ISSN 0026-2617, Microbiology, 2008, Vol. 77, No. 5, pp. 587–594. © Pleiades Publishing, Ltd., 2008.
Original Russian Text © S.Yu. Maksimenko, T.I. Zemskaya, O.N. Pavlova, V.G. Ivanov, S.P. Buryukhaev, 2008, published in Mikrobiologiya, 2008, Vol. 77, No. 5, pp. 660–667.

EXPERIMENTAL ARTICLES

Microbial Community of the Water Column of the Selenga River–Lake Baikal Biogeochemical Barrier

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Received July 10, 2007

Abstract—The microbial communities of the estuarine zone and the mixing zone of river and lake waters in the Selenga River estuary were studied using the fluorescence in situ hybridization (FISH) method. The microorganisms belonging to the phylogenetic group *Gammaproteobacteria* were found to predominate in the river estuary, constituting up to 17% of the total bacterial community. Among cultivable microorganisms, organotrophic bacteria were predominant (2040 CFU/ml) in this zone, which results in high rates of microbial production (6.0 μg C/(l day). The microbial community structure changed with distance from the river estuary; representatives of the *Alpha*-, *Beta*-, and *Gammaproteobacteria* were present in equal proportions; psychrotolerant and oligotrophic bacteria were numerous. The rate of heterotrophic carbon dioxide assimilation decreased to 3.8 μg C/(l day). At 5–7 km from the river estuary, where the hydrologic, physical, and chemical conditions are similar to those of lake waters, members of the *Betaproteobacteria*, which are typical of the open waters of Lake Baikal, are the major representatives of planktonic microorganisms.

Key words: Lake Baikal, Selenga shallow waters, microbial communities, heterotrophic CO_2 assimilation, fluorescence in situ hybridization (FISH).

DOI: 10.1134/S0026261708050123

The results of comprehensive studies of the zones of river inflow into seas and oceans indicate that active processes responsible for the transformation of fresh water into seawater occur in these zones. Upon entering saline water bodies, fresh river water changes under the influence of the salinity, hydrodynamic, and biological barriers (marginal filter); microbial communities greatly contribute to the transformation processes [1–3]. We have much less information on the processes that occur in the mixing zone of river and lake waters [4, 5].

More than 300 rivers and creeks (of which the Selenga is the largest) flow into Lake Baikal. This river provides for up to 30% of the water runoff and up to 50% of the water inflow into Lake Baikal. Where the river meets Lake Baikal, it forms a large estuary consisting of many branches, including the Kharauz, which is the largest of them. The physical properties, water chemistry, and the suspended matter composition of the Selenga River differ from those of Lake Baikal [6]. The results of air photographic survey and orbital imagery of the Selenga shoal demonstrate a pronounced boundary between river, mixed, and lake waters, which differ in color, physical transparency, and temperature [7].

The qualitative and quantitative assessment of phytoplankton, performed earlier in the mixing zone of the Selenga and Baikal waters [8], has demonstrated that the algae composition of the river differs from that of the lake. Due to shallow depths and the Selenga influence, the phytoplankton composition of the Selenga shoal is much richer in species diversity and quantitative characteristics of the algae than that of the open waters of Lake Baikal [9]. The results of a study of cultivable microorganisms indicate that there are some changes in the bacterioplankton composition in the zone of the Selenga inflow into Lake Baikal. It is well known that this method reveals no more than 1% of the total number of microbial calls [10]. Application of modern molecular ecology techniques that do not require cultivation offers much greater possibilities for the study of the microbial community structure. Fluorescence in situ hybridization (FISH) with rRNA-specific fluorescence-labeled oligonucleotide probes [11] is one of such techniques. The advantages of this method include its high sensitivity and the technological simplicity of its assessment tools.

