Journal of Oceanography
Vol. 52, pp. 1 to 15. 1996

Biological Community and Sediment Fatty Acids Associated with the Deep-Sea Whale Skeleton at the Torishima Seamount

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(Received 7 March 1995; in revised form 7 July 1995; accepted 14 July 1995)

A whale skeleton was discovered on the flat-topped summit of the Torishima Seamount, 4037 m deep, northwest Pacific Ocean, during a dive by the submersible *Shinkai 6500* in 1992. The skeleton was encrusted with mytilid mussels and harbored benthic animals such as galatheid crabs, echinoderms, sea anemones, and unidentifiable tube worms. The whale skeleton was revisited in 1993. Sediment samples were collected to outline the chemical-microbial distribution in the sediment associated with the skeleton. In the sediment, there was a gradient of sulfide concentration with the peak of 20 n moles per gram sediment just beneath a bone. Corresponding gradients were observed in thiosulfate-oxidizing enzyme activity, bacterial colony counts and fatty acid amounts. Direct analysis of the sediment fatty acid composition suggested the occurrence of methane-oxidizing bacteria and sulfur-reducing bacteria in close association with the whale skeleton. These observations imply that the methane and sulfides were formed during the saprogenic process and utilized for the chemosynthetic bacterial production to feed the whale skeleton-animal community.

1. Introduction

Deep-sea biological oases, that provide reducing chemical energy for chemosynthesis-based communities, have been found and characterized at hydrothermal vents (reviewed in Tunnicliffe, 1991, 1992; Lutz and Kennish, 1993) and cold seeps (e.g., MacDonald *et al*., 1990). The vestimentiferan tube worm community at submarine volcanic vent in the euphotic zone (82 m deep; Hashimoto *et al*., 1993) is thought to be a variety of, or a shallow-water counterpart of the deep-sea hydrothermal vent communities. Examples of other types of reducing environments include the dense clam beds in Laurentian Fan (Mayer *et al*., 1988) and the shipwreck tube worms in the northeastern Atlantic Ocean (Dando *et al*., 1992).

The discovery of a whale skeleton-associated biological community (Smith *et al*., 1989) added a new site of deep-sea oases for chemosynthesis-based fauna. However, the hypothesis proposed by Smith *et al*. (1989) that whale carcasses serve as "stepping stones" for the dispersal of some vent organisms was controversial, because the carcass-associated fauna was not necessarily vent-specific (Tunnicliffe and Juniper, 1990; Tunnicliffe, 1992); and because large whales and vent/seep communities appeared at different geological/evolutionary times (Squires *et al*., 1991). Yet, the co-occurrence of fossil whale bones and fossil *Calyptogena-Lucina* clams (Hachiya,

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1993) suggests those whale bones as chemosynthetic habitats for those bivalves, whose modern species are specifically adapted to vents, seeps and other reducing environments (e.g., Turner, 1985).

A whale skeleton was found near the eastern summit of the Torishima Seamount (4037 m deep), in the Western Pacific Ocean, during a dive by the DSV *Shinkai 6500*, Japan. According to the initial observation (Fig. 1; Fujioka *et al*., 1993; Wada, 1993), the skeleton consisted of 22 vertebrae and an assemblage of jawbones. The vertebrae were colonized by mytilid mussels, and harbored galatheid crabs resembling the species found in the Western Pacific back-arc vents such as in the North Fiji Basin (e.g., Jollivet *et al*., 1989; Desbruyères *et al*., 1994), Mariana Trough (e.g., Williams and Baba, 1989; Hessler and Lonsdale, 1991) and the Mid-Okinawa Trough (Naganuma *et al*., 1990). Thus, we assumed that the whale skeleton serves as a deep-sea oasis for benthic animals including vent taxa as well as more cosmopolitan ones.

A year after the discovery in 1992, the whale skeleton was revisited, and the bone-associated sediment cores were collected. This communication reports the chemical and the corresponding microbial gradients in the sediment, assuming the gradients formed in association with the whale carcass degradation. Also the involvement of bacteria metabolizing methane and sulfur in the whale carcass degradation is discussed.

2. Materials and Methods

2.1 Observation and sample collection

The whale skeleton at the Torishima Seamount (30°55.45′ N, 141°49.72′ E, 4037 m deep) was revisited during the 174th dive of the DSV *Shinkai 6500* (September 18, 1993; observer, H. Wada). Identification of the whale species was made by examining an ear tympanic bone collected by the submersible. Preliminary identification of the invertebrates associated with the skeleton was made according to Brusca and Brusca (1990) and Barnes (1980), with some collected samples and video/photo records.

