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EXPERIMENTAL ARTICLES

Virus Impact on Heterotrophic Bacterioplankton of Water Reservoirs

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Abstract—The quantitative distribution of viruses and their impact on heterotrophic bacterioplankton were studied in mesotrophic and eutrophic reservoirs of the Volga and Volga–Baltic waterway. The abundance of planktonic virus particles ranged from 9.4×10^6 to 120×10^6 ml^{-1} and was from 2.5 to 9 times greater than the bacterial numbers. Production of virioplankton varied from 2.1×10^6 to 132×10^6 particles (ml day)$^{-1}$ and the population turnover time values were between 0.3 and 11.6 days. The maximum values of numbers and production of virio- and bacterioplankton were observed in the eutrophic Ivan'kovo reservoir. Distribution of the viruses in the Volga reservoirs depended to a significant degree on the number and activity of heterotrophic bacterioplankton. The infected bacteria accounted for 5.5–33.5% of the total bacterial abundance. Phages were an important factor of bacterial mortality. During July to September virus-induced bacterial mortality varied between 6.1 and 40.6% (20.2% on average) of daily bacterioplankton production.

Keywords: quantitative distribution of viruses and bacterioplankton, viral lysis, bacterial mortality, lowland reservoirs.

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Studies performed during the last two decades demonstrated that viruses are constant, the most abundant, and versatile components of marine and freshwater bodies, which infects all groups of aquatic microorganisms, plants, and animals. This discovery changed significantly our perception of the structure and functioning of water ecosystems. The number of viral planktonic particles (virioplankton) in most water bodies was found to be in the range of 10^6–10^8 particles ml^{-1}, i.e., one order of magnitude higher than that of bacterioplankton. Most aquatic viruses turned out to be bacteriophages parasitizing on bacteria. Phages play an important role in controlling bacterial quantity and production, as well as in formation of the structure of bacterial communities, and thus influence significantly the biogeochemical cycles of carbon and other biogenic elements. Viruses are an important component of the microbial loop controlling the flows of matter and energy in the trophic nets of water bodies [1–6].

However, freshwater bacterioplankton is at present much less studied than that of seawater [7]. In the literature, there are data on the dynamics and function of viruses in a number of lakes [4, 5], but few data are available on virioplankton of water reservoirs, a widely occurring group of water bodies [8–12]. Water reservoirs are artificial water bodies in which the biological regime depends upon river water drain and a great number of abiotic factors connected directly or indirectly to the river regulation [13]. In particular, the differences between reservoirs and lakes include their shorter period of water exchange, considerable amplitude of water level alterations (several times higher than the seasonal alterations in lake water level), more distinct gradients of distribution of matter and hydrobionts caused by incoming river waters, and incomparably smaller age.

High values of virioplankton numbers, bacteria infection rate, and virus-induced bacterial death were detected in tropical reservoirs [10]. Studies of the seasonal dynamics of virus number and activity in the Rybinskoe (Russia) and Sep (France) mesotrophic reservoirs demonstrating that most bacteria (up to 40–60% of the daily bacterial production) died of virus infections revealed a positive correlation between virus number and water temperature, bacteria number, and production [11, 12]. In these reservoirs, virus-induced death of bacterioplankton was comparable to the rate of bacterioplankton consumption by protozoa. Experimental studies in the Rimov reservoir (Czech Republic) revealed that virus concentration and viral infection rate among bacteria correlated considerably with bacteria grazing, which allowed it to be assumed that grazing by protozoa stimulated viral activity [9]. Ecological studies of viruses in the reservoirs of Upper and Mid-Volga regions differing in morphometry, hydrological regime, and trophic status are limited to individual works [12]. Meanwhile, information on virus numbers and their contribution to the hydrobiont death rate is necessary for studying the trophic struc-

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ture and functional patterns of plankton communities in various water ecosystems, including reservoirs.

The aim of the present work was to evaluate the numbers and activity of planktonic viruses in seven reservoirs of the Volga–Baltic waterway differing in levels of phytoplankton primary production and anthropogenic influence. Specifically, the goals were (1) to study the spatial distribution of virioplankton and bacterioplankton, as well as the ratio of infected cells in the bacterial communities; (2) to determine the virus contribution to the death rate of heterotrophic bacteria; and (3) to reveal the importance of the relationship between viruses and other biotic and abiotic environmental factors.

