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Variation in Microbial Biomass and Community Structure in Sediments of Peter the Great Bay (Sea of Japan/East Sea), as Estimated from Fatty Acid Biomarkers

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Abstract – Variation in the microbial biomass and community structure found in sediment of heavily polluted bays and the adjacent unpolluted areas were examined using phospholipid fatty acid analysis. Total microbial biomass and microbial community structure were responding to environmental determinants, sediment grain size, depth of sediment, and pollution due to petroleum hydrocarbons. The marker fatty acids of microeukaryotes and prokaryotes - aerobic, anaerobic, and sulfate-reducing bacteria - were detected in sediments of the areas studied. Analysis of the fatty acid profiles revealed wide variations in the community structure in sediments, depending on the extent of pollution, sediment depth, and sediment grain size. The abundance of specific bacterial fatty acids points to the dominance of prokaryotic organisms, whose composition differed among the stations. Fatty acid distributions in sediments suggest the high contribution of aerobic bacteria. Sediments of polluted sites were significantly enriched with anaerobic bacteria in comparison with clean areas. The contribution of this bacterial group increased with the depth of sediments. Anaerobic bacteria were predominantly present in muddy sediments, as evidenced from the fatty acid profiles. Relatively high concentrations of marker fatty acids of sulfate-reducing bacteria were associated with organic pollution in this site. Specific fatty acids of microeukaryotes were more abundant in surface sediments than in deeper sediment layers. Among the microeukaryotes, diatoms were an important component. Significant amounts of bacterial biomass, the predominance of bacterial biomarker fatty acids with abundance of anaerobic and sulfate-reducing bacteria are indicative of a prokaryotic consortium responsive to organic pollution.

Key words – microbial community structure, sediments, fatty acids, biomarkers

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1. Introduction

Prokaryotic and eukaryotic microorganisms, – by their ubiquity, metabolic diversity and large contribution to biomass, – dominate production and fluxes of organic and inorganic matter in aquatic ecosystems. Benthic microbial communities are important to marine ecosystem function for a variety of reasons. Microorganisms in sediments are food resources for deposit-feeding invertebrates. The surface sediments are the site of nutrient regeneration. The distribution and abundance of microorganisms are determined by environmental factors and biotic interactions (Paerl 1998). Anthropogenic nutrients and other pollutant enrichment of the coastal waters are causing unprecedented changes in microbial community structure and function (Rajendran *et al.* 1997; Macalady *et al.* 2000; Langworthy *et al.* 2002). Biogeochemical and trophic consequences of such alterations are expanding on a local, regional, and global scale. The prevalence of organic pollution within the environment and the major role of microorganisms in its decomposition reveal the importance of increased understanding of their effects on microbial communities.

In environments such as soil and sediment, despite high microbial diversity, the difficulties in culturing native organisms make the culture-based methods inadequate or inefficient for differentiating microbial communities. Within such environments, lipid analysis has become an important ecological tool. An accurate measure of the microbial biomass and the microbial community structure of marine sediments can be obtained through the biochemical

analysis of phospholipid fatty acids (PLFA) (Perry *et al.* 1979; Gillan and Hogg 1984; White 1988). The method, which overcomes some limitations (of culture-based or molecular biological methods), is based on lipid extraction and further analysis of cellular phospholipids fatty acids. Fatty acids are useful biomarkers, since they are essential components of bacterial cells and eukaryotic organisms. Analysis of biomarker fatty acids has been successfully used for the interpretation of microbial community structure since certain fatty acids are specific to bacteria and different groups of bacteria have different fatty acid compositions (Guckert *et al.* 1985; Findlay and Dobbs 1993). Analysis of the fatty acids of sediments provides information on the living organisms, since the phospholipids in dead organic matter are rapidly hydrolyzed (White *et al.* 1979). Phospholipids are not storage lipids and have rapid turnover in sediments; therefore, the analysis of PLFA provides a measure of the viable microbial biomass. Though this method allows no identification of microbial taxa in complex communities, it has proven efficient in identifying various groups of microorganisms in environmental samples (Findlay and Watling 1998; Boschker *et al.* 1998; Langworthy *et al.* 2002).

The extensive coastal zone of Peter the Great Bay includes areas with ecologically contrasting conditions. Some pristine bays within Peter the Great Bay are part of the Marine Reserve, whereas bays near industrial zones are the draining areas of industrial and domestic wastewater, marine transportation, and agricultural effluents. Anthropogenic discharges to coastal waters comprise a broad spectrum of chemicals in addition to nutrients. Included are industrial, urban and agricultural petrochemicals, petroleum hydrocarbons, chlorinated hydrocarbons, chlorinated pesticides, and heavy metals (Tkalin 1996; Vaschenko 2000; Naumov 2003). Coastal sediments act as a waste sink to accumulate these pollutants. Diverse microorganisms play an important role in the degradation of pollutants and the decomposition of organic matter in the sediments and cycling of nutrients in the aquatic environment.

