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MARINE BIOLOGY

Virio- and Bacterioplankton in the Estuary Zone of the Ob River and Adjacent Regions of the Kara Sea Shelf

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Abstract—The distribution of structural and functional characteristics of virioplankton in the north of the Ob River estuary and the adjacent Kara Sea shelf (between latitudes 71°44′44″ N and 73°45′24″ N) was studied with consideration of the spatial variations in the number (N_B) and productivity (P_B) of bacteria and water properties (temperature, salinity, density) by analyzing samples taken in September 2013. The number of plankton viruses (N_V), the occurrence of visible infected bacteria cells, virus-induced mortality of bacteria, and virioplankton production in the studied region varied within (214–2917) × 10^3 particles/mL, 0.3–5.6% of N_B, 2.2–64.4% of P_B, and (6–17248) × 10^3 particles/(mL day), respectively. These parameters were the highest in water layers with a temperature of +7.3–7.5°C, salinity of 3.75–5.41 psu, and conventional density (σ_τ) of 2.846–4.144. The number of bacterioplankton was (614–822) × 10^3 cells/mL, and the N_V/N_B ratio was 1.1–4.5. A large amount of virus particles were attached to bacterial cells and suspended matter. The data testify to the considerable role of viruses in controlling the number and production of heterotrophic bacterioplankton in the interaction zone of river and sea waters.

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INTRODUCTION

The estuary regions of large rivers play a leading role in regulating the effect of continental processes on sea ecosystems and on the transformation of allochthonous material carried by rivers [4, 15]. This is the case for the Kara Sea, which annually receives about 1100 km^3 of fresh water through the estuaries of the Ob and Yenisei rivers [25, 28].

In estuary zones, fresh water and sea water interact, and a unique high-gradient pelagic biotope is formed. Estuaries are the habitats of specific plankton communities, which play a large role in the ecological aspects of interaction between river and sea and in the formation of biological production. They are also an example of adaptation to a strongly variable pelagic medium [9].

Viruses are the most numerous components of freshwater and marine microbial communities, and virus lysis kills a considerable amount of heterotrophic bacteria, which play the main role in the destruction of organic matter [29, 32]. Studies of the ecology of viruses in different freshwater and marine Arctic ecosystems have shown that their role in controlling the number and productivity of heterotrophic bacteria is significant is some habitats [1–3, 18, 26, 27, 31]. Structural and functional characteristics of virioplankton in the mixing zone of fresh and sea waters in the Arctic Basin have not been comprehensively studied. In several works devoted to the ecology of viruses in river estuaries of temperate and subtropical latitudes, the high number of virioplankton and specific features of its distribution in the fresh- and seawater mixing zone are discussed [11, 12, 16, 22].

The aim of this work is to determine the parameters of the number, production, and structure of virioplankton and to evaluate the infection rate of bacteria by bacteriophages and the level of virus-induced mortality of bacterioplankton in the interaction zone of fresh and sea waters. We also formulate the problem of revealing the specific features of the spatial distribution pattern of the structural–functional parameters of plankton viruses with respect to biotic (number and production of bacterioplankton) and abiotic (water temperature, salinity, and density) factors.

MATERIALS AND METHODS

The investigations were performed on September 3–7, 2013, during cruise 125 of the R/V *Professor Shtokman* at stations 17 (71°44′44″ N, 72°47′27″ E, depth 20 m) and 19 (72°34′18″ N, 73°49′30″ E, depth 18.5 m) located in the mouth of Ob Bay and stations 25 (72°44′24″ N, 73°27′43″ E, depth 26 m), 4 (72°57′27″ N, 73°16′90″ E, depth 29.4 m), and 26 (73°45′24″ N, 72°54′65″ E, depth 30.1 m) located on the adjacent shelf of the Kara Sea.

The samples were taken from four to five horizons with 5 or 10 L Niskin bottles of a Rosette system (Sea Bird Equipment, United States) with CTD sounding with respect to the depth of the site and temperature, electric conductivity, and fluorescence data.

Water samples were fixed with a neutral formaldehyde solution (the final concentration in the sample was 1%) and kept in the dark at +4°C. The total number of bacteria was calculated under a Leica DM 5000V luminescent microscope at ×1000 magnification (the samples were preliminarily stained by DAPI fluorochrome (4,6-diamidino-2-phenylindol) [23]. Fresh bacterial biomass was calculated from the volume of bacterial cells, using the Image Scope Color (the analytic program of images). The bacterial biomass was calculated in carbon units according to the volumes of bacterial cells by the equation: fgC/cell = $133.754 \times V^{0.438}$, where fgC/cell is carbon content (femtogram) in a cell and V is cell volume, μm^3 [6].