The aim of the present work was to study the structure of the microbial community inhabiting the nearshore area of the estuarine zone of the Selenga River flowing into Lake Baikal by cultivation on nutrient

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Table 1. Oligonucleotide probes and hybridization conditions used in the work

Probe	Nucleotide sequence	Phylogenetic group	Formamide concentration in hybridization buffer, %	NaCl concentration in wash buffer, M
EUB338	GCTGCCTCCCGTAGGAGT	*Eubacteria*	0	0.9
ALF986	GGTAAGGTTCTGCGCGTT	*Alphaproteobacteria*	20	0.225
BET42a	GCCTTCCCACTTCGTTT	*Betaproteobacteria*	35	0.08
GAM42a	GCCTTCCCACATCGTTT	*Gammaproteobacteria*	35	0.08
PLA886	GCCTTGCGACCATACTCCC	*Planctomycetes*	35	0.08
CF319a	TGGTCCGTGTCTCAGTAC	*Cytophaga-Flavobacterium*	15	0.08
ARCH915	GTGCTCCCCCGCCAATTCCT	*Archaea*	20–35	0.08
NON338	ACTCCTACGGGAGGCAGC		0	0.225

media and by the fluorescence in situ hybridization (FISH) technique.

MATERIALS AND METHODS

Water samples were collected in July–August, 2005 in the zone of the Selenga inflow into Lake Baikal and at distances of 1, 3, and 7 km from the river estuary from depths of 0, 5, 10, 25, and 50 m, as well as from the near-bottom layer. The samples were collected with a bathometer in sterile flasks using the traditional technique.

The water temperature was measured with a high-precision CTD probe MCTD3 (Falmouth Scientific Inc., United States); the measurement accuracy was 0.005°C.

Total numbers of microorganisms were determined by DAPI staining and subequent microscopy using an automated counting system [13].

Organotrophic microorganisms were grown at 20°C on tenfold diluted fish peptone agar containing dry nutrient agar and agar–agar (5 and 13.5 g/l, respectively). Oligotrophic microorganisms were grown on a medium containing the following (g/l): peptone, 0.1; yeast extract, 0.1; and agar, 10 [12]. Psychrotolerant microorganisms were grown at 4°C on the R_2A medium (Becton Dickinson, United States) containing the following (g/l): yeast extract, 0.5; peptone, 0.5; casamino acids, 0.5; dextrose, 0.5; soluble starch, 0.5; sodium pyruvate, 0.3; potassium hydrophosphate, 0.3; magnesium sulfate, 0.05; and agar, 15.

The functional activity of microbial communities was determined by radiocarbon analysis (using $NaH^{14}CO_3$ with an activity 0.5 μCi) by measuring the rate of heterotrophic CO_2 assimilation [12, 14]. The samples were incubated at in situ temperature. The filter radioactivity was measured in a RackBeta liquid scintillation counter (LKB Wallac, Finland).

For hybridization, cells were fixed using the standard technique proposed in [15]. For hybridization with the probes, water samples were filtered through 0.22 μm white polycarbonate filters (Millipore) using a peristaltic pump and treated with a cold 4% paraformaldehyde solution (PFA, Sigma) in phosphate buffer (PBS, pH 7.2) at room temperature for 30 min. The fixing solution was then filtered off. The filters were successively washed with PBS buffer and distilled water, which were then filtered off. The filters were air-dried and stored at –20°C until use. For fluorescence in situ hybridization (FISH), oligonucleotide probes labeled with the CY3 fluorescent dye (Sintol, Russia) were used.

The following standard probes were used: EUB338 for *Eubacteria*, ARCH915 for *Archaea*, ALF986 for *Alphaproteobacteria*, BET42a for *Betaproteobacteria*, GAM42a for *Gammaproteobacteria*, CF319a for *Cytophaga–Flavobacterium*, PLA886 for *Planctomycetes*, as well as the nonspecific NON338 probe, which exhibits no complementarity to any 16S rRNA gene fragment (Table 1) [15, 16]. In addition, unlabeled oligonucleotide competitive probes BET42a and GAM42a were used in order to avoid cross-detection of microorganisms belonging to the above groups.

The filters were placed on slides, treated with 20 μl of hybridization buffer (pH 7.4) containing the following: NaCl, 0.9 M; Tris–HCl, 20 mM; SDS, 0.01%; and formamide in concentrations depending on the probe used, and incubated in a humidified incubator at 46°C for 30 min. The hybridization buffer was then supplemented with 2.0 μl of the fluorescent labeled probe (50 ng/μl) and incubated at the same temperature for 90 min. The optimal time of hybridization has been determined experimentally for each oligonucleotide probe.

