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EXPERIMENTAL ARTICLES

On the Vertical Distribution of Microorganisms in Lake Baikal during Spring Deep-Water Renewal

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Abstract—The vertical distribution of microorganisms during spring deep-water renewal in Lake Baikal was studied. The downward advection of trophogenic waters was found to create conditions for the extensive growth of microorganisms capable of decomposing and mineralizing organic carbon, nitrogen, and phosphorus in deep water layers. These processes occur annually at spring thermal bars near the underwater slope of Lake Baikal, whereas in its pelagic zone, the deep intrusions of waters rich in organic material are observed only in the years when enhanced deep-water renewal is accompanied by a high spring yield of phytoplankton.

Key words: bacterial population, phytoplankton and organic matter, deep-water renewal processes

Lake Baikal is the world's largest (23500 km^3) and deepest (1640 m) freshwater lake. About 65% of its top-quality freshwater with a high oxygen content is concentrated at depths below 300 m. It is believed that the processes of decomposition and remineralization of organic matter in Lake Baikal are most active in its upper layers, whereas they are slow at depths below 300 m, and that the population of microorganisms decreases with depth, as occurs in other deep oligotrophic lakes [1]. However, there is evidence that, in some cases, the microbial population in Lake Baikal increases with depth [2–5].

Relevant studies showed that the surface waters of Lake Baikal may rapidly move downward [6–9], carrying with them oxygen, phytoplankton [10, 11], and, as Votintsev believed [12], organic matter produced by phytoplankton in the trophogenic layer. Obviously, such processes, which take place every spring and late autumn, must lead to an increase in the microbial population of Baikalian deep waters.

The present work was undertaken to prove this supposition by studying the distribution of microorganisms during spring deep-water renewal in the pelagic zone and underwater near-slope regions of Lake Baikal.

MATERIALS AND METHODS

Water samples were collected in June 1992 and 1993 and in July 1997 at stations along the section in the middle of Lake Baikal shown in Fig. 1. The thermal bar in the eastern part of Lake Baikal, observed from May 28 to June 20, was due to the input of the warm mineralized waters of the Selenga River. Samples, which were taken with bathometers from various depths (0, 25, 50, 100 m, and then at 100-m intervals down to the bottom), were analyzed for the content of microorganisms, chlorophyll, dissolved oxygen, and biogenic elements. Water temperature during sampling was measured with high-precision SBE-9 and SBE-25 temperature probes with an instrumental error of ± 0.005°C. The particular groups of microorganisms chosen for analysis (heterotrophic, ammonium-producing, phosphorus-fixing, and oligotrophic bacteria, as well as actinomycetes, fungi, and yeasts) allowed one to evaluate the transformation of organic matter and biogenic elements.

The total bacterial count was carried out by the epifluorescent filter technique. Before filtration, samples were stained with a 0.015% solution of acridine orange in phosphate buffer (pH 6.9). Green luminous bacterial cells were counted under a luminescence microscope [13]. Heterotrophic microorganisms, whose presence in water is an indication of abundant organic matter, were enumerated on two media, undiluted and tenfold diluted fish nutrient agar (FNA); oligotrophic bacteria were enumerated on a medium containing small amounts of organic and mineral substances [14]; and saprophytic microflora, which is identifiable with the physiological group of ammonium-producing bacteria, on nutrient agar. Actinomycetes, fungi, and yeasts were counted on Czapek medium [15]. Microorganisms involved in the phosphorus cycle were enumerated on a

