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MARINE BIOLOGY

Heterotrophic Bacteria of the Ob River Estuary during Growing Season: Spatial and Temporal Variability

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Abstract—Analysis of the distribution of abundance and activity of bacterioplankton in the estuary area of the Ob River in July 2016 and September 2013, as well as environmental factors, made it possible to distinguish between riverine, brackish water, and marine zones. In summer, the abundance of bacterioplankton varied from $2604 \pm 436 \times 10^3$ cells/mL in brackish waters to $468 \pm 91 \times 10^3$ cells/mL in seawater. The average values of bacterial production in waters with a salinity of less than 8 and more than 22 PSU were 17.43 and 4.91 mgC m^{-3} day^{-1}, respectively. In autumn, the bacterial abundance decreased towards the sea from $1289 \pm 385 \times 10^3$ cells/mL in freshwaters to $85 \pm 37 \times 10^3$ cells/mL in the offshore part; the value of production decreased by more than an order of magnitude. With an increase in salinity, the proportion of cells with active electron transport chain in the abundance of bacterioplankton decreased from 5.8 to 0.6%. Thus, the distribution of river runoff, marked by salinity, proved to be the main factor affecting the spatial distribution and activity of bacterioplankton. However, the mechanism of such regulation remains unclear.

Keywords: Arctic estuaries, bacterioplankton, temporal changes

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INTRODUCTION

River estuaries are characterized by the active contribution of the microbial community to the formation of a biological pump in the area of the marginal filter [6]. Annually, the Arctic Basin receives approximately 11% of the world river runoff, while its volume is only 1% of the volume of the World Ocean [25]. The average annual freshwater runoff into the Kara Sea is about 41% of the total Arctic runoff (1300–1400 km^3; [2, 7]), while the average runoff into the Gulf of Ob is 500 km^3/year [32]. Thus, the processes occurring in the mixing zone at the river–sea boundary of the Ob estuary affect not only coastal ecosystems but also the entire Arctic Basin [31].

Most studies considering the riverine organic matter of the Arctic estuaries have noted its highly refractoty state [23, 33]. Only during the flood period, the share of labile dissolved carbon in river runoff can increase to 20–40% [27]. Thus, the main carbon source for heterotrophic bacterioplankton in the estuarine areas of the Arctic shelf seas during most of the growing season is organic matter synthesized by primary producers. At the same time, probably, nutrients are not a limiting factor in the development of heterotrophic microplankton throughout most of the year. Only during the period of active snow melting, their concentration decreases significantly [23]. Nevertheless, hydrochemical parameters can serve as a marker of the activity of a heterotrophic community [5].

Under conditions of pronounced seasonality, a long polar night, as well as temporal and spatial limitation of phytoplankton bloom, which are characteristic of the Siberian Arctic seas, the microbial loop [24], and heterotrophic bacterioplankton, in particular, become the most important member of the pelagic community. Even at low temperatures, bacterioplankton plays a crucial role in the remineralization of nutrients and the transformation of organic matter [17, 29]. Climate change and active industrial development of the Arctic shelf have attracted attention to the microbial cenoses of the Arctic seas. However, the general understanding of the dynamics of microbial distribution and development, as well as the factors affecting them, is absent [10, 13, 14, 32, 36].

In this study, we attempted to assess the scale and nature of the variability of microbial communities in the zone of active frontogenesis at the river–sea boundary [18], using the example of one of the largest Siberian rivers, the Ob River. The goal was also to identify patterns for the distribution and activity of microorganisms in the estuary and adjacent shelf area based on expeditionary results and literature data.

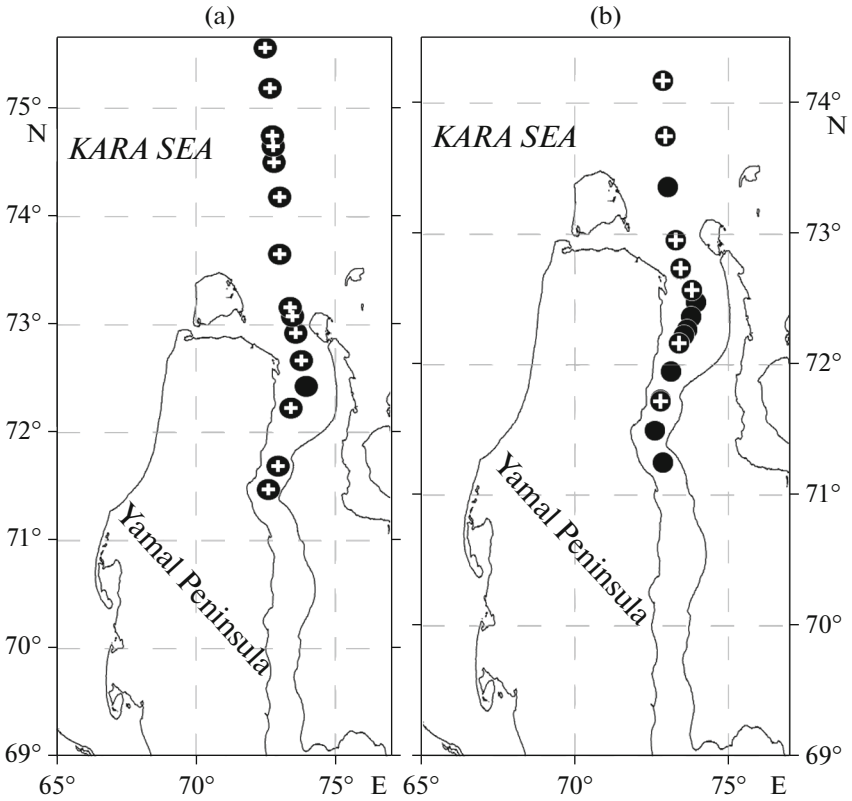

Fig. 1. Sketch map of study area in Ob estuary in (a) summer 2016 and (b) autumn 2013. (●) Stations at which hydrochemical parameters of water were analyzed; (+), points at which samples were collected to determine quantitative parameters of bacterioplankton.

