
**WATER QUALITY AND PROTECTION:
ENVIRONMENTAL ASPECTS**

State of Heterotrophic Bacterioplankton of Yenisei Estuary and the Zone of Ob–Yenisei Discharge in Autumn in Relation with Environmental Factors

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Abstract—In September 2011, variations of the total abundance of bacteria were studied, including, for the first time, the abundance of bacteria with intact membranes and active electron transport chains, as well as chlorophyll *a* concentrations and some hydrological and hydrochemical characteristics of water in Yenisei estuary and the zone of Ob–Yenisei river discharge. The study found abundant, viable, and actively functioning bacterioplankton to exist in the region. The absolute values of all microbiological characteristics were found to decrease with the distance from the freshened waters of Yenisei estuary toward the seawater of the Ob–Yenisei river discharge, while the percentage of bacteria with intact membranes and actively functioning microorganisms in bacterioplankton increased in the same direction. Positive correlations were found to exist between the values of all examined microbiological characteristics and the concentration in water of chlorophyll *a*, silicium, organic nitrogen, and oxygen, as well as water temperature. The same biological characteristics showed negative correlations with water salinity and phosphates. The spatial distribution of bacteria in the examined water body was supposed to be due mostly to the intensity of hydrodynamic processes, rather than the biogenic matter content of water.

Keywords: heterotrophic bacterioplankton, total bacteria abundance, the abundance of bacteria with intact membranes, the abundance of actively functioning bacteria, chlorophyll *a*

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INTRODUCTION

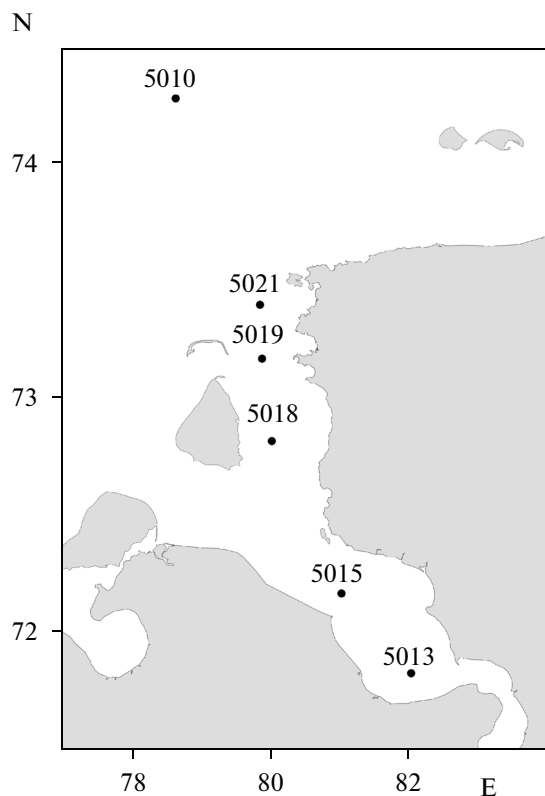
The estuaries of large rivers are important boundary structures through which terrigenous matter is exported into the ocean [3]. In addition, these are unique biotopes with considerable gradients of abiotic and biotic environmental factors. The current large-scale construction of hydrocarbon-production facilities on the shelves of arctic seas increases the anthropogenic impact on arctic estuarine systems. Heterotrophic bacterioplankton is an important component of biocenoses of any aquatic ecosystems, its major function being the assimilation and transformation of organic compounds, including pollutants of anthropogenic origin. However, the abundant evidence obtained by now suggests that the share of actively functioning microorganisms in natural microbiocenoses is not stable, varying within wide limits—from 3 to 65% [11, 18]. It is the pool of actively functioning microorganisms that ensures the development of heterotrophic processes in aquatic ecosystems; therefore, the knowledge of their abundance is required to assess the environmental conditions of water areas. The actively functioning bacterioplankton should be taken into account in studying both micro-

organisms production and their role in organic matter (OM) destruction, as well as in integral environmental estimates of the state of aquatic ecosystems.

Another important characteristic of the state of heterotrophic bacterioplankton is the percentage of cells with intact membranes in its composition, as the intactness of cell membrane is a key condition for the viability of any cell. At the same time, viable cells are not obligatory permanent contributors to the processes of OM production and destruction. They become such only under favorable environmental conditions.

Data on the abundance and occurrence of bacteria with active metabolism, as well as on the abundance of bacteria with intact cell membranes (ABIM), in estuarine water areas of large arctic rivers, in particular, in the Yenisei estuary and nearby water areas, are completely unavailable now. Also unknown are the key environmental factors that can have their effect on the abundance and occurrence of such microorganisms in estuarine waters.

The objective of this work is to study the abundance and spatial distribution of bacterioplankton with active metabolism and to determine ABIM in the



Schematic region of sampling in period from September 15 to 29, 2011. The black circles are six stations in the section along Yenisei estuary and in the Kara Sea in OYRD zone.

composition of bacteriocenosis in Yenisei estuary and the zone of Ob–Yenisei river discharge (OYRD) in September 2011.

An attempt is made to determine the key environmental factors that govern the character of spatial distribution of the examined microbiological characteristics.

MATERIALS AND METHODS

Samples for determining the microbiological characteristics were taken during the 59th voyage of the *r/v Akademik Mstislav Keldysh* in period from September 15 to 29, 2011 at six stations located in water areas with most contrast environmental conditions (figure): at the section through the Yenisei estuary and in the Kara Sea in the zone of OYRD. Stations 5013 and 5015 were situated in the strongly freshened water of the estuary; stations 5018 and 5019, in the frontal zone of river and sea water mixing (the zone of maximal water salinity gradient); and stations 5021 and 5010 in OYRD zone.

The depths of stations varied from 12 to 31 m. Water samples for microbiological analysis were taken at several horizons; their number depended on station depth and varied from 3 to 5 m. Rosetta complex,

which was used for sampling, was equipped by a set of bathometers and a CTD-probe. The total number of water samples taken for microbiological analysis was 23, the volume of each sample being 15 mL. The samples were placed in polystyrene test tubes and processed onboard of the ship immediately after they are delivered into the laboratory.

The microbiological characteristics determined in the samples included total bacterial abundance (TBA), ABIM, the number of bacteria with active electron-transport chain (CBTC + B).