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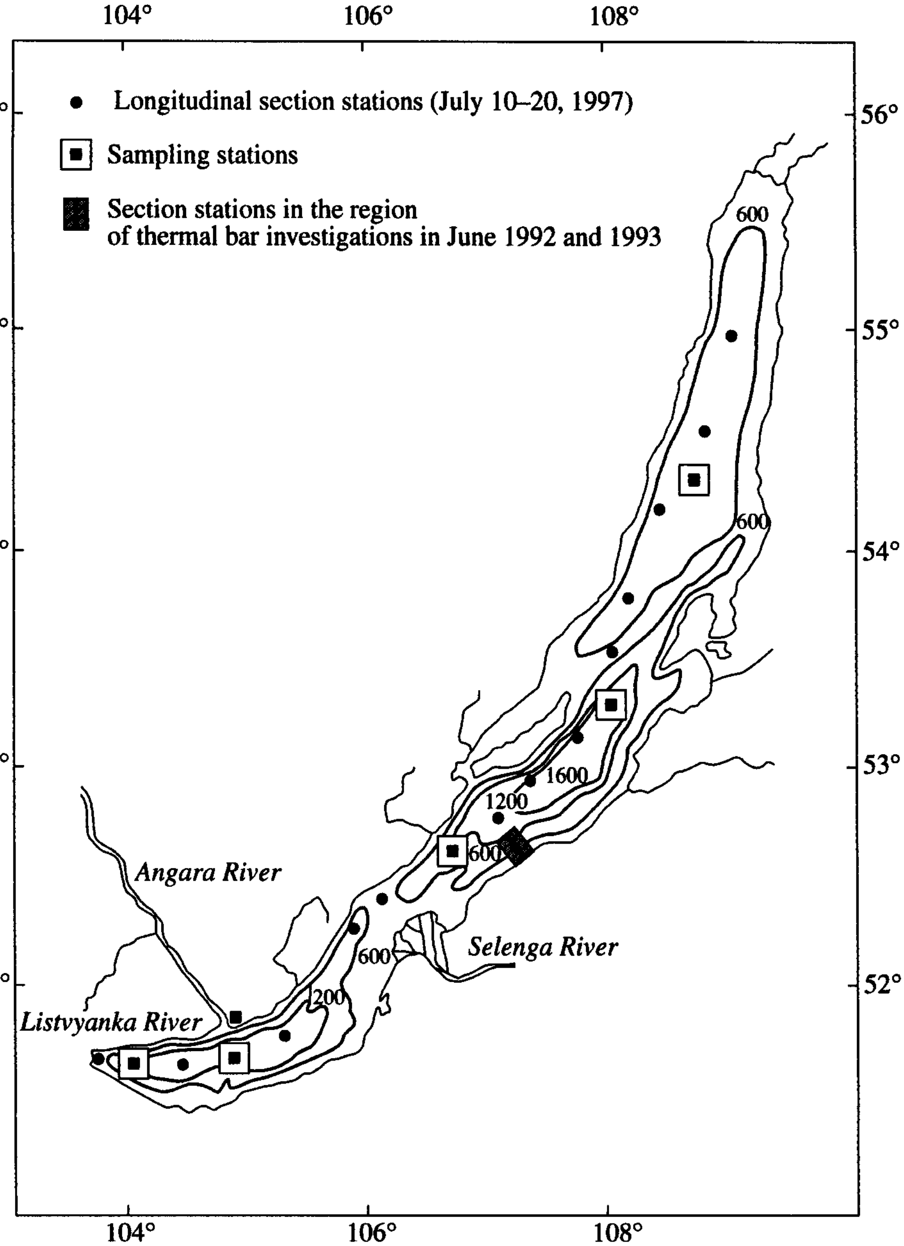

Fig. 1. Map showing the locations of stations during the complex investigation of Lake Baikal in June 1992 and 1993, and July 1997.

medium with yeast ribonucleic acid as the source of organic phosphorus [16]. This medium was inoculated immediately after water sampling.