The current study focuses on identifying spatial variations in sedimentary microbial community structure and the consequences of urban and industrial pollutant input. Analysis of phospholipid fatty acids, which are useful biomarkers of bacteria, was used to identify and quantify the viable microbial biomass in sediments and microbial community structure.

2. Materials and Methods

Study area and sample collection

Sediment samples were collected from 3 stations in Nakhodka Bay and from 5 stations in Vostok Bay of Peter the Great Bay, Sea of Japan/East Sea (Fig. 1). In Nakhodka Bay, station 1 was located at the top of the bay; station 2 was situated in the middle part; and station 3 was in the open part of the bay. Nakhodka Bay is receiving practically untreated wastewater from Nakhodka City, one of the Primorye's largest industrial cities, and from five major ports. Industrial and domestic sewage, marine transportation, and agricultural effluents are considered the major sources of pollution in the bay. The top of Nakhodka Bay is more heavily polluted: the content of oil hydrocarbons (petrochemicals) in sediments reaches 11.4 $\mu g \cdot g^{-1}$ dry weight (Naumov 2003). In Vostok Bay, stations 4, 5, and 6 were located in the inner, middle, and open parts of the harbor of the shipyard, which is heavily polluted by petroleum hydrocarbons. In the inner part of the harbor, the surface water was covered with a petroleum film. The environmental characteristics of the present study areas have already been described elsewhere (Vaschenko 2000; Khristoforova *et al.* 2001; Naumov 2003). Long-term environmental monitoring indicates that the major pollutants in the investigated areas are petroleum derivatives; the contents of heavy metal salts, pesticides, and herbicides have also increased. For comparison, bottom sediments were collected from the adjacent unpolluted areas in Vostok Bay (stations 7 and 8) with a low probability of

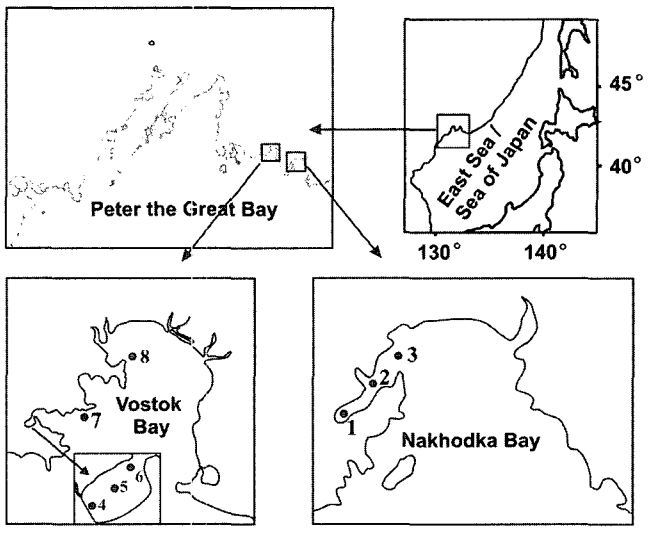

Fig. 1. Map showing the study area and the sampling stations.

exposure to anthropogenic stress.

Sediment cores were collected using scuba. Sediments were sampled by inserting a core (10 cm in diameter) into the sediments. The core was extruded, and the surface layer up to 0.3 cm thick, as well as 3–5 cm and 8–10 cm layers were sampled and stored at –20°C prior to analysis. Triplicate sediment cores were collected and worked up separately as replicate samples.

Fatty acid analysis

Sediment samples were stored at –20°C until analysis. Lipids were extracted by the method of Bligh and Dyer (1959). The extracted lipids were fractionated into neutral lipids, glycolipids, and phospholipids by column chromatography on silica gel by using chloroform, acetone, and methanol, respectively. The fatty acid methyl esters (FAME) were obtained from phospholipids fractions according to Carreau and Dubacq (1978) and were purified by thin-layer chromatography in benzene. The fatty acid methyl esters were analyzed on a Shimadzu GC-17A gas chromatograph with a flame ionization detector, using a Supelcowax-10 fused silica capillary column (30 m×0.25 mm i.d.) at 205°C. Helium was used as a carrier gas. Additionally, $AgNO_3$-TLC was used for the identification of unsaturated fatty acids; also for identification, part of the samples was preliminarily hydrogenated over PtO_2 in methanol for 30 min. Fatty acids were identified using standard mixtures and the known equivalent chain length (ECL) values. For the determination of absolute abundance (μg FAME·g^{-1} dry weight of sediment) before transesterification, 25 μg per probe of 23:0 was added as an internal standard. Fatty acids were designated as the number of carbon atoms, the number of double bonds. The position of the first double bond (n-x) was counted starting from the terminal methyl group; double bonds in PUFA are separated by a methylene group.