Chlorophyll *a* concentration was determined by fluorometric method [14] (with the use of MEGA-25 fluorimeter (Russia)). Water samples (500 mL), taken at different horizons, were filtered through fiberglass filters GF/F (Whatman). The filters were placed into 90% acetone; chlorophyll *a* was extracted at temperature of +4°C under darkroom conditions within 18–24 h with subsequent fluorimetry of the extract.

The main hydrological–hydrochemical characteristics of the environment were determined by researchers from the hydrochemical team (Shirshov Institute of Oceanology, RAS), headed by P.N. Makkaveev.

The obtained data were treated by statistical methods with the use of STATISTICA-10 software package. Spearman's coefficient of rank correlation was used for the correlation analysis.

Assessing the Total Abundance of Bacteria and the Amount of Bacterial Cells in Intact Membranes

TBA was evaluated with the use of a set of dyes LIVE/DEAD BacLight Bacterial Viability Kits 13152 (Molecular Probe, Eugene, Oregon, USA), consisting of fluorochrome SYTO 9, which binds with nucleic acids of cells with intact membranes, and fluorochrome of iodide propidium—a dye, which cannot penetrate through intact cell membrane and, therefore, accumulates only in cells with broken membrane. After dyeing, bacterial cells with intact membranes acquire bright green fluorescence, while cells with broken membrane show red fluorescence. TBA was calculated as the sum of the number of cells with intact and broken membranes.

To determine ABIM, unfixed freshly taken water samples, 6 to 8 mL in volume, were incubated in the presence of Live/Dead BacLight kit 13152 solution in concentration of 3 μL/mL within 15 min; next, bacteria from it were concentrated on a black Nuclepore filter (with pore diameter of 0.22 μm). The filter was dried on filter paper in darkness under the room temperature [10].

When ready for use, the preparation was immediately viewed under Leica DM-5000B microscope (with a magnification of 1000×) with a system of filters 13 (exciting filter BP 450–490, barrier filter LP 515). Not less than 20 arbitrarily chosen fields of vision were

counted with not less than 300 bacterial cells taken into account.

The Abundance of Bacteria with Active Electron-Transport Chain

CBTC + B was analyzed with the use of a tetrazolium salt—5-cyano 2,3-bis(4-methylphenyl) tetrazolium chloride (CBTC). The process solution of CBTC was prepared in accordance with [16]. Water samples were incubated in the presence of CBTC in concentration of 5 mM within 4 h in situ in an open basin on ship deck. After incubation, the sample was fixed by formaline solution (4%). Next, the cells from the sample were concentrated on a black Nuclepore filter (with pore diameter of 0.22 μm). The filter was dried on filter paper in darkness under the room temperature, and the preparation was made with the use of non-fluorescent immersion oil Olympus (Japan). The obtained preparation was immediately viewed under a Leica DMR-5000B microscope (magnification of 1000 \times) with a system of filters I3 (exciting filter BP 450-490, barrier filter LP 515). Not less than 20 view fields were counted for each preparation, with not less than 200 bacterial cells taken into account.

RESULTS OF THE STUDY

*Hydrological—Hydrochemical Characteristics and Chlorophyll *a* Concentration in Yenisei R. Estuary and the zone of Ob—Yenisei River Discharge in Autumn of 2011*

From September 15 to 29, 2011, the highest mean values of water temperature (8.19 $^{\circ}\text{C}$) were recorded in freshened waters (stations 5013 and 5015), while its lowest values (1.46 $^{\circ}\text{C}$) at stations 5021 and 5010 in the Kara Sea in OYRD zone (Fig. 1; Table 1). Minimal values of water salinity (on the average, 4.35 PSU) were recorded in the freshened-water zone (stations 5013 and 5015); and its maximal values (on the average, 28.49 PSU), on stations 5021 and 5010 (OYRD zone) (Table 1). The largest gradients of water salinity (5.51–31–72 PSU) were recorded in water at stations 5018 and 5019. The freshened and warmest waters showed the lowest mean concentrations of major biogenic substances compared with other regions (stations 5013 and 5015), in particular: phosphates (0.34 $\mu\text{g-at P/L}$) and nitrates 1.39 $\mu\text{g-at N/L}$, as well as oxygen (7.31 $\text{mL O}_2/\text{L}$) = (Table 1). These stations showed the highest mean concentrations of chlorophyll *a* (2.94 $\mu\text{g/L}$), nitrites and ammonium (0.19 $\mu\text{g-at N/L}$ and 2.40 $\mu\text{g NH}_4/\text{L}$), total nitrogen and total phosphorus (3.24 $\mu\text{g-at N/L}$ and 24.65 $\mu\text{g-at P/L}$), organic nitrogen (20.23 $\mu\text{g-at N/L}$), and silicium (98.32 $\mu\text{g-at Si/L}$). These characteristics decreased considerably toward the seaward part of the estuary, while the concentrations of phosphates and nitrates increased (Table 1).

In the section along the Yenisei estuary, the highest values of mean water temperature (6.95 $^{\circ}\text{C}$) and the concentrations of chlorophyll *a* (1.97 $\mu\text{g/L}$) and silicon (61.87 $\mu\text{g-at Si/L}$) were recorded in the surface water layer (0–5 m) (Table 2). The mean values of other characteristics, except for the concentrations of phosphates and ammonium in surface water layer, were much lower than those in underlying horizons.

The bottom water layer (17–31 m) showed the lowest mean concentrations of chlorophyll *a* (1.01 $\mu\text{g/L}$) and some other examined hydrological—hydrochemical characteristics: water temperature (0.91 $^{\circ}\text{C}$) and the concentrations of oxygen (7.24 $\text{mL O}_2/\text{L}$), ammonium nitrogen (1.26 $\mu\text{g-at N/L}$), and silicium (27.36 $\mu\text{g-at Si/L}$) (Table 2). The mean values of many hydrological—hydrochemical characteristics, such as water salinity (27.01 PSU) and the concentrations of phosphates (0.74 $\mu\text{g-at P/L}$), nitrates (3.38 $\mu\text{g-at N/L}$), nitrites (0.18 $\mu\text{g-at N/L}$), and organic nitrogen (23.98 $\mu\text{g-at N/L}$) in bottom water layers were higher than those in higher horizons.

The vertical distribution of the concentrations of ammonium and total nitrogen in the water mass was nearly the same in the section along the estuary: the largest mean concentrations of those biogenic elements were recorded in an intermediate (6–16 m) layer of water (Table 2).

