ISSN 0001-4370, Oceanology, 2015, Vol. 55, No. 4, pp. 561–572. © Pleiades Publishing, Inc., 2015.
Original Russian Text © A.I. Kopylov, A.F. Sazhin, E.A. Zabotkina, N.D. Romanova, 2015, published in Okeanologiya, 2015, Vol. 55, No. 4, pp. 620–631.

MARINE BIOLOGY

Virioplankton in the Kara Sea: the Impact of Viruses on Mortality of Heterotrophic Bacteria

A. I. Kopylov[a], A. F. Sazhin[b], E. A. Zabotkina[a], and N. D. Romanova[b]

[a] *Papanin Institute for Biology of Inland Waters, Russian Academy of Sciences, Borok, Nekouzskii raion, Yaroslavl oblast, 152742 Russia*
e-mail: kopylov@ibiw.yaroslavl.ru

[b] *Shirshov` Institute of Oceanology, Russian Academy of Sciences, Nakhimovskii pr. 36, Moscow, 117997 Russia*

Received September 04, 2014

Abstract—Studies were conducted in shallow and deepwater areas of the Kara Sea. The abundance of bacteria (N_B) and the abundance of viruses (N_V) ranged within (19.4–2215.1) × 10^3 cells/ml and (97.6–5796.8) × 10^3 particles/ml, respectively. The virus to bacteria ratio varied from 1.4 to 29.1. A positive correlation was found between N_B and N_V ($R = 0.87$, $n = 45$, $p = 0.05$. Using electron transmission microscopy it was detected that the frequency of visibly infected cells of bacteria (FVIC) varied from 0.2 to 1.9% of N_B. The maximum values of FVIC were recorded in the estuary of the Yenisei River. The infected cells of bacteria contained from 4 to 127 (an average of 12) phages/cell of mature viruses. Virus-mediated mortality of bacteria was 0.5% and varied from 1.4 to 16.1% of the total mortality of bacterioplankton. This indicates a minor role of viruses in the control of overabundance and production of bacterioplankton in the Kara Sea during the surveyed period.

DOI: 10.1134/S0001437015040104

INTRODUCTION

Viruses can be found in all marine ecosystems and are the most abundant component in planktonic communities [19, 20, 23]. Viral lysis of planktonic heterotrophic bacteria can cause more than 60% of bacterioplankton mortality, considerably affecting the values of energy and carbon fluxes, as well as the composition of bacterial communities in marine ecosystems [7, 8, 20, 22]. Sparse studies on ecology of viruses in Arctic waters demonstrate that the abundance of planktonic viruses at high latitudes is two orders lower that at temperate latitudes [1, 9, 10, 12, 18]. Bacterial mortality caused by viral lysis was determined using electron transmission microscopy. It varies from fractions of a percent to 40% of bacterioplankton mortality in different regions of the Arctic [2, 12, 17, 18]. The information on the ecology of planktonic viruses in the Kara Sea is based only on the results of our studies of virioplankton in coastal regions of the sea [3]. All the above-mentioned facts determine the aim of our study, which is to assess the abundance, spatial distribution, and size structure of virioplankton, the number of bacterial cells infected by phage viruses, and the virus-induced mortality of bacterioplankton in the pelagial of the Kara Sea.

MATERIALS AND METHODS

The studies were conducted during the 59th cruise of the research vessel *Academician Mstislav Keldysh* in 2011. Water samples were collected at stations 5010, 5013–5021 along the mouth zone of the Yenisei River from September 17 to September 22; at stations 5032–5034, 5037, 5039–5042 (the transect through the eastern slope of the St. Anna Trough (ESSAT)) and at stations 5043–5048 (the transect through the western slope of the St. Anna Trough (WSSAT) on September 28–29 (Figure). Water samples were collected from 2–5 layers using 5-L and 10-L Niskin bottles incorporated into a "Rosette" complex equipped with a CTD-probe (Sea Bird Equipment, United States). The sampled water layers were determined after the analysis of the temperature, conductivity, and fluorescence.

For the analysis of the total abundance of bacteria water samples, 25 or 50 mL in volume were fixed immediately after sampling in a pH-neutral formaldehyde solution (the final concentration was 1%) and were stored in polystyrene bottles. The water samples were stored at 4°C in the dark. The total abundance of bacteria was counted using a Leica DM 5000B with ×1000 magnification. Bacterial cells were stained with DAPI prior to the analysis [14]. The bacterial wet biomass was calculated according to the cell volume by means of the ImageScopeColor software. The bacterial biomass in carbon units was recalculated according to the cell volume as fg C/cell = $133.754V^{0.438}$, where fg C/cell is the carbon (C) content per one cell, fg; and V is the cell volume, μm^3 [3].

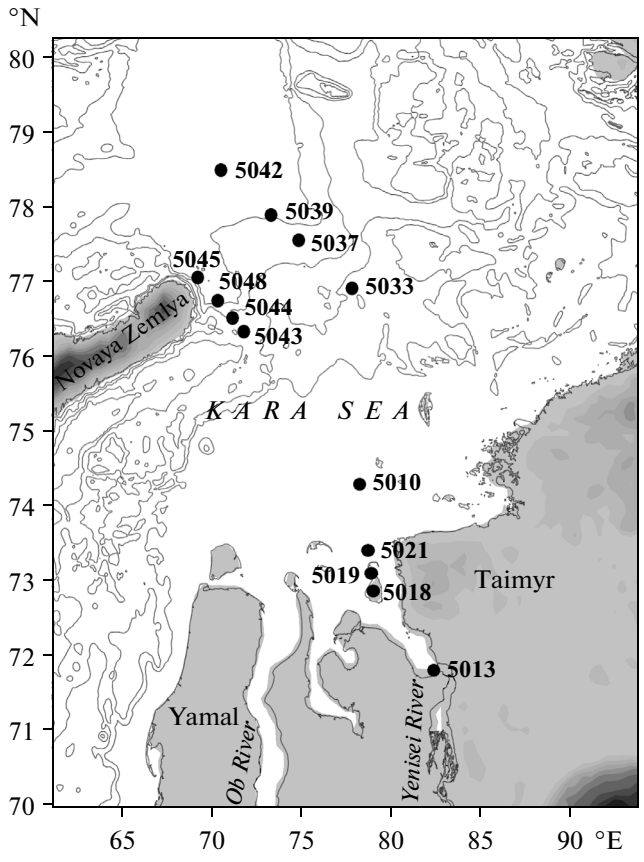

Diagram of station locations.