MATERIALS AND METHODS

The studies were carried out in July to September 2005 in seven mesotrophic and eutrophic reservoirs of the Volga River and the Volga–Baltic waterway (below, the Volga reservoirs)—namely, in the Ivan'kovo (11 stations), Uglich (10 stations), Rybinsk (34 stations), and Gorky (16 stations) reservoirs; the upper part of the Cheboksary reservoir (4 stations); and the Sheksna (12 stations) and Novinki (1 station) reservoirs. Virus and bacteria counting was carried out in integrated water samples obtained by mixing of the samples collected at a depth increment of 1 m from the surface to the bottom. Immediately after collection, the samples were fixed with 2% glutaraldehyde, then stored at 4°C in the darkness, and treated within 1 month.

Planktonic virus particles were counted by epifluorescence microscopy using SYBR Green I fluorochrome and Anodisc (Watman) aluminum oxide filters with pores of 0.02 μm [14]. Heterotrophic bacteria were enumerated using DAPI as a fluorochrome and black nuclear filters with a pore diameter of 0.2 μm (Nucleopore) [15]. The samples were examined under 1000-fold magnification in an Olympus BX51 epifluorescence microscope (Japan) with image analysis software. On each filter, at least 400 viral or bacterial particles were counted in 10–20 fields of view and dimensions of at least 100 bacteria were measured. Bacterial volume was approximated as spheres, cylinders, or ellipsoids. Organic carbon in wet bacterial biomass was calculated according to the equation $C = 120 \times V^{0.72}$, where C stands for carbon content, fg C cell^{-1}, and V is cell volume, μm^3 [16]. The accepted value for carbon content in a single viral particle was 10^{-10} μg C [17].

The bacterial specific growth rate (μ, h^{-1}) was evaluated by the frequency of dividing cells (FDC) according to the formula $\ln\mu = 0.299 \times FDC - 4.961$ [18]. Dividing cells were counted in parallel with the total bacteria counts. At least 30 dividing cells were counted on each filter. Bacterial production was calculated as the bacterial number (or biomass) multiplied by the specific growth rate. Primary phytoplankton production was determined by the radiocarbon method [19].

To determine the frequency of visibly infected cells (FVIC, % of the total bacteria count) and the average number of mature phages in infected bacteria (burst size, BS, particle cell^{-1}), transmission electron microscopy was used. Bacterial cells containing five and more mature phages were considered infected. Viruses and bacteria were precipitated by centrifugation at 35000 *g* for 1 h using an OPTIMAL L-90k (Beckman Coulter) ultracentrifuge with a 45i rotor onto 400-mesh nickel grids for electron microscopy covered with Pioloform with carbon coating. The grids were inspected under a JEM 100C (Jeol, Japan) electron microscope at 50000–150000× magnification. On each grid, 1100–1200 bacteria were analyzed. The error for counting of the visibly infected bacterial cells was 25–63%.

Mature phages become clearly visible in the host cell only by the end of the latent period, immediately before cell lysis. To calculate the total frequency of infected cells (FIC, % of the total bacteria number), the equation $FIC = 7.1 \times FVIC - 22.5 \times FVIC^2$ was used [20]. Viral-mediated mortality of bacteria (VMB, %) was determined according to the formula $VMB = (FIC + 0.6 \times FIC_2)/(1 - 1.2\,FIC)$ [20]. The grazing rates for infected and noninfected bacteria were taken to be equal, and the latent period was assumed to be equal to the bacterial generation time [21]. The total bacterial population was considered constant. Virus-induced mortality (VIM, cell (ml day)$^{-1}$) was calculated using the equation $VIM = VMB \times P_{BAC}$, where P_{BAC} is bacterioplankton production, cell (ml day)$^{-1}$. Viral plankton production was estimated as BS multiplied by VIM [9, 22]. The viral and bacterial population turnover periods were calculated as their total numbers divided by production of either viruses or bacteria, respectively.

Statistical processing of the data was performed using the Statistica 6.0 software package. Spearmen's rank coefficient was used to establish the correlation dependences between the parameters at a 0.05 significance level.

RESULTS

During the period of studies in July to September, intense development of phytoplankton was observed in the Volga reservoirs. The reservoir sites under study differed significantly in water transparency and photosynthesis rate per both volume and square unit (Table 1). The minimum and maximum values of these parameters in each reservoir differed by 1.6–3.0, 1.9–12.0, and 1.7–7.6 times, respectively. The highest values of phytoplankton primary production were registered in the eutrophic Ivan'kovo reservoir and the lowest in the Novinki reservoir.