3. Results and Discussion

Total microbial biomass

Total microbial biomass measured as total concentration of phospholipid fatty acids (PLFA) varied from 9.0 to 77.6 μg · g^{-1} of dry weight for sediment samples (Fig. 2). Similar values of microbial biomass were reported for marine sediments from Cartean Cove (the Gulf of Fos, France) (Aries *et al.* 2001). The increased PLFA concentration

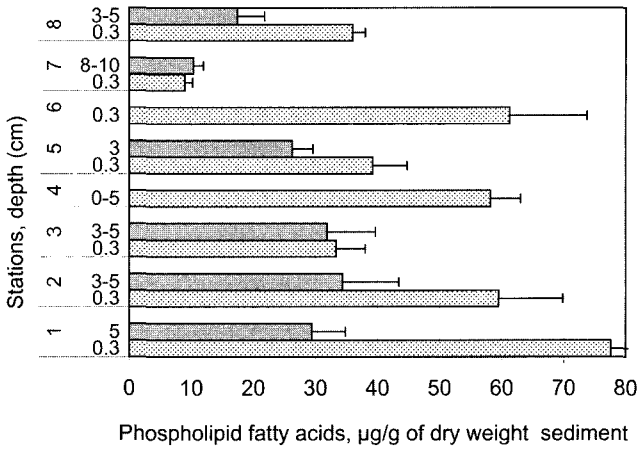

Fig. 2. The variations in the total sedimentary microbial biomass with depth (horizon) and at stations. Biomass is given as μg total PLFA · g^{-1} dry weight per sediment sample. Mean ±SD, n=3.

indicated an increase in the lipid-contributing microbial biomass in sediments, being a response to a wide range of environmental determinations and pollutants. Total microbial biomass decreased with sediment depth, and the vast majority of phototrophic microorganisms and bacteria present in surface sediments were less abundant in lower sediment horizons (Fig. 2). In a tidal flat in the Wadden Sea, microbial biomass expressed as PLFA abundance in surface sediments (0.5 cm) was 26 times higher than in deeper layers (50 cm), 10 and 0.4 μg·g^{-1} dry weight, respectively (Rutters *et al.* 2002). For near shore sediments from Bowling Green Bay (North Queensland), from an environment with low bacterial population and a significant relict sediments input, microbial biomass was estimated to vary from 199 μg · g^{-1} at 3–4-cm depth to 8 μg·g^{-1} at 11–13 cm depth (Gillan and Sandstrom 1985).

Total microbial biomass in muddy sediments was significantly higher than in a sandy bottom. Thus, in surface sediments of unpolluted areas, total microbial biomass measured as PLFA concentration was 4 times greater at the muddy bottom of station 8, as compared to that in sandy sediments of station 7. The low values of total microbial biomass were also reported for sandy beaches in northern Florida (Findlay and Dobbs 1993).

As shown in Fig. 2, petroleum-polluted areas (stations 1–3 in Nakhodka Bay and 4–6 in Vostok Bay) showed higher microbial biomass in sediments than in unpolluted areas (stations 7 and 8). Total PLFA content in sediments was the highest at the top of the two heavily polluted

bays (stations 1, 2 and 4). Similar amounts of microbial biomass were also found for the open part of harbor (station 6). Analysis of absolute abundance of different microbial groups shows that higher microbial biomass at station 6 was accounted for by high input of microeukaryotes (microalgae and protozoa) into the sediment of this area. High concentration of specific fatty acids of the microalgae, mainly diatoms, points to the origin of these PLFA. Similarly, river sediments polluted by polycyclic aromatic hydrocarbons (PAHs) in moderate concentrations exhibited higher total microbial biomass in comparison with the neighboring clean area (Langworthy *et al.* 1998). In contrast, much

Table 1. Composition of the PLFA (wt % of the total fatty acids) of the sediments of Nakhodka Bay taken at two depth (mean ± SD; n=3)