The chlorophyll *a* content of the water samples was determined using a three-wave laboratory fluorimeter devised at the Institute of Biophysics, Siberian Division, Russian Academy of Sciences and the Krasnoyarsk State University. For this purpose, water samples were successively illuminated with light at wavelengths of 420, 510, and 560 nm, and the induced fluorescence of phytoplankton pigments was recorded at 680 nm. The thus-derived values of fluorescence intensity (Fl_{420}, Fl_{510}, and Fl_{560}) were introduced into the following system of three linear equations, whose solution allowed the estimation of the chlorophyll concentration with respect to particular algal groups (diatoms, blue–green and green algae) [17]:

$$Fl_{420} = K^{b-g}_{420}C^{b-g}_{chl\cdot a} + K^{di}_{420}C^{di}_{chl\cdot a} + K^{gr}_{420}C^{gr}_{chl\cdot a},$$

$$Fl_{510} = K^{b-g}_{510}C^{b-g}_{chl\cdot a} + K^{di}_{510}C^{di}_{chl\cdot a} + K^{gr}_{510}C^{gr}_{chl\cdot a},$$

$$Fl_{560} = K^{b-g}_{510}C^{b-g}_{chl\cdot a} + K^{di}_{560}C^{di}_{chl\cdot a} + K^{di}_{560}C^{di}_{chl\cdot a},$$

where K^{di}, K^{b-g}, and K^{gr} are specific fluorescence yields of diatoms, blue–green algae, and green algae illuminated by excitation light with the respective wave-