The correlation analysis of abiotic parameters found the closest correlation to exist between water temperature and salinity ($R = -0.99$) (Table 3). These two characteristics showed a higher correlation with the majority of the examined nonbiological characteristics, except for the concentrations of total phosphorus, nitrogen, nitrites, and ammonium nitrogen in water. The closest, though oppositely directed correlations were established between the temperature and salinity, on the one hand, and the concentrations in water of biogenic substances, on the other hand, i.e., phosphates ($R = -0.83$ and 0.79, respectively) and nitrates ($R = 0.76$ and 0.73, respectively). Weaker correlations were observed between water temperature and salinity, on the one hand, and silicium content of water, on the other hand ($R = 0.61$ and -0.63 , respectively).

The correlation of the concentration of chlorophyll *a* in water with nonbiological characteristics was strong and positive only with silicon ($R = 0.71$) and weaker with water temperature ($R = 0.57$) (Table 3). A correlation with similar magnitude by opposite sign ($R = -0.58$) was recorded between chlorophyll *a* and water salinity.

Total Abundance of Bacteria

The values of TBA in the period of study in water of Yenisei estuary in different layers of the water mass varied from 29.87 thous. (st. 5018, horizon 20 m) to 1247.52 thous. cell/mL (station 5013, horizon 30 m) at the mean value over all stations and horizons of

Table 1. Distribution of hydrological--hydrochemical and biological characteristics in the section along the Yenisei estuary in September 2011 (here and in Table 2, the number above the line is the mean value, and below the line are the minimal and maximal values)

| Characteristic | Freshwater area (stations 5013 and 5015) | Frontal zone (stations 5018 and 5019) | OYRD* zone (stations 5021 and 5010) |
|--|---|--|--|
| TBA, thous. cell/mL | $\frac{743.54}{311.88-1247.52}$ | $\frac{175.64}{29.87-89.84}$ | $\frac{57.54}{37.29-97.46}$ |
| ABIM, thous. cell/mL | $\frac{611.19}{284.76-1006.83}$ | $\frac{163.75}{24.37-498.33}$ | $\frac{52.55}{32.84-89.8}$ |
| Share of ABIM in TBA, % | $\frac{84.22}{71.84-91.3}$ | $\frac{92.04}{81.56-97.75}$ | $\frac{90.81}{87.73-94.56}$ |
| CBTC + B, thous. cell/mL | $\frac{105.05}{80.09-130.94}$ | $\frac{19.20}{2.33-41.53}$ | $\frac{20.55}{5.7-40.68}$ |
| CBTC + B share in TBA, % | $\frac{17.38}{7.78-35.46}$ | $\frac{13.57}{1.79-31.19}$ | $\frac{36.03}{12.01-78.41}$ |
| Chlorophyll <i>a</i> concentration, µg/L | $\frac{2.94}{0.41-4.68}$ | $\frac{0.71}{0.28-1.6}$ | $\frac{0.72}{0.11-1.97}$ |
| Water temperature, °C | $\frac{8.19}{4.29-9.6}$ | $\frac{2.8}{0.62-7.02}$ | $\frac{1.46}{1.44-5.52}$ |
| Water salinity, PSU | $\frac{4.35}{0.07-22.07}$ | $\frac{24.48}{5.51-31.72}$ | $\frac{28.49}{16.87-32.14}$ |
| Phosphates, µg-at P/L | $\frac{0.34}{0.02-1.09}$ | $\frac{0.52}{0.27-0.88}$ | $\frac{0.54}{0.20-1.11}$ |
| Total phosphorus, µg-at P/L | $\frac{3.24}{0.18-10.39}$ | $\frac{0.91}{0.54-1.82}$ | $\frac{0.86}{0.40-1.43}$ |
| Nitrates, µg-at N/L | $\frac{1.39}{0.11-7.30}$ | $\frac{1.91}{0.44-5.68}$ | $\frac{1.74}{0.02-4.07}$ |
| Nitrites, µg-at N/L | $\frac{0.19}{0.09-0.27}$ | $\frac{0.07}{0.04-0.12}$ | $\frac{0.11}{0.03-0.29}$ |
| Ammonium nitrogen, µg-at N/L | $\frac{2.40}{0.0-6.81}$ | $\frac{1.45}{1.19-1.79}$ | $\frac{1.17}{0.56-1.67}$ |
| Total nitrogen, µg-at N/L | $\frac{24.65}{9.45-45.6}$ | $\frac{16.30}{12.46-20.40}$ | $\frac{12.98}{2.9-22.15}$ |
| Organic nitrogen, µg-at N/L | $\frac{20.23}{5.47-34.49}$ | $\frac{11.28}{0.32-18.65}$ | $\frac{9.98}{1.68-19.23}$ |
| Silicium, µg-at Si/L | $\frac{98.32}{51.72-113.59}$ | $\frac{23.69}{8.11-67.9}$ | $\frac{13.10}{5.46-35.35}$ |
| Oxygen, mL O ₂ /L | $\frac{7.31}{4.68-8.03}$ | $\frac{7.67}{6.88-8.05}$ | $\frac{7.41}{6.62-8.19}$ |

* OYRD is Ob–Yenisei river discharge.

Table 2. Vertical distribution of hydrological–hydrochemical and biological characteristics in the section along the Yenisei estuary in September 2011

