#### 3-4 Environmental Survey

#### 3-4-1 Survey Site

The survey area is located at the Triple Junction of the Central Spreading Ridge, where the existence of hydrothermal vents has been expected (Figure  $3 \cdot 4 \cdot 1 \cdot 1$ ). Samples for the water quality and microorganism survey were collected at five stations: 01SFRO01, 01SFRO02, 01SFRO03, 01SFRO04 and 01SFRO05. For the sediment and benthic organisms survey were collected at three stations: 01SFMC04, 01SFMC05 and 01SFMC07.

#### 3-4-2 Study for the Water Quality and Microorganism

#### 3-4-2-1 Water Quality

#### (1) Water Temperature

There was no significant difference in vertical profiles of water temperature among the stations: remarkable decreases from the surface to 500<sup>-</sup>m depth and moderate decreases from the 500<sup>-</sup>m to 2,000<sup>-</sup>m depth (Figure 3·4·2·1a).

#### (2) Salinity

Salinity increased from the depth of 30m and reached its maximum concentration at 300m. From there, it remarkably decreased to the depth of 600m, and it gradually increased again to the depth around 2000m. Same as the case of water temperature, vertical profiles of salinity were similar among the five stations (Figure 3-4-2-1b),

#### (3) Light transmission

Dissimilar to water temperature and salinity, significant differences in vertical profiles of light transmission were observed in the water columns deeper than 100m among the stations. Particularly, around the depth of 1,800m at 01SFRO03, there was a layer where the light transmission was anomalously low (Figure 3-4-2-1c).

#### (4) Methane

Regarding the concentration of methane in the water column, the lower concentration (lower than 0.8nmol/kg) was observed in the upper layer (1760 to 1860m), while the higher one (1.4 to 3.7nmol/kg) was in the deeper layer (1900m; 100m above the sea bottom). The concentrations of methane in overlying water at 01SFMC04, 05 and 07 were from 2.1 to 2.7nmol/kg, which were similar value to the 1900m (Table. 3-4-2-1)

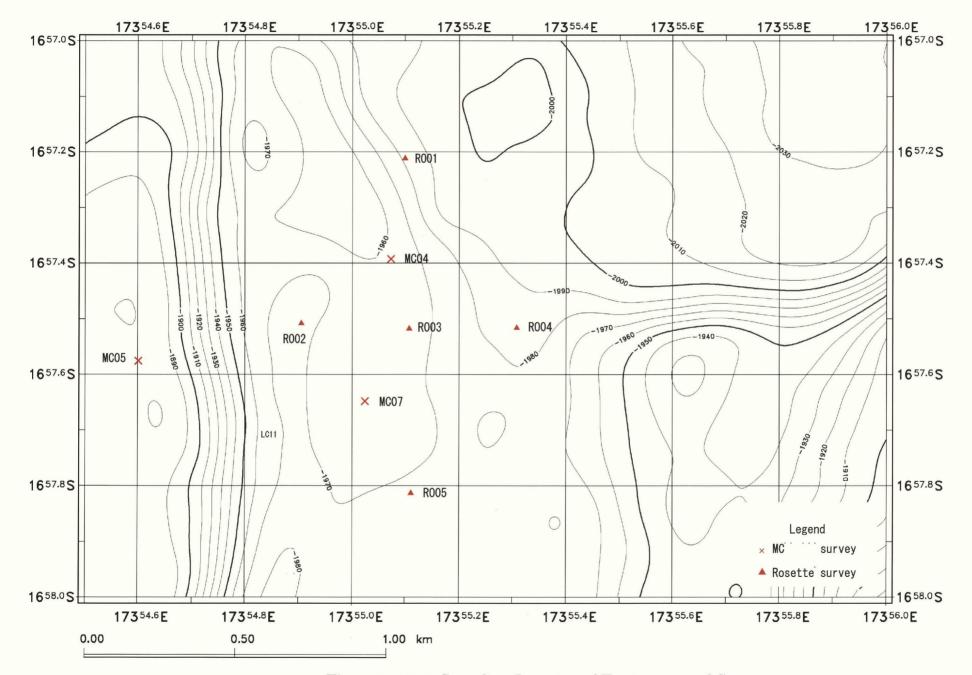
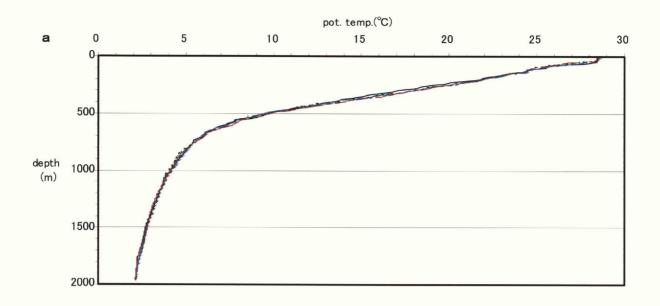
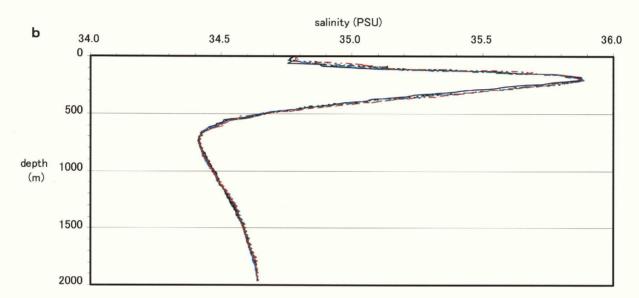
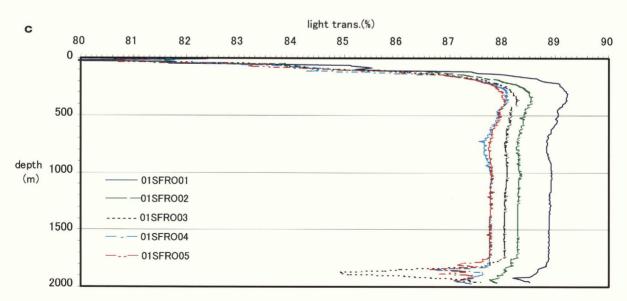