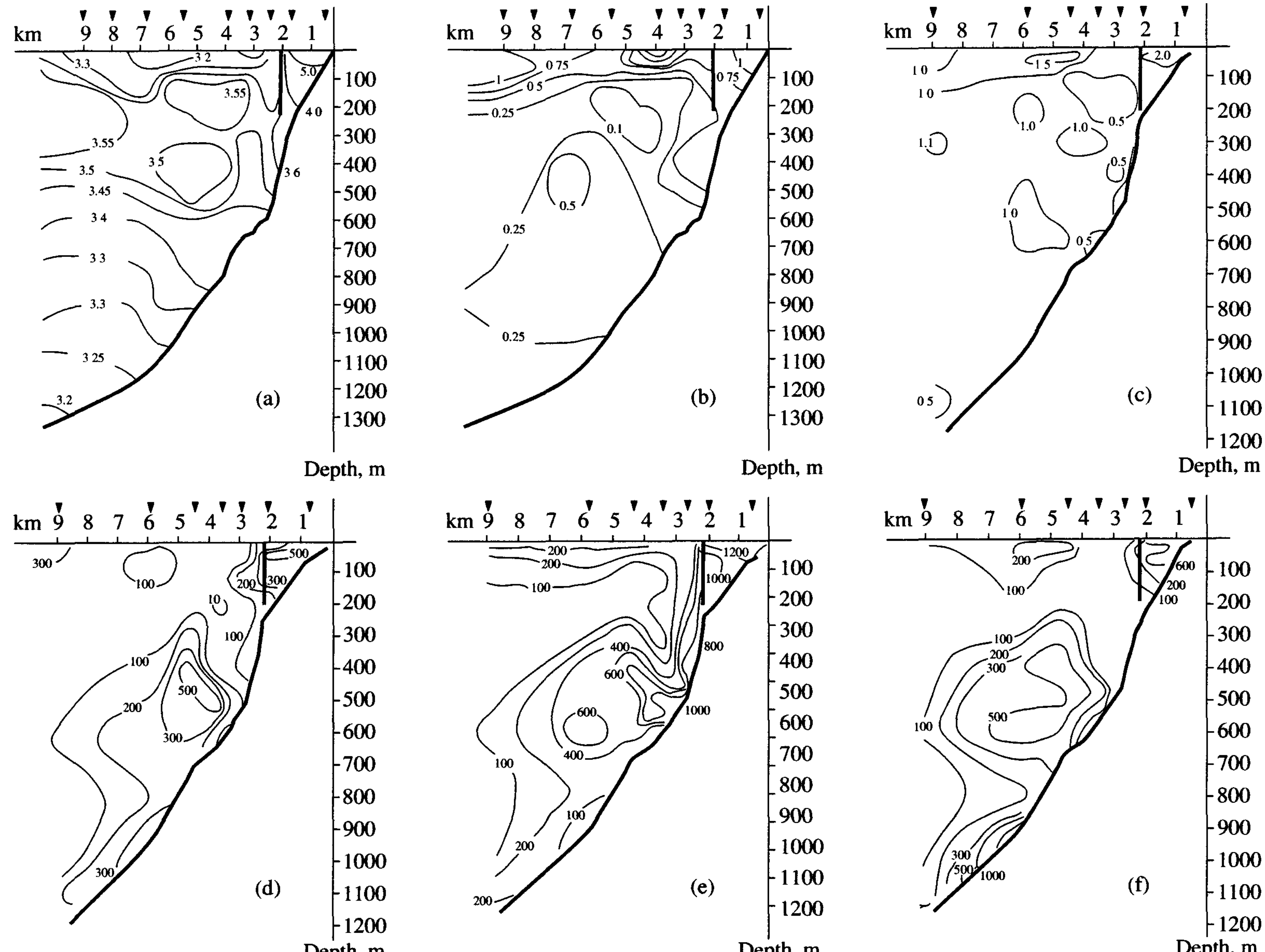

Fig. 2. Vertical profiles of some limnological characteristics and microbial population along the section near the southeastern coast of Lake Baikal on June 5, 1993 (solid lines show the position of the thermal bar, and arrowheads mark the stations of microbiological sampling): (a) water temperature, *C*; (b) chlorophyll concentration, μg/l; (c) total number of microorganisms, million cells/ml; (d) heterotrophic bacteria counted on undiluted FNA, cells/ml; (e) heterotrophic bacteria counted on tenfold diluted FNA, cells/ml; (f) ammonium-producing bacteria, cells/ml; (g) phosphorus-fixing bacteria, thousand cells/ml; (h) oligotrophic bacteria, cells/ml; (i) actinomycetes, fungi, yeasts, thousand cells/ml.