Sediment core samples were collected at the points of 1.5 m, 1 m, and 0.2 m from a posterior vertebral bone (Fig. 1). The points were arranged on a line to the vertebral axis. Another core sample was taken from beneath a mid-vertebral bone (0 m; Fig. 1). The core samples, about 15 cm long, were divided into three depth parts of 0–5 cm (upper), 5–10 cm (middle) and 10–15 cm

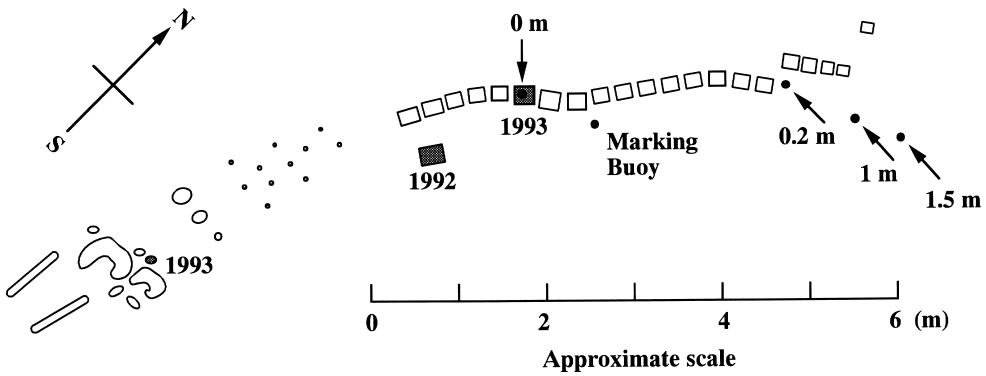

Fig. 1. Map of whale skeleton, reconstructed from *Shinkai 6500* photographs and videotapes. Sites of sediment collection are shown. Shaded bones were collected.

(lower) below the sediment surface. The reference sediment sample was collected at a site about 180 m southeast from the skeleton.

2.2 Chemical and biochemical measurement

About 2 g of sediment samples was suspended in (i) 10 ml of autoclaved, deoxygenated distilled water for determining sulfide concentration, or (ii) 10 ml of GF/C-filtered, autoclaved seawater for determining the activity of rhodanese (thiosulfate sulfurtransferase, EC 2.8.1.1).

Sulfide concentration was determined by a standard colorimetric method based on Lauth's violet formation from *p*-phenylendiamine (Strickland and Parsons, 1972). After brief centrifugation at 2000 × *g* for 5 min, the absorbance at 670 nm of the supernatant was measured. The absorbance-to-concentration conversion factor was calibrated using known concentrations of sodium sulfide. The sulfide concentration was expressed as n moles per g (wet) sediment. Contamination by gaseous sulfide from the laboratory atmosphere into the samples was limited to less than the detection level by handling the samples in a draft chamber.

Rhodanese activity was measured as the rate of thiocyanate formation from thiosulfate and cyanide (Sörbo, 1953; Weng *et al*., 1978). Each 1.2 ml of the suspension (240 mg sediment) was incubated with: 0.125 M $Na_2S_2O_4$, 0.4 ml; 0.2 M KH_2PO_4, 0.2 ml; and 0.25 M KCN, 0.2 ml, at 15°C for 30 min. The incubation was stopped by adding 0.2 ml of formalin and 1 ml of $Fe(NO_3)_3$ solution [100 g of $Fe(NO_3)_3 \cdot 9H_2O$ and 200 ml of 65% HNO_3 in 1 liter of deionized water]. After brief centrifugation at 2000 × *g* for 5 min, the absorbance at 460 nm of the supernatant was measured, and the absorbance reading 0.104 was taken equivalent to 1 μmoles SCN^- as slightly modified from Sörbo (1953). One unit of rhodanese activity was defined as the amount of the enzyme that forms 1 μmoles SCN^- per 30 min. The rhodanese activity was thus determined and expressed as units per g wet sediment.

2.3 Bacterial viable counts

Part of the sediment suspension in GF/C-filtered, autoclaved seawater was used to estimate the bacterial viable counts, expressed as the colony-forming units (CFUs). Each 0.1 ml (containing 20 mg sediment) of the suspension was spread on an agar plate and incubated at 4°C for 6 weeks. Two types of agar plates were used. One was ZoBell 2216E Marine Agar plates (Difco) for heterotrophic bacterial viable counts. The other was thiosulfate-based agar plates for autotrophic bacterial viable counts, modified from Naganuma *et al*. (1989), Seki and Naganuma (1989) and Naganuma and Seki (1994) as follows: Solution A, 10 g of $Na_2S_2O_3 \cdot 5H_2O$ and 15 g of agar, dissolved in 600 ml of aged, GF/C-filtered seawater added with 200 ml distilled water; Solution B, 0.25 g of K_2HPO_4 and 0.25 g of KH_2PO_4, dissolved in 100 ml of distilled water; and Solution C, 0.5 g of NH_4Cl and 0.25 g of $MgSO_4 \cdot 7H_2O$, dissolved in 100 ml of distilled water. The solutions A, B and C were autoclaved separately, cooled to 50°C, mixed and poured before solidification. The salinity of the mixed solidifying medium was 19–20 ‰, which was almost equal to that of the ZoBell 2216E medium (19.45 g NaCl per liter).

