

Methane seepage in the Japan Sea?-Sediment fatty acids from the Northern Okushiri Ridge, compared those from a methane seep and a submarine volcano

T. Naganuma and Y. Fukuda

Faculty of Applied Biological Science, Hiroshima University, Higashi-hiroshima, 739 Japan

Katsunori Fujikura and Jiro Naka

Deep Sea Research Department, JAMSTEC, 2-15 Natsushima-cho, Yokosuka, 237 Japan

Yukihiro Kato

Hydrographic Department of Japan, Tsukiji, Tokyo, 104 Japan

Akira Takeuchi

Department of Earth Science, Toyama University, 3190 Gofuku, Toyama, 930 Japan

Abstract. A biological community was discovered in the Northern Okushiri Ridge, north-eastern Japan Sea. The community was associated with seafloor fissures, and presumed to be dependent on chemosynthesis. Sediments in and nearby the fissures were collected, and their fatty acids were analyzed in amounts and compositions. The fatty acids compositions were compared with those from a nearby seamount, and a known methane seep and a submarine volcano. As a result, close relationship between the Northern Okushiri Ridge and the known methane-seep was found. This finding suggests that methane-seepage and the associated biological community were first discovered in the Japan Sea. This also support the hypothesis that the eastern margin of the northern Japan Sea is at the early stage of new subduction.

1. Introduction

The Northern Okushiri Ridge is located in the area where the eastern margin of the Japan Sea (= hypothesized incipient plate boundary) and the Suttsu tectonic zone (= the northern end of the northeastern Japan arc) are meeting (Fig. 1; Miyashita et al., 1989; Takeuchi and Tanaka, 1989, 1990). Subduction with back thrust, or obduction, is presumed to the area (Tamaki and Honza, 1985; Kuramoto, 1989; Tokuyama et al., 1989, 1992). Fissures with bacterial colonies and small biological communities of snails and amphipod-like plankton were observed in the western, frontal slope of the ridge (Takeuchi et al., 1992). Swarms of amphipod-like plankton were observed in the

eastern, back-thrustal slope of the ridge where high heat flux was measured (ODP site 795, Tamaki et al., 1990; Tokuyama et al., 1991). These observations were suggested to be linked to the cold seepage which is often associated with subduction. In a series of the Shinkai 6500 divers conducted in July 1995, the fissure-associated biological communities were re-observed, and the dependence on microbial chemosynthesis was strongly suggested (Fujikura et al., 1995). This suggestion can be confirmed by the detection of chemosynthetic microorganisms occurring in the sediment associated with the fissures. For this purpose, the amounts and compositions of sediment fatty acids are known to characterize effectively the sediment microflora (eg., Rajendran et al., 1994; Naganuma et al., 1996).

This communication reports the amounts and compositions of the sediment fatty acids from the northern Okushiri Ridge, in comparison with those from a well-characterized methane-seep, a seamount, a submarine volcanic vent, and a vestimentiferan tubeworm. The fatty acid analysis suggested the methane-seepage in the Northern Okushiri Ridge is likely to occur and form biological communities dependent on chemosynthesis. This possibility supports the hypothesis of incipient subduction in the Okushiri Ridge (Nakamura, 1983; Tamaki and Honza, 1985).

2. Materials and Methods

2. 1 Collection of sediment samples

(1) Northern Okushiri Ridge

Sediment samples of the Northern Okushiri Ridge (NOR) were collected during the *Shinkai* 6500 dives (Nos. 265-271, July 1995; Table 1). The sediment samples of NOR 1-6 were collected at the fissures in southern part of the Northern Okushiri Ridge (42-42'N to 42-46'N), where epicenter of the Hokkaido Nansei-oki Earthquake in July 1993 was located. The sediment samples of NOR 7-16 were collected at fissures on the westward steep slope in northern part of the Northern Okushiri Ridge (44-14'N).

Top 0-5 cm of the cores generally were taken and used for fatty acid analysis, except otherwise specified. Sediments other than the NOR samples were treated likewise.

