

# Microbial carbon isotope fractionation to produce extraordinarily heavy methane in aging hydrothermal plumes over the southwestern Okinawa Trough

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Within neutrally buoyant hydrothermal plumes derived from the Yonaguni Knoll IV hydrothermal field (24°51' N, 122°42' E, D = 1,370–1,385 m) in the southwestern Okinawa Trough back-arc basin, we obtained profiles of dissolved manganese (Mn), helium isotopes (<sup>3</sup>He/<sup>4</sup>He), methane (CH<sub>4</sub>) and its stable carbon isotope ratio ( $\delta^{13}\text{C}_{\text{PDB}}$ ) in young to old plume waters. We first mapped the spatial distribution of hydrothermal plumes by towing a fixed array of optical sensors (Miniature Autonomous Plume Recorders: MAPRs) above the field. We then made water column observations and samplings using a CTD (Conductivity, Temperature, and Depth sensors)-Carousel package with a transmissometer and Niskin-X bottles at five locations, with distances between 0.6 and 6.1 km from the hydrothermally active center so far discovered. Vertical profiles of light transmission and chemical tracers indicated triple-layered plumes, the centers of which were at depths of 700–800 m, ~1,050 m and ~1,200 m. The CH<sub>4</sub> concentrations and  $\delta^{13}\text{C}$  values for the 1,200-m plume ranged between 1,026 and 10 nmol/kg, and between –22.4 and +40.4‰ (the highest  $\delta^{13}\text{C}$  value yet reported for oceanic CH<sub>4</sub>), respectively, indicating active microbial CH<sub>4</sub> oxidation accompanied by the  $\delta^{13}\text{C}$  increase for residual CH<sub>4</sub>. The  $\delta^{13}\text{C}(\text{CH}_4)$  was shown to be useful for tracing such a “microbial plume” whose CH<sub>4</sub> concentration has already fallen to almost the background level. By applying the Rayleigh distillation equation for a closed system, we estimated the kinetic isotope fractionation factor of 1.012 for the CH<sub>4</sub> oxidation process occurring within the hydrothermal plumes deeper than 1,000 m.

Keywords: hydrothermal plume chemistry, microbial methane oxidation, carbon isotope fractionation, southern Okinawa Trough

## INTRODUCTION

Submarine hydrothermal fluids generally contain high amounts of methane (CH<sub>4</sub>), which is thought to be biogenic (bacterial and thermocatalytic origins) or abiogenic (outgassing from the mantle or inorganic synthesis) (e.g., Welhan, 1988). Just after the ejection of hydrothermal fluids above the seafloor, they are rapidly diluted more than 3 to 4 orders of magnitude by ambient bottom seawater to form neutrally buoyant hydrothermal plumes over the venting area. Methane is one of the useful com-

ponents that can be used to trace the special distribution of such hydrothermal plumes, because hydrothermal discharge fluids are generally 10<sup>5</sup> to 10<sup>7</sup> times enriched in CH<sub>4</sub> relative to ambient deep seawaters.

Since CH<sub>4</sub> is not a conservative component in seawater, being consumed by microbial CH<sub>4</sub> oxidation, the concentration of CH<sub>4</sub> in a hydrothermal plume has been known to be decreased not only by dilution but also by *in situ* microbial consumption as the plume ages (Kadko *et al.*, 1990; De Angelis *et al.*, 1993). The latter process is associated with an increase of <sup>13</sup>C/<sup>12</sup>C ratio (or  $\delta^{13}\text{C}$  value) of the remaining CH<sub>4</sub> in seawater, because the microbial CH<sub>4</sub> oxidation process preferentially consumes <sup>12</sup>C compared to <sup>13</sup>C (Coleman *et al.*, 1981; Tsunogai *et al.*, 2000; Cowen *et al.*, 2002).

In this study our goal was to compare the concentration of dissolved CH<sub>4</sub> and its  $\delta^{13}\text{C}$  value among various aging stages of a hydrothermal plume originating from

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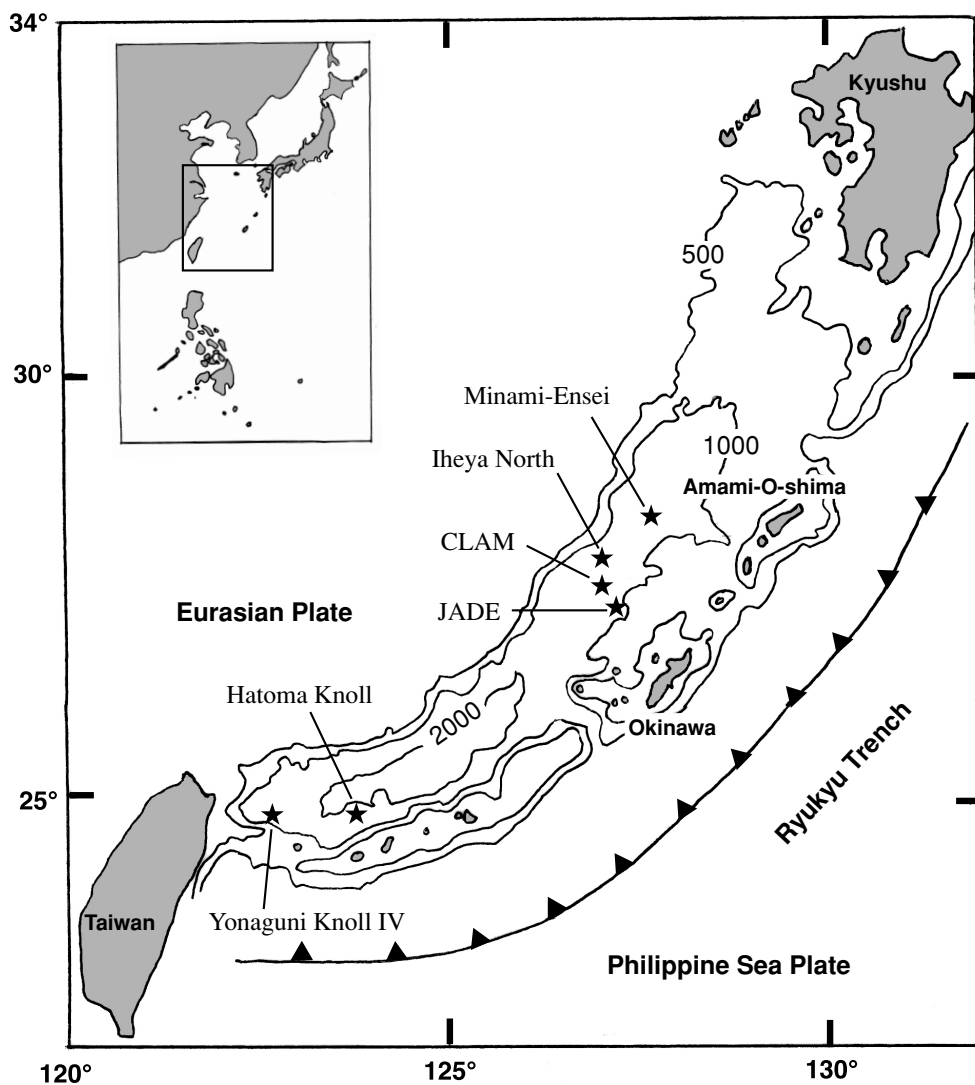