Table 1. Characteristics of the observation conditions and primary phytoplankton production in the reservoirs in August to September 2005

Reservoir	Date	D, m	Tr, cm	*T*, ×C	P_{PHY}	
					mg C $(m^3\ day)^{-1}$	mg C $(m^2\ day)^{-1}$
Ivan'kovo	August 24–26	3.0–16.0 / 8.8 ± 1.4	40–120 / 90 ± 8	18.3–27.8 / 20.6 ± 0.8	482–5414 / 1775 ± 925	1012–5685 / 2664 ± 1192
Uglich	August 22–24	4.0–19.0 / 10.8 ± 1.4	100–160 / 113 ± 6	19.9–23.0 / 20.5 ± 0.3	367–931 / 620 ± 98	920–1955 / 1426 ± 134
Rybinsk 1	July 20–31	5.5–16.5 / 11.2 ± 0.8	100–170 / 128 ± 5	19.6–23.0 / 22.2 ± 0.2	166–2005 / 834 ± 115	431–3294 / 1673 ± 210
Rybinsk 2	September 10–22	5.0–13.0 / 9.0 ± 0.6	100–190 / 122 ± 6	12.3–16.0 / 14.6 ± 0.3	57–194 / 140 ± 12	224–488 / 364 ± 24
Gorky	September 2–4	6.7–13.0 / 8.6 ± 0.5	90–150 / 114 ± 4	15.4–17.4 / 16.6 ± 0.2	173–989 / 522 ± 88	363–2077 / 1146 ± 164
Cheboksary	September 7	3.0–8.0 / 5.1 ± 1.1	90–140 / 112 ± 11	17.0–17.4 / 17.2 ± 0.1	253–489 / 358 ± 69	531–924 / 765 ± 119
Sheksna	August 3–9	4.0–13.0 / 6.4 ± 0.9	60–160 / 106 ± 9	20.5–21.7 / 21.1 ± 0.1	104–739 / 332 ± 54	276–1706 / 708 ± 111
Novinki	August 6	10.4	50	20.3	83	87

Notes: D, depth, Tr, transparency, T, water surface temperature; and P_{PHY}, phytoplankton production. Here and elsewhere, the range of the value variation is underlined; the average ± error value is below.

The bacterioplankton population fluctuated across the water bodies within the range of (3.2–31.0) × 10^6 cell ml^{-1} (Table 2). The range of variation of bacterial biomass was also significant, from 201 to 3782 mg ml^{-3}, i.e., from 53 to 818 mg C m^{-3}. The mean values of bacterial cell volume were within the range of 0.065–0.107 μm^3. The highest quantitative development of bacterioplankton was registered in the most productive Ivan'kovo reservoir. The number of planktonic viral particles was (9.4–120.3)× 10^6 particles ml^{-1}, 2.5–9.0 times higher than the number of bacteria (Table 2). The highest numbers of virioplankton were registered in the Ivan'kovo reservoir.

The values of the bacterial specific growth rate varied significantly between different regions of the same reservoir, as well as between the reservoirs (Table 3). The average turnover time for bacterioplankton (in September) varied from 1.8–1.9 days in the eutrophic Cheboksary and Ivan'kovo reservoirs to 2.9 days in the mesotrophic Rybinsk reservoir. The values of bacterial production varied significantly in different parts of the Volga reservoirs. The highest bacterioplankton productivity was registered in the Ivan'kovo reservoir and the lowest in the Rybinsk reservoir.

Virioplankton production was highest in the Ivan'kovo reservoir and lowest in the Rybinsk and Sheksna reservoirs (Table 4). The turnover time for viruses varied between different parts of the reservoirs in a wider range, from 0.3 to 11.6 days, than that for bacterioplankton. In the northernmost reservoirs (Rybinsk, Sheksna, and Novinki), the average values of turnover time for viruses were higher than those for bacteria, while, on the contrary, in more southern reservoirs they were lower.

During the period of the study, the numbers and production of virioplankton exhibited weak correlation with the primary phytoplankton production (Table 5). Weak negative correlation was established between the amount of viral particles and water turbidity. At the same time, a strong relation was revealed between the viral particle number and number, biomass, and production of heterotrophic bacteria.