The CFU numbers from triplicated plates were averaged and expressed as heterotrophic or autotrophic CFUs per g wet sediment.

2.4 Direct analysis of sediment fatty acids

Part of sediment was frozen and preserved in liquid nitrogen during the cruise, and lyophilized for extended preservation. Fatty acids were recovered from the lyophilized samples by saponification, methylation and organic extraction (Sasser, 1990a). Saponification reagent

was 45 g NaOH dissolved in 150 ml methanol and 150 ml distilled water. Methylation reagent was 325 ml of 6 N HCl mixed with 275 ml methanol, and used to form fatty acid methyl esters (FAMEs). The FAMEs were then extracted into the mixture of hexane and methyl tert-butyl ether (1:1 by volume). The FAME profiles were analyzed on a gas chromatograph (Hewlett-Packard 5890 Series II) equipped with a phenylmethyl silicone capillary (HP 19091B-102, Ultra 2), a flame ionization detector, and an automatic sampler (HP 7673A#201) (e.g., Rajendran *et al.*, 1994). The analysis was done at Acculab Inc., Newark, Delaware. Total fatty acid contents were expressed as ng per g dry sediment, and the fatty acid compositions were cluster-analyzed to calculate the relatedness or similarity within the sediment samples (Sasser, 1990b).

3. Results

3.1 Skeletal articulation and preservation

The length of the whale skeleton from the jawbones to the posterior end of the vertebrae was 10–11 m. The jawbone (Fig. 2b) morphology was typical of a mysticete cetacean, or a baleen whale. The ribs were missing. Close examination of the collected ear tympanic bone indicated that the whale belonged to a Bryde's whale (*Balaenoptera edeni*, identified by Dr. Hidehiro Kato, National Institute of Far Seas Fisheries, Japan). The whale skeleton was heading to southwest (about 220–230°; Figs. 2 and 3a), from which direction the bottom-water current of 0.1 knots (about 0.05 m/s) was flowing at the times of observation. The water temperature was 1.4°C.

The whale skeleton and the associated fauna was observed to be almost unchanged during the one-year interval between the two dives. There was little burial of the skeleton, probably due to slow sedimentation process around present-day Torishima Seamount (Fryer *et al.*, 1990; Fujioka *et al.*, 1991). The vertebral bones were mostly cube-shaped; the vertebral bone spines, which are typical of whales, were missing.

3.2 Whale skeleton-associated biological community

The fauna associated with the whale skeleton is listed in Table 1. The vertebrae, jawbones

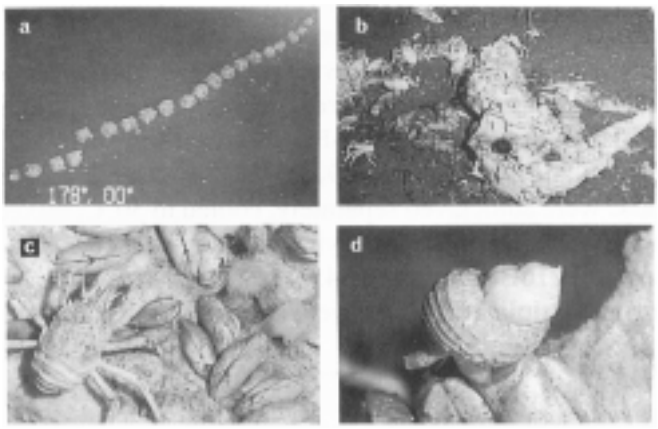

Fig. 2. Whale skeleton and associated animals: vertebrae (a) and jawbones (b) of the whale skeleton; a galatheid crab, *Munidopsis* sp., on a vertebra colonized by *Idasola* mussels and sea anemones (c); and a buccinid snail on a jawbone (d).

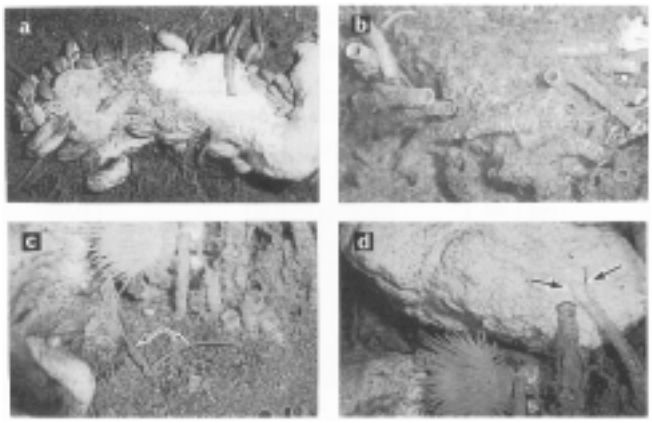

Fig. 3. Tubicolous polychaetes colonizing in association with whale skeleton. Worms inhabit around and beneath a rib fragment (a). Tubes were 1–2 mm wide and had "ridges" on the surface (b). Paired palps (arrowed) appeared out of the tube opening (c), (d).