The filters were then washed free from unbound and nonspecifically bound probes. For this purpose, they were incubated for 15 min at 48°C in a wash buffer (pH 7.4) containing the following: Tris–HCl, 20 mM; EDTA, 5 mM; SDS, 0.01%; and NaCl in concentrations

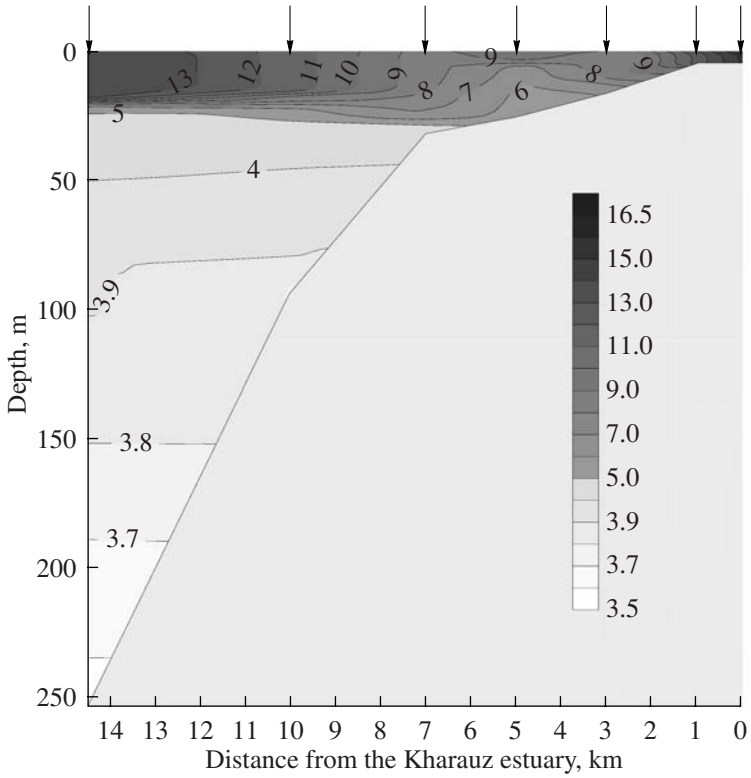

Fig. 1. Temperature distribution in the Selenga shallow waters.

corresponding to the probe used. After washing, the filters were dried on filter paper, stained with DAPI solution (1 μg/ml), and incubated in the dark for 5 min. The stained filters were washed with germ-free distilled water and air-dried.

The obtained specimens were examined under an Olympus epifluorescence microscope (Japan) in ten fields of view; the excitation and emission wavelengths were 552 and 460 nm, respectively. Cell counting was carried out using a software system developed at the Limnological Institute, Siberian Branch, Russian Academy of Sciences [13].

RESULTS

Where the Selenga meets Lake Baikal, the water temperature in the surface layers during the period of investigation varied from 10 to 17°C. Warm Selenga waters spread along the surface to approximately 14 km from the river estuary. Along the whole transect, its penetration depth did not exceed 25 m; below this layer, the water temperature was 3.5–6.0°C through the whole water column (Fig. 1). Only at a distance of 3–7 km from the estuary, the surface layer temperature decreased to 8–10°C; however, at a 14-km distance from the estuary it increased up to 13°C. These changes were perhaps due to the inflow of cold bottom water caused by wind-induced mixing of water arriving from other zones of the lake.

The total number of microorganisms (TNM) varied from 0.9 million cells/ml (SD ± 0.003) to 3.8 million cells/ml (SD ± 0.019) (Table 2). The highest values (2.9 million cells/ml) were observed in the upper water layers of the river estuary and the estuarine zone, as well as at a distance of 1 km from the estuary, which often occur at the inflow zones of big rivers [17]. Microorganisms were usually attached to suspension particles; in the water column of the estuary zone, their cell diameters were larger (1.0–1.5 μm). As the concentration of suspended particles in the water column decreased and water clarity increased, an insignificant decrease in the total number of microbial cells (to 2.0–2.3 million cells/ml) and the predominance of smaller (0.2–0.3 μm) bacterial cells were observed. High TNM values (3.6–3.8 million cells/ml) were detected in the near-bottom layers at a 7-km distance from the estuary. The results obtained in this zone are comparable with the numbers of microbial cells (0.02–4.8 million cells/ml) observed in 1984–1992 when counting of erythrosine-stained cells was performed [18].