MATERIALS AND METHODS

The material was collected at the Ob estuary transects towards the continental shelf during cruise 125 of the R/V *Professor Shtokman* (September 4–7, 2013) and cruise 66 of the R/V *Akademik Mstislav Keldysh* (July 19–24, 2016) to the Kara Sea ("Marine Ecosystems of Siberian Arctic" program) (Fig. 1). Water samples were collected using 5 L Niskin bottles as part of the Rosetta complex equipped with a CTD probe (SBE 911 Plus by SeaBird Electronics, United States) from the horizons selected based on the profiles of temperature, salinity, and fluorescence.

Sampling for determination of hydrochemical parameters was carried out according to GOST 51 592–2000 (General requirements for sampling). To determine pH, nutrients (silicates, phosphates, and nitrogen forms), and alkalinity, samples were collected into 0.5 L plastic containers, without fixation. Due to the high concentration of suspended matter in the river–sea mixing area, samples for the determination of the nutrients content were preliminarily filtered through a filter with a pore diameter of 0.45 μm (Millero, 1995).

The dissolved oxygen content was determined by the Winkler method [34]. The technique for determining nitrate is based on the reduction of nitrates to nitrites and subsequent colorimetry [34]. The content of phosphates was determined by the Murphy-Riley method [34]. The ammonium concentration was determined as described in [40].

Water samples for enumeration of bacteria and calculation of their biomass were fixed immediately after sampling with a neutral solution of formaldehyde (final concentration in the sample, 1%) and stored until further treatment. In autumn 2013, the concentration of bacterioplankton was determined by staining samples with the DAPI fluorochrome on nuclepore black filters (0.2 μm, Trackpore Technology) and analyzing them under a fluorescent microscope [35]. Fixed samples were treated at least three months after sampling [41]. In the summer of 2016, the abundance of bacterioplankton was determined using flow cytometry. Fixed samples were stained with the SYBR GREEN I fluorescent dye for 30 min and processed using a BD Accuri C6 cytometer within a day after sampling [28]. For a more accurate assessment of the abundance of bacterioplankton, the filtrate that passed through a filter with a pore diameter of 0.2 μm was simultaneously counted. At some stations, samples for cytometric analysis were also analysed using fluorescence microscopy. Based on the cytometry results, the ratio of cells with a high (HNA) and low(LNA) content of nucleic acids (an indicator predicting the proportion of active

cells) was calculated [30]. The methods used for counting microbial numbers do not allow the differentiation between Archaea and Bacteria. Therefore, the term bacteria used in this study concerned all heterotrophic prokaryotes.

Due to the difference in the time preceding the treatment of samples in 2013 and 2016, we carried out an additional experiment to assess the effect of storage of fixed samples on the change in the concentration of bacteria in them. Several studies have reported a decrease in the number of bacterial cells in seawater samples during storage [26]. At the same time, the storage time did not affect the number of cells containing a visible nucleoid. The cell abundance decreased within 40–70 days after fixation. After that, it reached a plateau, coinciding with the number of cells containing a visible nucleoid [41]. For one year, we compared changes in the cell abundance in the fixed liquid samples and in the samples that were filtered immediately after collection and frozen. The data obtained were compared with the number of viable cells (with a visible nucleoid) determined immediately after sampling. All measurements were carried out in triplicate.

The linear dimensions of the cells were measured using the ImageScopeColor M software for analyzing images. Bacterial biomass (in carbon units) was calculated as described in [12].

The determination of bacterial production and grazing of bacterioplankton by consumers was carried out by a direct method using antibiotic inhibitors of bacterial growth [37] in modification for natural habitats [42]. Immediately after collecting, water samples were added to 100 mL vials. In order to determine the grazing of bacterioplankton by nano- and microphages, the water samples were supplemented with antibiotics (benzylpenicillin (1 mg/L) and vancomycin (200 mg/L)) that suppressed the growth of bacteria but did not affect their consumers [37]. The untreated samples were used as control. These samples were exposed for 8–10 h in a seawater pool located on the upper deck of the vessel. The exposure time was selected based on the preliminary experiment on the dynamics of antibiotic effectiveness in polar waters. All experiments were carried out in duplicate. The manipulations associated with the experiments were conducted on the open deck of the vessel at ambient temperature.

The proportion of cells with an active electron transport chain(ETC) was determined experimentally by exposing samples to CTC fluorochrome (5-cyano-2,3-ditolyl tetrazolium chloride) [38]. A solution of CTC fluorochrome in distilled water (final concentration, 100 μg/mL) was added to each aliquot (5.4 mL) of seawater. The samples were exposed for 4 h at 4°C. After that, they were fixed with 37% formalin (final concentration, 1%), stained with DAPI fluorochrome, and concentrated on a black nuclepore filter (a pore diameter of 0.2 μm; Trackpore Technology). The slides were examined using a Leica DM 2500 microscope (magnification, ×1000; wavelength, 450–490 nm) to count CTC-stained cells, as well as in ultraviolet radiation to calculate the total number of bacteria stained with DAPI.