(2) *Shiribeshi Seamount*

Shiribeshi Seamount (SSM), located in the Northern Okushiri Ridge, has the major median N-S fault with scarps on its southern and northern slopes. Biological abundance was relatively high on the summit plane and dense communities were sporadically formed (Fujikura et al., 1991; Naganuma, 1995). However, seamounts are generally known to have high biological density due to increased and upwelling bottom currents (e.g., Rogers, 1994). Thus, the SSK communities may not be necessarily associated with venting or seepage. The sediment samples of SSK 1-7 were collected during the *Shinkai* 2000 dives (Nos. 745-747, July 1994; Naganuma 1995; Table 1).

(3) *Off-Hatsushima Methane Seep*

Large-scale colonies of *Calyptogena* clams are known at the off-Hatsushima Methane Seep (HMS) in the western Sagami Bay (Table 1; Hashimoto et al., 1989; Masuzawa et al., 1992). The sediment samples of HMS 1-3 and vestimentiferan tubeworms were collected during the *Shinkai* 2000 dive (No. 722, November 1993; observer, M. Yosida). A tubeworm was also used for fatty acid analysis, with subsamples from gill, muscle, trophosomal epidermis and trophosomal content (prefixed with TW).

(4) *Teishi Volcanic Knoll*

Teishi Volcanic Knoll (TVK), western Sagami Bay, is a submarine volcano that erupted in July 1989. Active venting and high water

temperature of 90-100°C were observed in the crater. The volcanic venting was gradually slowing down, and the maximum sediment temperature was 33°C in March 15 (Naka, 1994), when the samples TVK 1-3 were collected from inside of the crater during the *Dolphin* 3K dives (Nos. 175 and 176, Table 1).

2. 2 Fatty acid analysis

(1) *Freeze-dry (lyophilization)*

The sediments from the Northern Okushiri Ridge were freeze-dried and preserved at -80°C on board immediately after the recovery of the submersible. Otherwise, sediment and tubeworm samples were preserved on board at -80°C and freeze-dried afterward.

(2) *Fatty acid contents and compositions*

Analysis of fatty acid contents and compositions was done by gas chromatography. Total fatty acids in weighted sediment sample were saponificated and methylated to form fatty acid methyl esters (FAMES). FAMES were extracted into mixture of hexane and tert-butyl ether, and used for gas chromatography (Sasser, 1990a). The results of the FAME profiles (i.e., fatty acid compositions) were cluster-analyzed to visualize relatedness of the sediment with the dendrogram (Sasser, 1990b; Fig 1).

3. Results and Discussion

3. 1 Total fatty acid content

Total fatty acid content is a measure of the abundance of living, dead or dormant organisms in the sediment. Total fatty acid content of the sediments from the Northern Okushiri Ridge ranged from below the detection limit (NOR 10) to the maximum of 28.41 ng/g dry sediment (NOR 9), which was also the maximum among the samples listed in this report (Table 1).

There was a general tendency that the sediment that was closer to a fissure contained more fatty acid. Interestingly, while the maximum fatty acid content was found in the sediment that was filling a fissure (NOR 9), the minimum content was also found inside of, but on the 30 cm-deep bottom of another fissure (NOR 10). Probably we collected the exposed, old sediment at 30 cm below surface (cmbs) by recent fissuring.

Sedimentation rate of this area is estimated to be about 5-6 cm/1000 years according to the ODP investigation (Sites 795 and 796; Tamaki et al., 1990). Thus the simple age estimation of the sediment 30 cmbs is about 5000-6000 years old. Fatty acids in the "old" sediment were thus likely to be below the detection level after degradation over a long period.

3. 2 Diversity of sediment fatty acids

The number of fatty acid forms is a measure of microfloral diversity in the sediment. The number of fatty acid forms in the sediment sample varied from 1 to 17-18. When only a single form was contained, it was palmitic acid (16:0; NOR 15 and SSK4) or palmitoleic acid (16:1 w7c; NOR 1), both of which commonly occur in marine sediments and organisms.