The rates of heterotrophic CO_2 assimilation in the water column of the above-mentioned zones were measured simultaneously. The results indicated that the peak values (3.4–6.0 μg C/(l day)) were detected in the surface water layers of the estuary, up to the 3-km zone. On drawing near the open waters, the rates of heterotrophic CO_2 assimilation decreased to 2.6 μg C/(l day). However, at a distance of 5–10 km from the estuary, microbial activity

Table 2. Total numbers of microorganism in the Selenga shoal

Distance from the river estuary, km	Depth, m	TNM, million cells/ml	Heterotrophic CO_2 assimilation, μg C/(l day)	Percentage of cultivable organotrophs in the total number of microorganisms, %
Estuary	0	2.90 ± 0.02	6.0	0.07
1	0	1.80 ± 0.01	6.0	0.09
1	4.5	0.90 ± 0.00	6.4	0.03
3	0	2.30 ± 0.00	3.8	0.02
3	12	2.00 ± 0.01	2.9	0.02
7	0	2.40 ± 0.01	3.2	0.01
7	5	2.70 ± 0.02	3.4	0.01
7	10	3.60 ± 0.02	3.0	–
7	35	3.80 ± 0.02	2.8	0.00

intensified at a depth of 20–30 m; in the same zone, the water temperature increased to 10–13°C (Fig.2).

The horizontal distribution of microbial cell numbers determined by plating on rich and poor media did not correlate with temperature and was rather irregular. Organotrophic bacteria were most abundant in the estuary waters and at a 7-km distance from the estuary (2040 ± 120 CFU/ml (Fig. 2) and up to 223 ± 1 CFU/ml, respectively). Their distribution depended directly on the production activity of microorganisms (Fig. 2). High numbers of cultivable organotrophic bacteria were observed in the deep water layers at a distance of approximately 14 km, which can be probably attributed to the presence of easily mineralized organic matter entering the near-bottom layers after decomposition of spring algae [8]. The proportion of cultivable organotrophic bacteria in the total number of microorganisms was 0.002–0.7% and uniformly increased from the bottom up.

The vertical distribution of cultivable oligotrophic and psychrotolerant bacteria was nonuniform as well; the numbers of the former in the Kharauz estuary was 360 ± 15 CFU/ml, while at a 5-m depth it was 9720 ± 300 CFU/ml. A similar distributional pattern was observed in the case of cultivable psychrotolerant bacteria. This fact can be attributed to the dependence of the above physiological groups on water temperature or to the organic matter content required to maintain the population viability.

Phylogenetic analysis of the microbial community carried out using the FISH technique demonstrated that as many as half of all microorganisms inhabiting the Selenga shoal belong to the domain *Bacteria* (41–51% of the total number of microorganisms); they are uniformly distributed along the whole transect. A decrease in the number of microorganisms belonging to this domain was observed on drawing near the open waters (Fig. 3), as the temperature decreased.

The results obtained demonstrate that the proportion of eubacteria in the total number of microorganisms (43–59%) was relatively uniform at all sampling sites, which had been previously observed in the pelagic zone of Lake Baikal [15]. In the 2004–2006 summer seasons, similar results (38–50%) were obtained at the deep-water station of Southern Baikal [19]. On the whole, within the microbial communities under study, the proportions of microorganisms belonging to the domain *Bacteria* in the total number of microbial cells do not change significantly both in the pelagic zone of Lake Baikal and in Selenga shallow waters.

In these zones, most eubacteria belong to the classes *Gamma-* and *Betaproteobacteria* (6–17% and 10–14%, respectively). They are relatively uniformly distributed in surface waters from the estuary to the open waters of Lake Baikal. As the distance from the estuarine zone increased, the proportions of microorganisms belonging to the above classes decreased insignificantly in the deep layers, from 8 to 17% and from 12 to 14% of the total number of bacterial cells for *Gammaproteobacteria* and *Betaproteobacteria*, respectively (Fig. 3). In the case of the *Alphaproteobacteria*, a similar distributional pattern was observed.

