and $\delta^{13}C$ of DIC in the pore waters at the *Calyptogena* patch is quite different from those of Core BX-10 (Fig. 6). $\Delta^{14}C$ values of DIC in pore waters of the *Calyptogena* patch change linearly with those of $\delta^{13}C$, especially in the case of Dive 720, where four data points are plotted almost along a line through that of the bottom water. The lines for Dives 521 and 593 connect only two points but the slopes seem to be similar to that of Dive 720. This means that ^{14}C and ^{13}C of DIC behave conservatively with each other in the pore waters in the *Calyptogena* patch, as if mixing between two end members: the BW and DIC produced by oxidation of dead methane.

Paull *et al.* (1989) reported positive correlation between $\Delta^{14}C$ and $\delta^{13}C$ values of soft tissues of mussels and vestimentifera and of shells and carbonate deposits from abyssal brine seeps at the Florida Escarpment. The values of tissues showed a mixing between planktonic organic carbon and biogenic methane; those of carbonate, a mixing between bottom water and inorganic carbon that originated from the oxidation of methane (Paull *et al.* 1989). The linear relations between ^{14}C and ^{13}C values of DIC in pore waters from the Hatsushima site (Fig. 6) are plotted within the region of those of carbonates from the Florida Escarpment with very similar slopes, although carbonate deposition is not active in the *Calyptogena* patch at the Hatsushima site (Masuzawa *et al.* 1992). This indicates that DIC in the pore waters in the *Calyptogena* patch is the direct product of oxidation of dead methane through microbial sulfate reduction (Eq. 1).

If we assume the $\Delta^{14}C$ value of oxidized methane is −1000‰, the $\delta^{13}C$ value of DIC oxidized from dead methane is estimated to be *ca.* −45‰ from the linear relation for Dive 720 (Fig. 6). A kinetic isotope effect arises during oxidation of methane because methane containing the lighter isotopes of carbon and hydrogen is oxidized slightly faster than methane containing the heavier isotopes, and isotope fractionation factors of anaerobic oxidation of methane were reported to be 1.004 (Alperin and Reeburgh 1984) and 1.0088 ± 0.0013 (Alperin *et al.* 1988) in anoxic shelf sediments. If these isotope fractionation factors could be applied to the pore waters at the Hatsushima site, the $\delta^{13}C$ value of oxidized methane might be in the range of –41 to –35‰. The $\delta^{13}C$ values of typical thermogenic methane are –50 to –20‰; of typical microbial methane, –110 to –50‰ (Whiticar *et al.* 1986). Thus, the estimated $\delta^{13}C$ value of oxidized methane falls in the range of thermogenic methane.

$\Delta^{14}C$ and $\delta^{13}C$ values of shells of *Calyptogena soyoae* collected from the same *Calyptogena* community at the Hatsushima site were –222 to –216‰ and –0.9 to + 0.1‰, respectively (Sakai *et al.* 1987), and very similar to those of the pore waters at 0 cm by ISPS-S. Values for its soft tissue were –220 and –32.5‰, respectively (Sakai *et al.* 1987), and far from the straight lines in Figure 6. These findings suggest that metabolic behavior of carbon differs between shell and soft tissue of *Calyptogena soyoae,* as reported for soft tissues of mussels and vestimentifera at the Florida Escarpment (Paull *et al.* 1989).

CONCLUSION

By *in-situ* squeezing of pore waters at 6 depths between 0 to 45 cm from a deep-sea "cold seep" *Calyptogena* patch in the largest *Calyptogena* community at the Hatsushima site in Sagami Bay, Japan and by measuring $\Delta^{14}C$ and $\delta^{13}C$ of DIC as well as chemical compositions, we draw the following conclusions:

1. We confirmed that microbial sulfate reduction using methane as the reductant proceeds actively at *ca.* 20–40 cm depth under the living giant clams.
2. We observed extremely low $\Delta^{14}C$ values of DIC, such as –938 ± 20‰ at the 18 cm depth of Dive 593 (December 1991) and –938 ± 4‰ at the 36 cm depth of Dive 720 (November 1993), which indicate that the oxidized methane was virtually dead and supplied from the deep layer.
3. $\Delta^{14}C$ and $\delta^{13}C$ of DIC behave conservatively with each other and are plotted almost along a mixing line between the BW and DIC produced by oxidation of dead methane.
4. The $\delta^{13}C$ value of DIC oxidized from dead methane ($\Delta^{14}C$ = –1000‰) is estimated to be *ca.* –45‰.

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