Fatty acids	Station 1 (inner part)		Station 2 (middle part)		Station 3 (open part)	
	0.3 cm	5 cm	0.3 cm	3-5 cm	0.3 cm	3-5 cm
12:0	0.2 ± 0.2	0.6 ± 0.1	1.1 ± 0.7	0.6 ± 0.4	0.7 ± 0.3	0.6 ± 0.3
13:0	0.2 ± 0.1	0.2 ± 0.1	0.2 ± 0.2	0.2 ± 0.1	0.2 ± 0.2	0.2 ± 0.0
13:1	0.5 ± 0.4	0.2 ± 0.3	0.4 ± 0.5	0.1 ± 0.2	0.1 ± 0.1	0.0 ± 0.0
14:0-iso	0.6 ± 0.6	1.5 ± 0.0	0.4 ± 0.4	0.8 ± 0.1	1.7 ± 1.8	0.7 ± 0.2
14:0	5.0 ± 0.4	4.3 ± 0.3	5.4 ± 1.8	6.2 ± 1.4	5.8 ± 1.0	5.5 ± 0.5
14:1	1.3 ± 0.7	1.9 ± 0.0	1.4 ± 0.7	1.4 ± 0.5	1.4 ± 0.9	1.7 ± 1.1
15:0-iso	3.6 ± 0.4	4.4 ± 0.4	1.8 ± 0.7	1.8 ± 0.2	2.5 ± 0.9	2.0 ± 0.8
15:0-anteiso	4.1 ± 3.5	8.8 ± 0.8	3.3 ± 0.9	5.3 ± 1.8	4.5 ± 1.5	4.4 ± 1.2
15:0	2.4 ± 0.3	2.3 ± 0.2	2.8 ± 0.3	3.4 ± 1.2	3.3 ± 0.3	3.1 ± 0.4
15:1	0.5 ± 0.1	0.8 ± 0.1	1.1 ± 0.5	0.8 ± 0.1	0.7 ± 0.3	0.9 ± 0.2
16:0-iso	1.1 ± 0.2	1.6 ± 0.1	0.8 ± 0.2	0.9 ± 0.2	0.8 ± 0.1	0.8 ± 0.2
16:0-anteiso	0.1 ± 0.1	0.3 ± 0.1	0.2 ± 0.1	0.3 ± 0.1	0.3 ± 0.1	0.2 ± 0.1
16:0	21.2 ± 3.4	15.2 ± 0.6	22.1 ± 3.7	22.8 ± 4.0	20.8 ± 2.7	21.0 ± 3.4
16:1(n-7)	13.8 ± 3.0	16.8 ± 0.8	19.2 ± 6.4	16.6 ± 4.3	17.6 ± 6.2	20.2 ± 8.9
16:1(n-5)	1.2 ± 0.4	1.8 ± 0.4	0.9 ± 0.9	1.2 ± 0.4	1.6 ± 0.5	1.7 ± 0.7
17:0-iso	0.7 ± 0.1	1.2 ± 0.4	0.6 ± 0.2	0.8 ± 0.3	0.8 ± 0.4	0.7 ± 0.2
17:0-anteiso	1.1 ± 0.2	1.6 ± 0.1	0.9 ± 0.2	1.1 ± 0.1	1.4 ± 0.3	1.2 ± 0.2
16:2(n-6)	0.5 ± 0.2	0.8 ± 0.1	0.3 ± 0.2	0.7 ± 0.5	0.6 ± 0.3	0.5 ± 0.2
10Me16:0	0.7 ± 0.1	0.9 ± 0.1	0.7 ± 0.1	0.9 ± 0.1	0.8 ± 0.1	0.9 ± 0.1
17:0	1.2 ± 0.2	1.2 ± 0.1	1.2 ± 0.1	1.4 ± 0.1	1.2 ± 0.2	1.4 ± 0.4
17:1(n-8)	1.3 ± 0.3	1.7 ± 0.1	1.6 ± 0.5	1.5 ± 0.3	1.3 ± 0.3	2.3 ± 1.0
17:0-cyclo	0.8 ± 0.1	1.5 ± 0.4	0.6 ± 0.2	0.6 ± 0.3	0.7 ± 0.2	0.9 ± 0.2
18:0-iso	0.2 ± 0.1	0.3 ± 0.4	0.2 ± 0.2	0.3 ± 0.2	0.5 ± 0.6	0.1 ± 0.1
18:0-anteiso	0.2 ± 0.1	1.0 ± 1.3	0.9 ± 0.7	0.9 ± 0.9	0.8 ± 0.7	0.1 ± 0.2
18:0	11.5 ± 3.3	4.2 ± 0.3	9.7 ± 4.9	7.5 ± 2.3	9.0 ± 3.9	7.5 ± 4.3
18:1(n-9)	9.2 ± 1.0	9.0 ± 0.6	6.5 ± 1.3	7.3 ± 1.6	7.0 ± 1.9	7.4 ± 4.3
18:1(n-7)	6.8 ± 1.2	8.0 ± 0.2	5.5 ± 2.1	5.6 ± 3.2	5.2 ± 2.2	6.2 ± 2.4
18:2(n-6)	2.6 ± 1.0	3.3 ± 0.6	2.0 ± 0.4	2.5 ± 0.5	2.5 ± 0.4	1.8 ± 0.3
19:0	0.3 ± 0.1	0.4 ± 0.1	0.2 ± 0.2	0.2 ± 0.3	0.2 ± 0.3	0.4 ± 0.2
18:3(n-3)	0.4 ± 0.3	0.8 ± 0.1	0.8 ± 0.3	1.0 ± 0.8	0.4 ± 0.4	0.5 ± 0.2
19:0cyclo	0.6 ± 0.1	0.7 ± 0.2	0.3 ± 0.3	0.3 ± 0.2	0.3 ± 0.5	0.4 ± 0.4
18:4(n-3)	0.2 ± 0.0	0.0 ± 0.0	0.3 ± 0.3	0.1 ± 0.1	0.3 ± 0.3	0.1 ± 0.2
20:0	0.7 ± 0.6	0.6 ± 0.1	0.7 ± 0.4	0.5 ± 0.2	0.7 ± 0.4	0.3 ± 0.2
20:1	0.9 ± 0.2	0.7 ± 0.0	0.8 ± 0.2	0.8 ± 0.8	0.6 ± 0.5	0.7 ± 0.1
20:3(n-6)	0.3 ± 0.3	0.1 ± 0.1	0.3 ± 0.3	0.2 ± 0.3	0.1 ± 0.1	0.0 ± 0.0
20:4(n-6)	0.7 ± 0.2	0.7 ± 0.1	1.0 ± 0.6	0.9 ± 0.1	0.7 ± 0.2	0.8 ± 0.1
20:5(n-3)	2.4 ± 1.9	0.9 ± 0.1	1.4 ± 0.4	1.4 ± 0.1	1.2 ± 0.2	1.6 ± 0.8
22:5(n-3)	1.3 ± 1.1	0.4 ± 0.1	0.8 ± 0.8	0.4 ± 0.4	0.5 ± 0.2	0.4 ± 0.4
22:6(n-3)	1.2 ± 0.2	0.6 ± 0.0	1.2 ± 0.1	0.7 ± 0.4	1.2 ± 0.5	0.6 ± 0.2