Table 1. Macrobenthic animals observed in association with the whale skeleton at Torishima Seamount.

Phylum	Class	Family	Note
Cnidaria	Anthozoa	Actiniidae	Figs. 2c, 3c and 3d
Mollusca	Bivalvia	Mytilidae	*Idasola*? Figs. 2c and 3a
	Gastropoda	Buccinidae	Fig. 2d
Annelida	Polychaeta	Spionidae	Paired palps. Tube. Figs. 3a–d
Arthopoda	Crustacea	Galatheidae	*Munidopsis* . Figs. 2b and 2c
Echinodermata	Ophiuroidea		
	Echinoidea		

and rib fragments were heavily colonized by mytilid bivalves (Figs. 3c and 4a). The encrusting mytilid population seemed to be composed of a single mytilid species, and distinguished from typical deep-sea mytilids, *Bathymodiolus* species (Hashimoto and Okutani, 1994). The mytilid was tentatively identified as a certain species of the genus *Idasola* (by Dr. J. Hashimoto, Japan Marine Science and Technology Center, personal communication). An *Idasola* species colonizing another whale skeleton was reported to bear endosymbiotic bacteria (Smith *et al*., 1989). The size distribution of total 139 mytilid individuals from a vertebra was unimodal with the mode range of 20–25 mm in shell length and the maximum of 43 mm (Fig. 4).

Buccinid snails, common deep-sea carnivores, were also observed (Fig. 2d). The population density was, however, far lower than those of mytilild bivalves. The shell height of the snails was estimated to be 5–20 mm based on video records.

Galatheid crabs belonging to the genus *Munidopsis* were also abundant in the community (Table 1; Figs. 2b and 2c), and morphologically resembled the species found in the western Pacific hydrothermal vent areas (Williams and Baba, 1989). Their feeding behavior was not observed, however, their distribution in association with the whale bones suggested that they may

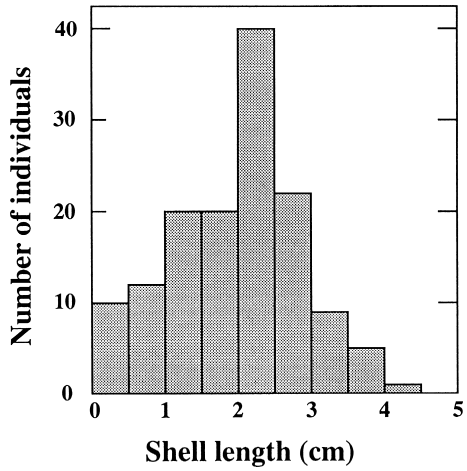

Fig. 4. Size distribution of the *Idasola* mussel population that encrusted a vertabra.

depend on bone-based microbial food chain, or may feed directly on the whale carcass and bones.

Unidentifiable tubed annelid worms (Figs. 3a–d) occurred directly beneath and in the close vicinity of the whale bones. The tubes of the worms had corrugation on the surface and a paired antenna-like organ which is called palps (Figs. 3b–d). The tube worms that have paired palps are mostly known in the spionid polychaetes (family Spionidae). Morphology and habit of the spionid worms (Barnes, 1980, p. 500–502) seems most closely fit to that of the whale skeleton-associated worms. A significant number of the spionid-like tube worms were observed and thus, thought to be a major component of the whale skeleton community.

Echinoderms such as ophiuroids and echinoids (Table 1) were also found in the community, though they are common and not necessarily vent-specific benthos. However, their population density in the community was clearly higher than that of ambient deep-sea floor, indicating that the whale carcass serves as the oasis not only for vent animals but also for more common deep-sea benthos.

3.3 Distribution of sulfides and microbial parameters

Sulfide concentration decreased with the distance and depth from the whale skeleton, and the overall distribution revealed to be a gradual one. The peak level of 20 n moles per gram wet sediment in the gradient was observed just beneath a bone (Table 2). This peak sulfide concentration was at the same level (20 nM in the pore water) as previously reported from the whale skeleton in Santa Catalina Basin (Smith *et al*., 1989). The bone-associated sulfide gradient shown in our study is strong evidence that a reducing environment was formed through the saprogenic decay of the whale skeleton.