The bacterial production and grazing of bacterioplankton by consumers were calculated by an approach that uses antibiotics [24], modified for natural habitats [30]. The approach is described in detail in [7].

Virus particles were calculated by epifluorescence microscopy using a SYBR Green I fluorochrome and Anodisc (Wathman) aluminum oxide filters with pores 0.02 μm in diameter [20]. Filters with viruses were analyzed under an Olympus BX51 (Japan) epifluorescence microscope at ×1000 magnification with a Cell-F analytic image system.

No less than 400 virus particles were calculated on each filter. The carbon content in one particle was taken as 0.055 fgC $virus^{-1}$ [26].

The frequency of visibly infected cells (FVIC) of heterotrophic bacteria, the share of the total number of bacteria, and the mean amount of mature phages in infected bacteria (Burst size (BS) particles/cell) were determined by electronic transmission microscopy. Viruses and bacteria were precipitated by centrifugation at 100000 g (35000 rpm) for 2 h with an OPTIMA L-90k (Beckman Coulter, United States) ultracentrifuge, on nickel grids with a density of 400 mesh covered by pioloform with a carbon coating. The grids were analyzed under a JEM 1011 (Jeol, Japan) electronic microscope at ×50000–150000 magnification. No less than 800 bacteria cells were calculated on each specimen. The frequency of all infected heterotrophic cells bacteria (frequency of infected cells (FIC), percentage of total heterotrophic bacteria) was calculated by the equation $FIC = 7.1FVIC - 22.5FVIC^2$ [13]. The viral-mediated mortality of bacteria (VMB) and the share of total mortality or production of bacterioplankton was determined by the equation $VMB = (FIC + 0.6FIC^2)/(1-1.2FIC)$ [13]. It is assumed that bacterial production is equal to the total mortality. The number of bacteria that died as a result of viral lysis (viral-induced mortality of bacteria, VIM), cells/(mL day) or mg C/(m^3 day) was calculated as VIM = VMB × P_B, where P_B is bacterioplankton production. We also determined virioplankton production (P_V, particles/(mL day)) by the equation P_V = BS × VIM, where BS is the number of viruses per one bacterial cell and VIM is given in cells/(mL day). The rotation period of the number of viruses was calculated by dividing their number by production. The amount of easily digestible organic matter of lysed bacterial cells (mg C/(m^3 day) supplied to the water medium as a result of viral lysis of bacterioplankton was determined as the difference between VIM and P_V. Our data were obviously somewhat overestimated, because the energy expenditure of viruses for the synthesis of capsid proteins and replication of nucleic acids were not taken into consideration. These data are still unknown.

The correlation between the parameters was determined with the Spearman range correlation coefficient for a significance level of 0.05. The range of mean data was evaluated by the error of mean.

RESULTS OF INVESTIGATION

The investigated area of the Ob River estuary was characterized by a very uneven distribution of physicochemical parameters (temperature, salinity, density, and turbidity) [5, 10] in the water column (Table 1). The low water salinity pointed to the effect of strong freshwater flow into the area of the studied stations.

In the interaction zone of the waters of the Ob River and the Kara Sea, the physicochemical water parameters varied widely (Table 1). The difference between the minimum and maximum data was tenfold for temperature, 85-fold for salinity, 18-fold for conventional density, and 38-fold for turbidity.

In the water column of the investigated region, the number (N_B) and biomass (B_B) of bacterioplankton varied significantly (Table 2). The difference between the minimum and maximum N_B and B_B was 43-fold and 65-fold, respectively. The mean volume of bacterial cells alternated from 0.014 to 0.042 μm^3 and averaged 0.027± 0.002 μm^3. Bacterioplankton production considerably differed with respect of the depth of the station (Table 2). The P/B coefficient varied within 0–2.87 day^{-1} for a mean of 1.50 ± 0.17 day^{-1}.

The mean of the N_B, B_B, and P_B parameters for the water column sharply dropped from the southernmost to the northernmost stations (17 and 26, respectively) (Fig. 1). Their values also decreased contrary to water salinity and parallel to water temperature (Fig. 2).

The variations in the amount of free viruses (N_V) were smaller: the minimum and maximum N_V differed 14 times and averaged (1033 ± 179) × 10^3 particles/mL (Table 3). The N_V/N_B ratio alternated from 0.6 to 21.9 for a mean of 4.3 ± 1.0. Positive correlations were revealed between N_V and N_B, between N_V and P_B, and between N_V and water temperature ($R = 0.54$, $p = 0.05$, $R = 0.75$, $p = 0.05$, and $R = 0.78$, $p = 0.05$, respec-

tively). There were also strong negative correlations of N_V with water salinity ($R = 0.78$, $p = 0.05$) and water turbidity ($R = 0.58$, $p = 0.05$).