| Characteristic | Surface layer (0–5 m) | Intermediate layer (6–16 m) | Bottom layer (17–31 m) |
|--|-------------------------------|-------------------------------|--------------------------------|
| TBA, thous. cell/mL | $\frac{362.47}{51.81-663.59}$ | $\frac{261.40}{51.79-999.20}$ | $\frac{227.94}{29.87-1247.52}$ |
| ABIM, thous. cell/mL | $\frac{323.83}{45.45-572.06}$ | $\frac{218.99}{47.04-717.83}$ | $\frac{188.16}{24.37-1006.83}$ |
| Share of ABIM in TBA, % | $\frac{89.92}{84.93-94.23}$ | $\frac{97.75}{71.84-97.75}$ | $\frac{93.33}{80.71-93.33}$ |
| CBTC + B, thous. cell/mL | $\frac{53.29}{13.98-104.88}$ | $\frac{45.74}{2.33-130.94}$ | $\frac{27.39}{5.30-97.04}$ |
| CBTC + B share in TBA, % | $\frac{20.87}{7.85-41.74}$ | $\frac{20.43}{1.79-44.35}$ | $\frac{31.63}{7.78-78.41}$ |
| Chlorophyll <i>a</i> concentration, µg/L | $\frac{1.97}{0.39-4.68}$ | $\frac{1.09}{0.28-4.42}$ | $\frac{1.01}{0.12-2.69}$ |
| Water temperature, °C | $\frac{6.95}{4.9-9.63}$ | $\frac{3.65}{0.19-9.64}$ | $\frac{0.91}{-1.44-9.57}$ |
| Water salinity, PSU | $\frac{10.52}{0.07-26.26}$ | $\frac{22.61}{0.07-30.82}$ | $\frac{27.01}{0.07-32.14}$ |
| Phosphates, µg-at P/L | $\frac{0.54}{0.18-2.19}$ | $\frac{0.47}{0.21-1.09}$ | $\frac{0.74}{0.23-1.11}$ |
| Total phosphorus, µg-at P/L | $\frac{1.06}{0.18-2.48}$ | $\frac{2.33}{0.40-10.39}$ | $\frac{2.32}{0.83-10.40}$ |
| Nitrates, µg-at N/L | $\frac{0.37}{0.02-0.75}$ | $\frac{1.55}{0.04-7.30}$ | $\frac{3.38}{0.19-5.68}$ |
| Nitrites, µg-at N/L | $\frac{0.08}{0.03-0.27}$ | $\frac{0.09}{0.04-0.26}$ | $\frac{0.18}{0.11-0.29}$ |
| Ammonium nitrogen, µg-at N/L | $\frac{1.48}{0-3.6}$ | $\frac{1.87}{0.8-6.8}$ | $\frac{1.26}{0.15-1.67}$ |
| Total nitrogen, µg-at N/L | $\frac{14.38}{3.00-21.45}$ | $\frac{20.64}{10.07-45.62}$ | $\frac{15.36}{7.50-24.46}$ |
| Organic nitrogen, µg-at N/L | $\frac{12.45}{2.14-21.17}$ | $\frac{14.96}{0.32-34.49}$ | $\frac{23.98}{1.68-23.98}$ |
| Silicium, µg-at Si/L | $\frac{61.87}{8.21-113.59}$ | $\frac{33.47}{5.46-112.50}$ | $\frac{27.36}{10.25-105.05}$ |
| Oxygen, mL O ₂ /L | $\frac{7.71}{6.8-8.1}$ | $\frac{7.49}{4.68-8.19}$ | $\frac{7.24}{6.63-7.76}$ |

Table 3. Significant values of coefficients of correlation between biological and biotic characteristics of water mass in the section along Yenisei estuary in September 2011 (given in parentheses is the significance level)

| Characteristics of water medium | TBA | ABIM | ABIM share in TBA | CBTC + B | CBTC + B share in TBA | Chlorophyll α | Salinity | Temperature |
|---|------------------|------------------|-------------------|------------------|-----------------------|----------------------|------------------|------------------|
| TBA, thous. cell/mL | 1.00 | 0.998 (0.001) | – | 0.72 (0.001) | –0.51 (0.05) | 0.47 (0.05) | –0.83 (0.001) | 0.83 (0.001) |
| ABIM, thous. cell/mL | 0.998 (0.001) | 1.00 | – | 0.72 (0.001) | –0.51 (0.05) | 0.48 (0.05) | –0.83 (0.001) | 0.82 (0.001) |
| Share of ABIM in TBA, % | – | – | 1.00 | – | 1.00 | –0.42 (0.05) | – | – |
| CBTC + B, thous. cell/mL | 0.72 (0.001) | 0.72 (0.001) | – | 1.00 | – | 0.66 (0.001) | –0.77 (0.001) | 0.74 (0.001) |
| CBTC + B share in TBA, % | –0.51 (0.05) | –0.51 (0.05) | – | – | 1.00 | – | – | – |
| Chlorophyll α concentration, $\mu\text{g/L}$ | 0.47 (0.05) | 0.48 (0.05) | –0.42 (0.05) | 0.66 (0.001) | – | 1.00 | –0.58 (0.01) | 0.57 (0.01) |
| Water salinity, PSU | –0.83 (0.001) | –0.83 (0.001) | – | –0.77 (0.001) | – | –0.58 (0.01) | 1.00 | –0.99 (0.001) |
| Water temperature, $^{\circ}\text{C}$ | 0.83 (0.001) | 0.82 (0.001) | – | 0.74 (0.001) | – | 0.57 (0.01) | –0.99 (0.001) | 1.00 |
| Phosphates, $\mu\text{g-at P/L}$ | –0.61 (0.01) | –0.60 (0.01) | – | –0.47 (0.05) | – | – | 0.79 (0.001) | –0.83 (0.001) |
| Total phosphorus, $\mu\text{g-at P/L}$ | – | – | –0.53 (0.01) | – | – | – | – | – |
| Nitrates, $\mu\text{g-at N/L}$ | –0.48 (0.05) | –0.47 (0.05) | – | – | – | – | 0.73 (0.001) | –0.76 (0.001) |
| Nitrites, $\mu\text{g-at N/L}$ | – | – | –0.59 (0.01) | – | – | – | – | – |
| Ammonium nitrogen, $\mu\text{g-at N/L}$ | – | – | – | – | – | – | – | – |
| Organic nitrogen, $\mu\text{g-at N/L}$ | 0.48 (0.05) | 0.48 (0.05) | – | 0.54 (0.01) | – | – | –0.55 (0.01) | 0.51 (0.05) |
| Silicium, $\mu\text{g-at Si/L}$ | 0.66 (0.001) | 0.66 (0.01) | –0.49 (0.05) | 0.76 (0.001) | – | 0.71 (0.001) | –0.63 (0.01) | 0.61 (0.01) |
| Oxygen, mL O_2/L | 0.43 (0.05) | 0.43 (0.05) | – | – | –0.45 (0.05) | – | –0.43 (0.05) | 0.42 (0.05) |