Fig. 1. A bathymetry map showing the location of the Yonaguni Knoll IV hydrothermal field in the southwesternmost Okinawa Trough. Other high temperature hydrothermal field yet located in the Okinawa Trough are also shown by star marks.

the same fluid venting site, to i) characterize non-conservative behavior of  $\text{CH}_4$  as the plume ages, ii) evaluate the usefulness of  $\delta^{13}\text{C}$  of  $\text{CH}_4$  as a tracer for hydrothermal plume dispersion, and iii) estimate the isotope fractionation factor for the microbial  $\text{CH}_4$  oxidation within the plume. We focused on the Yonaguni Knoll IV hydrothermal field in the Okinawa Trough, northwestern Pacific Ocean, as a  $\text{CH}_4$ -rich hydrothermal venting site favorable for accomplishing the purposes of this study.

#### YONAGUNI KNOLL IV HYDROTHERMAL FIELD

The Yonaguni Knoll IV hydrothermal field ( $24^\circ 51' \text{ N}$ ,  $122^\circ 42' \text{ E}$ , water depth: 1,370–1,385 m) is located in the southwesternmost part of the Okinawa Trough (SPOT)

back-arc basin behind the Ryukyu arc-trench system (Fig. 1). The Okinawa Trough, a typical sediment-hosted hydrothermal system covered with a several-km-thick layer of terrigenous materials from the Asian Continent, is thought to be in a rifting phase prior to the back-arc spreading (Sibuet *et al.*, 1987). Several submarine hydrothermal fields have been located along the Okinawa Trough as shown by star marks in Fig. 1, and geochemical studies on hydrothermal fluids have been conducted in this area since the 1980s (Sakai *et al.*, 1990a, b; Gamo *et al.*, 1991; Chiba *et al.*, 1993; Ishibashi *et al.*, 1995).

Hydrothermal activity at Yonaguni Knoll IV was discovered in 2000 by diving surveys made by manned submersibles *Shinkai 6500* and *Shinkai 2000* (JAMSTEC) (Matsumoto *et al.*, 2001; Fujikura *et al.*, 2001), and the

chemical characteristics of hydrothermal fluids with a maximum temperature of 328°C have been revealed in detail in previous works (Konno *et al.*, 2006; Hongo *et al.*, 2007; Suzuki *et al.*, 2008). It has been suggested that the seafloor phase separation plays an important role in the diverse range of fluid chemistry and mineralization in the Yonaguni Knoll IV field (Konno *et al.*, 2006; Suzuki *et al.*, 2008).

The CH<sub>4</sub> concentrations (extrapolated to [Mg<sup>2+</sup>] = 0) and their δ<sup>13</sup>C values of the endmember fluids were reported to be 1.2 to 13.5 mmol kg<sup>-1</sup> and -26.4 ± 1.6‰, respectively, for four active chimney sites in the Yonaguni Knoll IV hydrothermal field (Konno *et al.*, 2006). It should be noted that the endmember CH<sub>4</sub> concentrations at the Yonaguni Knoll IV field are one to two orders of magnitude higher than those at unsedimented mid-oceanic ridge hydrothermal sites in the Pacific, Atlantic, and Indian Oceans (e.g., Welhan and Craig, 1983; Charlou *et al.*, 1996; Gamo *et al.*, 2001), which is favorable for conducting detailed studies of the microbial CH<sub>4</sub> consumption in hydrothermal plumes.

#### OBSERVATION AND CHEMICAL ANALYSIS

Extensive water column surveys at and around the Yonaguni Knoll IV hydrothermal field were carried out in June 2002 during the KH-02-1 cruise leg-3 of the R/V *Hakuho Maru* (Ocean Research Institute, The University of Tokyo) (Okino *et al.*, 2002) for the purpose of detailed mapping of hydrothermal plumes and taking plume water samples for chemical analyses. First, the areal extent of neutrally buoyant hydrothermal plumes was mapped using light backscattering nephelometers known as Miniature Autonomous Plume Recorders (MAPRs) (Baker and Milburn, 1997) during towing operations of the side-scan sonar *Wadatsumi*. We used four MAPRs, three of which were attached to the towing cable spaced vertically at 1 m, 50 m, and 100 m above the depressor of the *Wadatsumi*. The other MAPR was suspended 50 m below the depressor. Pressure data of the MAPRs were calibrated by comparing them with those of the CTD (Conductivity, Temperature, and Depth sensors) system described later. Figures 2(a) and (b) show the towing lines of the *Wadatsumi* around the Yonaguni Knoll IV field, and a horizontal distribution of the MAPR data expressed as raw voltage values at a depth of 1,070 m, respectively. The raw voltage output of the MAPR (light backscattering anomaly) was shown to be proportional to the mass concentration for typical deep-sea particles (Baker *et al.*, 2001).