The mean N_V for the water column differed more weakly as compared to bacterioplankton (Fig. 1). The N_V/N_B ratio rose from 1.3 to 3.1–4.5 in the direction from the south (station 17) to the north (stations 4 and 26). The variations between mean N_V for water layers with different salinity and temperature were more significant (Fig. 2).

The diameter of the capsid of free viruses (D_V) alternated from 16 to 367 nm. The minimum and maximum mean D_V calculated for water sample differed 1.6 times and averaged 76 ± 5 nm (Table 4). Viral particles with capsid diameter of 40–100 nm predominated virioplankton in all the water samples. In water layers with high salinity and low temperature, the share of viruses with capsid diameter <40 nm was higher contrary to the participation of viruses with capsid diameter >200 nm (Table 4).

The amount of bacteria with attached virus particles varied from 6×10^3 cells/mL (station 4, 15-m-deep horizon) to 255×10^3 cells/mL (station 25, 5-m-deep horizon) and averaged $(94 \pm 20) \times 10^3$ cells/mL, which was 8.9% of N_B (station 4, 27-m-deep horizon) and 38.9% of N_B (station 26, surface horizon) or 21.0 ± 1.6% of N_B on average. Each bacterial cell could carry up to 13 virus particles, which averaged 1.2–2.0 (1.5 ± 0.04 particles/cell). The minimum and maximum amount of viruses attached to bacterial cells (N_{VB}) differed 13 times and averaged $(165 \pm 35) \times 10^3$ cells/mL (Table 3). The N_{VB}/N_V ratio alternated from 1.0% of N_V (station 4, 15-m-deep layer) to 51.5% of N_V (station 4, 5-m-deep layer) and averaged 14.8 ± 2.8% of N_V. The N_{VB} and N_{VB}/N_V mean for the water column dropped from the south to the north: station 17—295×10^3 particles/mL (26.3%), station 19—257×10^3 particles/mL (13.6%), station 25—150×10^3 particles/mL (16.3%), station 4—99×10^3 particles/mL (6.3%), and station 26—142×10^3 particles/mL (12.3%). The highest mean N_{VB} was revealed in water with a salinity of 3.75–5.41 psu and temperature of +7.3–7.5°C, while the mean N_{VB}/N_V was typical of water with a salinity of 7.46–13.45 psu and temperature of +5.3–7.2 °C (Fig. 2).

The capsid diameter of viruses attached to bacterial cells varied from 22 to 175 nm (62 ± 1 nm on average). Bacteriophages, which attack bacteria, were predominated by virus particles with capsids <70 nm in diameter (67% of the total amount of viruses attached to bacteria). Large virus particles with a capsid diameter of 160–175 nm were attached to bacilli 0.5 × 0.25 μm in size.

The amount of virus particles (N_{VP}) attached to suspended particles from 190 to 6387 nm in size varied widely and averaged $(142 \pm 329) \times 10^3$ particles/mL (Table 3). The N_{VP}/N_V ratio alternated from 1.9% to 22.2% (14.0 ± 3.4% on average). Up to 56 viruses were attached to one suspended particle, which averaged 1.5–12.4 for the water sample (3.8 ± 0.6 viruses/particle on average). The mean for water column N_{VP} and N_{VP}/N_V decreased from the south to the north: 232×10^3 particles/mL at station 17 (19.0%), 147×10^3 particles/mL (13.1%) at station 19, 124×10^3 particles/mL (14.0%) at station 25, 83×10^3 particles/mL (9.3%) at station 4, and 34×10^3 particles/mL (3.6%) at station 26. The highest mean N_{VP} and N_{VP}/N_V were seen in the freshest water (Fig. 2). The capsid diameter of viruses attached to suspension particles was 40–200 nm (90 nm on average).

The infection rate of bacteria by bacteriophages differed between various depths at the same station and between the stations (Table 5). For the water samples, the mean FVIC was 1.0 ± 0.3%. The FVIC values were the highest in water layers with a relatively high bacterial concentration and water temperature, and they were the lowest in bottom water layers with a small bacterioplankton concentration, low water temperature, and high salinity (Tables 1, 4). There was a positive FVIC correlation with the number of bacteria

Table 1. Temperature (T), salinity (S), conventional compactness (σ_τ), and conventional turbidity (TUR) of water