Spearman's rank correlation coefficient was calculated to identify correlations between community characteristics. Calculations were performed using the PAST 3.14 software package.

RESULTS

An experiment on the effect of sample storage on changes in the number of bacteria confirmed the conclusions [41] that the number of bacterial cells in the fixed liquid samples during the year decreased only in the first three months to the values of the abundance of bacteria with a visible nucleoid. At the same time, the abundance in the samples concentrated on a filter changed insignificantly during the entire observation period (Fig. 2).

July 2016. In 2016, the studies were carried out when the values of the runoff of the river Ob were high, shortly after the seasonal ice cover melted [39]. In contrast to the observations of previous years [13], the impact of the river runoff on the characteristics of the surface layer of the Kara Sea was mainly accumulated in the southern region of the basin adjacent to the estuaries [20]. The estuarine area of the river Ob and the adjacent shallow shelf were characterized by a high level of horizontal unevenness, primarily in the distribution of salinity (Fig. 3a). The scale of local increases in the gradients of hydrophysical parameters was 10–50 km, forming a pronounced frontal zone in the area of interaction between the river and sea waters [20].

During our observations, the transect along the Ob estuary was an example of the so-called "complex front" [7], consisting of a vertical front in the southern part of the transect (station nos. 5323 and 5324) and a horizontal front extending seawards from station no. 5319. The presence of riverine waters can be traced in surface waters almost throughout the entire length of the transect.

The relative content of dissolved oxygen was rather high (Fig. 3e). In surface waters, despite the relatively high temperature (11–12°C and higher), the dissolved oxygen content almost reached 9 mL/L. The saturation of water with oxygen increased up to 129% and did not decrease below 80%. The highest dissolved oxygen content and the level of water saturation with oxygen were observed near the surface at station nos. 5320 and 5321, where the position of the front changed from vertical to horizontal. The highest content of phosphates (Fig. 3b) and nitrates (Fig. 3f) was observed at the surface of the southernmost station of the transect (no. 5324). In the northern direction, their content decreased rapidly. North from station 5313, the content of dissolved inorganic phosphorus in the surface

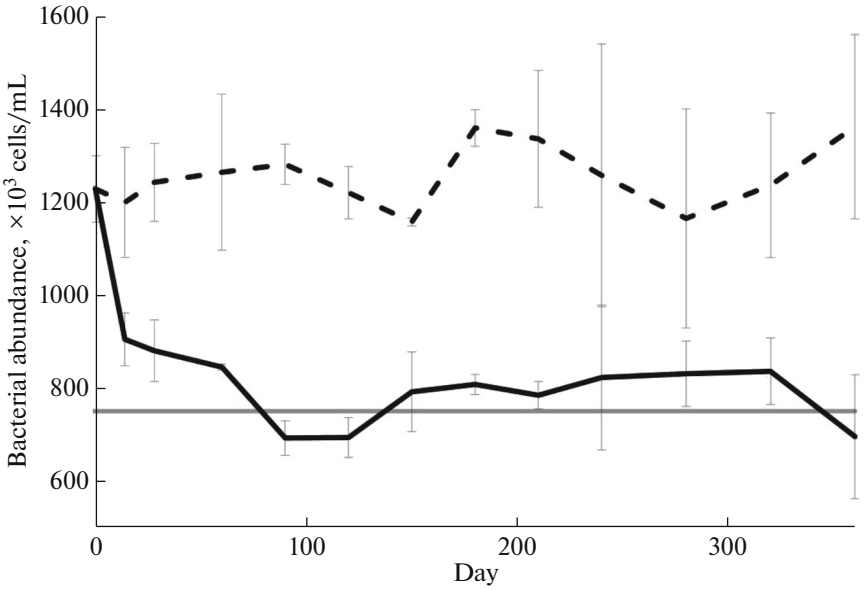

Fig. 2. Results of experiments on long-term storage of samples. Dynamics of changes in number of bacterial cells in (¦) samples concentrated on filter and (|) samples stored in liquid form. Gray line marks number of cells containing visible nucleoid.