Table 4. Distribution of microbiological characteristics in the section along Yenisei R. estuary in September 2011

| Station number | Horizon, m | TBA, thous. cell/mL | ABIM, thous. cell/mL | Share of ABIM in TBA, % | CBTC + B, thous. cell/mL | CBTC + B share in TBA, % |
|----------------|------------|---------------------|----------------------|-------------------------|--------------------------|--------------------------|
| 5013 | 5 | 643.26 ± 0.06 | 572.06 ± 0.05 | 88.93 | 104.88 ± 0.04 | 16.30 |
| | 15 | 999.20 ± 0.07 | 717.83 ± 0.06 | 71.84 | 106.79 ± 0.05 | 10.69 |
| | 30 | 1247.52 ± 0.09 | 1006.83 ± 0.08 | 80.71 | 97.04 ± 0.05 | 7.78 |
| Mean | | 963.33 | 765.58 | 80.49 | 102.90 | 11.59 |
| 5015 | 0 | 663.59 ± 0.05 | 563.59 ± 0.04 | 84.93 | 80.09 ± 0.05 | 12.07 |
| | 7 | 595.79 ± 0.06 | 522.06 ± 0.03 | 87.62 | 130.94 ± 0.05 | 21.98 |
| | 12 | 311.88 ± 0.04 | 284.76 ± 0.04 | 91.30 | 110.60 ± 0.05 | 35.46 |
| Mean | | 523.76 | 456.80 | 87.95 | 107.21 | 23.17 |
| 5018 | 0 | 189.84 ± 0.05 | 173.74 ± 0.02 | 91.52 | 38.56 ± 0.04 | 20.31 |
| | 8 | 159.33 ± 0.04 | 153.40 ± 0.02 | 96.28 | 17.80 ± 0.01 | 11.17 |
| | 15 | 65.89 ± 0.04 | 64.41 ± 0.02 | 97.75 | 20.55 ± 0.03 | 31.19 |
| | 20 | 29.87 ± 0.02 | 24.40 ± 0.02 | 81.56 | 5.30 ± 0.01 | 17.73 |
| Mean | | 111.23 | 103.98 | 91.78 | 20.55 | 20.10 |
| 5019 | 5 | 528.84 ± 0.05 | 498.33 ± 0.04 | 94.23 | 41.53 ± 0.04 | 7.85 |
| | 12 | 166.96 ± 0.04 | 155.09 ± 0.004 | 92.89 | 13.35 ± 0.01 | 7.99 |
| | 16 | 129.67 ± 0.02 | 119.50 ± 0.02 | 92.16 | 2.33 ± 0.01 | 1.80 |
| | 25 | 134.75 ± 0.01 | 121.20 ± 0.01 | 89.94 | 14.20 ± 0.01 | 10.53 |
| Mean | | 240.05 | 223.53 | 92.30 | 17.85 | 7.04 |
| 5021 | 1 | 97.46 ± 0.02 | 89.84 ± 0.01 | 92.17 | 40.68 ± 0.01 | 41.74 |
| | 16 | 57.42 ± 0.02 | 54.03 ± 0.01 | 94.10 | 8.69 ± 0.003 | 15.13 |
| | 23 | 57.21 ± 0.02 | 53.39 ± 0.01 | 93.33 | 24.37 ± 0.01 | 42.59 |
| | 31 | 37.29 ± 0.05 | 32.84 ± 0.04 | 88.07 | 29.24 ± 0.01 | 78.41 |
| Mean | | 62.34 | 57.52 | 91.92 | 25.74 | 44.47 |
| 5010 | 5 | 51.81 ± 0.03 | 45.45 ± 0.01 | 87.73 | 13.98 ± 0.01 | 26.99 |
| | 10 | 75.95 ± 0.05 | 71.83 ± 0.01 | 94.56 | 33.69 ± 0.01 | 44.35 |
| | 16 | 51.80 ± 0.04 | 47.04 ± 0.02 | 90.81 | 12.71 ± 0.01 | 24.54 |
| | 20 | 41.31 ± 0.03 | 36.55 ± 0.01 | 88.48 | 15.90 ± 0.01 | 38.48 |
| | 30 | 47.64 ± 0.02 | 41.95 ± 0.01 | 88.06 | 5.72 ± 0.004 | 12.01 |
| Mean | | 53.70 | 48.56 | 89.93 | 16.40 | 29.3 |

277.58 thous. cell/mL (Table 4). The highest values in different horizons were recorded in water of stations 5013 and 5015 (the most freshened zone of the estuary near the Yenisei mouth) (Table 4). The mean TBA for all samples at those two stations was 743.5 thous. cell/mL with variations from 311.88 to 1247.52 thous. cell/mL (Table 1).

The minimal TBA in the section was recorded in water at stations 5021 and 5010 in OYRD zone (Fig. 1; Table 4). The mean TBA for water column at these stations was 57.54 thous. with variations from 37.29 to 97.46 thous. cell/mL (Table 1).

The mean TBA at stations 5018 and 5019 (a domain of the frontal zone of river and sea water mixing) was 175.64 with variations from 29.87 to 528.84 thous. cell/mL (Fig. 1; Table 1), which is 4 times lesser than that in freshened water at stations

5013 and 5015 and 3 times larger than that in water mass at stations 5021 and 5010 (OYRD zone).

The vertical distribution of TBA in water in Yenisei estuary was uneven. The maximal mean TBA in water layer 0–5 m was 362.47 with variations from 51.81 to 663.59 thous. cell/mL was recorded in the surface layer (Table 2).

The minimal mean TBA equal to 227.94 with very wide variations from 29.87 to 1247.52 thous. cell/mL was recorded at the deepest horizons (17–31 m) in the section along Yenisei estuary. In the intermediate water layer (6–16 m) the abundance of bacteria averaged 261.40 thous. with variations from 51.79 to 999.20 thous. cell/mL (Table 2).

Thus, the abundance of heterotrophic bacterioplankton in the water mass in the section along

Yenisei estuary in autumn 2011 was mostly high. The maximal TBA was recorded in water mass at stations 5013 and 5015 (the freshened part of the Yenisei estuary). The minimal TBA was found in water samples from stations 5021 and 5010 (OYRD zone) with a steady decrease in TBA with the distance from the zone of river water toward the sea shelf.

The analysis of the vertical distribution of bacterioplankton in estuarine water mass showed that the TBA was maximal in the surface 0–5-m layer. The underlying water layers show lesser bacterial abundance. A tendency toward a decrease in TBA over depth is generally typical of all stations in the section, except for stations 5013 and 5019, where highest TBA values were recorded in the bottom (30 m) layers (Table 3).

TBA showed significant positive correlation with biological and hydrochemical characteristics of the water medium (Table 3). The closest correlation with TBA was found for ABIM ($R = 0.99$) and the amount of CBTC + B ($R = 0.72$). A weaker positive correlation was also found between TBA and the concentration of chlorophyll *a* in water ($R = 0.47$). A correlation with similar magnitude, but the opposite sign was found to exist with the share of CBTC + B in TBA ($R = -0.51$). Among nonbiological characteristics, the highest correlation with TBA was found for water temperature ($R = 0.83$) and its silicium content ($R = 0.66$), and weaker correlation, with the concentrations of organic nitrogen ($R = 0.48$) and oxygen ($R = 0.43$). The strongest negative correlation was found between TBA and water salinity ($R = -0.83$), and weaker correlations, with phosphates ($R = -0.61$) and nitrates ($R = -0.48$).