Single infected bacterial cells were shown by means of transmission electron microscopy to contain up to 403 phage particles (PSs), although the sample-average amount typically did not exceed 100 particles $cell^{-1}$ (Table 4). The viruses were found to infect morphologically diverse heterotrophic bacteria: cocci, rods, vibrios, and filaments. In the Ivan'kovo reservoir, 41% of the rods and 45% of the vibrios, on average, were

Table 2. Water-column mean value of number (N_{BAC}), mean cell volume (V), biomass (B_{BAC}) of bacteria, and number of viruses (N_{VIR})

Reservoir	N_{BAC}, 10^6 cell ml^{-1}	V, μm^3	B_{BAC}		N_{VIR}, 10^6 particles ml^{-1}	$N_{VIR}/N_{ÂÀÑ}$
			mg/m^3	mg C m^{-3}		
Ivan'kovo	6.2–31.0 / 12.0 ± 2.2	0.077–0.122 / 0.107 ± 0.004	545–3782 / 1283 ± 283	132–818 / 288 ± 60	15.7–120.3 / 55.2 ± 9.9	2.5–7.0 / 4.5 ± 0.4
Uglich	5.4–15.3 / 10.2 ± 1.1	0.060–0.129 / 0.092 ± 0.008	389–1862 / 940 ± 144	98–397 / 220 ± 30	21.7–73.8 / 42.9 ± 5.3	3.1–5.0 / 4.2 ± 0.2
Rybinsk 1	3.2–6.7 / 4.7 ± 0.3	0.047–0.084 / 0.065 ± 0.003	201–445 / 302 ± 21	53–107 / 78 ± 5	14.7–45.9 / 27.1 ± 2.4	3.3–9.0 / 6.0 ± 0.5
Rybinsk 2	3.3–12.8 / 7.7 ± 0.3	0.055–0.079 / 0.065 ± 0.001	214–768 / 496 ± 42	65–202 / 127 ± 11	11.4–67.7 / 33.3 ± 3.6	2.6–6.6 / 4.4 ± 0.3
Gorky	4.4–13.6 / 9.9 ± 0.6	0.065–0.114 / 0.087 ± 0.003	370–1127 / 855 ± 57	89–259 / 203 ± 13	21.2–85.6 / 49.1 ± 4.7	3.2–6.9 / 4.9 ± 0.3
Cheboksary	7.7–9.8 / 8.8 ± 0.4	0.062–0.109 / 0.088 ± 0.010	560–952 / 763 ± 85	146–220 / 181 ± 15	26.3–36.6 / 30.6 ± 2.3	2.9–3.7 / 3.5 ± 0.2
Sheksna	3.5–8.0 / 6.2 ± 0.4	0.036–0.143 / 0.083 ± 0.008	231–1158 / 523 ± 79	59–240 / 125 ± 15	9.4–24.8 / 20.4 ± 1.3	2.5–5.0 / 3.4 ± 0.2
Novinki	6.2	0.098	608	140	23.2	3.7

infected; in the Uglich reservoir, 56% of the vibrios were infected; and in the other reservoirs, high rates of rod-shaped bacteria were infected (40–64%) (Fig. 1). The reservoir-average infection rates for cocci and filaments did not exceed 18 and 36%, respectively.

The frequency of virus-infected cells (FVIC)—the amount of bacterial cells containing mature phage particles—varied across the water area from 0.8% in the Sheksna reservoir to 5.8% in the Uglich reservoir (Table 6). The number of bacteria dying due to cell lysis varied significantly between parts of the reservoirs. The maximum rate of cell lysis (VIM, cell (ml day)$^{-1}$) exceeded the minimum value by 2.2–10.7 times and, when expressed in percent of the daily bacterioplankton production (VMB), by 2.8–6.2 times (Table 6). The average VIM value across all reservoirs was 793×10^3 cell (ml day)$^{-1}$ and the VMB mean value was 20.2%.

DISCUSSION

Viruses are the most abundant plankton component in the reservoirs of the Volga–Baltic waterway. During the period of studies in July to September, the numbers of viruses were within the range of values normally registered in mesotrophic and eutrophic water bodies. In the latter case, the virioplankton numbers are $1.0–39.0 \times 10^7$ particles ml^{-1}. The ratio between the numbers of planktonic viruses and bacteria (N_{VIR}/N_{BAC}) in the reservoirs fell within the range observed in other aquatic ecosystems (0.4–32) [4, 5, 7, 9, 23–25]. As a rule, higher values of the parameter imply higher ratios of infected cells and higher lysis rates of the bacterioplankton. However, in our studies, the reservoir-mean value $N_{VIR}/N_{BAC} = 4$ was lower than the value calculated for a number of Canadian lakes ($N_{VIR}/N_{BAC} = 20$) [23].