Figure 3-4-1-1 Sampling Location of Environmental Survey

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a: potential temperature , b: salinity , c: light transmission

## Figure 3-4-2-1 Vertical Distribution of Physical Observations in Water Column

#### (5) Suspended Solid (SS)

Suspended solid in this site were too little to measure in the most of the stations and layers. Therefore it was impossible to compare the value between the stations and layers (Table. 3-4-2-2).

#### 3-4-2-2 Bacterioplankton

Concerned to the abundance of bacterioplankton (Table  $3 \cdot 4 \cdot 2 \cdot 3$ ) in the water column, the cells number was poor in the in the upper layer (1660 to 1720 m) ,which were from  $4.7 \times 10^3$  to  $1.1 \times 10^4$  cells/ml, however in the layer from 1760 to 1860m, it were rather rich, which is  $7.4 \times 10^3$  to  $3.3 \times 10^4$  cells/ml. The most abundant bacterioplankton, which is  $2.4 \times 10^4$  to  $4.2 \times 10^4$  cells/ml, were observed at the 1900m (Fig.3·4·2·2a).

On the other hand, regarding the cells number of bacteria in overlaying water were from  $4.3 \times 10^4$  to  $2.0 \times 10^5$  cells/ml, it is more abundant than that of water column. However it is uncertain either sedimentary bacteria were contained in the overlying water (Fig.3-4-2-2b).

#### 3-4-2-3 Conclusion of the Study for the Water Quality and Microorganism

In order to recognize the plume, which was derived from the hydrothermal vent, the T-S diagram, which applied to the data deeper than 1760m, was shown in the Figure.3-4-2-3. The regression line calculated the data of potential water temperature (T) and salinity (s) between the 1,600m and 1,760m, and actual data from deeper than 1760m. The larger difference between them was observed in the part of low temperature and high salinity, which suggested the water temperature shifted to the higher at the benthic area. The difference between the actual data sets and the data estimated by the regression, were indicated in the Figure  $3\cdot4\cdot2\cdot4$ . As the result, the larger differences were indicated in the deeper than 1760m, and it strongly demonstrated the existence of water mass, which characterized abnormal water temperature. Still more, the negative correlation between the light transmission and potential water temperature was also reveals (Figure  $3\cdot4\cdot2\cdot5$ ), and that the water mass, which indicate abnormal water temperature, was correspond to the maximum layer of the concentration of methane and the abundance of bacterioplankton.