Station	Depth, m	T, °C	S, psu	σ_τ	TUR, ftu
17	0	+8.137	0.385	0.148	487
	11	+6.813	7.456	5.800	1240
	15	+5.287	13.449	10.616	1492
	18	+5.303	13.403	10.579	2452
19	0	+7.360	3.746	2.846	294
	5	+7.199	11.156	8.676	174
	12	–0.577	29.500	23.687	1558
	16	–0.613	29.619	23.784	1632
25	0	+7.318	4.090	3.120	363
	5	+7.315	4.135	3.156	378
	12	–1.132	31.081	24.981	1030
	23	–1.219	31.464	25.294	2350
4	5	+7.526	5.410	4.144	241
	10	–0.734	30.290	24.330	221
	15	–1.463	32.365	26.031	537
	20	–1.545	32.669	26.280	704
	27	–1.553	32.706	26.310	978
26	0	+6.768	11.062	8.636	72
	12	+6.445	11.916	9.331	65
	20	–1.498	32.501	26.142	2451
	27	–1.519	32.655	26.268	2450

Table 2. Number (N_B), biomass (B_B), and daily production (P_B) of bacterioplankton

Station	Horizon, m	N_B, 10^3 cells/mL	B_B, mg C/m^3	P_B, 10^3 cells/mL	P_B, mg C/m^3
17	0	995	23.0	1065	24.6
	11	1102	25.9	1234	28.9
	15	489	10.0	621	12.7
	18	1247	22.4	0	0
19	0	797	11.9	606	9.1
	5	596	12.8	584	12.5
	12	222	2.2	493	4.9
	16.5	153	1.9	153	1.9
25	0	614	7.0	883	10.1
	5	822	10.4	123	1.6
	12	74	0.8	167	1.8
	23	49	0.4	123	1.0
4	5	734	10.7	1395	20.3
	10	204	2.5	212	2.6
	15	40	0.6	27	0.4
	20	29	0.4	73	1.0
	27	74	1.5	212	4.3
26	0	471	6.6	805	11.3
	12	433	7.0	784	12.7
	20	97	0.9	226	2.1
	27	66	0.6	121	1.1

($R = 0.34$, $p = 0.05$), bacterial production ($R = 0.58$, $p = 0.05$), the share of bacteria with attached viruses in the total number of bacterioplankton ($R = 0.60$, $p = 0.05$), the amount of viruses attached to bacterial cells ($R = 0.68$, $p = 0.05$), and water temperature ($R = 0.47$, $p = 0.05$); there was a negative FVIC correlation with water salinity ($R = -0.46$, $p = 0.05$), water density ($R = -0.46$, $p = 0.05$), and water turbidity ($R = -0.43$, $p = 0.05$). The mean FVIC values for the water column were higher at stations 25 (1.1% of N_B) and 4 (1.5% of N_B) then at the other stations (0.7–0.8% of N_B). The mean frequency of infected bacterial cells (FIC) for water samples was 6.7 ± 1.5% of N_B. The regularities of the spatial distribution of FIC in the studied region are similar to those of FVIC (Table 5). The mean FVIC and FIC in water layers with a salinity of 3.75–5.41 psu and temperature of +7.3–7.5 °C were 2.8–5.0 times higher than those in other water layers (Fig. 2).

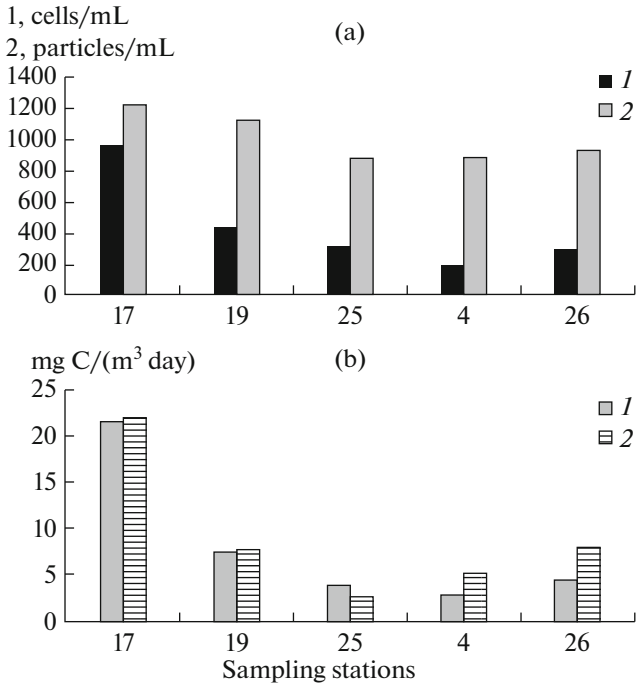

Fig. 1. Distribution of means for water column: bacterioplankton number (*1*—N_B, cells/mL) and virioplankton number (*2*—N_V, particles/mL) (a) and biomass (*1*—B_B, mg C/m^3) and production (*2*—P_B, mg C/(m^3 day)) of bacterioplankton (b) at the investigated stations.