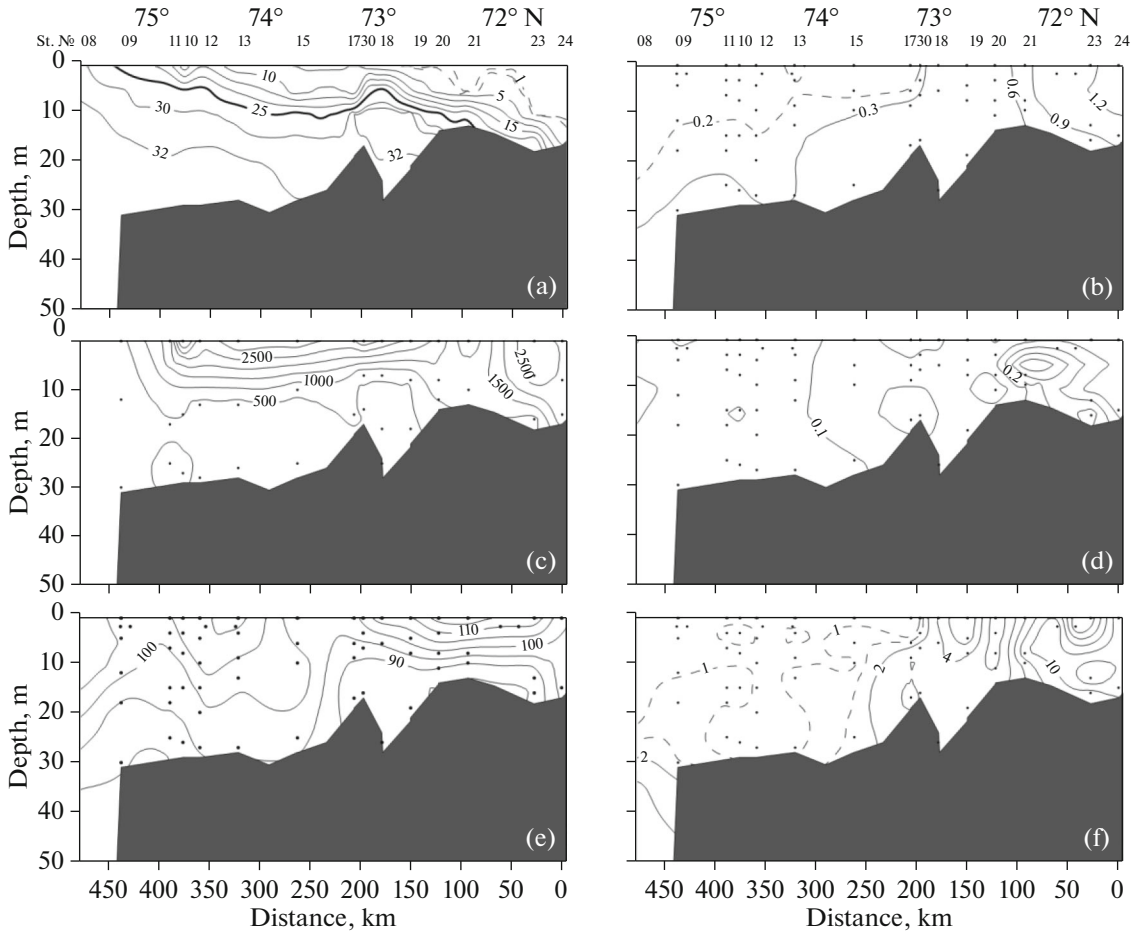

Fig. 3. Transect along Ob estuary (July 2016): (a) salinity, PSU; (b) concentration of inorganic phosphorus, μM; (c) abundance of bacteria, × 10^3 cells/mL; (d) concentration of ammonium, μM; (e) oxygen saturation, %; (f) concentration of nitrates, μM.

water layer could decrease to 0.02 μM, while nitrogen concentration reached analytical zero. It is also noteworthy that the content of ammonium nitrogen in the transect was unusually low: from 0.04 to 0.52 μM (Fig. 3d). The content of nitrites, on the contrary, reached 0.8 μM; in some cases, its concentration even exceeded that of nitrogen in the nitrate and ammonium forms. The near-bottom maximum of phosphates and nitrate, which was typical for the studies of previous years [1], was not detected.

The abundance of bacterioplankton in the transect along the Ob estuary varied from 98 to 4500 × 10^3 cells/mL (Fig. 3c). In the waters with a salinity of 0.15–17 PSU, the number of bacteria ranged from 500 to 4467 × 10^3 cells/mL, with an average value of 2604 ± 436 × 10^3 cells/mL. At salinity values of >22 PSU, the average value of this indicator was 468 ± 91 × 10^3 cells/mL, varying from 98 to 1263 × 10^3 cells/mL.

The proportion of HNA cells ranged from 11 to 87%. In the southern part of the transect (to station 5317), the proportion of HNA cells did not exceed 65%, with an average value of 44 ± 6%. To the north, this parameter varied from 48 to 81%, with average values of 54 ± 6% in the surface water layer and 76 ± 3% below the pycnocline.

The value of bacterial biomass varied from 1.4 to 53.65 mgC/m^3. In the waters with a salinity of less than 17 PSU, the average value of this indicator was 28.7 ± 8.62 mgC/m^3. At a salinity of more than 22 PSU, the average value of the bacterioplankton biomass decreased to 6.19 ± 1.06 mgC/m^3.

The maximum value of the bacterial production was observed on the surface horizon of station 5313, at which the values of biomass and specific growth rate (2.81 day^{-1}) were high, while the value of the bacterial production reached 150.73 mgC m^{-3} day^{-1}. At other sites, the production value varied from 0.18 to 26.3 mgC m^{-3} day^{-1}, and the specific growth rate changed from 0.05 to 3.29 day^{-1}. The average values of bacterial production in waters with a salinity of less than 8 and more than 22 PSU were 17.43 and 4.91 mgC m^{-3} day^{-1}, respectively. However, based on a large scatter of values, these differences were considered unreliable. On the contrary, the values of specific growth rate that were averaged for the water column increased towards the sea from 0.8 to 1.52 day^{-1}.

September 2013. In early September 2013, the salinity gradient at the boundary of the Ob estuary and the adjacent shelf of the Kara Sea was relatively small. Surface salinity in the estuarine frontal zone increased from 0.1–0.2 to 9–10 PSU (Fig. 4a) [19]. As in the summer of 2016, both vertical and horizontal fronts were observed in the transect. The vertical front was located in the area of stations 125-14 and 125-17, and it was distinguished by an abrupt increase in salinity from freshwater to 1 PSU.