lower microbial biomass occurred in sediments of eutrophic coastal bays in Japan, and the total PLFA concentration was less than 1 $\mu g \cdot g^{-1}$ dry weight of sediments (Rajendran *et al.* 1992, 1997). The authors attributed this to physiological stress within microbial communities caused by high levels of pollutants. Langworthy *et al.* (2002), studying the effect of different concentrations of PAHs on the microbial community of sediments, experimentally showed that the greatest microbial biomass occurred in sites with an intermediate concentration of PAHs, while the site with high PAHs had a smaller microbial biomass. Thus, negative deflection in community biomass can occur when the stressor is a toxic input and when the stressor is a useful input but at concentrations where toxic effects override stimulatory effects.

Microbial community structure

The fatty acid composition of sediments sampled in the inner, middle, and open parts of Nakhodka Bay is shown in Table 1. About 50 fatty acids detected in the sediments included saturated, branched, hydroxy, cyclopropyl, monounsaturated, and polyunsaturated fatty acids were in the range of C12-C22. Most of the PLFA found in sediments are considered to be of bacterial origin. It is known that branched-chain, cyclopropyl and certain straight-chain saturated and monounsaturated fatty acids present in sediments derive almost exclusively from *in situ* bacterial populations (White *et al.* 1979; Gillan and Hogg 1984).

Analysis of PLFA is a sensitive and convenient chemical method for the determination of community structure in sediments because certain fatty acids are specific to bacteria, and different groups of bacteria have different fatty acid compositions (Gillan and Hogg 1984; White 1988; Findlay and Dobbs 1993). This method has been successfully used for the determination of sedimentary microbial community structure in various environments, near shore and deepwater, estuarine and riverine, as well as in eutrophic and polluted areas. Findlay *et al.* (1990) have suggested to group together suites of the microorganisms in sediment that share biochemical characteristics, and defined four distinct functional groups based on biomarker fatty acids specific for these groups: I–microeukaryotes (microalgae and microzoobenthos), II–aerobic prokaryotes and eukaryotes, III–gram-positive prokaryotes and other anaerobic bacteria, and IV–sulfate-reducing and other anaerobic bacteria.

The functional group approach (Findlay *et al.* 1990; Findlay and Dobbs 1993) was used to estimate microbial community structure in sediments of Vostok and Nakhodka Bays. Fatty acid biomarkers, which are well known to correlate with certain groups of microorganisms, were identified in all samples in different proportions (Table 2, Fig. 3). The microbial community structure of the study areas exhibited wide variations, as determined from the variations in the PLFA proportions (Fig. 4). Detailed examination of the fatty acid profiles revealed wide variations in the community structure in sediments depending on the extent of pollution of the bays, sediment depth, and sediment grain size (sand-mud).

The indicators of microeukaryotes input in the microbial

Table 2. Relative proportions of different microbial groups in sediments detected as percentage of the total fatty acids. Data are means and SD (in parentheses), n=3.