The *Cytophaga-Flavobacterium* group, whose known representatives are able to degrade high-molecular-weight organic compounds, constitute from 1.5 to 7% of the total number of microorganisms; members of this group were most abundant at a depth of 5–15 m at a distance of 1–3 km from the estuary; their numbers were lowest in surface waters at a 7-km distance from the estuary. Members of this group were abundant in the open waters of Lake Baikal during the 2005–2006 summer seasons (5–7% and up to 3% of the total number of microorganisms, respectively) [19]. It should be

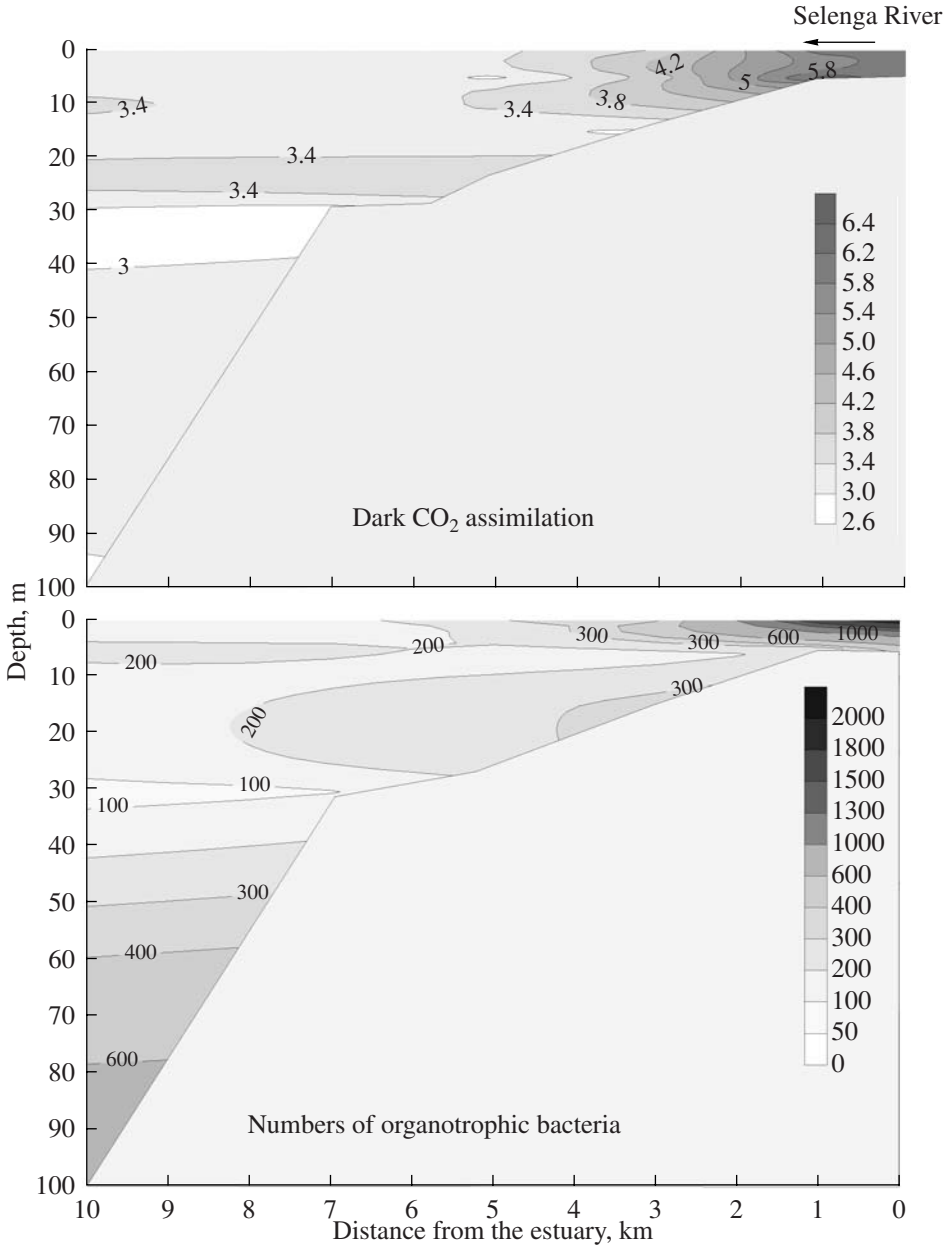

Fig. 2. Distribution of organotrophic bacteria (CFU/ml) and heterotrophic CO_2 assimilation in the Selenga shallow waters (μg C/(l day).

noted that their proportions in the total number of microbial cells determined earlier (August, 1995–1996) were larger, ranging from 4% to 18% [15]. These data indicate that, as the distance from the Selenga estuary increased, a sharp decrease in the proportion of microorganisms belonging to the *Cytophaga-Flavobacterium* group and inhabiting the estuary was observed. They were most abundant in the mixing zone of river and lake waters; their distributional patterns showed no association with temperature changes. This group, being at a low level in the trophic chain of degraders of high-molecular-weight organic compounds, predetermines the numbers of other bacterial groups utilizing easily mineralized organic matter [20].