The share of infected bacterial cells of various morphology differed. The total amount of virus-infected cells included bacilli (53.8 ± 5.8%), cocci (27.9 ± 5.4%), vibrios (16.5 ± 3.8%), and filamentous bacteria (1.8 ± 0.7%).

The amount of phages in infected bacterial cells (BS) (mean for water sample) significantly differed at various stations and depths and averaged 13.3 ± 2.3 particles/cell (Table 5). The maximum and mean BS for all the infected bacteria were 25 (8.9 ± 1.8) particles/cell (station 17), 131 (17.0 ± 7.9) particles/cell (station 19), 41 (11.0 ± 3.5) particles/cell (station 25), 101 (19.0 ± 6.5) particles/cell (station 4), and 14 (9.1 ± 0.8) particles/cell (station 26). The number of phages in bacteria cells living in water with a salinity of 3.75–

5.41 psu and temperature of +7.3–7.5°C was twice as high as BS in other waters (Fig. 2).

The capsid diameter of viruses inside bacterial cells varied from 18 to 81 nm (35 ± 1 nm). Among intracellular bacteriophages, those with a capsid diameter less than 70 nm constituted 94% of their total amount.

The variation in VMB was also considerable: 29-fold between the maximum and minimum parameters and 9.0 ± 3.0% of P_B on average for all water samples (Table 5). The average bacterial mortality for the water column as a result of virus lysis was higher at stations 25 and 4 (9.4 and 15.5% of P_B, respectively) compared to the other stations (5.0–6.2% of P_B). The mean VMB in water layers with different salinity and temperature varied significantly and were the highest for a water salinity of 3.75–5.41 psu and temperature of +7.3–7.5 °C (Fig. 2).

In general, bacteriophages lysed from 1×10^3 particles/mL or 0.01 mg C/m^3 (station 4, 15-m horizon) to 898×10^3 particles/mL or 13.07 mg C/m^3 (station 4, 5-m horizon) or, on average, $73 \pm 44 \times 10^3$ particles/mL or 1.12 ± 0.64 mg C/m^3. The majority of bacteria that underwent virus lysis were in water layers with a salinity of 3.75–5.41 psu and temperature of +7.3–7.5°C (Table 6).

The daytime virus production varied from 6×10^3 particles/mL (station 4, 15-m horizon) to $17\,248 \times 10^3$ particles/mL (station 4, 5-m horizon) and averaged $(1256 \pm 850) \times 10^3$ particles/mL. There was a positive correlation between the production of bacteria and viruses: $R = 0.55$, $p = 0.05$. The rotation period of the number of viruses varied less widely than that of their production: from 0.2 to 146 days (12.7 ± 7.1 days on average).

The greatest increase in the number of free viruses was seen in water layers with a salinity of 3.75–5.41 psu and temperature of +7.3–7.5°C (Table 6). The water with these parameters was also characterized by the smallest rotation period of the number of viruses (Table 6).

DISCUSSION

The abundance of virioplankton in the mixing zone of fresh water of the Ob River and saline water of the Kara Sea ($(1033 \pm 179) \times 10^3$ particles/mL) was lower