Microbial groups: their fatty acid biomarkers	Nakhodka Bay							Vostok Bay						
	Stations													
	1		2		3		4	5		6	7		8	
	Horizon (cm)													
	0.3	3-5	0.3	3-5	0.3	3-5	0.3-3	0.3	3-5	0.3	0.3	8-10	0.3	3-5
Microeukaryotes: C16, C18, C20, C22 PUFA	9.6 (2.0)	7.4 (0.7)	8.1 (1.5)	7.9 (1.5)	7.4 (0.3)	6.4 (1.3)	7.1 (1.0)	6.6 (1.3)	6.0 (1.0)	11.2 (1.8)	11.5 (1.2)	7.6 (0.4)	9.4 (1.4)	7.8 (1.9)
Aerobic prokaryotes and eukaryotes: 16:1, 17:1, 18:1, 18:2	34.9 (4.8)	40.5 (2.7)	35.7 (4.4)	34.7 (3.4)	35.1 (3.9)	39.6 (4.5)	32.4 (4.0)	36.8 (3.5)	37.9 (3.9)	37.3 (3.6)	39.0 (3.8)	33.1 (3.8)	43.0 (3.0)	37.5 (3.4)
Gram-positive prokaryotes and other anaerobic bacteria: 15:0, 15:0i, 15:0ai, 16:0i	11.2 (3.2)	17.0 (1.1)	8.7 (1.8)	11.4 (1.0)	11.1 (1.2)	10.4 (1.8)	10.9 (0.7)	14.9 (1.2)	12.4 (1.3)	9.7 (1.5)	5.4 (0.6)	8.3 (1.3)	11.7 (1.6)	13.3 (1.2)
Sulphate reducing and other anaerobic bacteria: 17:0, 17:0i, 17:0ai, 17:0cy, 19:0cy, 10Me16:0	4.8 (0.4)	6.9 (1.1)	4.3 (0.3)	5.1 (0.6)	5.1 (0.6)	5.6 (0.5)	6.5 (0.3)	7.3 (1.0)	5.9 (0.2)	5.7 (0.3)	4.6 (0.2)	4.2 (0.2)	5.2 (1.4)	5.2 (1.1)

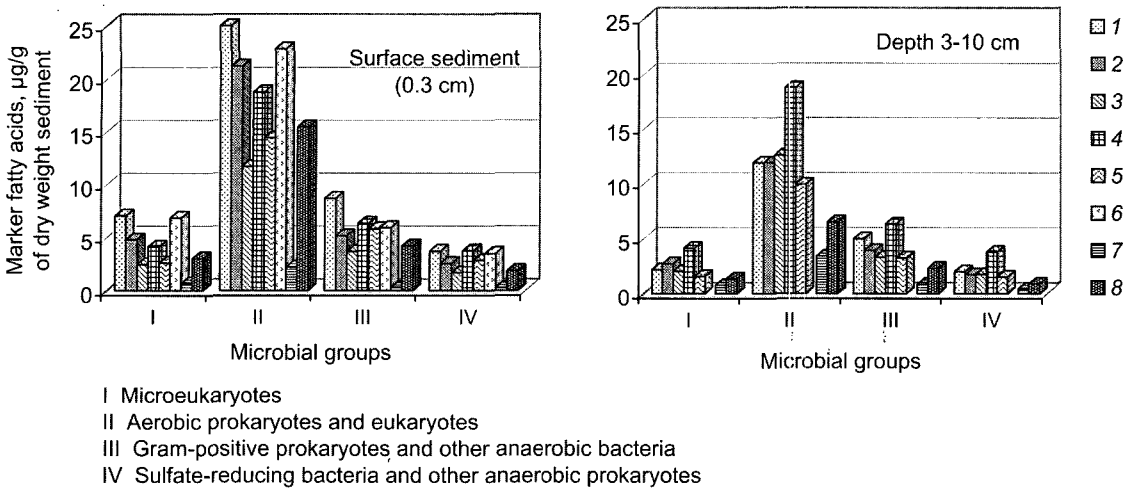

Fig. 3. Absolute abundance of the microbial groups (µg PLFA · g^{-1} dry weight of sediment) in sediment samples extracted from 8 stations.