Among the studied reservoirs, the minimum values of virioplankton numbers and production were registered in the Novinki reservoir and the maximum ones in the Ivan'kovo reservoir (Table 4). The values of virioplankton production and number of the turnover period were close to those characteristic of most freshwater and marine ecosystems, $10^5–10^8$ particles (ml day)$^{-1}$ and 0.05–30 days, respectively [5, 7].

Virioplankton distribution in the Volga reservoirs was determined to a significant extent by the level of the quantitative development and activity of heterotrophic bacterioplankton, indicating that most of the viruses in the reservoirs are bacteriophages. Phages dominate in the virioplankton of many other aquatic

Table 3. Specific growth rate (μ), turnover period for bacterial number (T_{BAC}), and production (P_{BAC}) of bacterioplankton

Reservoir	μ, h^{-1}	T_{BAC}, day	$Ð_{BAC}$	
			10^6 cells $(ml\ day)^{-1}$	mg C $(m^3\ day)^{-1}$
Ivan'kovo	0.012–0.041 / 0.026 ± 0.003	1.0–3.4 / 1.9 ± 0.2	3.5–12.9 / 6.8 ± 1.0	81–320 / 160 ± 28
Uglich	0.012–0.031 / 0.021 ± 0.002	1.5–3.4 / 2.2 ± 0.2	1.6–7.8 / 5.0 ± 0.6	29–166 / 107 ± 14
Rybinsk 1	0.013–0.031 / 0.019 ± 0.001	1.3–3.2 / 2.3 ± 0.1	1.5–3.0 / 2.1 ± 0.1	24–54 / 34 ± 2
Rybinsk 2	0.010–0.023 / 0.015 ± 0.001	1.8–4.2 / 2.9 ± 0.2	0.9–4.8 / 2.9 ± 0.3	15–80 / 48 ± 5
Gorky	0.006–0.042 / 0.018 ± 0.002	1.0–6.5 / 2.7 ± 0.3	1.8–7.0 / 4.0 ± 0.3	38–132 / 83 ± 7
Cheboksary	0.021–0.032 / 0.025 ± 0.003	1.3–2.0 / 1.8 ± 0.2	4.3–5.9 / 5.2 ± 0.4	74–144 / 109 ± 17
Sheksna	0.013–0.025 / 0.018 ± 0.001	1.7–3.2 / 2.5 ± 0.2	1.4–4.5 / 2.6 ± 0.2	29–81 / 50 ± 5
Novinki	0.018	2.3	2.7	60

Table 4. Production (P_{VIR}), turnover period for the viral number (T_{VIR}), and average amount of mature phage particles in cells of heterotrophic bacteria (BS)

Reservoir	P_{VIR}, 10^6 particles $(ml\ day)^{-1}$	T_{VIR}, day	BS, particles $cell^{-1}$
Ivan'kovo	11.4–131.5 / 72.8 ± 16.1	0.3–2.8 / 0.9 ± 0.3	17–83 / 58 ± 9
Uglich	12.3–111.4 / 47.3 ± 10.0	0.3–2.0 / 1.2 ± 0.2	23–109 / 45 ± 9
Rybinsk 1	4.0–25.2 / 12.2 ± 1.6	1.0–11.6 / 3.5 ± 0.9	8–43 / 28 ± 2
Rybinsk 2	2.1–75.4 / 16.2 ± 3.9	0.9–7.7 / 3.2 ± 0.4	7–39 / 20 ± 2
Gorky	9.8–95.9 / 34.4 ± 6.9	0.4–6.8 / 2.5 ± 0.5	17–72 / 41 ± 4
Cheboksary	28.0–57.3 / 39.4 ± 6.7	0.6–1.1 / 0.8 ± 0.1	23–58 / 35 ± 8
Sheksna	2.3–35.8 / 11.3 ± 4.2	0.5–8.7 / 3.9 ± 0.8	12–47 / 23 ± 3
Novinki	7.7	3.0	24

ecosystems [5], in particular, in freshwater reservoirs situated in the moderate [11] and tropical [10] climate zones.

Analysis of virioplankton of lakes of different types revealed a strong correlation between the virus number and the trophic status of the water body [23]. In our study, in a number of regions of the Volga reservoirs, the ratio between bacterioplankton production and primary phytoplankton production calculated per square unit of the water body surface was 0.7–1.7, suggesting that phytoplankton production is not the only source of organic substrates for bacteria. Apparently, this is the reason why, in our work, unlike the results of lake ecosystem investigation, only a weak positive correlation was found between virioplankton number and the primary production of phytoplankton, i.e., the trophic status of the reservoir.