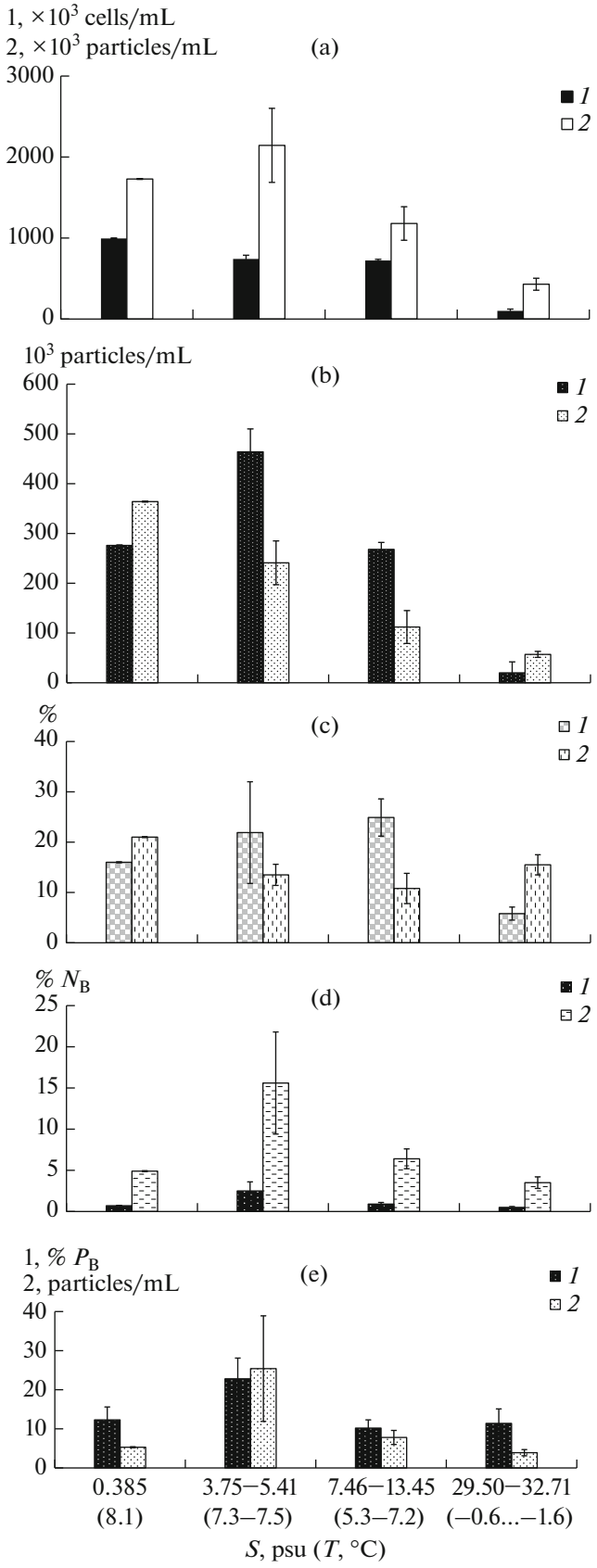

Fig. 2. Bacterial number (*1*—N_B, 10^3 cells/mL) and number of free viruses (*2*—N_V, 10^3 particles/mL) (a), number of viruses attached to bacterial cells (*1*—N_{VB}, 10^3 particles/mL) and to suspended particles (*2*—N_{VP}, 10^3 particles/mL) (b), shares of N_{VB} in N_V (*1*—N_{VB}/N_V, %) and of N_{VP} in N_V (*2*—N_{VP}/N_V, %) (c), frequency of visible (*1*—FVIC, % of N_B) and all infected (*2*—FIC, % of N_B) bacterial cells (d), virus-induced mortality of bacteria (*1*—VMB, % of P_B), and number of viruses inside bacterial cells (*2*—BS, particles/cell) (e) in water layers with various salinity (*S*, psu) and temperature (*T*, °C).

Table 3. Number of free viruses (N_V) and number of viruses attached to bacterial cells (N_{VB}) and suspended particles (N_{VP})

Station	Horizon, m	N_V, 10^3 particles/mL	N_V/N_B	N_{VB}, 10^3 particles/mL	N_{VP}, 10^3 particles/mL
17	0	1729	1.7	276	364
	11	1273	1.2	380	224
	15	498	1.0	150	87
	18	745	0.6	277	122
19	0	1992	2.5	281	368
	5	1921	3.2	311	186
	12	225	1.0	29	50
	16.5	340	2.2	33	50
25	0	2751	4.5	164	197
	5	915	1.1	471	169
	12	748	10.1	15	121
	23	214	4.4	13	46
4	5	2917	4.0	464	231
	10	432	2.1	47	91
	15	876	21.9	9	38
	20	253	8.7	9	49
	27	292	3.9	8	42
26	0	1447	3.1	326	28
	12	1198	2.8	166	28
	20	321	3.3	22	47
	27	597	9.0	14	36

Table 4. Capsid diameter of viruses (D) and shares of various size groups of virus particles of total number of free viruses in water layers of different salinity (S) and temperature (T)

S, psu	T, °C	D, nm	Shares, %					
			<40	40–60	60–100	100–150	150–200	>200
0.385	8.1	71 ± 4	3.4	50.0	32.8	10.3	1.7	1.7
3.746–5.410	7.3–7.5	90 ± 4	2.7 ± 0.9	20.1 ± 3.4	44.2 ± 5.2	23.7 ± 2.2	7.1 ± 2.4	2.2 ± 1.0
7.456–13.449	5.3–7.2	79 ± 5	7.5 ± 1.2	31.4 ± 2.9	40.4 ± 2.9	14.4 ± 2.9	4.1 ± 1.4	2.2 ± 1.3
29.500–32.706	–0.577–(–1.553)	77 ± 4	7.3 ± 2.3	30.9 ± 3.5	41.6 ± 2.9	15.6 ± 2.4	3.9 ± 1.2	0.7 ± 0.4

than N_V in shallow waters (at a depth of about 1 m) near the islands and Kara Sea coast ((11112 ± 2334) × 10^3 particles/mL), similar to N_V in the estuary zone of the Yenisei River ((975 ± 422) × 10^3 particles/mL), and higher than N_V in deep-water areas of the Kara Sea ((421 ± 50) × 10^3 particles/mL) [2, 3].