Judging from these results, it is possible to infer that hot water, which contain methane, was discharged in this site, and then abnormal water temperature and peaks of methane was observed at the neighborhood of the seafloor, consequently bacterioplankton, which use methane for its energy, was increased. However, although abnormal value was observed in the light transmission, it is not confirmed by the data of suspended solid. It is suggested to understand the hydrothermal plume in this site, the concentrations of Mn or Fe in the water should be analyzed.

Station	Water depth	Methane
	(m)	nmol/Kg
01SFR001	1953	2.4
	1913	1.7
	1863	2.6
	1763	1.0
	1713	0.8
	1663	<0.5
01SFR002	1963	2.6
	1923	3.2
	1873	3.2
	1773	1.4
	1723	<0.5
	1673	<b>&lt;</b> 0.5
01SFR003	1953	2.2
	1913	3.7
	1863	3.5
	1762	1.3
	1713	<b>&lt;0.5</b>
	1663	<0.5
01SFR004	1968	2.2
	1928	1.4
	1878	2.2
	1778	0.9
	1728	0.5
	1678	0.5
01SFR005	1956	2.7
	1916	3.5
	1866	1.5
	1766	
	1716	1.6 0.6
	1666	<0.5

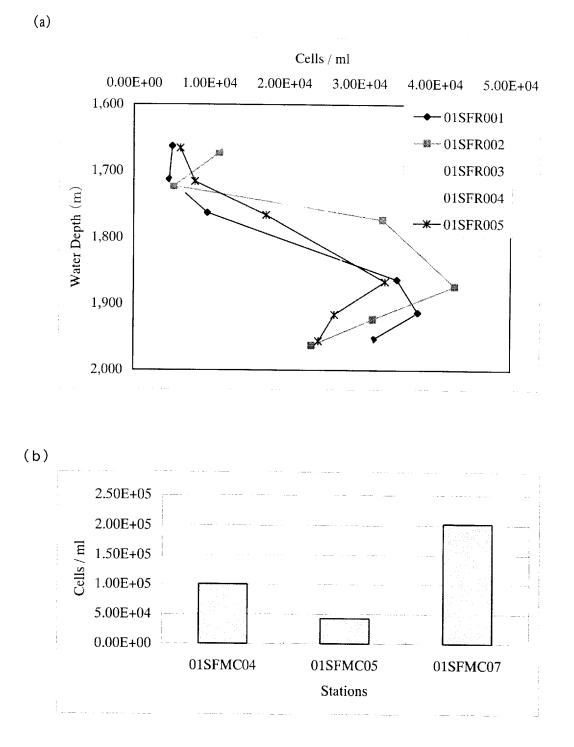
Table 3-4-2-1 The concentrations of methane in the water column

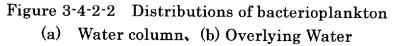
Stations	Water Depth	SS
	(m)	mg/L
01SFR001	1953	<1
	1913	<1
	1863	<1
	1763	<1
	1713	<1
	1663	<1
01SFR002	1963	<1
	1923	<1
	1873	<1
	1773	<1
	1723	<1
	1673	<1
01SFR003	1953	<1
	1913	1
	1863	<1
	1762	<1
	1713	1
	1663	1
01SFR004	1968	<1
	1928	<1
	1878	1
	1778	<1
	1728	1
	1678	<1
01SFR005	1956	<1
	1916	<1
	1866	<1
	1766	<1
	1716	<1
	1666	<1