The maximum concentrations of dissolved oxygen were confined to the vertical frontal zone (Fig. 4e). Its content on the surface reached 8.9 mL/L. The level of oxygen saturation at this point was 105–110% in the layer up to 5 m. The minimum oxygen content (less than 70%) was recorded in the cold bottom layer at 73° N (station 125-17), as well as in the zone below the pycnocline, in the area of the Ob bar (station 125-17). Relatively high concentrations of phosphates (1.53–2.05 μM), as well as nitrates and ammonium (2.82–3.44 μM and 2.8–4.1 μM, respectively), were also observed at these points.

The distribution of nutrients concentration was rather conservative. The maximum concentrations of phosphates (Fig. 4b) and nitrate (Fig. 4f) coincided with the orographic features of the Ob transect. The maximum concentration of nitrites in the surface layer (1.06 μM, station 125-16) was associated with river waters. When approaching the frontal zone (station 125-17), their concentration decreased to analytical zero. The second (near-bottom) maximum (1.14 μM) at station 125-26 was associated with the northern boundary of the frontal zone, which was identified according to pH. At the same points of the surface and bottom layers, the concentration maxima of ammonium were also noted (4.32 and 3.46 μM, respectively, Fig. 4d).

In September 2013, the abundance of bacteria in the area of the Ob estuary varied from 29 to 1875 × 10^3 cells/mL (Fig. 4c). The maximum values (994–1875 × 10^3 cells/mL) were observed in the upper mixed layer in the southern part of the transect. The minimum values (29–218 × 10^3 cells/mL) were registered in the seaward part of the transect in the near-bottom layer of cold salty water. The distribution of the abundance of bacterioplankton coincided with changes in salinity: the average abundance of bacteria at a salinity of less than 2.5 PSU was 1289 ± 385 × 10^3 cells/mL. In waters with salinity above 29 PSU, the total number of bacteria did not exceed 218 × 10^3 cells/mL, with an average value of 85 ± 37 × 10^3 cells/mL. The distribution of bacterioplankton biomass in the transect along the Ob estuary had a similar pattern. At the same time, the differences between the average values of this parameter in waters characterized by different salinity were even more pronounced. At a salinity of less than 2.5 PSU, the average biomass of bacterioplankton was 22.7 ± 8.5 mgC/m^3. Inbrackish waters with salinity of 3.5–22 PSU, this indicator decreased to 9.9 ± 2.5 mgC/m^3. At a salinity above 29 PSU, the average biomass value decreased to 1.2 ± 1 mgC/m^3. In the near-bottom water layer at the far northern station of the transect, high indicators of bacterioplankton biomass were observed, while the values of its abundance were relatively low (7 mgC/m^3 and 172 × 10^3 cells/mL, respectively). Probably, the high biomass value was associated with a significant proportion of large rod-shaped forms (38% of the total number). At other stations, their contribution did not