In the studied region, there was a positive correlation of the spatial distribution pattern of virioplankton with the distribution of the number of bacterioplankton and water temperature and a negative correlation with water salinity. Similar patterns were revealed for the estuary of the Charente River (France): a positive correlation of the number of virioplankton with the amount and production of bacterioplankton and water temperature and a negative correlation with water salinity [11]. Nevertheless, in the latter case, an increase in water salinity from 0.2 to 32.7 psu caused a drop in the ratio between the number of viruses and bacteria from 14.3 to 11.6. In our investigation, with an increase in water salinity from 0.385 to 29.50–32.71 psu, the N_V/N_B ratio increased from 1.7 to 6.6 ± 2.0.

Our studies have shown that the highest concentrations of plankton viruses were attributed to water layers with a salinity of 3.75–5.41 psu. A comprehensive study of the distribution pattern of phytoplankton in the Ob River estuary showed that the area of its highest concentrations coincided with the part of the frontal zone where the salinity of surface water layer above the piknocline varied from 2.5 to 7 psu. When water salinity increased to 9.5 psu, the amount of phytoplankton dropped sharply (by an order of magnitude; from 2.2–2.5 × 10^6 cells/L to 0.2–0.4 × 10^6 cells/L [8].

In the mixing zone of waters from the Ob River and the Kara Sea, the capsid diameter of free viruses (D_V) varied from 16 to 367 nm. In the estuary of the Charente River, this parameter alternated from 20 to 523 nm, but virioplankton was usually predominated by virus particles with a capsid diameter <65 nm (71 ± 5% of

Table 5. Frequency of visible infected bacterial cells (FVIC), frequency of infected bacterial cells (FIC), virus-induced mortality of bacteria (VMB), and the number of viruses inside bacterial cells (BS)

Station	Horizon, m	FVIC, % N_B	FIC, % N_B	VMB, % P_B	BS, phages/cell
17	0	0.7	4.9	5.3	12.3 ± 3.3
	11	0.7	4.9	5.3	5.0 ± 0.3
	15	0.5	3.5	3.7	6.5 ± 1.1
	18	0.7	4.9	5.3	11.7 ± 5.1
19	0	1.2	8.2	9.5	38.4 ± 16.6
	5	1.3	8.9	10.4	19.4 ± 8.1
	12	0.3	2.1	2.2	5
	16.5	0.3	2.1	2.2	5
25	0	0.7	4.9	5.3	14.3 ± 3.6
	5	2.5	16.4	22.3	19.3 ± 2.1
	12	0.7	4.9	5.3	4.7 ± 0.2
	23	0.5	3.5	3.7	5.5 ± 0.4
04	5	5.6	32.7	64.4	19.2 ± 4.01
	10	1.3	8.9	10.4	18.0 ± 9.0
	15	0.4	2.8	3.0	7.0 ± 0.9
	20	0.5	3.5	3.7	43.0 ± 20.0
	27	0.3	2.1	2.2	8
26	0	1.7	11.4	14.2	7.6 ± 1.2
	12	0.7	4.9	5.3	11.3 ± 1.0
	20	0.3	2.1	2.2	9
	27	0.5	3.5	3.7	8.5 ± 3.2

Table 6. Number of bacteria daily lysed by viruses (VIM), virus production (P_V) and rotation period of virioplankton number (t) in water layers with different salinity (S) and temperature (T).

Parameters		S, psu (T, °C)			
		0.385 (8.1)	3.75–5.41 (7.3–7.5)	7.46–13.45 (5.3–7.2)	29.50–32.71 (–0.6)–(–1.6)
VIM	10^3 particles/mL	56	27–898 / 258 ± 21	23–114 / 61 ± 15	1–22 / 7 ± 2
	mg C/m^3	1.30	0.36–13.07 / 3.71 ± 3.1	0.47–1.60 / 1.11 ± 0.22	0.01–0.27 / 0.08 ± 0.02
P_V, 10^3 particles/(mL day)		694	439–17248 / 5164 ± 405	150–1178 / 599 ± 187	6–403 / 78 ± 37
t_V, day		2.5	0.2–4.1 / 1.7 ± 0.8	1.6–3.9 / 2.6 ± 0.4	1.1–146 / 8.6 ± 2.2

the total number of virioplankton on average). Only sometimes were most (52–58%) represented by virus particles with a capsid diameter >65 nm. The group of small viruses was predominated (47–50%) by virus particles with a capsid diameter of 45–64 nm [12].