## Table 3-4-2-2The concentrations of Suspended Solid in the water column

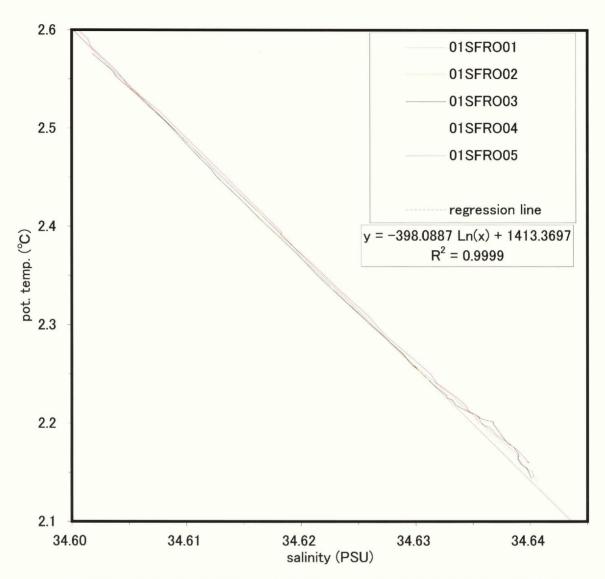
Sampling Site	Depth	Bacteria
Damping Dite	(m)	cells/ml
01SFR001	1953	3.20E+04
01SFR001	1913	3.78E+04
01SFR001	1863	3.50E+04
01SFR001	1763	9.77E+03
01SFR001	1703	4.68E+03
01SFR001	1663	4.08E+03
01SFR001 01SFR002	1963	$\frac{5.08E+03}{2.37E+04}$
01SFR002	1923	3.19E+04
01SFR002	1873	4.27E+04
01SFR002	1773	3.31E+04
01SFR002	1723	5.30E+03
01SFR002	1673	1.14E+04
01SFR003	1953	3.14E+04
01SFR003	1913	3.06E+04
01SFR003	1863	2.85E+04
01SFR003	1762	2.56E+04
01SFR003	1713	5.27E+03
01SFR003	1663	1.10E+04
01SFR004	1968	3.06E+04
01SFR004	1928	3.88E+04
01SFR004	1878	3.38E+04
01SFR004	1778	7.41E+03
01SFR004	1728	6.27E+03
01SFR004	1678	8.49E+03
01SFR005	1956	2.46E+04
01SFR005	1916	2.68E+04
01SFR005	1866	3.34E+04
01SFR005	1766	1.76E+04
01SFR005	1716	8.11E+03
01SFR005	1666	6.17E+03
01SFMC04	bottom water	1.01E+05
01SFMC05	bottom water	4.26E+04
01SFMC07	bottom water	2.02E+05

Table 3-4-2-3 Concentrations of Bacteria in the Water Column



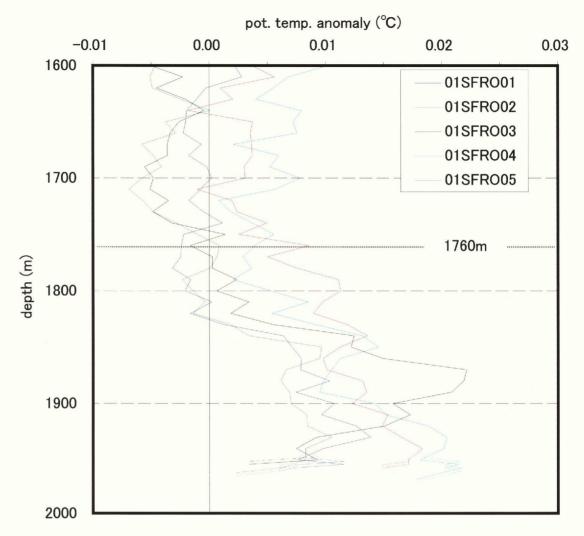


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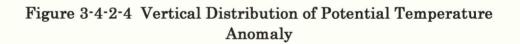


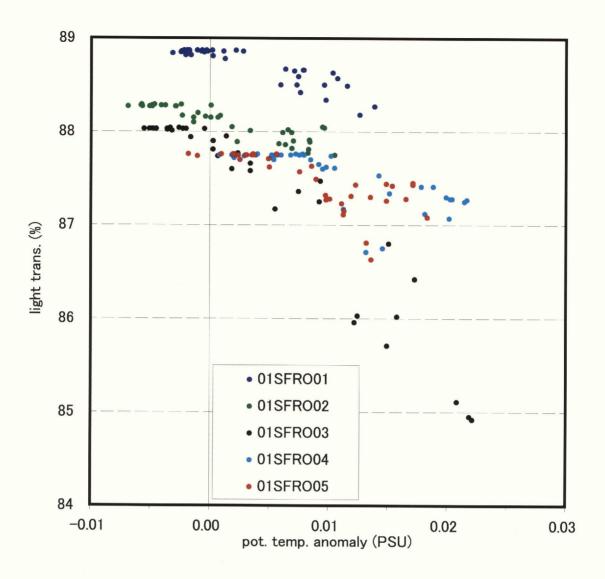
under 1600m depth at each station, with regression line to be expected from the real value in 1600-1760m depth.