Bacterial production and bacterioplankton consumption by grazers were estimated according to the method suggested by Sherr et al. [16], using antibiotics in a modification for the natural environment [21]. Immediately after water samples were collected, they were poured into 75-mL sterile transparent polystyrene bottles; antibiotics were added to some of them. The bottles were placed into large-size mesh containers and were exposed for 8 hours in an aquarium (4.85 × 2.58 × 2.5 m, 31 m^3 in volume) with running seawater, located on the upper deck. The period of exposure was chosen based on our experimental data on antibiotic activity dynamics in arctic waters. The container with bottles was placed 1 m below the water surface. The grazing of nano- and microphages on bacteria was estimated using samples with antibiotics (benzylpenicillin, 1 mg/L; vancomycin, 200 mg/L), which stopped bacterial growth but had no influence on the grazers [16]. Samples without antibiotics were exposed as control. All the sample preparations were performed on the deck at an air temperature which was close to the temperature of the surface water layer. The method of the experiment is presented in detail in the work by Sazhin et al. [24].

Viral particles were enumerated by epifluorescence microscopy using SYBR Green I and Anodisc aluminum oxide filters (Wathman) with a pore diameter of 0.02 μm [13]. The total amount of counted viral particles was not less than 400 for each filter. The carbon content per one viral particle was taken as 0.055 fg $virus^{-1}$ [18].

Filters with bacteria and viruses were examined under a ×1000 Olympus BX51 (Japan) with a Cell-F image-analysis system.

The frequency of visibly infected cells (FVIC), % of the total bacterial number, and the burst size (*BS*), particles/cell, were determined by transmission electron microscopy. Viruses and bacteria were precipitated on 400-mesh pioloform-covered carbon-plated nickel grids by centrifugation at 100000 g (35000 rpm) for 2 hours using an OPTIMA L-90k ultracentrifuge (Beckman Coulter, United States). The grids were examined under a JEM 1100 (Jeol, Japan) electron microscope at magnification ×50000–150000. On each grid, not less than 800 bacterial cells were examined. The frequency of infected cells (FIC, % of the total number of heterotrophic bacteria) was calculated according to the equation $FIC = 7.1FCVI - 22.5FVIC^2$ [6]. The viral-mediated mortality of bacteria (VMB, %) was determined according to the formula $VMB = (FIC + 0.6FIC^2)/(1-1.2FIC)$ [6]. The abundance of bacterial population was assumed to be constant, i.e., bacterial production was equal to mortality. The rate of virus-induced mortality (VIM), cell/(mL day) or mg C/(m^3 day) was calculated according to the equation $VIM = VMB \times P_B$, where P_B is the bacterioplankton production. The virioplankton production (P_V, particles/(mL day) was calculated according to the equation $P_V = BS \times VIM$, where VIM, cell/(mL day). Virus turnover time was determined by the division of their number by their production. The amount of easily oxidizable organic matter released from lysed bacterial cells, mg C/(m^3 day) into aquatic environment was determined as the difference between VIM and P_V. These values are, apparently, overestimates, since energy consumption of viruses for the synthesis of capsid proteins and replication of nucleic acid was not accounted for. The data on these issues are not found or are absent in the literature.

The Spearman's rank coefficient was used to determine the correlation between parameters at a significance level of 0.05.

RESULTS

The abundance (N_B) and biomass (B_B) of bacterioplankton in the surveyed shallow regions of the Kara Sea were considerably higher than in deepwater regions (Table 1). The values of N_B and B_B were on

average in the water column 2068.6 ± 943.0 × 10^3 cell/mL or 41.27 ± 3.23 mg C/m^3 in the mouth of the Yenisei River, 278.4 ± 89.0 × 10^3 cell/mL (6.87 ± 1.22 mg C/m^3) in the estuary, 55.8 ± 11.2 × 10^3 cell/mL or 1.97 ± 0.58 mg C/m^3 on the eastern slope of the St. Anna Trough (ESSAT), and 94.8 ± 39.0 × 10^3 cell/mL (1.53 ± 0.45 mg C/m^3) on the western slope of the St. Anna Trough (WSSAT). A high positive correlation was determined between water temperature and N_B ($R = 0.77$, $p = 0.05$). Experimental estimations of bacterioplankton production demonstrated a high reproduction rate of microorganisms at the mouth of the Yenisei River (Table 1). In the larger estuarine zone the growth of bacteria was not detected in most of experiments. In the ESSAT region, a relatively high bacterial production was recorded only at station 5042, both on the surface layer and at depths of 100 and 461 m; in the near bottom layer it was 3.85–4.03 mg C/m^3 (Table 1). The specific bacterioplankton production (P/B) was maximal in the surface layer and near the bottom (2.08 and 2.24, respectively). At other stations in the region P_B was low (0.02–1.43 mg C/m^3) or almost zero. In the western part of the St. Anna Trough, bacterial production was reliably recorded at all stations and the layers under survey except station 5043, where it constituted 7.38 mg C/m^3 but its values were within the range 0.02–0.087 mg C/m^3. P/B varied from 0.13 to 1.31 (Table 1).

The abundance of virioplankton (N_V) in the water of the surveyed regions of the sea ranged from 97.6 × 10^3 particles/mL to 5796.8 × 10^3 particles/mL, averaging 856.6 ± 201.5 × 10^3 particles/mL (Table 2). The maximum values of N_V in the period under study were recorded at surface layers, and the minimum values were recorded at depths of 150 to 250 m. The abundance of viruses at depths lower than 300 m (water temperature below 0°C) was 1.2–2.1 times higher than in the upper layers. The ratio N_V/N_B varied from 1.4 to 29.1, averaging 7.1 ± 1.0 (Table 2). A high positive correlation was found between N_B and N_V ($R = 0.87$, $p = 0.05$).