community are polyunsaturated fatty acids (PUFA): 16:2ω6, 18:2ω6, 18:3ω3, 18:4ω3, 20:3ω6, 20:4ω6, 20:5ω3, 22:5ω3, and 22:6ω3. Their content varied from 6.0 to 11.5% of the total fatty acids in the investigated areas (Table 1 and 2) and exceeded the earlier reported level for sediments of Vostok Bay (2–5% of the total fatty acids) (Imbs *et al.* 1994). The biomarkers of diatoms (Volkman *et al.* 1989, Zhukova and Aizdaicher 1995), 20:5ω3, 16:2ω6 and 16:4ω1 PUFA, were identified in the sediments. Therefore, diatoms appear to be visible contributors to eukaryotes of the sediments studied. High concentration of 16:0 and 16:1ω7 in surface sediment provides further evidence in support of diatom input. The fatty acids 18:3ω3 and 22:6ω3 may derive from flagellates and PUFA ω6 and ω3 are indicative of heterotrophic flagellates and protozoa (Zhukova and Kharlamenko 1999). PUFA, which are usually the indicators of microeukaryotes in sediments, were found in higher proportions in surface sediments than in deeper layers. For example, PUFA concentration in deeper sediment layers at station 7 was significantly lower than in surface sediments (7.6 and 11.5% of the total fatty acids, respectively). The absolute abundance of this group of microorganisms was also higher in surface sediments and decreased with sediment depth (Fig. 3). Thus, at the heavily polluted top of Nakhodka Bay (station 1), it decreased more than three times in deeper sediment layers (Fig. 3).

In our study, the highest concentrations of PUFA specific to microeukaryotes were detected in unpolluted areas (stations 7 and 8), as well as in the open part of harbor (station 6), where there are favorable environmental conditions for their development. However, rather high concentrations of PUFA were detected in sediments of polluted areas as well. In a pristine site with a low probability of exposure to anthropogenic stress, phototrophic microeukaryotes were found to be the most abundant in the benthic microbial community (Findly and Waitling 1998). The presence of fatty acids in the form of heterotrophic microeukaryotes (Zhukova and Kharlamenko 1999) may suggest the development of a microbial loop, in which abundance of bacteria as a result of organic pollution, serves as a food source for predatory heterotrophic microeukaryotes. Langworthy *et al.* (1998) found that sediments polluted by PAHs were enriched in fatty acids associated with heterotrophic eukaryotes. Carman *et al.* (1995) have documented development of the sediment food web in polycyclic aromatic hydrocarbon-polluted estuarine sediments. However, high concentrations of pollutants in a bay of the inland Sea of Japan led to a significant decrease in microeukaryotes in sediments (1.6–7.8%), as indicated by biomarker fatty acids (Rajendran *et al.* 1992, 1997).

Generally, the microbial community structure of the Vostok Bay and Nakhodka Bay sediments was dominated by prokaryotes, as evidenced from the amounts of characteristic bacterial fatty acids, branched-chain, odd, cyclopropyl, and *cis*-vaccenic acid (18:1ω7) (Table 1, 2). Among the bacterial fatty acids detected in the sediments, 18:1ω7 was predominant, its content reaching 8.3% of the total PLFA. *cis*-Vaccenic acid is usually considered to be a bacterial marker and, along with the branched-chain fatty acids, has been proposed for the estimation of

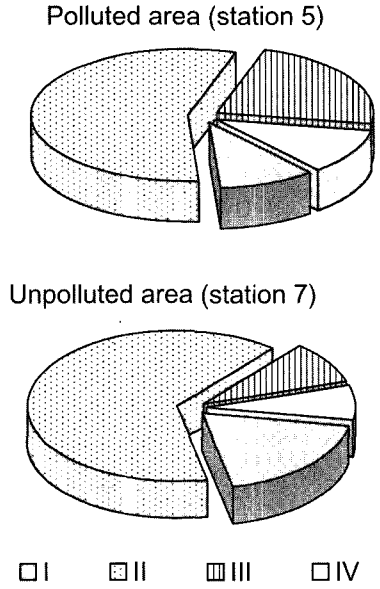

Fig. 4. Relative distribution of different groups of microorganisms in the sediment of polluted and unpolluted stations (weight % of the total PLFA).

bacterial biomass in sediments (Gillian and Hogg 1984).

Among prokaryotes, the aerobic bacteria were the predominant group found in the microbial community in all areas studied. The characteristic fatty acids of aerobic gram-negative bacteria, monounsaturated 16:1, 17:1, 18:1 and 18:2 fatty acids, comprised 32.4–43% of the total PLFA (Table 2). The relative abundance of fatty acid biomarkers of aerobic bacteria decreased according to sediment depth in unpolluted areas from 43 to 37.5% at station 8 and from 39 to 33.1% at station 7. However, in heavily polluted areas, the contribution of aerobic bacteria slightly increased in deeper sediment horizons (Table 2). The concentration of the biomarker fatty acids of aerobic bacteria differed insignificantly between unpolluted and polluted areas (Fig. 4). However, aerobic bacteria showed spatial variations in biomass (Fig. 3). The absolute abundance of aerobic bacteria decreased with sediment depth in all areas studied, excluding station 7 where that found in sand sediment increased. The biomass of aerobic bacteria was significantly higher in polluted areas. Among the areas studied, the top of Nakhodka Bay (station 1) is reported to be more heavily polluted (Naumov 2003). The microbial community here was dominated by aerobic bacteria, as evidenced from the highest amounts of biomarker fatty acids, 27.1 $\mu g \cdot g^{-1}$, dry weight (Fig. 3). High level of aerobic, gram-negative bacteria in petroleum-polluted sediments was not unexpected because these organisms are commonly isolated from polluted environments and it is well known that PAHs are rapidly degraded under aerobic conditions (Atlas 1981; Langworthy *et al.* 1998).