The mean virus biomass in the Volga reservoirs was 4.4 mg C m^{-3}, which corresponds to 2.3% bacterial biomass or 0.6% of the total biomass of the planktonic community (Fig. 2). The main contribution (52% on average) to the development of planktonic biomass was made by phytoplankton. The contribution of heterotrophic bacteria was almost half this, 28.2% on average. In the literature, there are reports of higher virioplankton biomass values than in the Volga reservoirs. For example, in the oligomesotrophic Sep reservoir (France), the virioplankton biomass reached 26 mg C m^{-3}, which was 25% of the bacterioplankton biomass [11]. However, when comparing these data with the results of the present work, one should take into account that the coefficient used in the calculations of virioplankton biomass by the authors of [11] was 22×10^{-10} μg C $particle^{-1}$ while we assumed the carbon content per virus particle of 10^{-10} μg C [17].

Infected cells (FIC) constituted 5.5–33.5% of the total heterotrophic bacterioplankton number in the Volga reservoirs (Table 6). The highest amount (2.7×10^6 cells ml^{-1}) was registered in the Uglich reservoir. Positive correlation was revealed between the number of viruses and the number of infected bacteria ($R = 0.75$, $F = 7.8$, $p < 0.05$). Analysis of the literature data shows that in most freshwater ecosystems phages infect from 5 to 25% bacteria, although in some lakes, especially in their anaerobic zones, the share of infected cells in the community may be higher [5, 7, 9, 24, 26]. For example, in an oligotrophic humified lake (Sweden) it reached 43% [27].

Our results demonstrate that bacteriophages are an important mortality factor for heterotrophic bacteria in the Volga reservoirs. From 6.1 to 40.6% of the daily bacterioplankton production was killed by viral infection in these aquatic ecosystems in August–September (Table 6). In some lakes virus-induced mortality of bacterioplankton during certain time periods reached 60–97% of its daily production [7, 24, 28]. Among the Volga reservoirs, the highest bacterial lysis by viruses was observed in the mesoeutrophic Rybinsk reservoir

Table 5. Characteristics of the correlation between the number (N_{VIR}) and production (P_{VIR}) of virioplankton and some characteristics of the water layer

Parameter	Units	N_{VIR}		P_{VIR}	
		r	*F*	*r*	*F*
Water transparency	cm	−0.21	3.7	−0.28	6.3
Phytoplankton production	mg C $(m^3\ day)^{-1}$	–	–	0.20	2.4
	mg C $(m^2\ day)^{-1}$	–	–	0.27	4.4
Bacterial number	10^6 cells ml^{-1}	0.85	223.6	0.55	36.0
Bacterial biomass	mg C m^{-3}	0.80	144.6	0.57	37.6
Bacterial production	cells $(ml\ day)^{-1}$	0.71	88.0	0.75	99.0
	mg C $(m^3\ day)^{-1}$	0.64	60.2	0.59	43.0

Note: *r*, pair correlation coefficient; *F*, Fisher criterion; $n = 88$; and $p = 0.05$. Dashes stand for no correlation.

Table 6. Frequency of visibly infected cells (FVIC), frequency of infected cells (FIC), virus-induced mortality of bacteria (VMB), and virus-induced lysis rate (VIM)

Reservoir	FVIC, % N_{BAC}	FIC, % N_{BAC}	VMB, % $Ð_{BAC}$	VIM, 10^3 cells $(ml\ day)^{-1}$
Ivan'kovo	1.2–3.6 / 2.1 ± 0.3	8.3–22.4 / 14.0 ± 2.0	10.5–34.8 / 19.1 ± 3.4	669–3059 / 1292 ± 320
Uglich	1.4–5.8 / 2.7 ± 0.4	9.4–33.5 / 17.3 ± 2.5	14.2–40.2 / 23.5 ± 3.0	372–2227 / 1122 ± 189
Rybinsk 1	1.0–3.3 / 2.4 ± 0.2	6.9–21.0 / 15.5 ± 1.0	7.8–31.6 / 21.2 ± 1.6	209–638 / 431 ± 35
Rybinsk 2	1.5–4.0 / 2.8 ± 0.2	9.5–24.8 / 17.5 ± 1.2	11.3–40.6 / 25.9 ± 2.4	212–1933 / 717 ± 116
Gorky	0.8–3.8 / 2.3 ± 0.2	5.5–23.7 / 15.1 ± 0.2	6.1–37.8 / 20.5 ± 2.3	196–2108 / 835 ± 124
Cheboksary	1.7–4.0 / 2.6 ± 0.5	11.4–24.8 / 16.8 ± 3.2	14.1–40.6 / 24.8 ± 6.0	832–1847 / 1215 ± 223
Sheksna	0.8–3.8 / 1.8 ± 0.3	5.5–23.7 / 11.7 ± 1.5	6.1–37.8 / 15.0 ± 2.4	161–1043 / 410 ± 88
Novinki	1.5	10.0	12.0	322