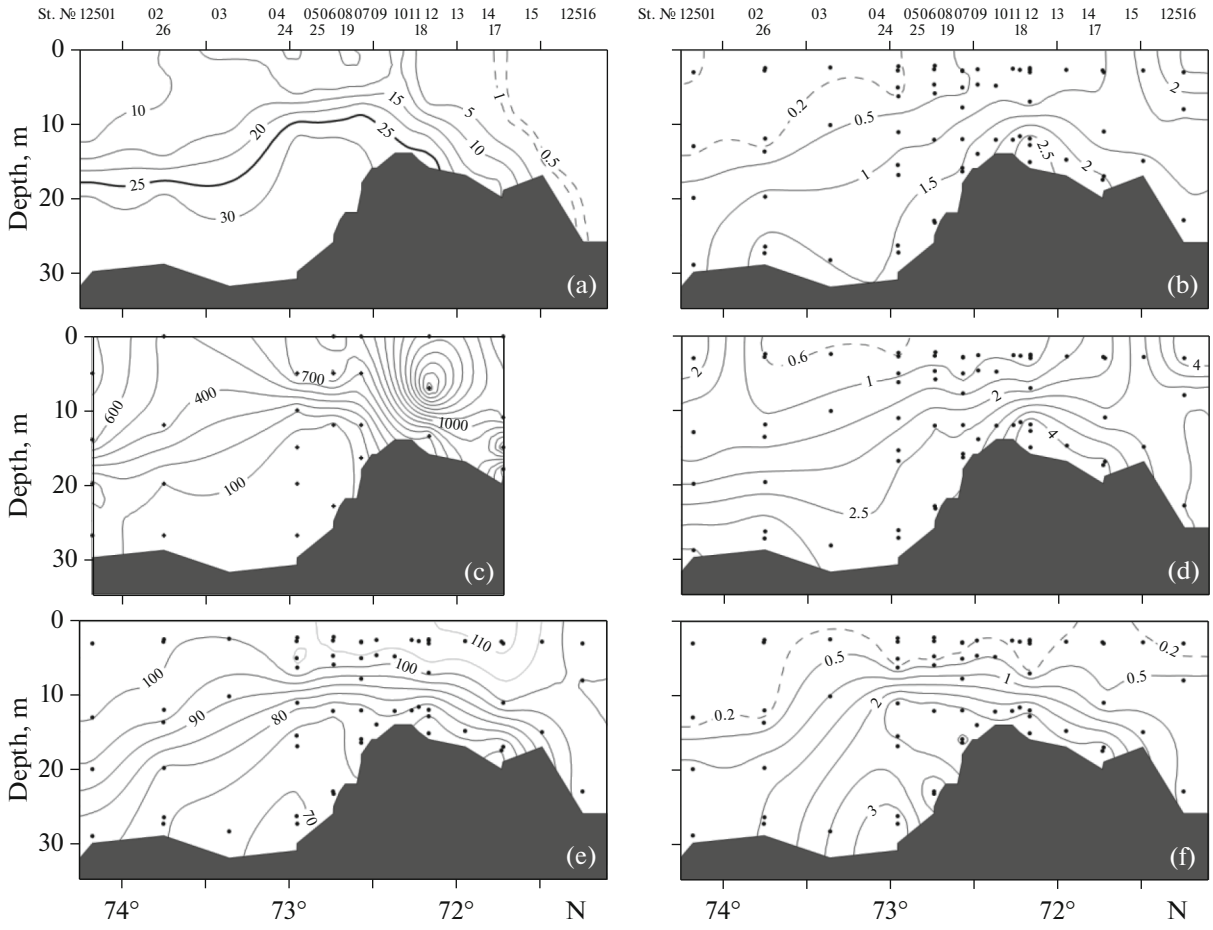

Fig. 4. Transect along Ob estuary (September 2013): (a) salinity, PSU; (b) concentration of inorganic phosphorus, μM; (c) abundance of bacteria, × 10^3 cells/mL; (d) concentration of ammonium, μM; (e) oxygen saturation, %; (f) concentration of nitrates, μM.

exceed 25%; most of the cells were represented by coccoid forms.

In autumn 2013, the proportion of bacterial cells with active ETC also decreased with an increase in salinity. In waters with a salinity of less than 2.5 PSU, this parameter was 5.8 ± 0.27%. Inbrackish waters, it was 2.6 ± 0.5%. At salinity values of more than 29 PSU, the proportion of actively respiring bacteria decreased to 0.6 ± 0.4%.

DISCUSSION

July 2016. The high values of the relative oxygen content in surface waters described in this study were not observed during previous studies in the Gulf of Ob in July-August 2010 [1, 15]. Possibly, this was evidence of the active growth of phytoplankton [16], which is indirectly confirmed by the extremely low concentrations of phosphates and nitrate at the outlet of the gulf. Probably, available forms of nutrients were consumed by phytoplankton in waters with a salinity range from 1 to 10 PSU. Low concentrations of ammonium and, conversely, a high contribution of nitrite to the content of dissolved inorganic nitrogen may indicate "immature" and incomplete processes of oxidation of organic matter.

Analyzing the data on the distribution of abundance and activity of bacterioplankton based on conventionally identified salinity intervals, it is important to take into account the fact that waters of different salinity in the estuarine area can be confined to different depths. However, the abundances of bacterioplankton had close values in brackish waters (3.5–22 PSU) at different stations of the transect both in the upper photic and near-bottom layers. No correlation between this parameter and depth was found ($r = 0.2$, $p > 0.5$).

In July 2016, no significant correlations between the distribution of bacterioplankton abundance and the considered environmental parameters were found in the waters with a salinity of less than 17 PSU. Changes in the composition and quantitative characteristics of the phytoplankton community along the river–sea gradient did not affect the abundance of bacterioplankton [16]. Bacterial numbers exceeding 1 million cells/mL were observed in the euphotic layer when the concentration of chlorophyll *a* varied from 0.46 to 33 μg/L. At the

same time, values below 600×10^3 cells/mL were noted, while the pigment concentration changed in the range of 0.04– 27.3 μg/L [22].

Below the pycnocline at depths down to 30 m (at which dissolved oxygen concentrations were measured), a negative correlation between the abundance of bacterioplankton and water oxygen saturation ($r = -0.64$, $p < 0.05$) was observed. At the same time, the average bacterial abundance in the waters with oxygen saturation of less than 95% was $614 \pm 128 \times 10^3$ cells/mL. At its concentration of more than 98%, the average abundance of bacteria decreased to $303 \pm 98 \times 10^3$ cells/mL. This fact suggests the active participation of bacterioplankton in the remineralization of organic matter. Changes in the concentration of dissolved organic carbon and an increase in the share of the colloidal form in its composition also indicated the transformation of organic matter of autochthonous origin into a dissolved form on the near-bottom horizons of the seaward part of the transect [20]. Moreover, high rates of specific production of bacterioplankton (more than 1 day^{-1}) in the seaward part of the transect were associated with the areas of increased concentration of colloidal organic matter. On the contrary, the maximum values of HNA cells were associated with more oxygen-rich water layers ($r = 0.74$, $p < 0.05$), with an average value of $76 \pm 6\%$ versus $54 \pm 11\%$ in waters with oxygen saturation less than 95%. A negative correlation between the proportion of HNA cells and the total concentration of dissolved inorganic nitrogen ($r = -0.77$, $p < 0.05$) was also noted.

The value of bacterial production poorly correlated with the number of HNAcells ($r = 0.45$, $p < 0.05$), as well as with the concentration of chlorophyll "a" ($r = 0.42$, $p < 0.05$).