Previous research has shown that high-temperature (220–328°C) fluid venting associated with sulfide–sulfate structures exists at four sites (named Tiger, Lion, Swallow, and Crystal) (Suzuki *et al.*, 2008; Inagaki *et al.*, 2006) that are located between 24°50.80′ N and 24°50.95′ N,

122°41.95′ E and 122°42.10′ E, as shown in figure 2 of Suzuki *et al.* (2008). This active center of the Yonaguni Knoll IV field is indicated by the filled square in Fig. 2(b). It is obvious that the hydrothermal plume, as indicated by the MAPR anomalies, was more significant closer to the active center (Fig. 2(b)). We occupied 5 hydrocast stations (SPOT-1 to -5) as shown in Fig. 2(b); station SPOT-5 (24°51.34′ N, 122°41.83′ E) was closest to the activity center (0.6 km to the north), while station SPOT-4 (24°53.65′ N, 122°43.76′ E) was farthest from the center at a distance of 6.1 km to the northeast.

We took seawater samples from surface to bottom (~10 m above seafloor) using 12-L Niskin-X bottles (General Oceanics) attached to a CTD-Carousel system (Seabird, model-9 plus and Carousel-32) for 1.5 days between 16 and 18 June, 2002. All the Niskin-X bottles were cleaned thoroughly with detergent, acid and Milli-Q water before use. A total of 24 Niskin-X bottles were attached to the CTD-Carousel system together with a sonar pinger (Benthos, 2216) for measuring the distance above the bottom. The CTD-Carousel package was connected to the end of a titanium armored cable. During the hydrocasts, the magnitude of hydrothermal plumes was monitored on board the ship in real time using a transmissometer (Seatech, 25 cm light path) installed on the CTD-Carousel system, which enabled us to surely take plume samples.

Upon recovery of the CTD-Carousel package, samples for CH<sub>4</sub> and carbon isotope measurements were transferred to glass vials (120 cm<sup>3</sup> in volume), sterilized with HgCl<sub>2</sub>, sealed and kept in a refrigerator before analysis on land. The concentration of CH<sub>4</sub> and its <sup>13</sup>C/<sup>12</sup>C ratio were measured with a continuous flow—<sup>13</sup>C/<sup>12</sup>C ratio mass spectrometer (CF-IRMS) system (Thermo, MAT-252) at Hokkaido University (Tsunogai *et al.*, 2000), and the δ<sup>13</sup>C<sub>PDB</sub> value was expressed in the usual delta notation (Craig, 1957). In addition, sub-samples for shorebased analyses of Mn concentration and helium isotope ratio (<sup>3</sup>He/<sup>4</sup>He) were taken in acid-cleaned low-density polyethylene bottles (Nalgene, 250 cm<sup>3</sup> in volume) and in copper tubes (~30 cm<sup>3</sup>; 9.52 mm O.D. with a thickness of 1.5 mm, ~100 cm long), respectively. The Mn samples (unfiltered) were acidified to pH 2 by adding ultrapure HNO<sub>3</sub> (TAMAPURE, AA-100). After adjusting the sample pH at 8.8 in the shorebased laboratory, total dissolvable Mn was measured using an automatic Mn analyzer based on chelating resin preconcentration and chemiluminescence detection (Doi *et al.*, 2004). <sup>3</sup>He/<sup>4</sup>He ratios were measured on a conventional noble-gas mass spectrometer (VG5400, Micromass Co.) according to the method of Sano and Wakita (1988). Dissolved oxygen was measured with a conventional Winkler method using an automatic titrator on board the ship.