The values of N_V averaged 4548.5 ± 816.2 × 10^3 particles/mL near the mouth of the Yenisei River and 974.8 ± 422.2 × 10^3 particles/mL in the estuary of the river. In the regions of the eastern and western slopes of the St. Anna Trough, N_V values were 374.2 ± 37.4 × 10^6 particles/mL and 479.6 ± 101.6 × 10^6 particles/mL, respectively. Average values of the N_V/N_B ratio were considerably smaller in shallow parts of the estuary of the Yenisei River (2.3 ± 0.5–3.0 ± 0.4) than in the deepwater parts of the eastern and western slopes of the St. Anna Trough (10.7 ± 2.0 and 7.9 ± 1.7, respectively).

The number of bacteria with viral particles attached to their cells (N_{BV}) varied from 1.6 × 10^3 to 330.5 ×10^3 cells/mL averaging 36.7 ± 11.8 × 10^3 cells/mL in the waters of the Kara Sea. The highest values were recorded in the region of the Yenisei River mouth. The portion of N_{BV} of the total bacterioplankton abundance (N_{BV}/N_B) varied in a narrower range (6.3–18.8%), averaging 12.7 ± 0.5%. The average values of N_{BV}/N_B in shallow parts of the Kara Sea (13.5–15.2%) were close to values in the deepwater regions (11.2–13.0%). From 1 to 30 viral particles were detected on the surface of one bacterial cell. The capsid diameter of attached viruses varied from 18 to 184 nm. The number of viruses attached to bacterial cells (N_{VB}) varied from 2.5 × 10^3 particles/mL to 469.6 × 10^3 particles/mL, averaging 54.2 ± 16.7 × 10^3 particles/mL. The N_{VB}/N_V varied within 0.5–33.7% and averaged 6.2 ± 0.9% (Table 2). A high positive correlation was found between N_V and N_{VB}: $R = 0.90$, $p = 0.05$. The average values of N_{VB} and N_{VB}/N_V were 428.2 ± 38.0 × 10^3 particles/mL and 9.8 ± 1.0%, respectively, at the mouth of the Yenisei River; 60.5 ± 20.6 × 10^3 particles/mL and 10.5 ± 2.2 in the Yenisei estuary; 9.3 ± 2.4 × 10^6 particles/mL and 3.1 ± 0.6% in the region of the eastern slope of the St. Anna Trough, and 17.0 ± 4.6 × 10^6 particles/mL and 4.7 ± 1.2% in waters of the western slope (Table 2).

Viruses without tail appendages predominate among virioplankton in all surveyed regions of the Kara Sea (Table 3). The proportion of such viruses and of viruses with short (to 62 nm) and long (to 560 nm) tail appendages in the total abundance of viruses averaged 79.7 ± 1.0%, 13.7 ± 1.0%, and 6.6 ± 0.7%, respectively, in all samples.

The diameter of capsids of detected viral particles varied from 18 to 389 nm. The average size of capsids of planktonic viruses varied within 31–89 nm and was on average 72 ± 2 nm (Table 4). In most cases, phages of 60–100 nm in size were the most numerous group in the composition of virioplankton (64% of the examined samples). Large phages of 200 to 289 nm in size were recorded in 20 water samples (44%), i.e., their size was comparable with the size of the smallest bacterial cells.

As the result, in all surveyed regions of the Kara Sea the contribution of viruses of different size groups 18–40, 40–60, 60–100, 100–150, 150–200 and viruses more than 200 nm to the total abundance of virioplankton was on average 11.9 ± 1.9%, 29.3 ± 1.7%, 41.4 ± 1.7%, 13.7 ± 1.1%, 2.7 ± 0.4%, and 1.0 ± 0.2%, respectively.

The frequency of visibly infected cells (FVIC), i.e., the portion of cells containing mature phage particles in the total abundance of bacterioplankton varied from 0.2 to 1.9% reaching maximum values in the Yenisei mouth

Table 1. Abundance ($N_B \times 10^3$ cells/mL), biomass (B_B mg C/m^3), and daily production (P_B) of bacterioplankton

No. of station	Horizon, m	T, °C	N_B	B_B	P_B		P/B
					10^3 cells/mL	mg C/m^3	
Mouth of the Yenisei River							
5013	5	9.6	2215.1	47.52	4829	103.60	2.18
	15	9.5	2098.2	33.52	3956	63.20	1.89
	28	9.5	1892.5	23.62	3674	45.86	1.94
Estuary of the Yenisei River							
5018	0	4.7	888.4	14.02	273	4.30	0.31
	8	3.6	99.0	3.39	0	0	0
	10	2.1	186.9	6.98	0	0	0
	20	0.2	121.6	5.06	0	0	0
5019	5	7.0	1045.0	10.93	0	0	0
	16	0.9	125.0	6.51	0	0	0
	25	–0.5	70.0	5.33	17	1.29	0.24
5021	2	5.5	440.0	16.66	0	0	0
	16	0.1	59.0	2.03	0	0	0
	23	–0.3	147.0	4.88	0	0	0
5010	5	4.8	142.8	5.42	0	0	0
	20	–0.6	136.4	2.36	0	0	0
	30	–1.4	157.8	5.73	0	0	0
Eastern slope of the St. Anna Trough							
5033	0	4.5	113.5	1.81	0	0	0
	9	4.9	140.2	8.97	0	0	0
	30	–0.4	19.4	0.74	0	0	0
	120	–1.1	28.1	1.69	0	0	0
5037	7	4.1	63.8	3.44	0	0	0
	25	1.7	21.1	0.82	37	1.43	1.74
	75	0.0	24.3	0.30	28	0.34	1.14
	315	–0.3	35.7	0.41	2	0.02	0.06
5039	0	3.4	58.7	1.23	31	0.64	0.52
	30	1.3	27.1	0.56	27	0.56	0.99
	75	0.5	29.2	0.51	8	0.13	0.26
	354	–0.3	27.6	0.98	0	0	0
5042	5	2.9	53.1	1.85	110	3.85	2.08
	25	0.9	36.3	0.74	0	0	0
	100	1.5	165.3	5.73	116	4.03	0.70
	461	–0.4	49.7	1.79	111	4.01	2.24
Western slope of the St. Anna Trough							
5043	0	5.3	549.2	6.35	638	7.38	1.16
5044	5	3.3	97.0	2.02	24	0.50	0.25
	20	3.5	29.8	0.60	39	0.78	1.31
	152	–0.3	44.0	0.95	20	0.43	0.45

Table 1. (Contd.)