The sediments having diverse fatty acids were generally high in the total fatty acid content. For example, the NOR 9 sample contained 17 fatty acid forms and the maximum fatty acid contents, and the tendency was found for other samples. Total fatty acid content [X] and the number of fatty acids [Y] of all but NOR 10 and TW samples were positively correlated with statistical significance, expressed by the following allometric equation:

$$Y = 4.614 X^{0.372} (r=0.745, n=29).$$

Applicability of allometric equation for sediment fatty acids is not clear. However, it can be at least concluded that the increase of total fatty acids should be associated with the entry of new fatty acid forms, not ascribed to the increase of a single or couple of forms. That is, the sediment having high microbial abundance is expected to have high microfloral diversity.

3. 3 Composition of fatty acids

The fatty acids characteristic of the sediments associated with biological communities were odd-numbered fatty acids (15, 17, 19). In contrast, the sediments not associated with methane seeps or biological communities consisted largely of even-numbered, saturated fatty acids (14:0, 16:0, 18:0) as shown with the NOR 1 and SSK 4 samples, as well as with the sediment from 4000 m deep (e.g., Naganuma et al., 1996). Gamma-linolenic acid (18:3 w6) was found in the Northern Okushiri Ridge sediments (NOR 2-6), which is generally of

animal origin. This suggests that the abundance of meio- and microfauna increased, but the source of organic input for the increase is unclear. Arachidonic acid (20:4 w6), possibly of plant and/or diatom origin, was found in the sample from Teishi Volcanic Knoll (TVK3). Considering the water depth of 108 m and distance from land of 4 km at the sampling site, it is very likely that the fatty acids of the sediment were influenced by the input from terrestrial plants and shallow-water phytoplankton.

3. 4 Dendrogram

Relatedness of the sediments based on fatty acid composition was schematically expressed by a dendrogram (Fig. 1). Sediments having similar fatty acid compositions are closely located each other on a dendrogram. On the contrary, Sediments of different fatty acid compositions are remotely located (Naganuma et al., 1996). The HMS samples were from the well-known methane seep and regarded as the references of the methane seep sediments, with HMS 1 being from inside of a *Calypotgena* colony and thus most associated with methane seep. The most closely related to HMS 1 were the samples NOR 8 and NOR 9, which were from inside of and at 0.5 m from a fissure, respectively (Fig. 1, Cluster 1). Coincidentally, NOR 9 was the highest both in the total fatty acid content and the fatty acid diversity, and NOR 8 was second to it.

The most closely related to HMS 2 and 3 were NOR 11, SSK1 and SSK6 (Fig. 1, Cluster 2). NOR11 was also collected at 1 m from a fissure. SSK 1 and SSK 6 was collected at the sites where swarms of planktonic crustaceans and chaetognaths were observed.

Interestingly, NOR 2 was closely related to tubeworm tissue samples (Fig. 1, Cluster 3). NOR2 was collected at a flame structure or an incomplete mud volcano in the Flame structure in the epicenter of the Hokkaido Nansei-oki Earthquake. Flame structure is a sedimentary structure consisting of flame-shaped plumes of mud that have been squeezed irregularly upward into an overlying layer. NOR2 was collected a flame structure that was covered with gelatinous material. The gelatinous material was most probably associated with certain geological

activity such as episodic methane seepage, but its origin and nature were unclear. At least, the material was assumed to have biological origin, considering the close similarity with tubeworm tissues in fatty acid composition.