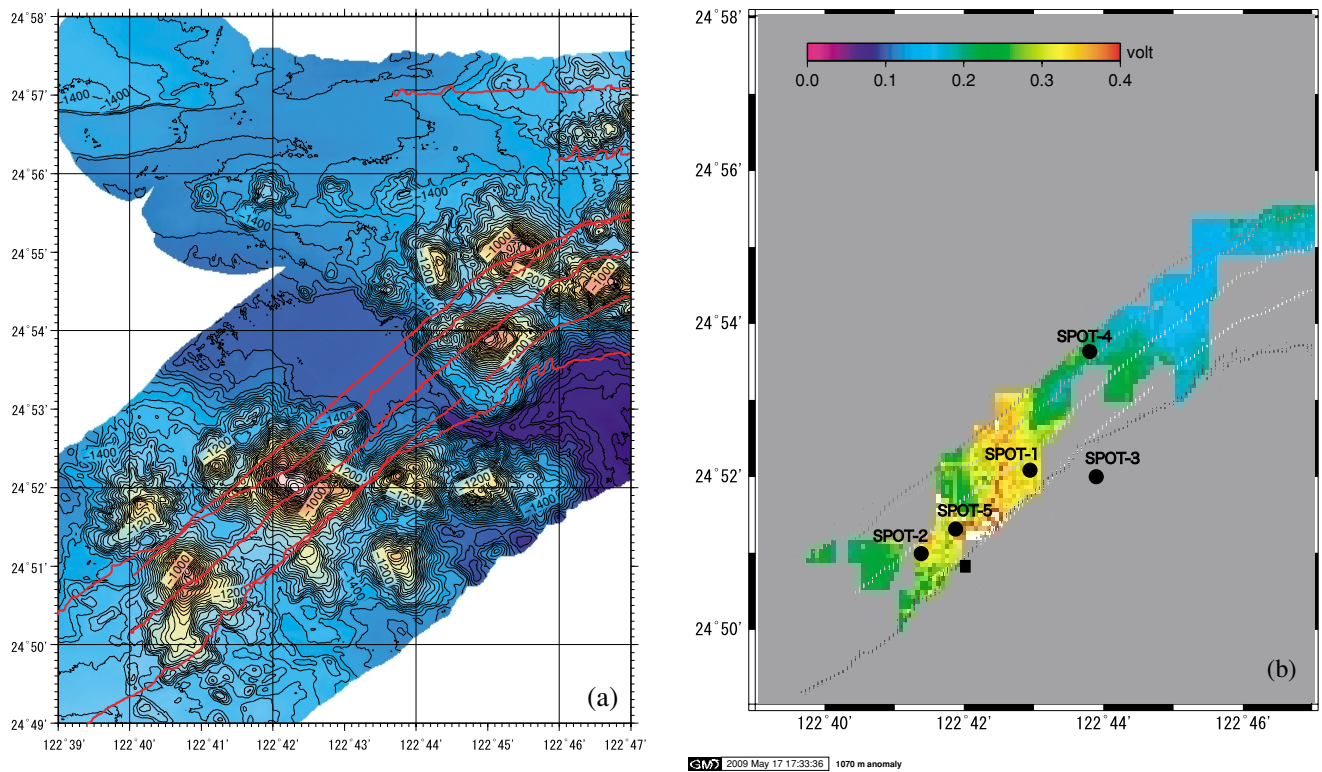


Fig. 2. (a) A bathymetric map around the Yonaguni Knoll IV hydrothermal field together with the tracks of the deep-tow side-scan sonar system Wadatsumi. (b) A map showing the hydrocast stations (SPOT-1 to -5) and the hydrothermal chimney site (shown by a filled square), together with spatial distribution of hydrothermal plumes at a depth of 1,070 m expressed by the anomalies (volt output) of MAPRs (Miniature Autonomous Plume Recorders) attached to the Wadatsumi. Northward to northeastward dispersion of hydrothermal plumes from the chimney site are suggested from red and yellow colored zones.

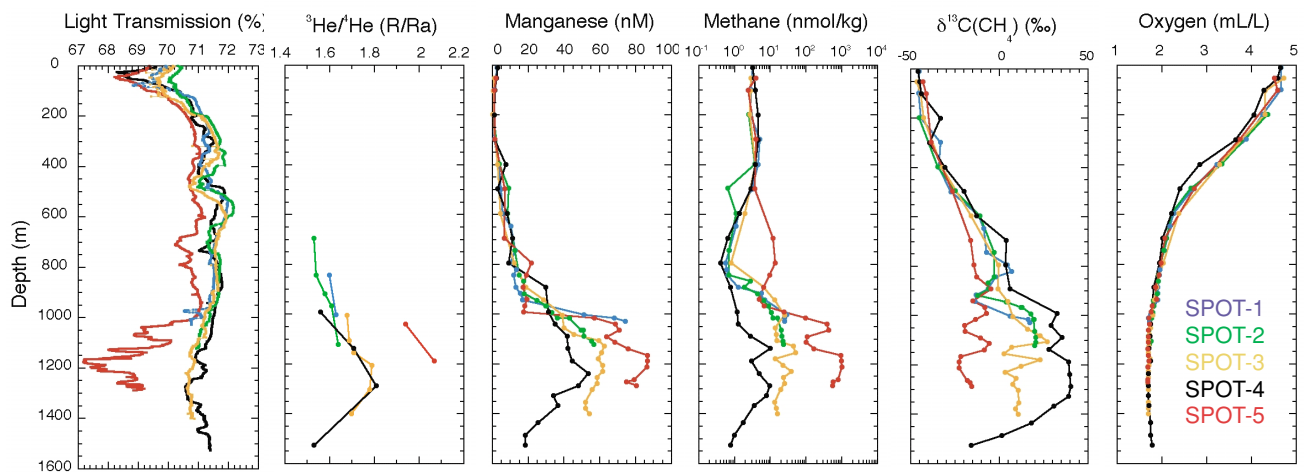


Fig. 3. Vertical profiles of light transmission, Mn,  $CH_4$ ,  $\delta^{13}C(CH_4)$  and  $O_2$  at stations SPOT-1, -2, -3, -4 and -5.

Table 1. Chemical composition of seawater samples collected at stations SPOT-1 (a), SPOT-2 (b), SPOT-3 (c), SPOT-4 (d), and SPOT-5 (e)

(a) SPOT-1, 16-June 2002, 08:03–09:21; 24°52.06' N, 122°42.90' E; Depth: 1060 m

Depth (m)	Temperature (°C)	Salinity	Oxygen (mL/L)	<sup>3</sup> He/ <sup>4</sup> He (R/R <sub>a</sub> )	Mn (nM)	CH <sub>4</sub> (nM)	δ <sup>13</sup> C (CH <sub>4</sub> ) (‰)
10	28.116	34.145	4.67		3.0	3.2	-45.1
98	22.387	34.850	4.67		1.3	2.9	-45.3
198	18.271	34.742	4.26		0.4	2.8	-44.1
297	14.629	34.582	3.89		1.3	5.3	-33.1
398	11.212	34.446	3.23		3.9	4.7	-33.9
497	8.673	34.382	2.69		5.1	3.2	-27.2
595	6.840	34.366	2.27		5.8	1.3	-11.4
645	6.509	34.374	2.19		10.4	1.1	-8.8
743	5.701	34.398	2.06		13.0	0.7	-7.2
793	5.494	34.404	1.98		10.1	0.6	4.7
819	5.286	34.404	1.96		13.7	0.6	7.3
843	5.255	34.406	1.95	1.60	12.0	0.7	-2.0
892	5.070	34.415	1.91		13.2	1.3	-6.1
916	5.050	34.411	1.91		15.7	5.7	-13.0
941	5.037	34.414	1.89		17.5	6.5	-13.4
941	5.032	34.416	1.91		17.1	7.0	-13.6
1001	4.550	34.439	1.76	1.63	51.2	29.2	6.3
1014	4.203	34.444	1.71		68.4	26.0	16.5
1027	4.196	34.445	1.73		74.4	25.4	17.4

## RESULTS AND DISCUSSION

Chemical data together with temperature and salinity for seawater samples taken at stations SPOT-1, -2, -3, -4 and -5 are summarized in Tables 1(a)–(e). <sup>3</sup>He/<sup>4</sup>He ratios were measured with the analytical error of about ±1% of the R/R<sub>a</sub> value, where R is the <sup>3</sup>He/<sup>4</sup>He ratio and R<sub>a</sub> is that of the atmospheric helium used as the standard. Analytical errors for O<sub>2</sub>, Mn, CH<sub>4</sub>, and δ<sup>13</sup>C (CH<sub>4</sub>) measurements are estimated to be less than ±0.02 mL/L, ±3%, ±0.3 nmol/kg, and ±0.3‰, respectively.