No. of station	Horizon, m	T, °C	N_B	B_B	P_B		P/B
					10^3 cells/mL	mg C/m^3	
5045	0	3.6	80.9	2.66	22	0.72	0.27
	20	3.4	58.1	0.83	61	0.87	1.04
	100	1.4	27.4	0.53	1	0.02	0.04
	527	–0.5	40.5	0.83	17	0.35	0.41
5048	0	4.8	146.9	2.49	3	0.05	0.02
	20	3.4	45.9	0.77	10	0.17	0.22
	60	3.2	25.0	0.38	6	0.09	0.25
	170	–0.3	41.1	0.54	16	0.21	0.40
	241	–0.1	46.5	0.89	6	0.11	0.13

and averaging 1.5 ± 0.2%. In the estuary of the Yenisei River FVIC averaged 0.6 ± 0.1%, and it was similar in the regions of the eastern and western slopes of the St. Anna Trough (0.5 ± 0.1%) (Table 4). The diameter of the capsids of viral particles found in bacterial cells ranged from 15 to 167 nm. A weak positive correlation between FVIC and N_{BV}/N_B was found in all surveyed waters ($R = 0.26$, $p = 0.05$). The ratio of the number of bacteria containing distinctly visible viruses to the number of bacteria with viruses attached to the cell surface was 7.6–11.5% (on average 10.2 ± 1.3) in the part adjacent to the Yenisei mouth and 1.4–14.7% (on average 4.8 ± 0.5) in other surveyed regions. At the same time, a high positive correlation was found between FVIC and N_{VB} ($R = 0.65$, $p = 0.05$). Based on estimations of FVIC we calculated that the frequency of infected cells (FIC) in the total bacterioplankton abundance varied within 1.4–12.7% (on average 4.1 ± 0.4).

According to our data, heterotrophic bacteria of different morphologies were infected by viruses to a variable degree. The total number of cells infected by viruses comprised bacilli (54.7%), cocci (29.3%), vibrio (12.0%), and filamentous bacteria (4%).

The phage's burst size (*BS*) on average in a sample differed considerably at different stations and depths, and constituted 12 ± 2 phages/cell. In the surveyed regions the maximum and average *BS* for all infected bacteria were 75 and 13 ± 6 phages/cell in the Yenisei mouth, 69 and 14 ± 4 phages/cell in the estuary of the Yenisei River, 57 and 9 ± 2 phages/cell in the eastern slope of the St. Anna Trough, and 127 and 12 ± 3 phages/cell on the western slope of the St. Anna Trough.

Virus-induced mortality of bacterioplankton (*VMB*) during the surveyed period was low and averaged 4.5 ± 0.5% in all regions (Table 5). Thus, the average *VMB* in the part adjacent to the Yenisei mouth constituted 12.6 ± 1.9%, which considerably exceeds *VMB* in other surveyed water areas: 4.4 ± 0.7% in the estuary of the Yenisei, 3.7 ± 0.7% in waters of the ESSAT, and 4.0 ± 0.5% in the waters of the WSSAT.

The daily virus-induced mortality of bacteria, cells/(mL day) and mg C/(m^3 day) (VIM) and the viral production (P_V) were calculated in the water samples in which the bacterioplankton production was determined experimentally. The highest VIM values were recorded at the shallow mouth of the Yenisei River; the VIM values were much lower in the deepwater parts of the Kara Sea and decreased with depth (Table 6). As is known, some amount of organic matter enters the aquatic environment from bacterial cells as the result of viral lysis. Assuming that the ratio of bacterial production to their ratio in the waters of the Kara Sea averages 0.27 [11], we calculated that daily demand of bacterioplankton for organic matter (C_B) varies within 169.8–333.7 mg C/m^3 (on average 245.9 ± 47.7) at the mouth of the Yenisei River and ranges from 0.3 to 27.3 mg C/m^3 (on average 5.9 ± 1.1) in other surveyed regions. During the period of our studies in the Yenisei River, the amount of organic matter (entering the aquatic environment as the result of viral lysis of bacteria) which could be repeatedly used by microorganisms constituted 2.6–4.3% (on average 3.4 ± 0.5) of the daily demand of bacterioplankton for organic matter. In other regions of the Kara Sea the amount of repeatedly consumed organic matter constituted 0.4–3.4 (on average 1.2 ± 0.1)% C_B.

Thus, this additional source of nutrients was insignificant for bacterioplankton in the Kara Sea during the period under survey.

The maximum viral production (P_V) and the minimum turnover time of virioplankton abundance (T_V) were typical for waters adjacent to the mouth of the Yenisei (4158–12026 × 10^4 h/(mL day) and 0.4–

Table 2. Abundance of virioplankton (N_V), abundance of bacteria with attached viruses (N_{BV}), and abundance of viruses attached to bacteria (N_{VB})