(25.9% on average) and in the eutrophic Cheboksary reservoir (24.8% on average) (Table 6). Averaged across all studied reservoirs, the value was 20.2% of bacterial production, which was close to the values registered in the oligomesotrophic Sep reservoir (21%) [11] and the mesotrophic Rimov reservoir (Czech Republic, 17%) [9]. On the whole, virus-induced bacterioplankton mortality in freshwater ecosystems was about 20% of its production [4, 5, 7]. A strong correlation was revealed between the reservoir-mean primary plankton production and the number of lysed bacteria, $R = 0.73$, $n = 7$, $F = 5.6$, and $p < 0.05$.

The average number of viral particles released during the lysis of a single heterotrophic bacteria (BS) was highest (58 particles $cell^{-1}$) in the most productive Ivan'kovo reservoir (Table 4). These results are in agreement with the previously published data on an increase in BS values with increase of the water trophicity level [4, 5, 7, 29]. Apparently, the reason for this is that, in productive ecosystems, the number of active and rapidly growing bacteria, which are preferentially targeted in the case of viral attack, is higher. Moreover, bacteria in eutrophic waters are usually larger than in oligotrophic ones.

Apart from inducing bacterioplankton death, the viruses also stimulate its development, since easily consumed organic substances are released into the environment along with the viruses during lysis of host cells. Carbon and other biogenic elements of the suspended organic matter (SOM) of the cell become soluble (dissolved organic matter (DOM)). These soluble compounds are actively metabolized by heterotrophic bacteria and thus remain in the planktonic microbial community, rather than reaching the higher levels of trophic nets; this phenomenon is termed the viral shunt. Moreover, bacterial mineralization of organic substances transforms nitrogen, phosphorus, silicon, and other biogenic elements into their mineral forms, which are easily available to phytoplankton [3, 5, 30].

The rate of viral lysis of heterotrophic bacteria varied between 7.1 in the Rybinsk reservoir (in July) to 29.1 mg C $(m^3\ day)^{-1}$ in the Ivan'kovo reservoir, with an average value of 15.5 mg C $(m^3\ day)^{-1}$ (Table 7). It was calculated that the processes of nucleic acid replication and synthesis of capsid proteins in bacteriophages consume 0.8–7.3 (3.0 in average) mg C $(m^3\ day)^{-1}$ of organic matter of the lysed bacteria. The rate of release of organic compounds into the water was 5.9–21.8 (12.5 in average) mg C $(m^3\ day)^{-1}$, i.e., 5.4–19.7% (11.2% on average) of the daily integral primary plankton production, the major source of organic matter in the reservoir pelagial (Fig. 3). The percentage was much higher (72.7%) in the Novinki reservoir due to low phytoplankton production resulting from the high content of mineral suspension and consequently low transparency of water.

Our studies showed that virioplankton is an important component of the microbial planktonic trophic nets of the Volga–Baltic waterway reservoirs. Viruses infect large amount of bacteria and cause mortality of