### Spatial variation of hydrothermal plumes

Figure 3 shows composite profiles of light transmission, <sup>3</sup>He/<sup>4</sup>He ratio (R/R<sub>a</sub>), Mn, CH<sub>4</sub>, δ<sup>13</sup>C (CH<sub>4</sub>), and O<sub>2</sub> at all the stations. Light transmission anomalies observed in deep seawater above hydrothermal zones generally indicate an excess of turbidity due to fine particles derived from the mixing of hot fluids and ambient seawater. Station SPOT-5 shows the strongest light transmission anomaly at ~1,170 m depth, while other stations show weaker anomalies as shown in Fig. 3. Station SPOT-5 also showed the highest He isotope anomaly with the maximum R/R<sub>a</sub> value of 2.07 at 1,187 m depth. This R/R<sub>a</sub> value is significantly higher than the background level (up to 1.25) in the East China Sea (Takahata *et al.*, 2004). The He isotope anomaly is evidence of the existence of mantle helium, confirming that the light transmission anomaly signals at SPOT-5 originated from hydrothermal fluid

venting.

Profiles of light transmission, Mn and CH<sub>4</sub> demonstrate triple-layered hydrothermal plumes, centered at 700–800 m, ~1,050 m, and ~1,200 m depths. Although the 700–800-m plume is less significant than the deeper plumes, Mn concentrations in this depth range at all the stations are significantly higher than its mean concentration in the open ocean of 0.3 nM (Bruland and Lohan, 2003), confirming hydrothermal input of Mn. The multi-layered structure of hydrothermal plumes suggests plural fluid venting sites and/or some temporal variation of the fluid venting pattern.

The profiles of Mn and CH<sub>4</sub> show that the highest and lowest numbers of anomalies were observed at stations SPOT-5 and SPOT-4, respectively, indicating that the former station is typical of a young plume and the latter is typical of an old plume. Suzuki *et al.* (2008) showed the endmember Mn concentration on the average for four hot fluids from the Yonagumi Knoll IV activity center was 1.01 ± 0.25 mmol/kg, from which the dilution factor for the 1,200-m plume at SPOT-5 ([Mn] = 87 nM) can be estimated to be an order of 10<sup>4</sup>. The central depth of this plume (~1,200 m at SPOT-5) becomes deeper by ~100 m at station SPOT-4 as shown in Fig. 3, indicating the plume is bending down as it ages spreading northeastward.

### CH<sub>4</sub> and its δ<sup>13</sup>C: useful non-conservative tracers for hydrothermal plumes

The CH<sub>4</sub> concentration in Fig. 3 is plotted in logarith-

Table 1. (continued)

(b) SPOT-2, 16-June 2002, 13:27–14:49; 24°50.56'N, 122°41.34'E; Depth: 1173 m

Depth (m)	Temperature (°C)	Salinity	Oxygen (mL/L)	<sup>3</sup> He/ <sup>4</sup> He (R/R <sub>0</sub> )	Mn (nM)	CH <sub>4</sub> (nM)	δ <sup>13</sup> C (CH <sub>4</sub> ) (‰)
198	18.521	34.767	4.33		0.7	2.6	-45.1
198	18.499	34.765	4.37		0.7	2.5	-45.0
395	11.842	34.448	3.35		4.0	3.8	-34.5
493	8.458	34.384	2.66		9.4	0.7	-24.8
595	6.635	34.369	2.22		8.8	1.1	-10.9
694	5.860	34.401	2.11	1.53			
743	5.573	34.401	2.05		12.6	0.7	-2.8
843	5.139	34.412	1.93	1.54	15.3	0.7	-2.6
867	4.999	34.417	1.93		17.5	2.8	-6.2
892	4.873	34.420	1.87		18.4	1.9	-5.7
918	4.768	34.422	1.90	1.58	17.9	4.6	-11.9
943	4.688	34.424	1.82		25.0	6.6	4.1
967	4.601	34.428	1.83		30.2	9.3	12.4
967	4.609	34.425	1.80	1.61	29.9	8.8	12.9
992	4.554	34.432	1.81		33.3	11.0	17.8
1015	4.523	34.433	1.78		36.5	12.2	19.7
1039	4.415	34.437	1.74		43.2	16.1	19.5
1062	4.344	34.438	1.72		49.8	20.7	20.0
1063	4.347	34.437	1.72		50.9	20.6	20.2
1088	4.327	34.437	1.70		51.4	21.4	20.8
1107	4.309	34.437	1.77		55.6	23.1	20.8
1121	4.293	34.439	1.73	1.64	56.9	24.0	20.2

(c) SPOT-3, 16-June 2002, 19:10–20:32; 24°51.54'N, 122°43.50'E; Depth: 1453 m

Depth (m)	Temperature (°C)	Salinity	Oxygen (mL/L)	<sup>3</sup> He/ <sup>4</sup> He (R/R <sub>0</sub> )	Mn (nM)	CH <sub>4</sub> (nM)	δ <sup>13</sup> C (CH <sub>4</sub> ) (‰)
50	24.140	34.861	4.73		1.27	2.9	-46.2
101	22.235	34.768	4.31		0.69	2.9	-44.2
198	18.560	34.770	4.31		0.44	2.8	-42.6
397	11.243	34.425	3.30		3.17	4.0	-32.0
595	7.218	34.356	2.38		4.5	2.0	-15.6
793	5.545	34.399	2.04		12.3	0.8	-0.4
892	4.920	34.415	1.89		18.8	6.5	-0.5
942	4.678	34.394	1.84		28.7	13.5	4.9
1004	4.484	34.432	1.81	1.68	39.2	25.8	7.6
1055	4.361	34.435	1.75		40.2	14.0	16.0
1080	4.329	34.438	1.73		45.8	15.1	23.2
1105	4.266	34.439	1.71	1.69	59.7	15.3	27.3
1128	4.209	34.443	1.72		62.8	44.8	7.3
1154	4.180	34.445	1.71	1.71	61.2	52.3	2.7
1178	4.141	34.447	1.74		59.3	14.2	23.2
1204	4.135	34.570	1.72	1.79	61.7	22.9	12.5
1228	4.107	34.449	1.70		61.7	39.8	3.5
1253	4.088	34.450	1.70		58.7	24.0	9.5
1278	4.088	34.451	1.69		58.7	26.0	7.6
1303	4.067	34.450	1.70	1.78	55.9	19.9	10.8
1352	4.046	34.451	1.70		52.3	13.7	11.2
1377	4.049	34.452	1.71		52.2	14.8	9.2
1399	4.045	34.4521	1.71	1.70	54.40	15.9	10.9