The *Planctomycetes* group was not detected in the Selenga shallow waters during the given periods. Their proportion in the total bacterioplankton community of the deep-water zone of open Baikal determined in 1995–1996 was also low (1–2% of the total number of microorganisms) [15]. This group was also detected in the pelagic zone of Lake Baikal in 2005–2006; however, members of this group were not abundant (1–4.5% of the total number of microorganisms) [19].

For studying bacterial plankton, a probe for *Archaea* as another component of the microbial com-

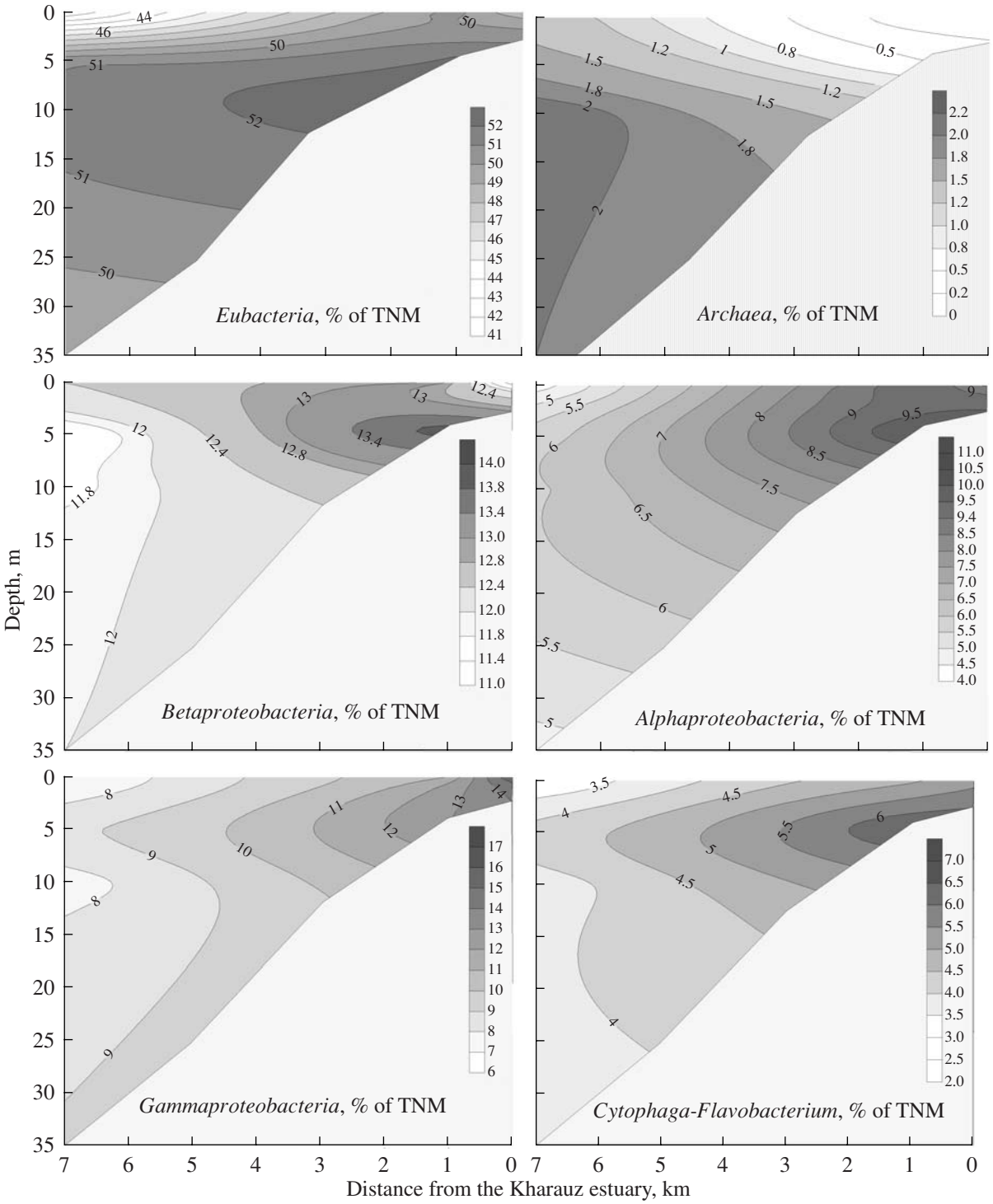

Fig. 3. Distribution of microorganisms of various phylogenetic groups in the Selenga shallow waters.