No. of station	Horizon, m	N_V, $\times 10^3$ particles/mL	N_V/N_B	N_{BV}		N_{VB}	
				10^3 cells/mL	% N_B	10^3 h/mL	% N_V
Mouth of the Yenisei River							
5013	5	3013.5	1.4	293.5	13.2	352.2	11.7
	15	4835.1	2.3	330.5	15.8	462.7	9.6
	28	5796.8	3.1	313.1	16.5	469.6	8.1
Estuary of the Yenisei River							
5018	0	5484.4	6.2	131.9	14.8	184.7	33.7
	8	251.6	2.5	11.2	11.4	15.7	6.2
	10	485.9	2.6	29.1	15.6	68.0	14.0
	20	255.4	2.1	12.6	10.3	34.5	13.5
5019	5	2821.5	2.7	138.5	13.2	256.8	9.1
	16	254.2	2.0	16.1	12.8	22.5	8.8
	25	374.6	5.4	10.8	15.4	15.1	4.0
5021	2	871.9	2.0	37.6	8.5	48.9	5.6
	16	126.4	2.1	11.1	18.8	17.8	14.1
	23	409.8	2.8	19.5	13.3	23.4	5.7
5010	5	346.0	2.4	15.7	11.0	20.4	5.9
	20	547.3	4.0	19.8	14.5	29.7	5.4
	30	441.8	2.8	24.6	15.6	48.6	11.0
Eastern slope of the St. Anna Trough							
5033	0	449.1	4.0	17.8	15.7	26.7	5.9
	9	364.5	2.6	12.0	8.6	27.0	7.4
	30	565.0	29.1	3.1	16.2	4.3	0.8
	120	554.5	20.0	3.8	13.3	4.2	0.8
5037	7	253.7	4.8	6.4	10.0	7.0	4.6
	25	407.0	19.3	2.5	12.0	4.2	1.0
	75	428.5	17.6	2.7	11.2	3.5	0.8
	315	522.2	14.6	5.3	14.8	6.9	5.6
5039	0	173.8	3.0	4.8	8.1	7.7	4.4
	30	457.2	16.9	2.1	7.9	2.5	0.5
	75	145.8	5.0	2.8	9.5	3.6	2.5
	354	289.0	10.5	3.8	13.9	4.6	1.6
5042	5	226.9	4.3	4.2	8.0	5.9	2.6
	25	174.1	4.8	3.5	9.7	4.2	2.4
	100	371.9	2.3	22.7	13.7	31.8	8.6
	461	604.1	12.1	4.2	8.4	5.0	0.8
Western slope of the St. Anna Trough							
5043	0	1129.1	2.0	37.1	6.8	48.2	4.3
5044	5	314.7	3.2	15.6	16.1	23.4	7.4
	20	163.0	5.5	5.4	18.0	10.8	6.6
	152	97.6	2.2	3.1	7.0	4.0	4.1

Table 2. (Contd.)

No. of station	Horizon, m	N_V, $\times 10^3$ particles/mL	N_V/N_B	N_{BV}		N_{VB}	
				10^3 cells/mL	% N_B	10^3 h/mL	% N_V
5045	0	935.3	11.6	14.0	17.3	22.4	2.4
	20	675.2	11.6	9.0	15.4	14.4	2.1
	100	181.5	6.6	3.4	12.2	4.4	2.4
	527	385.6	9.5	4.2	10.4	6.3	1.6
5048	0	381.9	2.6	21.4	14.6	53.8	14.1
	20	1071.0	23.3	7.7	16.8	10.8	1.0
	60	220.9	8.8	1.6	6.3	3.2	1.4
	170	570.4	13.9	4.8	11.8	6.2	1.1
	241	108.2	2.3	7.4	16.0	13.6	12.6

Table 3. Portion (%) of viruses without tail appendages (1), with short (2) and long (3) tail appendages in total abundance of virioplankton

Region	1	2	3
Mouth the Yenisei River	74.6–76.7 average 75.4 ± 0.7	13.6–14.3 14.1 ± 0.2	8.9–11.4 10.5 ± 0.8
Estuary of the Yenisei River	69.8–89.4 average 79.6 ± 1.6	6.0–22.1 13.4 ± 1.6	2.7–12.2 6.9 ± 0.9
Eastern slope of the St. Anna Trough	66.7–90.9 average 80.7 ± 1.8	0–33.3 13.9 ± 2.0	0–16.7 5.4 ± 1.3
Western slope of the St. Anna Trough	67.6–89.4 average 80.4 ± 0.8	0–31.2 13.9 ± 2.5	0–20.0 5.7 ± 1.5

0.8 days, respectively). In deepwater regions the values of P_V differed greatly in different layers from 4.2–1294.8 × 10^4 h/(mL day) in surface layers to 1.8–35 × 10^4 h/(mL day) at depths more than 30 m. The time of turnover of the virioplankton abundance ranged from 22 hours to 179 days, respectively (Table 6).

DISCUSSION

The analysis of the data demonstrated that the number of planktonic viral particles at the mouth of the Yenisei River in September 2011 was higher than in other surveyed regions of the Kara Sea and was similar to the abundance of virioplankton in other arctic coastal waters (Table 7). The number of virioplankton in the deepwater parts of the Kara Sea corresponds to a lower range of values of the abundance of planktonic viruses in other deepwater regions of the Arctic (Table 7). According to our and published data there is a positive correlation between the abundance of viruses and bacteria [15, 17, 18]. The virus/bacteria ratio is smaller in shallow parts of the Kara Sea where the average abundance of virioplankton is higher than in its deepwater part (1.64 ± 0.53 × 10^6 and 0.42 ± 0.05 × 10^6 particles/mL, respectively). In the deepwater part the ratio is greater, i.e., $N_V/N_B = 2.9 \pm 0.3$ and 9.4 ± 1.3, respectively.

Viruses with a capsid diameter of 60–100 nm (64% of samples) and 40–60 nm (29% of samples) prevailed among the virioplankton of the Kara Sea. According to other publications, viral particles with the diameter of capsids in the range from 30–60 nm can dominate in marine waters [5, 20]. It should be noted that viral particles with the diameter of capsids more than 0.2 μm, i.e., with sizes similar to the size of small bacteria were detected in 20 of 45 analyzed samples. The

Table 4. Average diameter of the capsid of viral particle (S, nm) and the portion of viral particles with capsids of different size in total abundance of virioplankton (%)