Table 1. (continued)

(d) SPOT-4, 17/18-June 2002, 23:59–01:27; 24°53.65' N, 122°43.76' E; Depth: 1564 m

Depth (m)	Temperature (°C)	Salinity	Oxygen (mL/L)	<sup>3</sup> He/ <sup>4</sup> He (R/R <sub>o</sub> )	Mn (nM)	CH <sub>4</sub> (nM)	δ <sup>13</sup> C (CH <sub>4</sub> ) (‰)
10	27.014	34.168	4.66		3.2	3.3	-45.7
50	25.006	34.487	4.59		2.4	3.6	-44.8
99	22.419	34.681	4.29		1.6	4.0	-43.8
199	17.023	34.686	4.06		1.3	4.6	-33.2
298	13.153	34.507	3.65		1.5	4.7	-39.3
397	9.472	34.401	2.85		7.7	3.9	-30.4
496	7.481	34.357	2.41		3.2	2.9	-19.8
595	6.521	34.372	2.23		8.4	1.4	-12.6
694	5.696	34.399	2.03		11.3	0.7	3.9
793	5.190	34.409	1.95		9.2	0.4	3.7
892	4.728	34.424	1.83		30.0	0.8	6.3
991	4.431	34.434	1.77	1.56	31.4	1.2	32.7
1039	4.361	34.438	1.76		35.3	1.3	29.4
1088	4.308	34.438	1.74		41.9	2.8	35.6
1137	4.282	34.440	1.72	1.71	42.4	10.0	28.0
1188	4.212	34.441	1.76		44.7	3.1	39.3
1237	4.112	34.448	1.71		53.6	5.0	39.7
1286	4.091	34.449	1.70	1.81	48.2	10.0	40.4
1327	4.066	34.450	1.71		34.3	8.0	39.4
1366	4.015	34.452	1.72		36.7	3.7	30.9
1435	3.994	34.455	1.76		25.8	1.8	18.2
1486	3.963	34.454	1.75		18.5	1.0	1.6
1526	3.941	34.456	1.79	1.53	18.6	0.8	-15.6

(e) SPOT-5, 18-June 2002, 19:12–20:20; 24°51.34' N, 122°41.83' E; Depth: 1344 m

Depth (m)	Temperature (°C)	Salinity	Oxygen (mL/L)	<sup>3</sup> He/ <sup>4</sup> He (R/R <sub>o</sub> )	Mn (nM)	CH <sub>4</sub> (nM)	δ <sup>13</sup> C (CH <sub>4</sub> ) (‰)
50	25.027	34.579	4.52		2.1	4.0	-42.8
100	22.465	34.844	4.59		1.2	2.4	-41.0
297	13.696	34.521	3.75		1.5	4.1	-38.3
496	8.731	34.385	2.73		7.1	3.8	-26.1
694	5.880	34.403	2.07		6.9	12.0	-15.9
793	5.416	34.410	1.99		22.1	14.0	-14.3
842	5.241	34.403	1.94		19.2	9.9	-12.5
892	4.897	34.415	1.87		17.3	6.7	-4.7
940	4.769	34.419	1.89		19.5	4.9	-14.8
966	4.697	34.419	1.79		18.4	6.9	-8.0
990	4.577	34.430	1.77		17.6	24.9	-7.0
1014	4.309	34.439	1.72		57.2	100.4	-13.2
1039	4.242	34.439	1.73	1.94	68.8	400.7	-19.5
1064	4.235	34.441	1.70		71.1	434.7	-19.2
1089	4.240	34.441	1.71		64.0	110.4	-10.4
1113	4.214	34.443	1.71		68.5	101.3	-5.6
1139	4.183	34.444	1.69		75.9	169.2	-8.4
1164	4.137	34.444	1.71		86.8	948.2	-21.7
1187	4.134	34.446	1.72	2.07	86.7	1005.6	-22.6
1212	4.157	34.444	1.69		86.4	1026.4	-22.4
1263	4.123	34.447	1.69		79.2	794.3	-18.0
1271	4.111	34.446	1.69		74.9	576.5	-16.4
1287	4.107	34.449			80.6	565.5	-15.3

mic scales, demonstrating that the residence time of CH<sub>4</sub> is much shorter than that of Mn, as previously observed for the Endeavour Ridge hydrothermal plume (Kadko *et al.*, 1990). As shown in Tables 1(d) and (e), the maximum CH<sub>4</sub> concentration at SPOT-4 (10 nM at 1,286 m depths) is no more than a hundredth that at SPOT-5 (1,026 nM at 1,212 m depth), while the maximum Mn concentration at SPOT-4 (53.6 nM at 1,237 m depth) is more than a half of that at SPOT-5 (86.8 nM at 1,164 m depth). Such a significant decrease of CH<sub>4</sub>/Mn ratios from the young plume to the old plume indicates that the CH<sub>4</sub> consumption by methane-oxidizing bacteria (methanotrophs) occurs much more rapidly than dilution with ambient seawater (De Angelis *et al.*, 1993).

The rapid decrease and increase, respectively, of CH<sub>4</sub> concentration and of δ<sup>13</sup>C (CH<sub>4</sub>) suggest a high level of microbial activity (CH<sub>4</sub> oxidation) on hydrothermal plume particles and/or in plume seawater, possibly supported by abundant CH<sub>4</sub> in Yonaguni Knoll IV hydrothermal fluids. Such microbial process consumes <sup>12</sup>C-CH<sub>4</sub> more rapidly than <sup>13</sup>C-CH<sub>4</sub>, which causes significant carbon isotope fractionation of CH<sub>4</sub> as the plume ages (Tsunogai *et al.*, 2000, 2005; Cowen *et al.*, 2002; Grant and Whiticar, 2002). This is clearly reflected in a big difference of δ<sup>13</sup>C (CH<sub>4</sub>) between the SPOT-5 and SPOT-4 hydrothermal plumes. The δ<sup>13</sup>C (CH<sub>4</sub>) value of the 1,200-m plume is increased by as much as >60‰, from -22.4‰ at SPOT-5 to +40.4‰ at SPOT-4. The value of +40.4‰ is the highest δ<sup>13</sup>C so far observed for CH<sub>4</sub> in seawater. The minimum δ<sup>13</sup>C (CH<sub>4</sub>) value observed for the 1,200-m plume at SPOT-5 (-22.6‰) was already heavier than the averaged value for the fluid endmembers, -26.4 ± 1.6‰ (Konno *et al.*, 2006), suggesting a progress of CH<sub>4</sub> oxidation between the fluid endmember and the young plume at SPOT-5.