September 2013. The maximum values of dissolved oxygen were associated with the vertical front at the boundary of the transition from freshwater to 1 PSU salinity and indicated the increased activity of primary producers. The concentration of nutrients was quite conservative and, probably, did not serve as a limiting factor in the development of plankton. At the same time, a decrease in the content of nitrites in the surface layer towards the frontal zone could indicate their active consumption. Moreover, the near-bottom maximum of nitrites could indicate an incomplete process of organic matter oxidation.

The distribution pattern of the abundance of bacterioplankton is similar to the variability of the concentration of chlorophyll *a* ($r = 0.88$, $p < 0.05$). The bacterial abundance also decreases with an increase in the proportion of pheophytin ($r = -0.83$, $p < 0.05$) [3]. However, based on the available data, it is not possible to conclude whether the distribution of bacteria is affected by the concentration of primary producers, or both parameters are regulated by the same environmental factors. It should be noted that while the maximum abundance of bacterioplankton in the surface layer was observed at salinities less than 2.5 PSU, the region of the highest phytoplankton concentrations coincided with the area of the estuarine frontal zone, in which water salinity above the pycnocline increased from 2.5 to 7 PSU. With an increase in salinity to 9.5 PSU, a sharp (an order of magnitude) decrease in the abundance of phytoplankton was recorded [16]. At the same time, the abundance and production of bacterioplankton in the water layer above the pycnocline decreased only two times.

In comparison with the data obtained in autumn 2011 in the area of the estuary of the Yenisey River [9], the proportion of cells with active ETC in autumn 2013 in the Ob estuary was one order of magnitude lower. Moreover, in 2011, an increase in the proportion of actively respiring cells was reported in the seaward part of the transect compared to the river area. During our observations, the situation was the opposite. Nevertheless, an increase in the proportion of HNA cells towards the sea was noted in the area of the Ob estuary in July 2016. Thus, it can be concluded that there is no unified pattern of changes in the distribution of active bacterioplankton cells in the estuarine area.

During our studies in autumn 2013, the values of bacterioplankton production, as well as the abundance indicators, changed by more than an order of magnitude, with an increase in salinity [4]. At salinity values of less than 2.5 PSU, the average value of bacterial production was 26.8 ± 4.2 mgC m^{-3} day^{-1}. In brackish waters (3.5–22 PSU), the average value of this indicator was 10 ± 4 mgC m^{-3} day^{-1}. In waters with a salinity of above 29 PSU, the daily production of bacteria did not exceed 5 mgC m^{-3} day^{-1}, with an average value of 2.1 ± 0.9 mgC m^{-3} day^{-1}. Despite this, the values of specific production were higher at a salinity of more than 29 PSU, with an average value of 1.9 ± 0.5 day^{-1}, while in more freshened waters, its average value was lower (1.1 ± 0.4 day^{-1}) [4]. In the water layer located under the pycnocline, the production values decreased with a decrease in the concentration of dissolved oxygen ($r = 0.82$, $p < 0.05$), which, probably, indicated the active participation of bacterioplankton in the decomposition of organic matter.

The number of viral particles in the transect along the Ob estuary [4] decreased significantly in the water layer under the pycnocline. Its average value was $1794 \pm 448 \times 10^3$ particles/mL (changing from 915 to 2917×10^3 particles/mL) above the salinity gradient, and decreasing to $453 \pm 153 \times 10^3$ particles/mL (from 214 to 876×10^3 particles/mL) at greater depths. The maximum values of phage-infected bacterial cells were in the upper 10-m layer ($12 \pm 6\%$ of the total number of bacterioplankton). This indicator significantly decreased with depth ($3.5 \pm 0.6\%$). In the water layer below the pycnocline, the value of the specific growth rate of bacterioplankton decreased with an increase in the number of viral particles ($r = -0.66$, $p < 0.05$),