No. of station	Horizon, m	S, nm	% of N_V					
			18–40	40–60	60–100	100–150	150–200	200–389
Mouth the Yenisei River								
5013	5	79 ± 5	7.5	26.4	49.1	9.4	1.9	5.7
	15	61 ± 3	17.7	46.9	27.8	6.3	0	1.3
	28	86 ± 4	7.6	18.1	43.7	22.9	6.7	1.0
Estuary of the Yenisei River								
5018	0	69 ± 5	7.7	31.4	43.6	12.8	2.6	1.9
	8	65 ± 5	20.0	33.3	33.3	13.4	0	0
	10	84 ± 7	12.9	25.8	30.6	21.0	6.5	3.2
	20	70 ± 4	12.5	25.0	52.1	10.4	0	0
5019	5	79 ± 7	8.5	36.6	36.6	12.7	2.8	2.8
	16	69 ± 3	1.2	40.9	48.1	7.4	2.4	0
	25	80 ± 9	4.1	13.5	56.7	23.0	2.7	0
5021	2	69 ± 3	9.3	40.8	36.4	9.3	3.4	0.8
	16	88 ± 6	6.1	15.2	42.3	30.3	6.1	0
	23	86 ± 4	4.3	12.5	37.5	31.9	9.7	4.1
5010	5	89 ± 3	4.3	10.6	53.8	25.9	5.4	0
	20	73 ± 4	23.5	19.8	39.5	11.1	4.9	1.2
	30	66 ± 3	27.6	29.0	32.9	7.9	2.6	0
Eastern slope of the St. Anna Trough								
5033	0	89 ± 9	0	22.2	51.9	14.8	7.4	3.7
	9	69 ± 4	6.0	46.0	38.0	10.0	0	0
	30	64 ± 4	6.0	42.0	48.0	4.0	0	0
	120	77 ± 4	3.6	28.6	44.6	19.6	3.6	0
5037	7	75 ± 10	10.7	35.7	42.9	7.1	0	3.6
	25	54 ± 2	20.0	48.9	31.1	0	0	0
	75	80 ± 4	4.3	19.1	53.2	21.3	2.1	0
	315	86 ± 10	6.4	28.7	44.6	14.9	4.3	1.1
5039	0	77 ± 4	6.4	12.8	65.9	12.8	2.1	0
	30	67 ± 7	11.0	38.9	38.9	5.6	5.6	0
	75	76 ± 4	5.6	33.3	36.1	23.6	0	1.4
	354	58 ± 8	40.0	33.4	13.3	13.3	0	0
5042	5	74 ± 5	5.6	29.6	49.9	13.0	0	1.9
	25	79 ± 7	6.7	25.0	53.3	13.3	1.7	0
	100	78 ± 11	13.5	32.4	29.7	20.7	0	3.7
	461	75 ± 7	5.3	21.1	52.5	15.8	5.3	0
Western slope of the St. Anna Trough								
5043	0	67 ± 3	0	50.0	43.8	6.2	0	0
5044	5	81 ± 8	14.6	18.4	36.6	19.5	7.3	3.6
	20	77 ± 8	9.1	31.8	36.4	13.6	9.1	0
	152	63 ± 15	42.9	0	42.8	14.3	0	0

Table 4. (Contd.)

No. of station	Horizon, m	S, nm	% of N_V					
			18–40	40–60	60–100	100–150	150–200	200–389
5045	0	70 ± 4	7.5	37.3	35.8	16.4	3.0	0
	20	60 ± 2	11.1	37.0	50.0	1.9	0	0
	100	70 ± 7	16.2	41.9	29.7	5.4	5.4	1.4
	527	72 ± 7	15.9	32.9	43.2	6.8	0	1.2
5048	0	88 ± 5	0	18.2	52.3	25.0	4.5	0
	20	73 ± 4	6.6	19.1	57.1	13.2	3.3	0.7
	60	67 ± 5	11.3	38.6	30.2	18.9	0	1.0
	170	31 ± 3	72.7	27.3	0	0	0	0
	241	69 ± 6	0	42.9	47.6	9.5	0	0

number of large capsids was 2–172 (on average 30 ± 10) × 10^3 particles/mL or 1.61–6.2 (on average 9.2 ± 1.0)% of the number of bacterioplankton. Thus, when enumerating microorganisms by epifluorescence microscopy, in some cases large viruses appear like small bacterial cells. Based on all available data the average diameter of viral capsids was 6.7 times smaller than the average diameter of a bacterial cell (72 ± 2 and 484 ± 16 nm, respectively).

The number of bacteria with viruses attached to the cell surface was 13.8 ± 0.7% of the bacterioplankton abundance in the shallow part of the Kara Sea and 11.5 ± 0.5% in its deepwater parts. Thus, bacteriophages attacked a rather large number of bacteria.

The number of bacterial cells containing visible viral particles (1.2–1.9% of the total abundance of bacteria) and virus-induced mortality of bacteria (9.5–16.1% of the total mortality) at the mouth of the Yenisei River were similar to the values which we obtained earlier in the coastal waters of the Kara Sea (1.6 ± 0.2% and 14.1 ± 2.6%, respectively) [2]. In other regions of the Arctic, the contribution of viruses to the total mortality of bacterioplankton was higher and constituted 9–37% in coastal waters of the Chuckchee and Bering seas [17] and 6–28% in the Baffin Sea [12]. In open parts of the water area in the Kara Sea FVIC (0.2–1.5%, on average 0.5%) and VMB (1.4–10.1%, on average 3.6%) were low.

The FVIC and VMB values obtained in the course of our studies were close to the values recorded in surface waters of the central Arctic—0–1.4% (on average 0.5%) and < 1.0–11.0% (on average 4.0%), respectively [18]. The contribution of viral lysis to the total mortality of bacterioplankton in deep waters in the northern part of the Chuckchee Sea varied from 2–16% averaging 9% [17]. In the period of our studies the number of mature bacteriophages in bacterial cells in the Kara Sea reached 127 (on average 12 ± 2) phages/cell, which was lower than in the central Arctic >200 (on average 35 ± 48) phages/cell [18]. The analysis of the size structure of attached and intracellular viruses has demonstrated that in the Kara Sea heterotrophic bacteria are infected by bacteriophage viruses with a capsid diameter of 15–184 nm.

The low percentage of virus-infected bacteria is caused by low abundance of virioplankton and the increase in water viscosity in cold arctic waters. This, in turn, determines the rate of contacts between viruses and hosts which is an order lower in the Arctic than in temperate waters. For example, such data are obtained for the Baffin Sea where virus-infected bacteria constitute 6–28% of the total abundance of microorganisms [18].