Figure 3-4-2-3 T/S Diagrams

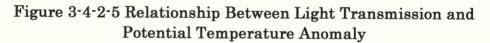


indicate the differences between real value and expectations by formula in Figure 3-4-2-3.





under 1600m depth at each station.



#### 3-4-3 Study for the Sediment and Benthic organism

#### 3-4-3-1 Sediment

#### (1) Total Sulfide (T-S)

Samples were collected from the three stations mentioned before. However, no samples were collected from 0<sup>-1</sup>, 1<sup>-2</sup> and 2<sup>-3</sup>cm layers at 01SFMC04, while the samples of 01SFMC05 and 01SFMC07 were collected from 0 to 5 cm. The concentrations of T-S in 3<sup>-4</sup>cm and 4<sup>-5</sup>cm layers at 01SFMC04 were 0.02 and 0.04mg/g(D). At 01SFMC05, they were equal to or less than 0.01 mg/g(D). At 01SFMC07, they were 0.02mg/g(D) in 0<sup>-1</sup>, 3<sup>-4</sup> and 4<sup>-5</sup> layers, while 0.01 mg/g(D) in the other layers (Table 3<sup>-4-3-1</sup>).

#### (2) Total Organic Carbon (TOC) and Inorganic Carbon

The samples for Total Organic Carbon (TOC) were collected from 3.4, and 4.5 cm at 01SFMC04, 0.10cm layers at 01SFMC05, and 0.5cm layers at 01SFMC07. TOC concentration, which was smaller than 1 mg/g (D), were observed all the station shallower than 5 cm, while at the 01SFMC05, where was only station collected the samples from deeper than 5 cm, varied within the range of 1.37 to 1.48 mg/g (D). Regarding inorganic carbon, similar to TOC, the remarkable difference was observed between 0.5 cm and 5-10 cm, i.e. the former varied 5.99 to 6.77 mg/g (D) and the latter did 15.31 to 41.37 mg/g (D) (Table 3.4.3.1, Figure 3.4.3.1).

#### (3) Total Nitrogen (TN)

The samples for Total Nitrogen (TN) was collected the same station and layers of TOC. TN concentration, which was smaller than 0.2 mg/g (D), were observed all the station shallower than 5 cm, while at the 01SFMC05, where was only station collected the samples from deeper than 5 cm, varied within the range of 0.22 to 0.32 mg/g(D) (Table  $3\cdot4\cdot3\cdot1$ , Figure  $3\cdot4\cdot3\cdot1$ ).

#### (4) CN ratio

All the data of CN ratio were listed in the Table 3-4-3-1. And then, CN ratio of 01SFMC05 was indicated also in the Figure 3-4-3-1. Although the slightly differences depend on the stations were observed, most of the data was extended between 4 to 5, and there was no tendency with the depth in the sediment.

Sampling	Layer	T-S	TOC	Inorganic carbon	TN	CN
Station	cm	mg/g(D)	mg/g(D)	mg/g(D)	mg/g(D)	ratio
01SFMC04	3 - 4	0.04	0.69	-	0.15	4.60
	4 - 5	0.02	0.98	-	0.2	4.90
01SFMC05	0 - 1	0.01	0.60	7.09	0.09	6.67
	1 - 2	0.01	0.46	6.08	0.13	3.54
	2 - 3	0.01	0.47	6.42	0.08	5.88
	3 - 4	< 0.01	0.50	5.99	0.13	3.85
	4 - 5	0.01	0.49	6.77	0.1	4.90
	5 - 6	-	0.75	15.36	0.22	3.41
	6 - 7	-	1.41	35.69	0.23	6.13
	7 - 8	-	1.37	36.23	0.23	5.96
	8 - 9	-	1.47	36.75	0.32	4.59
	9 - 10	-	1.48	41.37	0.32	4.63
01SFMC07	0 - 1	0.02	0.78	-	0.16	4.88
	1 - 2	0.01	0.45	-	0.08	5.63
	2 - 3	0.01	0.27	-	0.09	3.00
	3 - 4	0.02	0.28	-	0.12	2.33
	4 - 5	0.02	0.63	-	0.19	3.32