The Abundance of Bacteria with Intact Membrane

ABIM in the water mass in the section along the Yenisei estuary in September 2011 was relatively high. On the average over the section, it was 236.96 thous. with variations from 24.40 thous. (st. 5018, 20 m) to 1006.83 thous. cell/mL (st. 5013, 30 m) (Table 4). The maximal ABIM (as well as TBA) was recorded in water at stations 5013 and 5015 (the freshened part of the Yenisei estuary) (Tables 1, 4). Low ABIM values were more common in water of the OYRD zone (stations 5021 and 5010). In water of the frontal mixing zone of river and sea water (stations 5018 and 5019), ABIM was much lesser than that in the most freshened estuarine water, but higher than in the OYRD zone (Tables 1, 4).

The analysis of the vertical distribution of ABIM showed that its maximal value was recorded in the surface (0–5 m) water layer in the section along Yenisei estuary. The mean ABIM for this layer was 323.83 (with variations from 45.45 to 572.06) thous. cell/mL (Table 2). Minimal ABIM was recorded in the bottom (17–31 m) layer, where it averaged 188.16 thous. cell/mL at a very wide scatter of values from 24.37 to 1006.83 thous. cell/mL. In the intermediate water

layer (6–16 m), the mean ABIM was 218.99 (with a wide variations from 47.04 to 717.83) thous. cell/mL (Table 2).

Overall, the spatial distribution of ABIM was similar to that for TBA (Table 4). This is confirmed by the presence of very strong positive correlation between those characteristics ($R = 0.99$). All other correlations, established for ABIM had nearly the same values as TBA.

The share of ABIM in TBA for water all over the section averaged 89.5% with variations from 71.8 to 97.74%. Nearly all bacterioplankton in the water mass of Yenisei estuary, at stations of OYRD zone and beyond it was potentially viable. Note that the mean share of ABIM in TBA at stations with freshened water was lesser than those in the OYRD zone and the frontal zone (Table 1).

The share of ABIM in TBA correlated only with the concentrations in water of chlorophyll *a* ($R = -0.42$) among the biological factors and with the concentrations of total phosphorus ($R = -0.53$), nitrites ($R = -0.59$), and silicium ($R = -0.49$).

In summary, water of Yenisei estuary in autumn 2011 was found to contain abundant and, judging by the share of ABIM in TBA, potentially viable bacterioplankton, its abundance depending primarily on TBA and the concentrations of chlorophyll *a* and water temperature and silicium content.

The Abundance of Bacteria with Active Electron-Transport Chain

The abundance of bacteria with active electron-transport chain (CBTC + B) in water of the Yenisei estuary in autumn 2011 varied widely—from 2.33 (water layer at 16 m at st. 5019) to 130.9433 thous. cell/mL (water layer at 7 m at st. 5019) (Table 4). For the entire Yenisei section, the abundance of CBTC + B averaged 42.13 thous. cell/mL.

The percentage of CBTC + B in TBA was evaluated over the section of Yenisei estuary to compare the development of the active fraction of bacterioplankton in different biotopes. Its average value over the section was 23%.

The mean abundance of CBTC + B reached its maximum in freshened water (stations 5013 and 5015), where it was 105.5 thous. cell/mL with minimal and maximal values of 80.09 and 130.94 thous. cell/mL, respectively (Table 1). However, the relative number of actively functioning organisms in those freshened waters, judging by the mean value of CBTC + B share in TBA, was relatively low—17.38 with variations from 7.78 to 35.46%.

The minimal mean value of CBTC + B abundance (19.20 thous. cell/mL) was recorded in the frontal zone (stations 5018 and 5019) with considerable variations of the initial values (2.33–41.53 thous. cell/mL) (Table 1). The share of CBTC + B in TBA at stations

of the frontal zone was 13.57%, which is less than that in water of freshened stations.

At stations 5021 and 5010, the mean abundance of CBTC + B was slightly greater than that in water of the hydrological front (20.55 with variations from 5.7 to 40.68 thous. cell/mL). The relative value of CBTC + B in TBA at stations in OYRD zone was the highest compared with freshened waters of the estuary and the frontal zone; it was 36.03 with variations from 12.01 to 78.41%.

The vertical distribution of CBTC + B abundance was found to decrease with the depth of the examined stations. The largest mean amount of CBTC + B was recorded in the surface (0–5 m) water layer in the Yenisei section, where it was 53.29 with variations from 13.98 to 104.88 thous. cell/mL. The mean share of CBTC + B in TBA in surface water layer, which was similar to that in the intermediate layer, amounted to 20.87 with variations from 7.84 to 41.74%.

The mean abundance of CBTC + B in water layer of 6–16 m was 45.74 with very wide variations from 2.33 to 130.94 thous. cell/L. The relative amount of actively functioning microorganisms at stations of the frontal zone in water samples from horizons 6–16 m were the same as those in the surface water layer and averaged 20.43 with variations from 1.79 to 44.35%.

The minimal mean abundance of CBTC + B, equal to 27.39 with wide variations from 5.30 to 97.04 thous. cell/mL was recorded in the lower (17.31 m) water layer (Table 3). However, the relative amount (31.63%) of actively functioning microorganisms in the bottom water layer was found to be appreciably greater than that in the upper water layer with variations of the initial values of this share from 7.78 to 78.41%.

Therefore, in September 2011, bacterioplankton with active electron-transport chain was widespread in the water mass of Yenisei estuary, though its distribution was uneven. The share of those bacteria varied within wide limits from 1.79 to 78.41%. The mean values of this characteristics in different parts of the section are high, varying from 20 to 32%. The maximal shares of the population of actively functioning cells in bacterioplankton were recorded in water in OYRD zone at low absolute values of all microbiological characteristics.

The character of the spatial distribution of CBTC + B was partly similar to that of TBA and CBTC + B: the mean amounts of bacteria in all these groups were maximal in freshened waters. The mean amounts of CBTC + B in water of the frontal and OYRD zones were similar, though both were more than 5 times less than those in freshened waters. The mean TBA and ABIM in these zones also decreased; however, in water of the frontal zone, they were 4–5 times greater than in OYRD zone water. Therefore, the spatial distribution of CBTC + B in water of OYRD zone and the frontal zone differed from the distributions of TBA and ABIM.