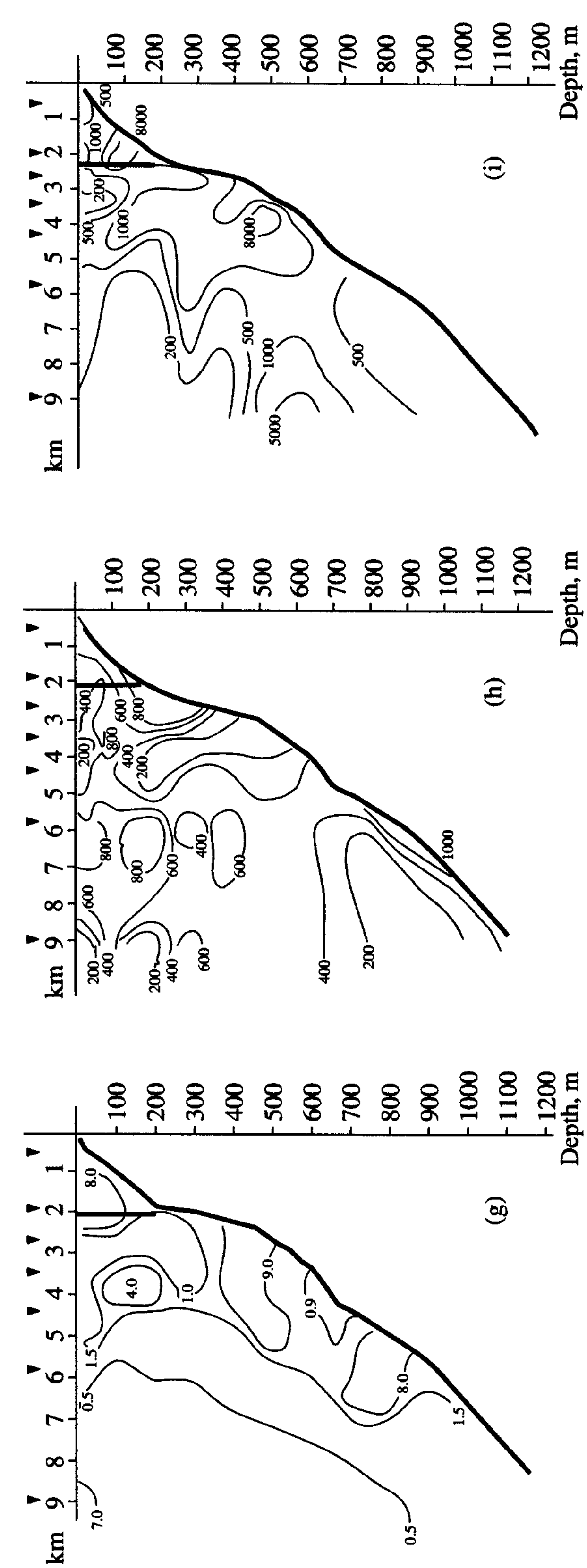

Fig. 2. (Contd.)

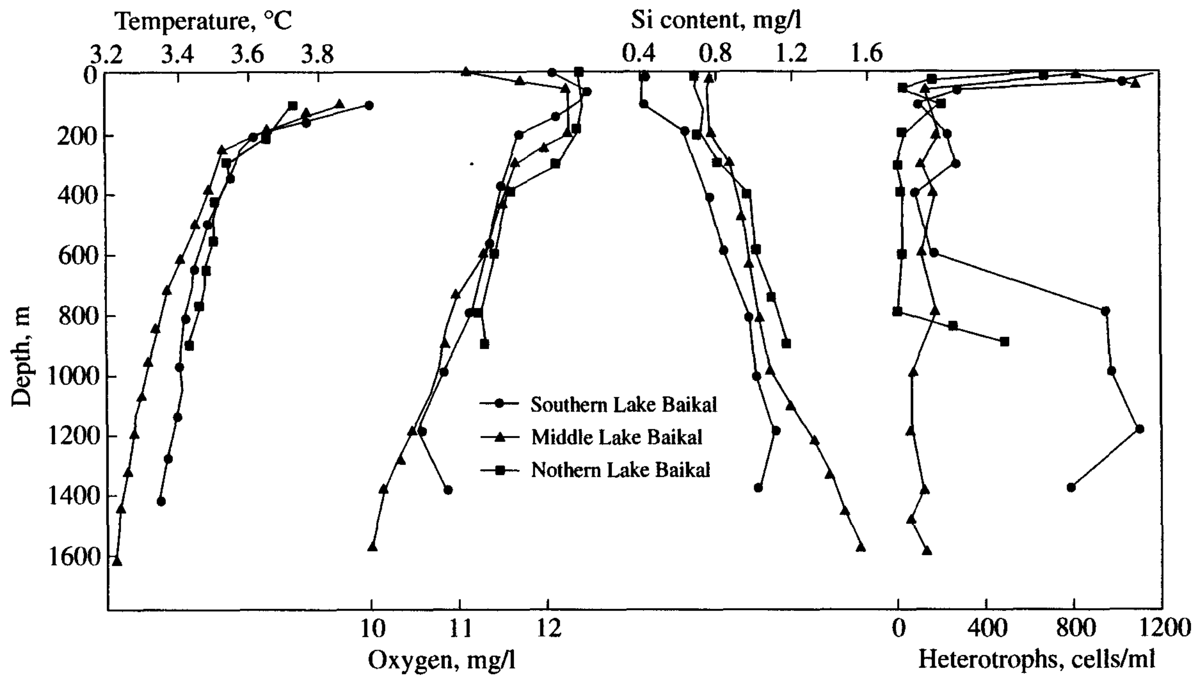

Fig. 3. Vertical profiles of some limnological characteristics and the population of heterotrophic bacteria counted on tenfold diluted FNA in the pelagic zone of Lake Baikal on July 10–20, 1997.

lengths and $C^{di}_{chl \cdot a}$, $C^{b\text{-}g}_{chl \cdot a}$, and $C^{gr}_{chl \cdot a}$ are the concentrations of chlorophyll *a* in these algal groups.