The distribution of rhodanese activity was similar to that of sulfide concentration, forming a gradient with the peak just beneath a vertebra (Table 2). However, patchy high activity was found in the gradient in the uppermost part of the sediment, 1.5 m from the vertebra. Patchy high levels of the heterotrophic bacterial viable counts and total fatty acid contents were also found in the same sample, and thus the patchy high levels in parallel were unlikely to be an artifact.

Autotrophic and heterotrophic bacterial viable counts distributed almost in parallel, and the highest levels were found just beneath the vertebra (Table 2). However, there was a distributional

Table 2. Chemical, biochemical and microbiological data of the sediment samples taken at different distances from the vertebral axis of the Torishima Seamount whale skeleton.

Distance (m)	Core depth (cm)	Sulfide (n moles/g)	Rhodanese (units/g)	TB agar* $\times 10^4$ (CFU/g)	2216 agar** $\times 10^4$ (CFU/g)	TB/2216 ratio	Fatty acids (dry) (ng/g)
0	0–5	20.0	4.5	3.3	11.5	0.29	3.7
0	5–10	4.5	4.1	0.58	1.3	0.45	1.8
0	10–15	0.2	2.0	0.52	1.5	0.35	1.3
0.2	0–5	3.7	3.2	2.1	3.6	0.58	1.5
0.2	5–10	1.3	2.0	0.43	1.8	0.24	0.5
0.2	10–15	0.3	0.4	0.53	1.9	0.28	0.6
1	0–5	0.7	0.7	1.0	2.5	0.40	0.6
1	5–10	0.5	0.5	0.48	1.7	0.28	0.1
1	10–15	0.3	0.4	0.35	1.3	0.27	0.4
1.5	0–5	0.5	1.5	0.46	3.4	0.14	3.0
1.5	5–10	0.3	0.5	0.35	1.1	0.32	0.4
1.5	10–15	0.2	0.4	0.23	1.3	0.17	0.6
Reference	0–5	<0.1	<0.1	<0.1	1.2	<0.08	0.1

*Autotrophic bacterial viable counts.
**Heterotrophic bacterial viable counts.

discrepancy in the upper sediment 1.5 m from the vertebra, where the heterotrophic viable counts were patchily high but the autotrophic viable counts were not. In contrast, rhodanese (assumed to be an autotrophy-related enzyme) activity was also high in the upper sediment 1.5 m from the vertebra, which suggests the potential rhodanese activity in heterotrophs.

Higher levels of bacterial viable counts, both autotrophic and heterotrophic, were limited to the upper parts of the sediment samples (Table 2). The total fatty acid content, a microbial biomass indicator, was better correlated with the viable counts, rather than the total counts.

3.4 Composition of the sediment fatty acids

The fatty acid compositions of the sediment samples are shown in Table 3. Fatty acids of C14-, C16- and C18-straight, saturated chains accounted for 28.6–100% of the total fatty acid composition. The sediment just beneath the vertebra has the greatest diversity in the fatty acid composition, having 13 fatty acid categories. This was followed by 8-category fatty acid composition from the sediment 5–10 cm below the vertebra. Co-occurrence of the odd-numbered fatty acids such as $C_{15:0}$ and $C_{17:0}$ was common to and characteristic of all samples from beneath the vertebra. In the sediment adjacent to the skeleton (i.e., the 0.2-m samples), the upper part had a diverse fatty acid composition, including unsaturated fatty acids such as $C_{16:1}$ and $C_{18:1}$ were otherwise seen only in the sediment just beneath the vertebra. The $C_{18:1}$ fatty acids consisted of 4 forms (ω7c, ω9c, ω9t and ω12t), however, separate determination of the 4 forms was difficult due to the limited resolution of the gas-chromotograms.

The diversity, as well as the total amount, of the fatty acids generally decreased with the increase and depth from the skeleton. However, there was patchy high diversity in the upper part of the 1.5-m sediment, where other microbial parameters showed similar patchy high levels (Table 3).

Table 3. Fatty acid compositions of the sediments at different distances and depths from the whale skeleton at the Torishima Seamount.