In the estuary of the Yangtze River, most plankton viruses were bacteriophages and only 5.4% of the total amount of virioplankton was represented by algae viruses [16]. Taking into consideration that in the investigated water area, the greatest size of the viruses that attack heterotrophic bacteria was 175 nm, the share of bacteriophage viruses in different water layers could be as high as 90.7–96.6% of the total amount of virioplankton.

Electron microscope studies have shown that in addition to free viruses, the water table of the investigated region contains a considerable amount of virus particles attached to bacterial cells and particles. Their total number was (47–695) × 10^3 ((289 ± 53) × 10^3, on average) particles/mL or 5.4–69.9% (29.0% ± 3.4 on average) of the amount of free viruses.

The number of virioplankton in natural waters depends on virus production and decay or removal from the water column [14, 19, 21]. Our data show that a significant amount of plankton viruses may be obviously removed from the water of the investigated region by heterotrophic nanoflagellates consuming bacteria with attached viruses, as well as by precipitation of large suspended particles with attached viruses.

The mean FVIC in the estuary of the Ob River was lower (1.0 ± 0.3%) than the FVIC in coastal waters near the islands and at the shore of the Kara Sea (1.6 ± 0.2% of N_B), but higher than the FVIC in the estuary zone of the Yenisei River (0.6 ± 0.1% of N_B) and in the deep-water region of the Kara Sea (0.5 ± 0.1% of N_B). Meanwhile, the maximum FVIC in the investigated region (5.6% of N_B) was the highest among the maximum data for other areas of the Kara Sea (1.2–4.3% of N_B) [2, 3].

In different Arctic habitats, virus-induced mortality of bacterioplankton (VMB) determined by the approach of transmission electronic microscopy significantly varied from <1% of P_B (in the Central Arctic) to 44.8% of P_B (in the coastal water of the Kara Sea) [1, 2, 18, 26, 27]. In our investigations, VMB alternated within 2.2–64.4% of P_B.

In the mixing zone of waters of the Ob River and Kara Sea, the lowest FVIC and VMB were typical of sea water with a temperature below zero, high salinity (29.50–32.71 psu) and density, low number of bacteria and production, and, as a consequence, a very low frequency of contacts between viruses and bacteria.

The largest percentage of virus-infected bacteria and their mortality as a result of virus lysis were seen in sea water diluted by fresh water (salinity of 3.75–5.41 psu). Virus production (P_V) and the number of bacteriophages (BS) were the greatest in this water. In the horizons with smaller (0.385 psu) or greater (7.46–13.45 psu) water salinity, FVIC, VMB, P_V, and BS were lower.

The mean rotation period of the amount of free viruses (t_V) for the mixing area of river and sea waters (12.7 ± 7.1 day) exceeded this parameter for the surface layer (0–25-m) of the deep-water area of the Kara Sea (32.8 ± 13.0 days) [3]. The minimum t_V was typical of the water layer with a salinity of 3.75–5.41 psu.

Part of the organic matter of lysed bacteria (VIM-P_V) enters water after virus lysis of their cells and the discharge of mature bacteriophages into the environment. This can be used again by active bacteria. Assuming that the ratio of bacterial production to their nutrition in the Kara Sea averages 0.27 [17], we calculated that the daytime need of bacterioplankton for organic matter (C_B) varied within 1.5–107.9 mg C/m^3 day with respect to the depth of the water layer (30.6 ± 7.0 mg C/m^3 day on average), and VIM-P_V alternated from 0.01 to 12.21 (1.06 ± 0.60 on average) mg C/m^3 day. As a result, the C_B/VIM-P_V ratio differed from 0.4% in bottom water to 16.2% in subsurface slightly saline water and averaged 3.4 ± 0.8%. Therefore, organic matter, which enters the water medium as a result of virus lysis of bacteria, could be a significant additional source of nutrition for bacterioplankton only in layers with a water salinity of 3.75–5.41 psu.

CONCLUSIONS

Our investigations have shown that the water interaction area of the Ob River and the Kara Sea is characterized by a very uneven spatial distribution pattern of the structural and functional parameters of virioplankton. The maximum number of viruses in surface desalinated water exceeded the minimum amount in the bottom water layer by an order of magnitude. In the study period, there was a positive correlation of the number of phytoplankton with the amount and production of bacterioplankton and water temperature, and a negative correlation with water salinity and density. In addition to free virus particles, a considerable number of viruses were attached to bacterial cells and suspended particles. In the studied waters of the Ob River estuary, the infection rate of bacteria, virus-induced mortality of bacterioplankton, and virus production were the highest (maximum for the Kara Sea) in water layers with a temperature of +7.3–7.5°C and salinity 3.75–5.41 psu.

In the study period, bacteriophage viruses played a considerable role in controlling the number and production of heterotrophic bacterioplankton.

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