As can be seen for the 700–800-m plume (Fig. 3), the CH<sub>4</sub> oxidation or the δ<sup>13</sup>C anomaly is still recognized after the CH<sub>4</sub> concentration has reached almost the background level (<1 nM), showing concave profiles between 600 and 1,000 m depth at stations SPOT-1, -2, -3, and -4. The concavity indications of the CH<sub>4</sub> profiles are similar to that observed in the Endeavour Ridge hydrothermal plume where the microbial CH<sub>4</sub> oxidation was accelerated (figure 11(A) of Kadko *et al.*, 1990). We could recognize hydrothermal plumes based on the positive δ<sup>13</sup>C anomaly as “microbial plumes,” even after the CH<sub>4</sub> anomaly has disappeared and becomes useless as the plume tracer.

#### Carbon isotope fractionation during the microbial CH<sub>4</sub> oxidation

Since the hydrothermal plumes of this study are in oxygenated seawater as shown in Table 1 and Fig. 3, aerobic CH<sub>4</sub> oxidation is expected to occur as shown in the

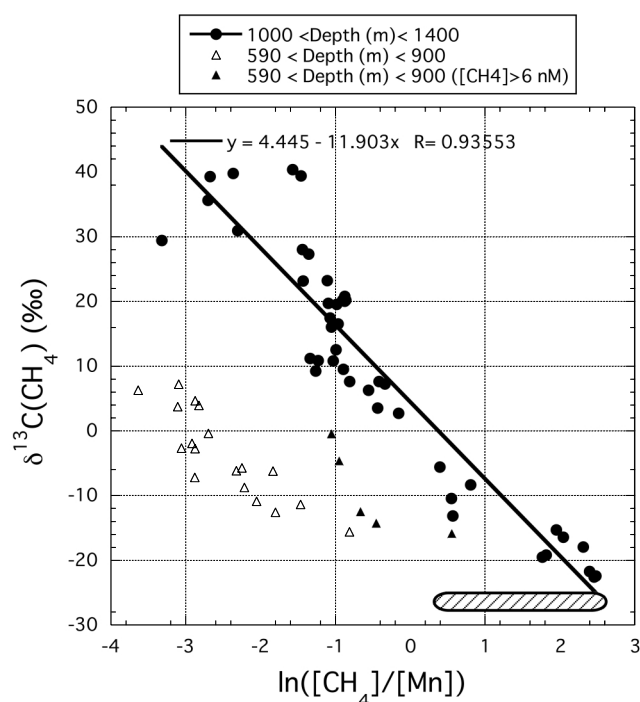


Fig. 4. Relationship between  $\ln([CH_4]/[Mn])$  and  $\delta^{13}C(CH_4)$  for the data between 590 m and 900 m depth (triangles) and the data between 1000 m and 1400 m depth (filled circles). The straight line is the linear regression result by applying the Rayleigh equation (3) to the deeper plume samples between 1000 m and 1400 m depth. The filled triangles show samples with relatively high CH<sub>4</sub> values (>6 nM). The shaded ellipse means a data area of endmember fluids so far observed at the Yonaguni Knoll IV hydrothermal field (Konno *et al.*, 2006; Suzuki *et al.*, 2008).

following reactions (1) and (2):



The kinetic isotope fractionation factor  $\alpha (=k_{12}/k_{13})$  can be estimated from the following Rayleigh distillation equation by assuming a closed system (Coleman *et al.*, 1981):

$$\begin{aligned} \delta^{13}C_t &= 1000(1/\alpha - 1)\ln(M_t/M_0) + \delta^{13}C_0 \\ &= 1000(1/\alpha - 1)\ln M_t + K \quad (K: \text{constant}) \end{aligned} \quad (3)$$

where  $\delta^{13}C_t$  and  $\delta^{13}C_0$  are the  $\delta^{13}C$  value of temporal (residual) CH<sub>4</sub> and that of the initial CH<sub>4</sub>, respectively, and  $M_t$  and  $M_0$  are the amounts of CH<sub>4</sub> in a system at the temporal and initial stage, respectively. If the CH<sub>4</sub> oxidation proceeds in a closed system, we could use the measured CH<sub>4</sub> concentration as  $M_t$ . However, the hydrother-



mal plume is diluted by mixing with ambient seawater. To correct for the effect of dilution, we used  $[\text{CH}_4]/[\text{Mn}]$  as  $M_t$ , by assuming that Mn could almost be regarded as a conservative entity or that it varies only by dilution with ambient seawater, because Mn is known to have much longer residence time than  $\text{CH}_4$  in hydrothermal plumes (Kadko *et al.*, 1990). Although this assumption should be definitely confirmed by a linear relationship between Mn and a conservative tracer such as  $^3\text{He}$ , we cannot give any quantitative discussion on this point this time due to the lack of He concentration data.

Figure 4 shows the relationships between  $\ln([\text{CH}_4]/[\text{Mn}])$  and  $\delta^{13}\text{C}(\text{CH}_4)$  for the data from all the stations in two depth ranges: (1) from 1,000 to 1,400 m depth to include most samples of the 1050-m and 1200-m plumes (shown by filled circles), and (2) from 590 to 900 m depth for the 700–800-m plume (shown by triangles). As for the latter samples, five data with relatively high  $\text{CH}_4$  concentration ( $[\text{CH}_4] > 6$  nM) are separately indicated by filled triangles, while the other data with less  $[\text{CH}_4]$  values by open triangles. A wide range of the endmember fluid data so far observed ( $\delta^{13}\text{C}(\text{CH}_4) = -26.4 \pm 1.6\text{‰}$  and  $\ln([\text{CH}_4]/[\text{Mn}]) = 0.3$  to 2.6; Konno *et al.*, 2006; Suzuki *et al.*, 2008) are also shown as a shaded ellipse.