Distance (m)	Depth (cm)	9:0	12:0	14:0	15:0	15:0 iso	15:0 anteiso	16:0	16:0 -OH	16:1 ω7c	16:1 ω7c,-OH	17:0	17:0 iso	17:0 10 Me	17:1 ω8c	18:0	18:1 *	20:2 ω6,9c
0	0–5	—	7.0	6.2	1.1	—	—	19.0	3.6	28.5	5.5	1.1	—	3.1	1.7	3.4	17.0	2.8
0	5–10	1.9	3.7	11.2	3.0	—	—	52.0	4.9	—	—	2.6	—	—	—	20.9	—	—
0	10–15	2.4	3.4	12.0	3.8	—	—	51.2	6.2	—	—	3.8	—	—	—	17.3	—	—
0.2	0–5	—	—	11.0	—	3.4	4.7	49.0	—	9.3	—	—	—	—	—	14.3	8.3	—
0.2	5–10	—	—	14.1	—	—	—	59.2	—	—	—	—	—	—	—	26.7	—	—
0.2	10–15	—	—	13.4	—	—	—	52.7	6.8	—	—	—	—	—	—	27.1	—	—
1	0–5	—	4.9	15.2	—	—	—	52.3	5.2	—	—	—	—	—	—	22.4	—	—
1	5–10	—	—	—	—	—	—	67.4	—	—	—	—	—	—	—	32.6	—	—
1	10–15	—	—	14.6	—	—	—	55.9	—	—	—	—	—	—	—	29.5	—	—
1.5	0–5	1.8	3.7	10.7	1.5	—	2.0	48.0	3.2	—	—	—	—	—	—	29.1	—	—
1.5	5–10	—	—	14.2	—	—	—	55.5	—	—	—	—	—	—	—	30.4	—	—
1.5	10–15	—	—	11.7	—	—	—	53.9	—	—	—	—	6.9	—	—	27.5	—	—
Reference	0–5	—	—	—	—	—	—	52.7	—	—	—	—	—	—	—	47.3	—	—

*Sum of 18:1 *ω*7c, *ω*9c, *ω*9t and *ω*12t.

4. Discussion

4.1 Whale skeleton preservation

The Torishima Seamount whale skeleton was observed to lack the whole rib part. In addition, its vertebral bones were mostly cube-shaped with their spines missing. The spines were possibly corroded, and the skeletal corrosion may explain why the ribs were missing. Whale ribs are generally degenerated and are subject to mechanical fragmentation and corrosion. Thus, the ribs might disappear faster than other parts of the skeleton. A similar observation was previously reported from the whale skeleton found in Santa Catalina Basin (1240 m deep), in which part of the ribs was missing but other parts of the skeleton were almost complete (Allison *et al*., 1991). The SCB whale skeleton was assumed to be of a blue whale (*Balaenoptera musculus*) or a fin whale (*B. physalis*) (Smith *et al*., 1989), and the Torishima Seamount whale skeleton is thought to be of a closely related species, Bryde's whale (*B. edeni*). Thus, the taphonomic similarity of faster disappearance of the ribs can be presumed for at least these baleen whales of the *Balaenoptera* species. Another possibility is that the rib parts, along with viscera, were eaten by large carnivores such as sharks and killer whales while the whales were dying and falling.

The whale carrion is thought to have been consumed by common deep-sea carnivores such as buccinid snails, galatheid crabs, and ophiuroids, which were associated with the skeleton. They may have fragmented the carrion, and thus, accelerated the carcass degradation and skeletonization. For instance, a few buccinids skeletonized a whole sardine carcass in 2 days in a container at 1200 m deep (T. N., personal observation).

Estimation of the age of the whale skeleton (period after death) will facilitate understanding of the taphonomic processes of the whale carcass. Measurement of the shell length of the mytilid mussels encrusting the whale bones may help the estimation. The largest mytilid shell from a vertebra was 43 mm long, and the average or median shell length range was 20–25 mm (Fig. 4). Previously estimated shell growth rates of mytilid mussels and vesicomyid clams at 2500 and 2600 m-deep vents were 26–33 mm per year and 2.7–40 mm per year, respectively (Rhoads *et al*., 1981; Turekian and Cochran, 1981; Turekian *et al*., 1983). These values may not be directly applied to the mytilids inhabiting the whale skeleton, which is different from the vent conditions in amount and duration of chemical energy supply, water depth and hydrostatic pressure, etc. However, if the growth rates at high-energy sites such as hydrothermal vents could be taken as the maxima for deep-sea mussels and clams, the minimum age estimates of the whale skeleton mytilids would be calculated. For example, the estimated age for a 43 mm-long shell was 1–16 years, based on the growth rates mentioned above and in Lutz and Kennish (1993).

4.2 Whale skeleton-associated chemical and microbial gradient

The whale skeleton was tentatively identified as an adult Bryde's whale, which is typically 12–15 m in length and about 12 tons by weight (e.g., Cohat, 1986). Degradation of this huge amount of biomass must have influenced the ambient and in situ environment, for example, by producing anaerobic, reducing conditions. The degradation might start with aerobic processes that consume a huge quantity of oxygen to oxidize the carrion. Then, under the resultant hypoxic condition, anaerobic degradation processes might occur and eventually become dominant (Allison, 1988b). The typical products of anaerobic protein degradation are sulfides (Allison, 1988a). Sulfides were also possibly produced by sulfate-reducing bacteria, utilizing hydrogen produced through the anaerobic oxidation of lipids which is abundant in whale bones (Smith,

1992). Methane might possibly be co-produced with hydrogen, and utilized by methane-oxidizing bacteria. In addition, microbial sulfate reduction using methane was geochemically implicated (Masuzawa *et al*., 1992). The co-occurrence of sulfate-reducing bacteria and methane-oxidizing bacteria in the skeleton-associated sediment was biochemically suggested and is discussed later.