As was the case with the maximums of TBA and ABIM, the maximum abundance of CBTC + B in the vertical section was recorded in the surface water layer (0–5 m) with the lowest relative abundance of active bacteria. The only exceptions are stations 5010 (OYRD zone) and 5015 (freshened water), where the maximal abundances of CBTC + B were recorded in intermediate water layers (at 10 and 7 m, respectively) (Table 4). In layer 6–16 m, the characteristics of CBTC + B, TBA, and ABIM decreased (especially, TBA and ABIM). All three characteristics had even lesser values in bottom (17–31 m) water layers. However, the share of active microorganisms in TBA was highest in the bottom layers.

The abundance of CBTC + B was found to have most significant positive correlation with biological characteristics (i.e., with TBA, $R = 0.72$; and ABIM, $R = 0.72$), while the correlation with chlorophyll *a* concentration in water was somewhat lower ($R = 0.66$) (Table 3). The correlations between CBTC + B and water temperature ($R = 0.74$) and between CBTC + B and water silicium content ($R = 0.76$) were high, while the correlation between CBTC + B and the concentration of organic nitrogen in water was medium ($R = 0.54$). The values of CBTC + B show a strong negative correlation only with water salinity ($R = -0.77$) and a weak correlation with its phosphates content ($R = -0.47$).

The share of CBTC + B in TBA is not correlated with the majority of water medium characteristics; its correlation was negative with TBA ($R = -0.51$), ABIM ($R = -0.51$), and with water oxygen content ($R = -0.45$).

Chlorophyll a and Nonbiological Environmental Characteristics

Chlorophyll *a* concentrations were maximal in freshened waters where they varied from 0.41 to 4.68 with a mean of 2.94 $\mu\text{g/L}$. The mean values of chlorophyll *a* concentration in water of the frontal zone and OYRD zone were the same. The vertical distribution of chlorophyll *a* concentration was uneven. The maximal mean value of this characteristic (1.97 $\mu\text{g/L}$) was recorded in the surface layer, while its mean values in intermediate and bottom layers were nearly the same. Chlorophyll *a* showed a statistically significant positive correlation only with silicon ($R = 0.71$) and a weaker correlation with water temperature ($R = 0.57$). Its correlation with water salinity had a similar, but negative value ($R = -0.58$). No correlation was found to exist between chlorophyll *a* concentration and the concentrations of nitrates and phosphates. Similar results were obtained for chlorophyll *a* for the Kara Sea in 2007 [5].

The majority of hydrochemical characteristics, except for the concentrations of total phosphorus, nitrites, and ammonium nitrogen, have significant correlations with hydrological characteristics, i.e.,

water salinity and temperature (Table 3). Especially strong correlation with the latter two characteristics were detected for the biogenic substances that are of greatest importance for bacteria—nitrates and phosphates. Their correlations with water salinity were positive ($R = 0.73$ and $R = 0.79$, respectively) and those with the temperature were negative ($R = -0.76$ and $R = -0.83$, respectively). The correlation with water temperature and salinity was medium ($R = 0.61$ and $R = 0.63$, respectively). The correlation between these hydrological characteristics and organic nitrogen and oxygen were less significant.

DISCUSSION OF RESULTS

The published studies on the bacterioplankton of the Yenisei estuary are few [1, 2, 4, 6, 15] and do not contain data on the characteristics studied in this work, such as ABIM and the amount of CBTC + B.

TBA in water of the Yenisei varied within 29.87–1247.52 with a mean of 277.52 thous. cell/mL. These results are most close to those obtained for the region in August–October 2009, when TBA varied from 20 to 520 thous. cell/mL; the largest TBA values were recorded in the warmest (10.8°C) surface water; in addition, TBA was found to decrease with depth [6].

Some data on bacterioplankton of the Yenisei R. collected in the 1970s were generalized in [1, 2]. According to these data, TBA in the Yenisei R. at the Igarka section (the closest to st. 5013 with freshened water) in the mid-September reached 1 million cell/mL. Notwithstanding the considerable difference between the periods of studies and the methods of TBA assessment, these values are close to the mean values of TBA (966.33 thous. cell/mL), obtained by the authors of this article in water column at st. 5013.

The estimates of TBA in water of Yenisei estuary in August–October 1993 [4] are much lower than those obtained by the authors of this article. According to [4], the values of TBA in the section along Yenisei mouth, estimated by epifluorescent microscopy with dyeing bacteria by acridine orange, varied from 16 to 57 thous. cell/mL; and bacterioplankton concentration in surface layer was higher.

B. Meon and R.M.W. Amon [15] evaluated TBA in surface water of the Yenisei estuary by microscopy with dyeing bacteria by DAPI dye. They showed that TBA in autumn 2001 in the fresh surface water of the Yenisei estuary varied from 1400 to 1600 thous. cell/mL. With water salinity increasing from 5 to 15 PSU, the values of TBA also somewhat increased, varying within 1600–2400 thous. cell/mL. The TBA, evaluated by the authors in the surface freshwater layer in the Yenisei estuary (stations 5013 and 5015) varied from 643.26 to 663.59 thous. cell/mL, thus falling below those obtained in 2001 [15]. This may be due to the difference between the methods of dyeing bacteria and the considerably year-to-year variations of TBA in Yenisei water [2].

The high values of all microbiological characteristics in the surface layer of most freshened zones of the Yenisei estuary are quite explicable as the surface waters of the Ob and Yenisei estuaries in autumn refer to eutrophic water areas in terms of chlorophyll *a* content [7]. They typically show higher abundance of heterotrophic bacteria compared with meso- and oligotrophic waters of the Kara Sea. A positive contribution in this case may also be due to the discharge of large amounts of bacteria into the estuary by Yenisei runoff.

In September, the vegetation season in the study region ends; therefore, in this period, phytoplankton has no strong effect on the concentrations of biogenic substances as it does at the peak of its abundance. This can be seen from the high concentrations in the Yenisei estuary of nitrates and phosphates, which are of greatest importance for bacteria, as well as ammonium nitrogen. The high concentrations of nitrites in the freshened waters at stations 5013 and 5015 suggest the active processes of nitrification, for which ammonium is the source of nitrogen.