munity under study was used for the first time [21]. In the pelagic zone of the South Baikal, in the surface water layers, the proportion of *Archaea* in the total number of microorganisms reached 6%; according to the data obtained in 2004–2006, in ice-covered water it reached 10% of the total number of microbial cells [19], which is comparable to the numbers of the *Alphaproteobacteria*. According to the published data on other aquatic ecosystems, the proportion of marine archaea in the total plankton community may be up to 45% of the total number of microorganisms [22, 23].

In the Selenga shoal, microorganisms belonging to the domain *Archaea* were scarce. They were more abundant (up to 2.2%) at a 7-km distance from the estuary at a depth of 10 m, while in the Kharauz estuary they were not detected (Fig. 3). It should be noted that the amount of archaea in the water column depended inversely on the amount of eubacteria.

In the 2004–2006 summer seasons, the proportion of archaeal cells in the deep-water column of Southern Baikal was 0–5.5% of the total number of microorganisms and reached its peak (6.1%) in June 2006 [19].

DISCUSSION

Our investigation carried out by cultivation techniques and fluorescence in situ hybridization (FISH) revealed changes in the phylogenetic structure of the microbial community inhabiting the mixing zone of river and lake waters.

The hydrologic properties of the region under study, including the runoff regime, temperature contrasts (gradients) between the river and lake waters, mixing-induced water thickening, and the flow rates, play an important role in this process. At a distance of 2–3 km from the estuary, where the decrease in the flow rate results in a decrease in the bearing capacity of the water flux, the precipitation of mineral and organic fractions was observed resulting in an increase in the numbers of microorganisms of all physiological groups [24]. Beyond the mixing zone, the numbers of microorganisms were similar to those recorded in 2005–2006 in the open waters of Lake Baikal [19].

The rate of heterotrophic CO_2 assimilation, which can serve as an indicator of microbial activity, significantly decreased as the distance from the shallow increased and the deepwater zones drew nearer, in spite of the increase in the total number of microorganisms ($r = 0.8$). The CO_2 assimilation rates recorded were higher than those observed in the zones of the Yenisei and Ob inflow into the Kara Sea (0.3–1.44 μg C/(l day) [17].

The studies of microorganisms belonging to the domain *Archaea* demonstrated that the numbers of archaea were one order of magnitude lower than the numbers of eubacteria; in the estuarine zone, the proportion of cells hybridized with the ARCH915 probe was half as great as that in the open waters of Lake Baikal.

The total number of bacteria identified with the above oligonucleotide probes indicated that many microorganisms (42–57%) were not detected with these probes. A broader spectrum of oligonucleotide probes is possibly required, which would enable us to detect other groups of bacteria, including the *Actinobacteria*, *Deltaproteobacteria*, etc. The results obtained may also be due to changes in the metabolic state of bacterial cells, their rRNA content, and differences in the detection limits of the FISH technique [23]. It may be suggested that microbial cells not detected with these probes may represent some unknown, phylogenetically distinct groups of eubacteria and archaea endemic of Lake Baikal; to identify them, development of a new oligonucleotide probe is required [25].

Hence, the structure of the microbial community of the Selenga shoal changes as the river waters mix with lake water. Organotrophic microorganisms, which are replaced by psychrotolerant and oligotrophic microorganisms in the waters of Lake Baikal, are predominant in the Selenga estuary. As the distance from the estuary increases and the temperature decreases, the rate of heterotrophic CO_2 assimilation by bacterial cells decreases as well. The proportion of the *Gammaproteobacteria* that are predominant in the shallow water zone was significantly lower; at a distance of 1.5–3 km from the estuary, microorganisms belonging to the classes *Alpha- Beta-* and *Gammaproteobacteria* appear in equal proportions. At a distance of more than 3 km from the estuary, members of the *Betaproteobacteria* represent the predominant class of planktonic microorganisms, which is typical of the open waters of Lake Baikal.

ACKNOWLEDGMENTS

This work was supported by the research program of the Presidium of the Russian Academy of Sciences 18.10, grant no. NSH-4738.2006.4.

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