CONCLUSIONS

A high positive correlation was found between the abundance of virioplankton and bacterioplankton in the Kara Sea. In the surveyed period, the average size of capsids of viral particles was 7 times higher than the average size of bacteria. In addition to free viral particles, a large number of viruses were attached to bacterial cells. The portion of visibly infected bacterial cells in the total abundance of bacterioplankton in shallow and deepwater parts and at different stations and layers differed greatly. The viral infection of bacteria and virus-induced mortality of bacterioplankton was higher in the mouth of the Yenisei River than in other

Table 5. Frequency of visibly infected cells (FVIC, % of N_B), frequency of all infected cells of bacteria (FIC, % of N_B), bacterial mortality due to viral lysis (VMB, % of the total mortality), and burst sizes of mature bacteriophages inside infected cells (*BS*, phages/cell)

No. of station	Horizon, m	FVIC	FIC	VMB	*BS*
Mouth of the Yenisei River					
5013	5	1.5	10.1	12.3	7 ± 1
	15	1.2	8.2	9.5	32 ± 17
	28	1.9	12.7	16.1	17 ± 9
Estuary of the Yenisei River					
5018	0	1.2	8.2	9.5	9 ± 2
	8	0.6	4.2	4.5	45 ± 10
	10	0.6	4.2	4.5	8 ± 3
	20	0.2	1.4	1.4	7
5019	5	0.5	3.5	3.7	36 ± 19
	16	0.7	4.9	5.4	5 ± 0.5
	25	0.6	4.3	4.7	8 ± 1
5021	2	0.2	1.4	1.4	6
	16	1.0	6.9	7.8	35 ± 6
	23	0.5	3.5	3.7	8 ± 4
5010	5	0.5	3.5	3.7	6 ± 2
	20	0.2	1.4	1.4	5
	30	0.7	4.9	5.4	17 ± 8
Eastern slope of the St. Anna Trough					
5033	0	0.4	2.8	3.0	14 ± 10
	9	0.4	2.8	3.0	8 ± 4
	30	0.5	3.5	3.7	11 ± 5
	120	0.5	3.5	3.7	9 ± 5
5037	7	0.2	1.4	1.4	5
	25	1.5	10.1	12.3	35 ± 9
	75	0.3	2.1	2.2	9 ± 0.5
	315	0.6	4.2	4.5	10 ± 6
5039	0	0.3	2.1	2.2	6 ± 1
	30	0.4	2.8	3.0	7 ± 0.3
	75	0.4	2.8	3.0	9 ± 1
	354	0.9	6.2	7.0	7 ± 1
5042	5	0.8	5.5	6.1	8 ± 2
	25	0.2	1.4	1.4	7
	100	0.4	2.8	3.0	10 ± 3
	461	0.2	1/4	1/4	5
Western slope of the St. Anna Trough					
5043	0	1.0	6.9	7.8	26 ± 9
5044	5	0.4	2.8	3.0	8
	20	0.5	3.5	3.7	10 ± 2
	152	0.4	2.8	3.0	9
5045	0	0.4	2.8	3.0	18 ± 4
	20	0.7	4.9	5.4	48 ± 39
	100	0.2	1.4	1.4	7
	527	0.2	1.4	1.4	9
5048	0	0.4	2.8	3.0	14 ± 3
	20	0.5	3.5	3.7	25 ± 7
	60	0.8	5.5	6.1	8 ± 1
	170	0.8	5.5	6.1	17 ± 6
	241	0.5	3.5	3.7	8 ± 2

Table 6. The number of lysed bacteria (VIM), production of viruses ($P_V \times 10^4$ h/(mL day)) and the turnover time of viruses (T, day)

Depths, m; number of measurements, ( )	VIM		P_V	T
	10^3 cells/(mL day)	μm C/(m^3 day)		
Mouth of the Yenisei River				
0–28 (3)	375.8–594.0 average 520.4 ± 72.3	6004–12742 8710 ± 2055	4158–12026 8756 ± 2369	0.4–0.8 0.6 ± 0.1
Estuary of the Yenisei River and the St. Anna Trough				
0–25 (11)	0.4–49.8 average 8.7 ± 4.7	6–576 136 ± 54	4.2–1294.8 275.9 ± 83.2	0.9–107.1 32.8 ± 13.0
30–100 (5)	0.3–3.5 average 1.1 ± 0.6	4–121 31 ± 22	2.7–35.0 10.2 ± 6.2	10.6–93.3 61.3 ± 14.2
152–241 (3)	0.2–1.0 average 0.6 ± 0.2	4–13 10 ± 3.0	1.8–17.0 8.1 ± 4.6	18.1–60.1 37.2 ± 12.3
461–527 (2)	0.2–1.6 average 0.9	5–56 30	2.2–8.0 5.1	75.5–178.5 127.0

Table 7. Abundance of bacterioplankton ($N_B \times 10^5$ cells/mL) and abundance of virioplankton ($N_V \times 10^6$ particles/mL) in the surface waters of the Arctic

Area of studies	Month	N_B	N_V	N_V/N_B, %	Source
Coastal waters					
Beaufort Sea	05–08, 10	0.9–33.7	0.2–10.9	0.85–0.0	Clasen et al. [7]
Kara Sea	08–09	10.5–30.6	3.3–25.1	2.3–9.9	Kopylov et al. [2]
Kara Sea, mouth of the Yenisei River	09	18.92–22.15	3.01–5.80	1.4–3.1	Present study
Open waters					
Central Arctic	09–10	0.03–0.47	0.68–11.0	5–70	Steward et al. [18]
Barents sea	06–07	4.1–60.0	0.7–9.0	3 (average)	Howard-Jones et al. [9]
Barents sea	09	0.1–4.4	1.7–64.1	2.4–43.5	Venger et al. [1]
Chuckchee Sea	08	0.8–8.7	0.1–1.7	–	Hodges et al. [8]
Chuckchee Sea, northern part	08–09	2.1	2.5	–	Steward et al. [17]
Kara Sea, eastern part	09	0.6–10.4	0.1–5.4	2.0–6.2	Present study
Kara Sea, St. Anna Trough	09	0.25–.5	0.1–1.1	2.0–29.1	Present study

parts of the Kara Sea. In the deepwater part of the Kara Sea relatively high values of these parameters were characteristic both of surface and deep waters. Generally, bacteriophages played a minor role in the control over abundance and production of heterotrophic bacterioplankton in the period under survey.