The integrity of cell membrane is the key condition of cell viability; therefore, the count of ABIM in water samples from natural water bodies is a common procedure. The soundness of such approach has been shown in [17] and, of arctic water areas, in [21]. Commonly, ABIM is evaluated with the use of a standard set of dyes LIVE/DEAD BacLight Bacterial Viability Kits [11, 19]. As shown in [17], the sum of cells with intact and broken cell membranes are strongly correlated with TBA values ($R = 0.97$; $p < 0.01$), if the latter is determined by epifluorescent microscopy with bacteria dyed by acridine orange fluorochrome. On the other hand, some researchers report that the TBA values obtained with the use of LIVE/DEAD BacLight Bacterial Viability Kits dyes are commonly lower than that obtained with the use of DAPI fluorochrome [13].

Several studies with the use of dyes from the LIVE/DEAD BacLight Bacterial Viability Kits were carried out in the Sea of Japan in autumn 1995. The share of ABIM in TBA was shown to average 69.7% with variations from 61.8 to 75% [17]. In September 1996, the share of ABIM in TBA in water of the Sea of Japan varied from 40.5 to 80.3%, while in January of the next year, it increased to vary within 65.2 to 86.4% [11]. In the shelf water of the Baltic Sea, the share of ABIM in TBA averaged 55 with variations from 22 to 81% [19]. In the estuarine areas of this sea, the share of cells with intact membrane was somewhat higher than it was on the shelf: it averaged 64 with variations from 52 to 76%. According to the authors' data, the share of ABIM in TBA in the section along the Yenisei estuary was higher—89.5% with variations from 71.8 to 97.74%. At the same time, the share of ABIM in TBA was lower in freshened waters than in waters of the frontal zone and the zone of OYRD.

Although the integrity of the membrane is the key condition for the viability of a bacterial cell, some data suggest that this may not imply active cell metabolism [10]. Another drawback of the method for evaluating the amount of viable cells with the use of LIVE/DEAD BacLight Bacterial Viability Kits dyes is due to this set of fluorochromes failing to account for the cells with no nucleotide, though they are undoubtedly dead [10]. The results of assessing ABIM with the use of this method are also sometimes incorrect for laboratory bacterial cultures [13].

A reliable indicator of bacterial respiration and their utilization of substrates is the activity of their electron-transport chain [18]. Therefore, in addition to assessing ABIM, the authors also determined, in water samples, the amount of bacteria with active electron-transport chain (CBTC + B) and next evaluated the share of CBTC + B in TBA. Few data on the abundance of CBTC + B in aquatic ecosystems and the ratios between CBTC + B amount and TBA are available in the literature. According to the authors' data, the share of CBTC + B in TBA in the section along the Yenisei estuary averaged 23.35% with wide variations from 180 to 78.4%. In the case of another large estuary, Chesapeake Bay, it was shown that the share of CBTC + B in TBA varied from 29 (May) to 69% (September) in surface-water samples and from 29 (May) to 62% (September) in samples from the depth of 8.5 m [20]. In the case under consideration, the variation range of the abundance of the most active part of bacterioplankton was much wider (1.80–78.41%) than it was in Chesapeake Bay; this range extended toward both minimal and maximal values, which might be due to the significant difference between the bacterioplankton habitat conditions in the Yenisei estuary and Chesapeake Bay. According to [19], the share of bacteria with active metabolism is higher in weakly polluted water areas and lower in waters subjected to chronic anthropogenic pollution. Therefore, we can suppose that the higher share of the actively functioning bacterioplankton in its total abundance, as well as the wider range of its variations in the Yenisei estuary compared with Chesapeake Bay are due to its higher anthropogenic load.

The share of actively functioning bacteria in bacterioplankton in the Yenisei estuary was largest in the water mass at stations in the OYRD zone, where it averaged 36.03 with variations for 12.01 to 78.41%. Water in this zone showed higher salinity and low temperature (Table 1). In highly freshened and warmer waters of the estuary, which are rich in chlorophyll *a* and biogenic matter (stations 5013 and 5015), the share of CBTC + B in TBA is much lower, its mean value being 17.38 with variations from 7.78 to 35.46%. It can be supposed that the steep gradients of water salinity, which can be seen in the most freshened waters of the Yenisei estuary and in the frontal zone, have an adverse effect on the activity of riverine bacterioplankton that have entered this zone. This is not the

case in water of the OYRD zone because there are no steep gradients here, the conditions of the medium are much more stable, and the microbial population is mostly represented by marine species. Similar data have been obtained for water of the Baltic Sea [19]: the share of actively breathing bacteria was higher here (3–23%) than it was in the eutrophic water of estuarine areas (1–21%). The share of active bacteria was highest in oligotrophic river water (3–48%) [19].

The positive correlations between the abundance of active bacterial fraction and the concentration of chlorophyll *a* in water, silicium concentration, as well as water temperature and salinity were also established in [19, 20]. According to the authors' data, water temperature and salinity also show the closest correlation with another two microbiological characteristics—TBA and ABIM. They also increase with temperature and decrease with water salinity (the absolute values of all microbiological characteristics decrease with the passage from freshened estuarine water to seawater). The negative correlations of TBA, ABIM, and CBTC + B with phosphates, and TBA and ABIM also with nitrates, may be an indication that the spatial distribution of bacteria in the examined water area is primarily determined by the hydrodynamic processes in it, rather than the concentrations of biogenic substances. The less the effect of freshwater on seawater, the lower the absolute values of microbiological characteristics, and vice versa.

CONCLUSIONS

In autumn 2011, the water in the section along the Yenisei estuary contained mostly abundant, viable, and actively functioning heterotrophic bacterioplankton. The maximal values of the examined microbiological characteristics were recorded in water mass at stations 5013 and 5015 in the freshened part of the Yenisei estuary. Their minimal values were found in water samples taken in OYRD zone (at stations 5021 and 5010).

The absolute values of the examined microbiological characteristics decreased with the distance from the zone of freshened water of the Yenisei estuary toward marine water areas in the OYRD zone, while the relative abundance of the viable and active bacterioplankton increased in the same direction.

Positive correlation was found to exist between the values of the examined microbiological characteristics (TBA, ABIM, CBTC + B) and the concentration in water of chlorophyll *a*, silicium, and organic nitrogen, as well as water temperature and salinity; they showed negative correlations with phosphate concentrations; and TBA and ABIM, also with nitrate concentrations. These correlations may be an indication that the spatial distribution of bacteria in the examined water area is primarily governed by hydrodynamic processes and, partly, to the state of phytoplankton community. The

latter has the largest effect on the abundance of CBTC + B.

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