# Table3-4-3-1The vertical profiles of Total Sulfide (T-S), Total OrganicCarbon (TOC),Inorganic carbon and CN ratio

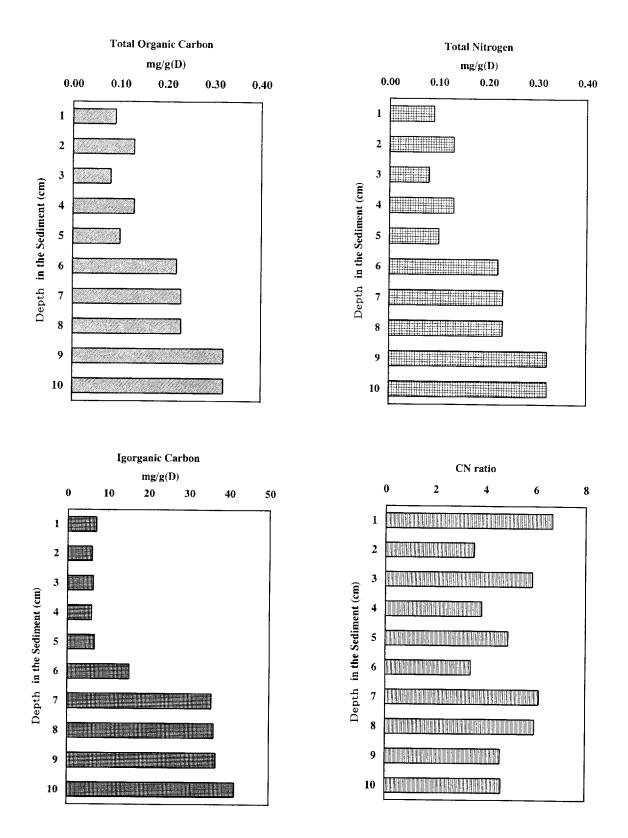


Figure 3-4-3-1 The vertical profiles of Total Organic Carbon, Total Nitrogen, Inorganic Carbon and CN ratio in the 01SFMC05.

#### 3-4-3-2 Benthic Organism

#### (1) Sedimentary Bacteria

Samples were collected from the three stations mentioned before. However, no sample was collected from 0-1, 1-2, and 2-3 cm layers at 01SFMC04, resulting in limited available samples only from 3-4 and 4-5 cm layers at the station. On the other hand, samples were collected at 01SFMC05 and 01SFMC07 from every 1 cm layer between 0 and 10 cm depth and between 0 and 5 cm depth, respectively. Due to a small number of samples and a lack of uniformity in the sample sets, a direct or strict comparison of the abundance of sedimentary bacteria among those three stations should be avoided. Therefore, the following descriptions are no more than the results of analyses for each station without a scientifically strict comparison among them.

Regarding  $3\cdot 5\cdot cm$  layers, the abundance of sedimentary bacteria in 01SFMC04 was fewer than that of other two stations (Figure  $3\cdot 4\cdot 3\cdot 2$ ). Neither increase nor decrease trends were not confirmed in the vertical profile of 01SFMC07, while a vertical reduction was confirmed in that of 01SFMC05. The maximum abundance of sedimentary bacteria observed in this study was  $5.44 \times 10^8$  cells/gD observed at the  $1\cdot 2\cdot cm$  layer in 01SFMC05, which was equal to or less than that in the abyssal plain, the Central Pacific Ocean. Based on the result, it is difficult to assume that there is either an ecosystem of chemosynthetic bacteria or at least an environment influenced by such an ecosystem in the study site.

#### (2) Meiobenthos

Six phyla, including protozoa, were confirmed as meiobenthos (Table 3-4-3-2). Typical deep-sea meiofauna such as Gatrotricha, Loricifera, and Gastropoda were not found, nevertheless it is uncertain whether such a result is attributable to the characteristics of the site because of the small number of samples.

Total number of meiobenthos, including Protozoa (foraminifera), was 78 inds./10cm<sup>2</sup>, 181 inds./10cm<sup>2</sup>, and 176 inds./10cm<sup>2</sup> at 01SFMC04, 01SFMC05, and 01SFMC07, respectively(Figure  $3\cdot4\cdot3\cdot3$ ). Similar to the results with respect to sedimentary bacteria, there was no distinct difference in the abundance of meiobenthos between the study site and the abyssal plain. Therefore, it is unlikely that a chemosynthetic community existed in the area.