ACKNOWLEDGMENTS

The work was supported by the Russian Foundation for Basic Research, projects nos. 14-04-00130a and 14-05-10055_K and the Russian Scientific Foundation, project no. 14-50-00095, processing of materials.

REFERENCES

1. M. P. Venger, T. I. Shirokolobova, P. R. Makarevich, and V. V. Vodop'yanova, "Viruses in the pelagic zone of the Barents Sea," Dokl. Biol. Sci. **446** (1), 306–309 (2012).
2. A. I. Kopylov, D. B. Kosolapov, E. A. Zabotkina, P. V. Boyarskii, V. N. Shumilkin, and N. A. Kuznetsov, "Planktonic viruses, heterotrophic bacteria, and

nanoflagellates in fresh and coastal marine waters of the Kara Sea Basin (the Arctic)," Inland Water Biol. **5** (3), 241–249 (2012).
3. N. D. Romanova and A. F. Sazhin, "Relationships between the cell volume and the carbon content of bacteria," Oceanology (Engl. Transl.) **50** (4), 522–530 (2010).
4. A. F. Sazhin, N. D. Romanova, and S. A. Mosharov, "Bacterial and primary production in the pelagic zone of the Kara Sea," Oceanology (Engl. Transl.) **50** (5), 759–765 (2010).
5. M. C. Alonso, G. F. Jimenez, T. Rodriguez, and J. J. Borrego, "Distribution of virus-like particles in an oligotrophic marine environment (Alboran Sea, Western Mediterranean)," Microb. Ecol. **42**, 407–415 (2001).
6. B. Binder, "Reconsidering the relationship between virally induced bacterial mortality and frequency of infected cells," Aquat. Microbial. Ecol. **18**, 207–215 (1999).
7. J. L. Clasen, S. M. Brigden, J. P. Payet, and C. A. Suttle, "Evidence that viral abundance across oceans and lakes is driven by different biological factors," Freshwater Biol. **53**, 1090–1100 (2008).
8. L. R. Hodges, N. Bano, J. T. Hollibaugh, and P. Yager, "Illustrating the importance of particulate organic matter to pelagic microbial abundance and community structure – an Arctic case study," Aquat. Microb. Ecol. **40**, 217–227 (2005).
9. M. H. Howard-Jones, V. D. Ballard, A. E. Allen, et al., "Distribution of bacterial biomass and activity in the marginal ice zone of the central Barents Sea during summer," J. Mar. Syst. **38**, 77–91 (2002).
10. R. Maranger, D. F. Bird, and S. K. Juniper, "Viral and bacterial dynamics in arctic sea ice during the spring algal bloom near Resolute, NWT, Canada," Mar. Ecol.: Progr. Ser. **111**, 121–127 (1884).
11. B. Meon and R. M. W. Amon, "Heterotrophic bacterial activity and fluxes of dissolved free amino acids and glucose in the Arctic rivers Ob, Yenisei, and the adjacent Kara Sea," Aquat. Microb. Ecol. **37**, 121–135 (2004).
12. M. Middelbore, T. G. Nielsen, and P. K. Biorsen, "Viral and bacterial production in the North Water in situ measurements batch-culture experiments and characterization of a viral-host system," Deep-Sea Res. **49**, 5063–5079 (2002).
13. R. T. Noble and J. A. Fuhrman, "Use of SYBR Green for rapid epifluorescence count of marine viruses and bacteria," Aquat. Microb. Ecol. **14**, 113–118 (1998).
14. K. G. Porter and Y. S. Feig, "The use DAPI for identifying and counting of aquatic microflora," Limnol. Oceanogr. **25** (5), 943–948 (1980).
15. C. Säwström, J. Laybourn-Parry, W. Graneli, and A. M. Anesio, "Heterotrophic bacterial and viral dynamics in Arctic freshwaters: results from a field study and nutrient-temperature manipulation experiments," Polar Biol. **30**, 1407–1415 (2007).
16. B. F. Sherr, E. B. Sherr, T. L. Andrew, R. D. Fallon, and S. Y. Newell, "Trophic interactions between heterotrophic Protozoa and bacterioplankton in estuarine water analyzed with selective metabolic inhibitors," Mar. Ecol.: Progr. Ser. **32**, 169–179 (1986).
17. G. F. Steward, D. C. Smith, and F. Azam, "Abundance and production of bacteria and viruses in the Bering and Chukchi seas," Mar. Ecol.: Progr. Ser. **131**, 287–300 (1996).
18. G. F. Steward, L. B. Fandino, J. T. Hollibaugh, T. E. Whitledge, and F. Azam, "Microbial biomass and viral infections of heterotrophic prokaryotes in the subsurface layer of the central Arctic Ocean," Deep Sea Res., Part I **54**, 1744–1757 (2007).
19. C. A. Suttle, "Viruses in the sea," Nature **437**, 356–361 (2005).
20. M. G. Weinbauer, "Ecology of prokaryotic viruses," FEMS Microbiol. Rev. **28** (2), 127–181 (2004).
21. T. Weisse, "The microbial loop in the Red Sea: dynamics of pelagic bacteria and heterotrophic nanoflagellates," Mar. Ecol.: Progr. Ser. **55**, 241–250 (1989).
22. L. E. Wells and J. W. Deming, "Significance of bacterivory and viral lysis in bottom waters of Franklin Bay, Canadian Arctic, during winter," Aquat. Microb. Ecol. **43**, 209–221 (2006).
23. K. E. Wommack and R. R. Colvell, "Viruses in aquatic ecosystems," Microbiol. Mol. Biol. Rev. **64**, 69–114 (2000).

Translated by N. Ruban