Figure 4 shows that there are two linear trends with different slopes, one for the deeper plume data (filled circles) and the other for the shallower plume data (triangles). The linearity is definite evidence that the  $\text{CH}_4$  distribution at SPOT-1 to -5 is controlled by the above-mentioned isotope fractionation associated with the aerobic  $\text{CH}_4$  oxidation. Separate trends between the deeper plumes and the shallower plume suggest i) that the endmember fluids (i.e., starting points somewhere in the shaded ellipse) may be different, i.e., a higher  $[\text{CH}_4]/[\text{Mn}]$  value for the source fluids of the deeper plumes, and ii) that the isotope fractionation factors are different between the deeper plumes and the shallower plume. The latter point will be discussed later in detail.

The linear least-square regression was applied to the deeper plume data between 1,000 and 1,400 m depth (filled circles) in Fig. 4, giving the following best-fitted equation:

$$\delta^{13}\text{C}_t = -11.903 \ln M_t + 4.445 \quad (R = 0.936). \quad (4)$$

Equation (4) is indicated by the straight line in Fig. 4. The obtained factor of  $-11.903$  has the standard deviation of 0.685. By substituting these values in Eq. (3), the kinetic isotope fractionation factor ( $\alpha$ ) is calculated to be  $1.012 \pm 0.001$  ( $\pm 2\sigma$ ). This value is within the wide range of  $\alpha$  (1.002 to 1.039) so far reported for microbial  $\text{CH}_4$  oxidation from both laboratory culture studies and field observations in freshwater and seawater, as summarized by Grant and Whiticar (2002) and Templeton *et al.* (2006).

We also obtained a smaller  $\alpha$  value ( $1.005 \pm 0.002$ ) for the shallower data shown by triangles in Fig. 4. Why are the kinetic isotope fractionation factors different between the shallow and the deep hydrothermal plumes from the same submarine hydrothermal field? Cowen *et al.* (2002) attributed the variation of  $\alpha$  values to temperature from a positive correlation between  $\alpha$  and temperature, but this is not the case for our result, because the shallower plume at higher temperatures ( $5\text{--}7^\circ\text{C}$ ) gave a lower  $\alpha$  value of 1.005, in contrast to the deeper plumes at lower temperatures ( $4.0$  to  $4.5^\circ\text{C}$ ) with an  $\alpha$  value of 1.012. Another possibility is that the carbon isotope discrimination by  $\text{CH}_4$ -oxidizing bacteria may vary significantly depending on several conditions, such as types of methanotroph, their cell densities, limited  $\text{O}_2$  availability, or other variables, according to Templeton *et al.* (2006).

On the other hand, we have to take it into consideration that the  $\text{CH}_4$  values of the shallower plume are relatively low, some of which are close to the background level of  $<1$  nM. Although we occupied no control station to define the background  $\text{CH}_4$  concentration and its  $\delta^{13}\text{C}$  value in this study area, the background  $\text{CH}_4$  concentration could be estimated to be  $\sim 0.5$  nM from the measured values in the mid-Okinawa Trough ( $27^\circ 22' \text{N}$ ,  $126^\circ 45' \text{E}$ ) (Watanabe *et al.*, 1995). The  $\delta^{13}\text{C}$  of background  $\text{CH}_4$  at 2,000 to 3,000 m depth measured in the world oceans has been  $-35$  to  $-50\text{‰}$  (Holmes *et al.*, 2000; Cowen *et al.*, 2002; Keir *et al.*, 2005, 2009), which is lighter than all the plume  $\delta^{13}\text{C}$  values shown in Fig. 4. It is therefore expected that continued mixing with background seawater should decrease the  $\delta^{13}\text{C}$  value as the hydrothermal plume disperses horizontally. While this decreasing effect is negligibly small where the  $\text{CH}_4$  concentration is much higher than the background level as those of the deeper plumes, the effect becomes more significant as the  $\text{CH}_4$  concentration decreases closer to the background value (Keir *et al.*, 2009). As a result, the slope of  $\delta^{13}\text{C}(\text{CH}_4) - \ln([\text{CH}_4]/[\text{Mn}])$  relationship of the shallower plume with low  $\text{CH}_4$  concentrations should become more gentle to give lower  $\alpha$  value than that expected from the Rayleigh distillation only. It seems reasonable that some shallower plume samples with relatively high ( $>6$  nM)  $\text{CH}_4$  concentration (filled triangles in Fig. 4) show a more steep slope than the other samples (open triangles), suggesting a higher  $\alpha$  value close to that of the deeper plume samples of 1.012.

## CONCLUDING REMARKS

1) Triple-layered hydrothermal plumes were recognized at 700–800 m,  $\sim 1,050$  m, and  $\sim 1,200$  m depths over the Yonaguni Knoll IV hydrothermal field, southwesternmost Okinawa Trough, from profiles of light transmis-

sion,  $^3\text{He}/^4\text{He}$  ratio, concentrations of Mn and  $\text{CH}_4$ , and  $\delta^{13}\text{C}$  of  $\text{CH}_4$ .

2) Methane concentration in the hydrothermal plumes decreases much more rapidly than that of Mn as the plumes become older, indicating microbial  $\text{CH}_4$  oxidation is actively occurring. Even after the  $\text{CH}_4$  concentration falls to almost the background level, the existence of the hydrothermal plume (microbial plume) can still be detected from positive anomalies of  $\delta^{13}\text{C}$  of methane.

3) The microbial  $\text{CH}_4$  consumption is clearly supported by the increase of  $\delta^{13}\text{C}$  ( $\text{CH}_4$ ) values as the plume becomes older. Inverse linear correlations between  $\ln([\text{CH}_4]/[\text{Mn}])$  and  $\delta^{13}\text{C}$  ( $\text{CH}_4$ ) gave a kinetic isotope fractionation factor of 1.012 for the plumes deeper than 1,000 m, as determined by applying the Rayleigh distillation equation to the linear trend.

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