Distributions of rhodanese activity, bacterial viable counts (both autotrophic and heterotrophic), and total fatty acid contents were generally similar to that of sulfide concentration. The analysis of linear correlations of these variables and sulfide concentration is shown in Table 4. It can be thus concluded that microbial processes in the whale skeleton system were affected by the concentrations of sulfides derived from the whale carcass degradation. However, it should be noted that the bacterial viable counts were the results of incubations at 4°C and 1 atm, which condition might have favored only a fraction of whole bacterial populations. For example, it was demonstrated that incubation at a high pressure increased the viable counts of deep-sea aerobic bacteria (Morita, 1972) and the degradation by deep-sea bacteria (Poremba, 1994).

Regardless of the generally parallel distributions, patchy high values of rhodanese activity, heterotrophic viable counts and total fatty acid contents were observed in the upper part of the sediment, 1.5 m from the skeleton (Table 2). Rhodanese was assumed to be primarily an autotrophy-related enzyme, however, the good correlation with heterotrophic viable counts (and the poor correlation with autotrophic viable counts) seemed discrepant. However, the occurrence of rhodanese is not limited to autotrophs but widespread among heterotrophs (e.g., Westley, 1973), though its primary role in heterotrophy is unlikely. The high rhodanese activity in the upper part of the 1.5 m-sediment was probably due to the presence of heterotrophic bacteria, whose patchy distribution in turn was confirmed by the amount of total fatty acid contents. Alternative explanation is that there may have been more autotrophs, most of which were uncultured.

Table 4. Correlations among sulfide concentration and microbial variables in the whale skeleton-associated sediments at the Torishima Seamount. Results from linear correlation analyses using the data in Table 1 were expressed as correlation coefficients.

Combination	Correlation coefficient (r)	r^2	$n-2$ (n, sample number)	Student's t***
Sulfide-rhodanese	0.75	0.57	10	3.62
Sulfide-autotrophs*	0.88	0.78	10	5.94
Sulfide-heterotrophs**	0.95	0.90	10	9.23
Sulfides-fatty acids	0.75	0.56	10	3.53
Autotrophs-rhodanese	0.70	0.49	10	3.12
Autotrophs-heterotrophs	0.91	0.82	10	6.80
Autotrophs-fatty acids	0.67	0.45	10	2.83
Heterotrophs-rhodanese	0.63	0.39	10	2.54
Heterotrophs-fatty acids	0.77	0.63	10	4.09
Fatty acids-rhodanese	0.75	0.57	10	3.61

*Autotrophic bacterial viable counts.

**Heterotrophic bacterial viable counts.

***$t_{0.1\%[10]} = 4.59$; $t_{1\%[10]} = 3.17$; $t_{2\%[10]} = 2.76$; $t_{5\%[10]} = 2.23$.

The ratios of autotrophic to heterotrophic viable counts were generally high, reaching 0.58 in the upper part of the sediment, 0.2 m from the skeleton (Table 2). These ratios compare favorably with those observed in hydrothermal plumes (up to 0.3) and contrast with the ratio of <0.001 in normal seawater (Naganuma and Seki, 1994), and with the low numbers of thiosulfate-oxidizers at hydrothermal plume boundaries (Naganuma *et al*., 1991). Thus, the high ratio of auto- to heterotrophic bacterial counts strongly indicates a similarity between hydrothermal plumes and the whale skeleton-associated sediment, in terms of microfloral chemosynthetic potential as well as by abundance.

4.3 Biomarker fatty acids

Microbial fatty acid compositions are known to be species-specific, and thus, the use of fatty acids as biomarkers is becoming popular in microbial identification (e.g., Yang *et al*., 1993), and characterizing microorganisms in the environment (e.g., Parkes, 1987; Mancuso *et al*., 1990). In this study, analysis of sediment fatty acids (Table 3) demonstrated the occurrence of biomarker fatty acids for sulfur-reducing bacteria ($C_{15:0}$, $C_{15:0\ iso}$ and $C_{17:1}$; Taylor and Parkes, 1983, 1985; Parkes and Calder, 1985) and for methane-oxidizing bacteria ($C_{16:1}$ and $C_{18:1}$; Nicholas *et al*., 1985). In particular, we found $C_{16:1\ \omega 7c}$ fatty acid, which is relatively abundant in Type I methane-oxidizers (Nicholas *et al*., 1985). Type II methane-oxiders are characterized by $C_{18:1\ \omega 7c}$ (Nicholas *et al*., 1985), whose occurrence was only suggested in our analysis, as we could only determine the mixture of $C_{18:1}$ fatty acids. Similar biomarker profiles of the deep-sea sediments were previously reported from the Venezuela Basin (3500–5000 m deep) and the Puerto Rico Trench (8375 m deep), where the occurrence of sulfur-reducing bacteria was also suggested (Baird and White, 1985). In the whale skeleton-associated sediment of the Torishima Seamount, the occurrence of the biomarkers for sulfur-reducing and methane-oxidizing bacteria was restricted to the close vicinity of the skeleton. Thus, the microbial sulfur-reduction and methane-oxidation are thought to be closely associated with the degradation of the whale carrion and lipid-rich bones, and play key roles in the formation of the deep-sea biological oasis at the whale skeleton. This seems in good agreement with the proposed taphonomic processes of whale carcasses, based on the data and observations other than fatty acid analyses (Allison *et al*., 1991; Smith, 1992).