In terms of the topography, 01SFMC05 was located on the top of the mount, while 01SFMC04 and 01SFMC07 were in the depression. However, the densities of meiobenthos in the 01SFMC05 and 01SFMC07 were two times higher than that of 01SFMC04. So, the density and topography is not proportional to one another, which indicates the differences in abundance were determined by other than topography.

The vertical distributions demonstrate that most of the organisms were concentrated upper than 1 cm depth, and the differences in vertical distribution were not so obvious in comparison with that in abundance between the stations (Figure  $3\cdot4\cdot3\cdot4$ ). In regard to organisms, nematodes were distributed even in deeper layers, while foraminifera were concentrated in upper layers.

According to the distributional nature of benthos described above, the evidence indicating the existence of a chemosynthetic community, which was derived from a thermal vent, was not confirmed. The non-parallel relationship between the biological abundance and the topography suggests the needs of further study to investigate other environmental factors influencing on the abundance of benthic organisms.

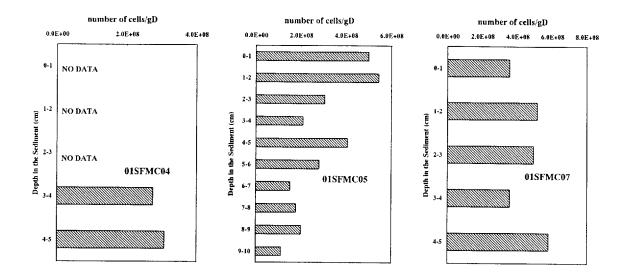
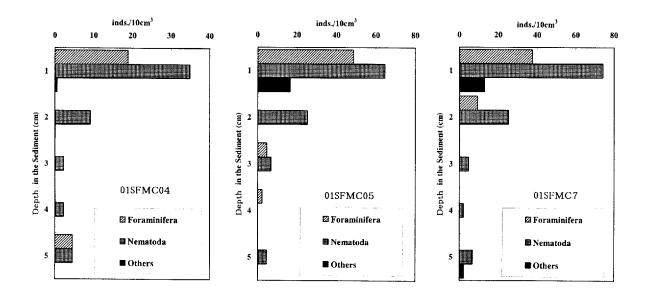


Figure 3-4-3-2 Vertical profiles of sedimentary bacteria

#### Table 3-4-3-2 List of appearance fauna

Ph y lum	Class	Order or Family
Protozoa	Rhizopoda	Foraminifera
Kinorhyncha	····	· · · · · · · · · · · · · · · · · · ·
Nematoda		
Mollusca	Bivalvia	····
Annelida	Polychaeta	
		Paraonidae
		Cirratulidae
Arthropoda	Ostracoda	
	Copepoda	Harpacticoida
	Isopoda	Ischnomesidae
	(Nauplius)	

Figure 3-4-3-3 Abundance of meiobenthos in the three stations



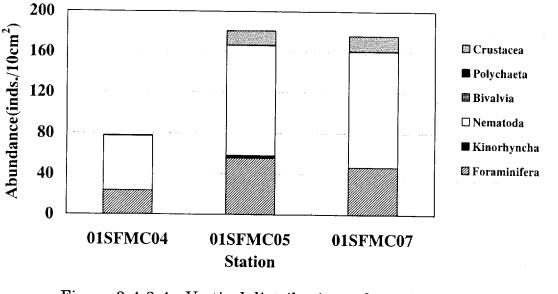


Figure 3-4-3-4 Vertical distributions of meiobenthos in the three stations

### 3-4-3-3 Conclusion of the Study for the Sediment and Benthic organism

Slight differences exist among the samples collected from each station at depths shallower than 5cm with respect to total sulfide, organic and inorganic carbon, and total nitrogen. However, those differences are not significant enough to discuss heterogeneities among the stations. However, obvious differences were found between depths shallower and deeper than 5 cm at 01SFMC05 with respect to total sulfide, organic and inorganic carbon, and total nitrogen.

Based on an assumption that CaCO<sub>3</sub> increased most significantly as a source of inorganic carbons, the increases in organic carbon and total nitrogen could be attributed to a chemosynthetic community established around hydrothermal vents. Furthermore, such an assumption could explain why CN ratios were constant at all the depths.

Combined the results obtained through this study with facts already known, it was strongly indicated that at least the environment at 01SFMC05 was under the influence of hydrothermal vent was estimated.