3. 5 Biomarker fatty acids

Certain fatty acids are present in notable quantity or as unique for specific species. Such fatty acids often serve as biomarkers to characterize the microflora of environmental samples (e.g., Rajendran et al., 1994). In this study, the biomarker fatty acids for the microorganisms involved in targeted biogeochemical processes were selected as follows: Sulfate reduction - 15:0, 15:0 iso/anteiso, 16:1 iso, 18:1 w7c, 17:0 cyclo, 17:1 w8c, and 17:1 iso/anteiso; Methane oxidation - 16:1 w5c, and 18:1 w7c; and Sulfide oxidation - 18:1 w7c, 19:0 cyclo. Also, the biomarker fatty acids for campylobacters (16:0 3-OH, 18:1 w7c, and 19:0 cyclo) were also selected. Campylobacters represent the epsilon-subdivision of Proteobacteria, many of which are microaerophilic and known to dominate hydrothermal vent microflora (e.g., Polz and Cavanaugh, 1995). They are likely to contribute to the co-existence of anaerobic sulfate-reduction and aerobic sulfide-oxidation (e.g., Teske et al., 1996).

Activities of sulfate-reduction and methane-oxidation were assumed for the samples of HMS 1, NOR 8, NOR 9 and SSK 1, based on the occurrence of biomarker fatty acids. HMS 1 was from a known methane seep, and thus reasonably characterized as such. The NOR 8 and NOR 9 was probably associated with methane seepage, although methane concentration in the water and/or sediment was not measured. SSK 1 was from a fault scarp with a dense planktonic community, but the association with methane seepage or other geological activities is not known. The planktonic community rather seemed to be associated with increased and upwelling bottom current typical of seamounts (e.g., Rogers, 1994), and thus not related to methane seepage. Possible sulfate reduction and methane-oxidation might occur in the local sediment receiving large organic inputs such as fecal pellets, molts and debris from the plankton community.

4. Conclusions

The fissure-associated sediments of the Northern Okushiri Ridge were very closely related to those of the off-Hatsushima Methane Seep, based on the fatty acid compositions and the occurrence of specific biomarkers. The close relatedness strongly suggests that the biological community of the Northern Okushiri Ridge is dependent at least in part on methane seepage associated with formation of the fissures. This conclusion supports the hypothesis of incipient subduction in the northern Okushiri Ridge.

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Table 1. Locations and total fatty acid contents of representative sediment samples.

Sample	Dive number*	Location	Water depth (m)	Water temp (°C)	Note (with reference to fissures)	Total fatty acids (ng/g dry sediment)
NOR 2	265 (C-2)	42°42.508'N 139°30.028'E	3307	0.3	Flame structure Gelatinous material	5.66
NOR 8	268 (C-2)	44°14.087'N 139°06.261'E	3110	0.3	0.5 m from a fissure Blackened surface	12.91
NOR 9	268 (C-3)	44°14.087'N 139°06.261'E	3109	0.3	Sediment over a fissure Whitened surface	28.41
NOR 11	269 (C-3)	44°13.947'N 139°06.362'E	3095	0.3	1 m from a fissure	3.47
SSM 1	745	Southern slope, Shiribeshi SM	477	0.6	Copepods (<i>Oithona</i> , etc) Chaetognaths; Scarp	1.68
SSM 6	747	Northern slope, Shiribeshi SM	282	1.4	Planktonic crustaceans Chaetognath swarm; Scarp	0.23
HMS 1	722	Off-Hatsushima methane seep	1169	2.4 [2.6]**	<i>Calypotogena</i> colony Reddish deposit	7.82
HMS 2	722	Off-Hatsushima methane seep	1169	2.4 [2.6]**	Tubeworms Carbonate concretion	3.10
HMS 3	722	Off-Hatsushima methane seep	1166	2.4 [2.6]**	Dead shells of <i>Calypotogena</i>	0.70
TW tr. cont.	722	Off-Hatsushima methane seep	1169	2.4 [2.6]**	Tubeworm trophosome content (mostly bacterial)	174.25 ng / g dry material
TVK 3	176	34°59.40'N 139°07.96'E	108	15 [33]**	South-western terrace of the crater wall	8.92