Branched fatty acids iso-15:0, anteiso-15:0 and iso-16:0, as well as 15:0, which are indicative of microbial group III, gram-positive and anaerobic bacteria, varied from 5.4 to 17% of the PLFA. The highest concentration of these biomarker acids was found in sediments at the top of Nakhodka Bay. These differences could be attributed to the environmental characteristics and pollution. As shown in Table 2 and Fig. 4, the contribution of this group of bacteria to the microbial community in polluted areas was higher than in unpolluted areas, it increased with sediment depth and depended on sediment grain size (sand or mud). For example, the relative abundance of anaerobic bacteria at the 3–5 cm depth was higher than in surface sediment, 17 versus 11.2% in the inner part and 11.4 versus 8.7% in the middle part of Nakhodka Bay. The concentration of specific fatty acids of anaerobic bacteria in muddy sediment was several times higher than that of sand sediment (5.4 and 11.7%, respectively). The lowest absolute amounts of anaerobic bacteria, 0.3 $\mu g \cdot g^{-1}$ of dry weight, were found in sand sediment, while in muddy sediment extracted from unpolluted areas, this value was 14 times higher (Fig. 3). The presence of anaerobic bacteria biomarkers in increased concentrations in polluted areas gives strong evidence that these organisms are responding to petroleum hydrocarbon pollution in these regions. The highest amounts of anaerobic prokaryotes were found in sediments of the inner part of heavily polluted Nakhodka Bay (17% or 8.7 $\mu g \cdot g^{-1}$, dry wt). This value is similar to the percentage of anaerobic bacteria in sediment found in the eutotrophic areas of the semi-enclosed inland Sea of Japan (Rajendran *et al.* 1992, 1997).

The signature fatty acids of sulfate-reducing bacteria and other anaerobic bacteria of microbial group IV are Me-16:0, iso-17:0, anteiso-17:0, 17:0, cyclo-17:0, and cyclo-19:0 (Findlay and Dobbs 1993). These biomarker fatty acids were detected in the sediment of all areas studied and varied from 4.2 to 7.3% of the total PLFA (Table 2, Fig. 4). Apparently, this group of prokaryotes formed a considerable proportion of microbial communities because the majority of the areas studied have muddy bottom sediment. This is supported by the very low content of

sulfate-reducing bacteria in sandy sediment, 0.3 μg versus 1.9 $\mu g \cdot g^{-1}$ in the muddy sands of unpolluted areas (Fig. 3). Their biomass in polluted areas was still higher, 3.8 $\mu g \cdot g^{-1}$ dry weight of sediment. The concentration of biomarker fatty acids of bacteria was similar to that reported for bottom sediment of the polluted Amursky Bay (Peter the Great Bay, Sea of Japan / East Sea), oil derrick sites on the shelf of Sakhalin Island (Imbs *et al.* 1994), and eutrophic bays in Japan (Rajendran *et al.* 1997; Rajendran and Nagamoto 1999). In addition, the concentration of fatty acids cyclo-17:0 and anteiso-17:0, which are the main indicators of sulfate-reducing bacteria, was markedly higher in deeper sediment layers and in more polluted areas (Table 1 and 2).

4. Conclusion

Total microbial biomass and microbial community structure were responding to a wide range of environmental determinants, sediment grain size, depth of sediment layer, and pollution – oil hydrocarbons. Rather high microbial biomass in the sediments of polluted areas of Vostok and Nakhodka Bays, a substantial contribution of characteristic fatty acids of anaerobic and sulfate-reducing bacteria, small proportions of polyunsaturated fatty acids, which are markers of microeukaryotes, were indicative of a highly developed microbial community exposed to organic pollution. However, no principal differences in the structure of microbial communities were found between unpolluted and polluted areas. Consequently, the microbial community was not experiencing any stress from pollution. The increase in total microbial biomass and marginal changes in microbial community structure of polluted areas, compared to the neighboring unpolluted areas, suggest an addition of organisms rather than replacement of organisms in the community.

The microbial community structure and total microbial biomass in sediments in the seaward parts of polluted areas showed similar distributions in unpolluted areas. Thus, natural intrinsic degradation potential may serve as an effective and nondisruptive mechanism for remediating organic pollution in the environment.

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