The concentration of dissolved oxygen was determined by the method of Winkler, and that of silicon, by the photometrical method based on the interaction between silicic acid and ammonium molybdate in acidic medium with the formation of yellow silicomolybdic acid detected at $\lambda = 410$ nm [20, 21]. Because of the instability of oxygen and silicon contents during sample storage, they were measured immediately after water sampling. The sensitivity of silicon determination was 0.01–0.02 mg Si/l with a standard deviation of 2%.

RESULTS AND DISCUSSION

The distribution of microorganisms in the near-slope zone was studied in the southeastern part of middle Baikal 70 km away from the Selenga River delta during expeditions in 1991–1993 and in 1995 [8, 10]. A total bacterial count was carried out on June 7, 1992 and on June 5, 1993. During the 1992 expedition, heterotrophic bacteria were enumerated at stations located 6, 9, and 12 km from the coast. In more detail, the distribution of different groups of bacteria was studied in 1993 (Fig. 2).

In the midlatitudinal belt, freshwater lakes are characterized by the occurrence of spring thermal bars, when the mixing of waters with temperatures above and below the temperature of maximum water density results in the downward advection of denser water [20]. In the majority of midlatitudinal freshwater lakes, thermal bars sink to the bottom and prevent the mixing of coastal and deep waters, as a result of which these waters considerably differ in physicochemical characteristics and the activity of the biological processes taking place within them [20]. In deep Lake Baikal, thermal bars do not reach the bottom, thus allowing for the near-slope circulation of coastal and deep waters due to thermal convection induced by the downward movement of cold waters [8] and density convection associated with a high mineralization of input riverine waters in the coastal zone [9, 10]. The circulation rate (0.2–0.3 cm/s) provides a rapid descending movement of surface waters to the slope foot.

On June 5, 1993, the thermal bar was located 2 km from the coast between 200- and 500-m depths, separating cold (3.2–3.5°C) deep water from warm (about 10°C) coastal water (Fig. 2). The total number of diatoms reached 2.45 million cells/l in coastal water, and no more than 1.2 million cells/l in deep water [11]. Circulation processes affected the temperature and chlorophyll concentration profiles near the slope (Figs. 2a and 2b) and gave rise to a second population maximum of some groups of microorganisms near the bottom.

The total bacterial population (TBP) was maximum in the trophogenic layer (0–50 m), changing typically from 0.5–1.5 million cells/l in the pelagic zone to 2 million cells/l in the coastal zone (Fig. 2c). Near the bottom, TBP was 0.3 million cells/l in deep waters and increased in the direction toward the coast, probably because of the sinking of bacteria-contaminated surface waters along the slope. The decrease in TBP at the thermal bar was due to the upward advection of deep waters to the convergence zone of the thermal bar.

Convection processes considerably influenced the distribution pattern of heterotrophic bacteria enumerated on diluted medium (Figs. 2d and 2e). High concentrations of such bacteria (200–1000 cells/l) were revealed in the near-slope waters. High concentrations of these bacteria (500–1000 cells/ml) observed in some regions at greater depths (400–600 and 900–1100 m) were probably due to the downslope sinking of warm coastal waters, which are rich in organic matter. This supposition was corroborated by the increased temperature and high chlorophyll content of deep waters in these regions.

The detection of outbreaks in the population of heterotrophic bacteria at depths of 400–600 m and distances of 4–7 km from the coast indicates the intrusion of waters from a sinking stream. The compensating upwelling of deep waters is easily distinguished by a decrease in the population of heterotrophic bacteria near the thermal bar to values typical of deep waters (< 100 cells/l). Similar changes were observed in the population of bacteria mineralizing proteins and organic compounds of nitrogen and phosphorus. As is evident from Figs. 2f and 2g, such mineralization processes occur not only in the trophogenic layer, but also at any depths above the underwater slope of Lake Baikal.