* 6K, DSV *Shinkai 6500*; 2K, DSV *Shinai 2000*; and 3K, ROV *Dolphin 3K*

** Sediment temperature (10-20 cm below surface)

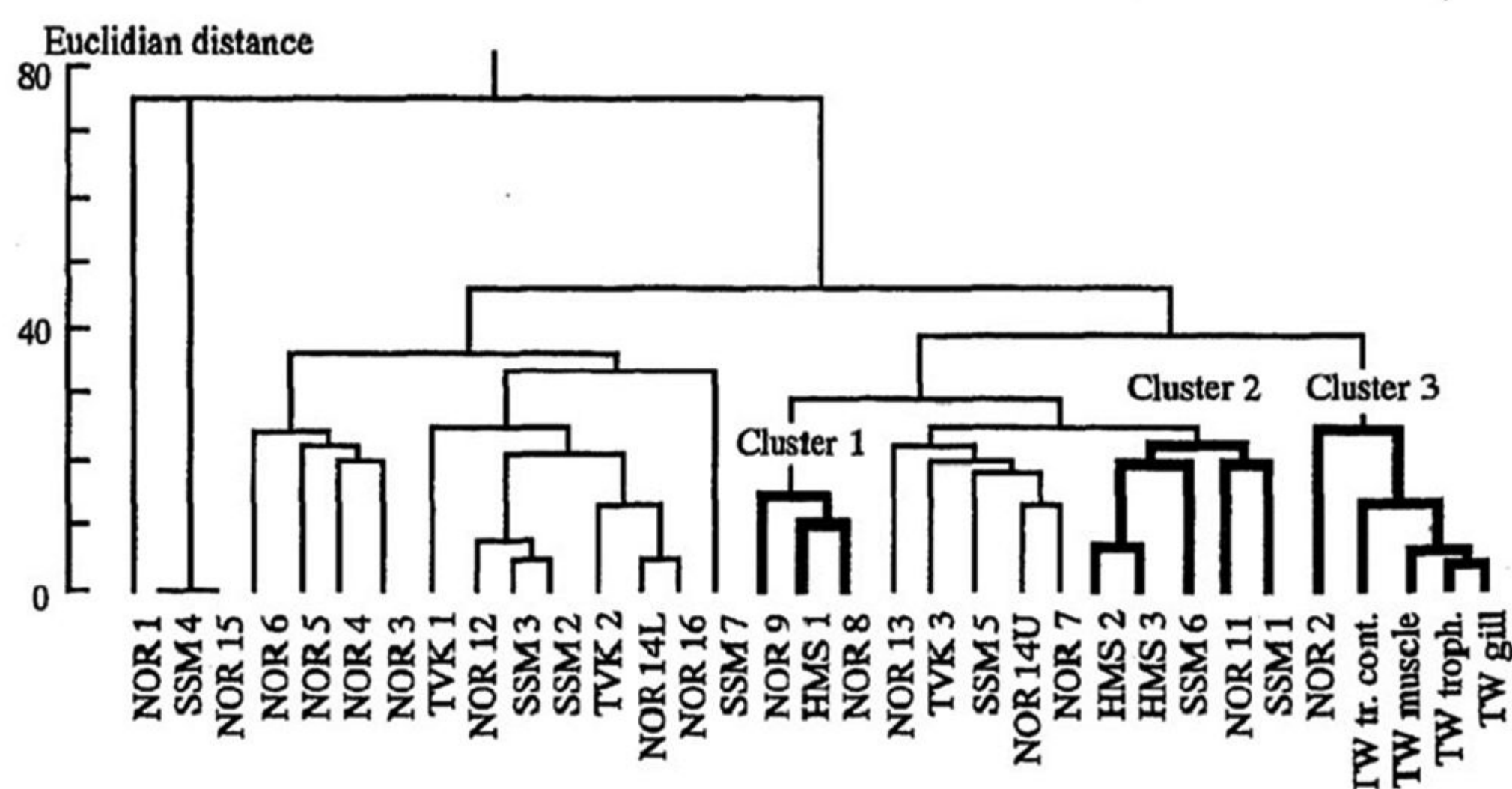


Fig. 2. Dendrogram based on fatty acid compositions of sediments and tubeworm tissues.