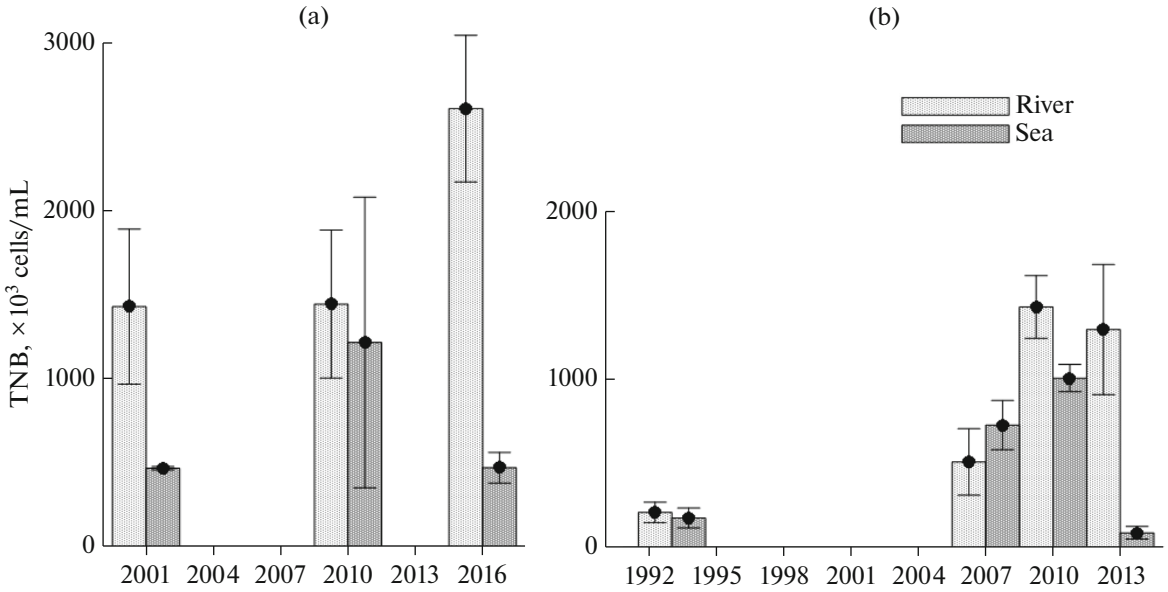

Fig. 5. Changes in total number of bacteria (average value for all stations) of surface layer in river and marine zones of estuarine area of Ob River in summer (a) and autumn (b).

which suggested the involvement of viral lysis in the regulation of the growth rate of bacteria [4].

Thus, despite the presence of correlations between the distribution of bacterioplankton and other biotic and abiotic parameters of the environment, the main factor affecting the distribution of bacterioplankton in the estuarine area was the distribution of river runoff, which was primarily marked by changes in salinity. Based on the available data, it was not possible to identify the exact mechanisms that determined this effect.

Interannual variability. Summarizing the currently published data on the distribution of bacterioplankton in the area of the Ob estuary in the summer–autumn period [8, 11, 13, 32], we can be confident only about a decrease in the abundance of bacteria towards the sea (Fig. 5). Interannual fluctuations in the abundance of bacteria were not explained by temperature changes or runoff volumes in the studied years [21, 39]. In autumn 1993, the abundance of bacterioplankton in the area of the Ob estuary was relatively low: 206×10^3 cells/mL in the river zone and 173×10^3 cells/mL in the shelf area [8]. At the end of summer 2001, the average abundance of bacterioplankton in the waters of the surface layer in the area of the Ob estuary was 1.4 million cells/mL in freshwater (<1 PSU), 2.1 million cells/mL in brackish water (5–15 PSU), and less than 0.5 million cells/mL in the marine (>20 psu) zone [32]. The average values of bacterial production also decreased in the direction of the sea, with average values of 7.14, 5.06, and 3.21 mgC m^{-3} day^{-1} for the freshwater, brackish water, and marine zone, respectively. For all zones, bacterioplankton was limited by the available carbon source [32]. In the autumn of 2007, the abundance of bacterioplankton in the area of the Ob estuary (in the layer above the pycnocline) in the samples stored for more than three months (potentially viable cells) was $505 \pm 197 \times 10^3$ cells/mL in the "river" part of the transect (salinity less than 5 PSU) and $723 \pm 146 \times 10^3$ cells/mL in the seaward part of the estuary [13]. In the water layer under the pycnocline, the total abundance of bacterioplankton decreased noticeably to $376 \pm 64 \times 10^3$ cells/mL. The specific growth rate of bacteria in the layer above the pycnocline was 0.54 day^{-1} in "river" waters versus 0.18 day^{-1} in the seaward part of the estuarine zone. In the water layer below the pycnoclines, the specific growth rate of bacteria tended to zero, slightly increasing to 0.06 day^{-1} in the bottom water layer [13]. In summer 2010, the indicators of the abundance of bacterioplankton in river and sea waters did not differ much: 1400 ± 440 and $1200 \pm 864 \times 10^3$ cells/mL, respectively [11]. In the autumn of the same year, a similar pattern was observed: the numbers of bacterioplankton in the "river" zone remained the same, while it slightly decreased to $1000 \pm 81 \times 10^3$ cells/mL in the marine part [11].

Based on these data, we may conclude that the main factor determining the distribution and activity of bacterioplankton in the estuary region of the Ob River was the distribution of river runoff, which was primarily marked by salinity. At the same time, it was not possible to identify the exact mechanism that played a key role in the regulation of these parameters, using the available data. Taking into account the discreteness of changes in the characteristics of bacterioplankton, which allowed the conditional differentiation between the riverine, brackish, and marine areas, we suggested the influence of geochemical processes occurring on certain salinity gradients.

CONCLUSIONS

Based on analysis of the literature data and our results, it should be noted that in most cases, the distribution of both the quantitative and productive characteristics of bacterioplankton in the estuarine area of the river is determined primarily by the distribution of river runoff, which is estimated based on salinity. At the same time, the boundaries of changes in the hydrochemical environmental characteristics, as well as the abundance and composition of different components of the plankton community, did not agree with each other during our observations. The comparison of the data obtained in the middle and at the end of the growing season indicated higher quantitative parameters for bacterioplankton in summer. Nevertheless, a similar pattern of the distribution of bacterioplankton abundance on the river–sea gradient was observed in both seasons. In the zone of the Ob estuary, it was almost always possible to distinguish the zones of freshened and sea waters that differed significantly in the considered parameters. In most cases, the characteristics of bacterioplankton changed discretely between them. However, if the abundance of bacteria decreased towards the sea in most cases, the indicators of the activity were less conservative. In summer, the proportion of cells with a high content of nucleic acids, as well as the value of specific production, increased towards the sea. In autumn, an opposite pattern was observed for the proportion of bacteria with active ETC. The correlation with other environmental factors was less clear. Probably, the search for the factors regulating interactions in the microplankton community requires the search and analysis of additional environmental characteristics, including geochemical processes at the river–sea boundary.

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