Fatty acids known to characterize sulfur-oxidizing bacteria (Kerger *et al*., 1986) were not detected. Biomarker fatty acids for heterotrophic bacteria have not been proposed. Thus, the contribution of sulfur-oxidizing and heterotrophic bacteria made to the sediment fatty acid compositions was not estimated.

Contributions of eukaryotic fatty acids in sediments are typically indicated by the high-ratio occurrence polyenoic fatty acids (Bobbie and White, 1980). The ratio of polyenoic fatty acids in the skeleton-associated sediment was only fractional, indicating that the fatty acid profiles of the skeleton-associated sediment consisted almost of prokaryotes (bacteria). In addition, the contribution of the sinking particles was thought to be insignificant. That is, the sinking particles (>50 μm in diameter) at the depth range of 2000–4700 m were typically shown to have polyenoic $C_{20:5}$ fatty acid (Saliot *et al*., 1982), which was not detected in the whale skeleton-associated sediment.

The fatty acid compositions have been used to compare and relate the sediment, as previously applied to the characterization of marine sediments and benthic microbial communities (e.g., Rajendran *et al*., 1992, 1994). The fatty acid-based comparison was applied to whale skeleton-associated sediments at different distances and depths, and the relatedness (similarity) of the sediments is schematized by a dendrogram in Fig. 5. The sediment just beneath the bone (0-U) was distinctly separated from other sediments, and the very adjacent sediment (0.2-U) was

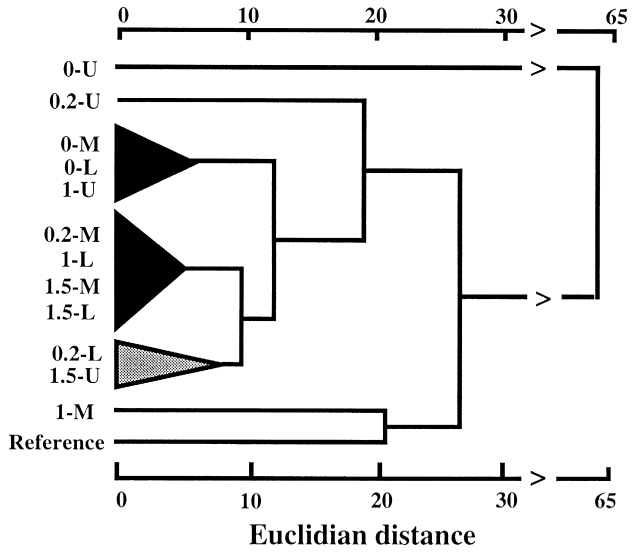

Fig. 5. Dendrogram of the sediments at different distances (0, 0.2, 1 and 1.5 m) and depth (0–5 (U), 5–10 (M), 10–15 (L) cm) from the vertebral axis. Euclidian distance was calculated by cluster-analyzing the fatty acid compositions of the sediments.

also distinct. Most of the skeleton-associated sediments were closely related with three subgroups, while they were distantly related to the reference sediment (180 m from the skeleton). Thus, it can be concluded that the whale skeleton had much influence on the microfloral structure of the deep-sea sediment by inputting high-energy organic material and yielding high, reducing chemical energy.

Acknowledgements

We are very much obliged to the crew of RV *Yokosuka* and the operation team of DSV *Shinkai 6500*, Japan Marine Science and Technology Center (JAMSTEC). Special thanks to Dr. C. J. Meisel, Acculab Inc., Delaware, USA, for the analysis of sediment fatty acids; to Dr. H. Kato, National Research Institute of Far Seas Fisheries, Japan, for identifying the species of the cetacean skeleton; and to Dr. J. Hashimoto, JAMSTEC, for preliminary identification of the bivalves attaching to the whale bones. Many thanks to Y. Kato, Chiba Prefecture Central Museum, Japan, and K. Aoike, Yokohama National University, Japan, for their technical assistance.

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