Observations performed in June 1992 also indicated high population densities of heterotrophic bacteria (up to 1000 cells/ml) near the underwater slope of Lake Baikal in comparison with their small densities (several cells/ml) in the middepth water layers. The similarity of the bacterial distribution patterns obtained in the springs of 1992 and 1993 suggests the annual character of the processes occurring near thermal bars. Due to spring thermal bars, warm coastal waters with a high content of organic matter and bacteria sink to great depths, where organic matter is subjected to bacterial destruction.

The increase in the abundance of oligotrophic microorganisms in middepth waters (Fig. 2h) was caused by vertical water exchange due to thermal bars and convective processes [10].

The distribution of actinomycetes, fungi, and yeasts in Baikalian waters (Fig. 2i) reflects the distribution of allochthonous organic matter, whose presence in the lake is due to riverine inputs and bank runoff. The high abundance of this group of microorganisms in coastal and near-slope waters in the region of bathymetry can be explained by the rapid transfer (in about four days) of Selenga River waters by Baikalian streams. Based on these observations, it can be concluded that waters in the near-slope region extending to 4–6 km from the coast resulted from the mixture of lake and riverine waters.

The effect of near-slope processes on the formation of deep waters in the pelagic zone of Lake Baikal is weak. However, these deep waters are partially renewed every spring due to thermobaric instability and associated convective processes, which often occur in the form of direct intrusions [6, 7, 18]. As a result, near-bottom and deep waters are replaced by cold surface waters with a low content of biogenic elements (e.g., silicon) and high concentrations of oxygen [6] and organic matter (as established by our observations carried out in 1995). Observations performed on July 10–20, 1997, in the pelagic zone of the lake also detected the current or recent (which occurred 1–3 weeks ago) processes of deep-water renewal. Water intrusions were well pronounced in northern and especially southern Baikal but were absent in middle Baikal (Fig. 3), thus indicating the different activity of deep-water renewal processes in different parts of the lake.

Accordingly, vertical bacterial distributions were also different. In 1997, the spring phytoplankton yield in Lake Baikal was high, reaching 1.5–2 g/m^3 in the trophogenic layer. Together with the enhanced vertical convection of waters, this provided good conditions for the transfer of organic matter to deep and near-bottom layers in southern and northern Baikal. As a result, in these regions of Lake Baikal, at depths below 400–600 m, the bacterial population was as high as 400–1000 cells/ml (Fig. 3), which is typical of the trophogenic layer. However, in middle Baikal, where deep-water renewal processes were weak, the vertical distribution of the bacterial population was normal: it decreased from 470–1600 cells/ml in surface layers to several cells/ml in near-bottom layers. Thus, the different intensity of water exchange processes in the different regions of Lake Baikal determined the 2- to 3-orders-of-magnitude difference in the vertical distribution of microorganisms.

Thus, due to water exchange, the distribution of microorganisms in deep Baikalian waters is complex. The increase in the microbial population of near-bottom waters in the pelagic and coastal–pelagic zones in the spring is associated with the rapid transfer of "fresh" organic matter from the trophogenic layer to great depths, where it is digested by bacteria. Therefore, the bacterial self-cleaning of Baikalian waters occurs at any depth.

This phenomenon occurs annually in the coastal zones of Lake Baikal due to spring thermal bars. The increase in the population of bacteria capable of decomposing organic matter at various depths is an indication of the enhanced circulation of organic matter and biogenic elements in the near-slope regions of the lake. In the pelagic zone, large water intrusions rich in organic matter and the associated increase in the bacterial population of deep and near-bottom waters can be observed only in the years when enhanced deep-water renewal coincides with a high spring phytoplankton yield.

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