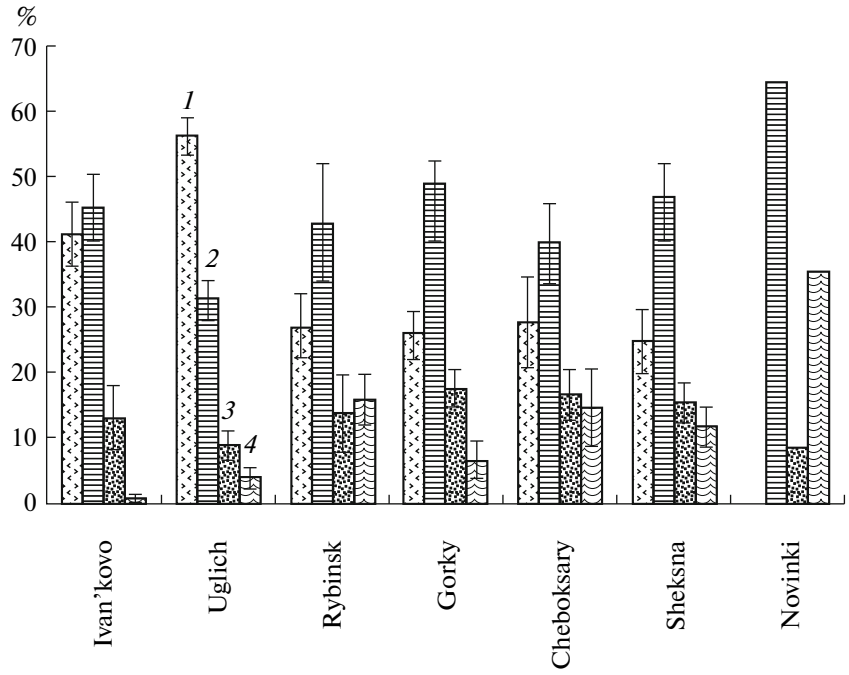

Fig. 1. Ratio of infected cells (%) among various morphological types of bacterioplankton of the reservoirs: vibrios (*1*), rods (*2*), cocci (*3*), and filaments (*4*).

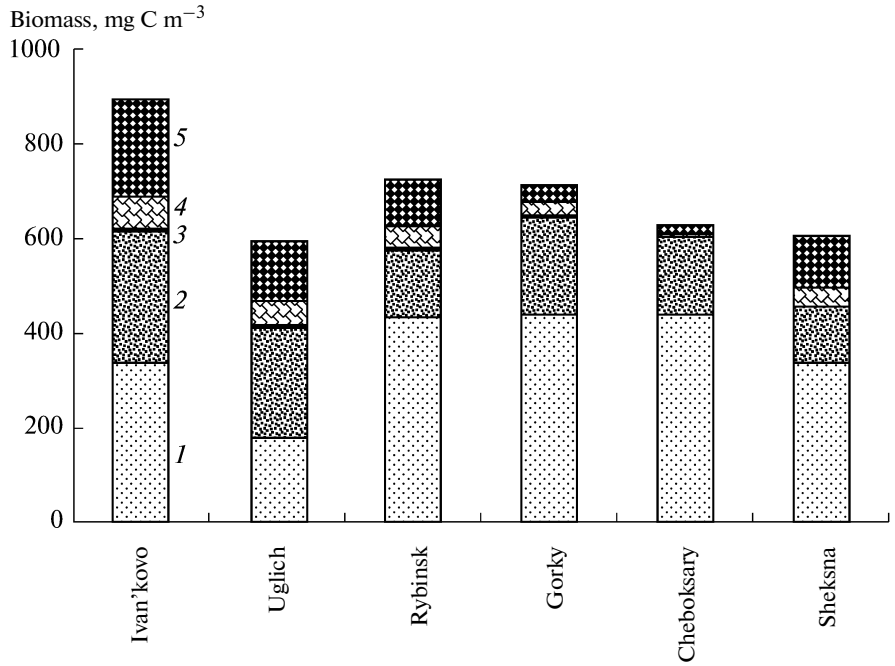

Fig. 2. Total plankton biomass of the reservoir and its contribution to the development of phytoplankton (*1*), bacteria (*2*), viruses (*3*), protozoa (*4*), and multicellular zooplankton (*5*).

Table 7. Virus-induced bacterial lysis rate (VIM); amount of organic substance of lysed bacteria used for synthesis of viral particles (C_{VIR}) and released into water (DOM = VIM – C_{VIR})

Reservoir	VIM	C_{VIR}	POB
	mg C (m^3 day)$^{-1}$		
Ivan'kovo	13.5–76.1 / 29.1 ± 8.3	1.1–13.2 / 7.3 ± 1.6	9.4–62.9 / 21.8 ± 7.0
Uglich	10.1–31.2 / 20.1 ± 2.6	1.4–9.5 / 4.3 ± 0.8	4.0–25.3 / 15.8 ± 2.2
Rybinsk 1	3.9–9.2 / 7.1 ± 0.6	0.4–2.5 / 1.21 ± 0.2	3.0–8.2 / 5.9 ± 0.5
Rybinsk 2	3.2–29.2 / 10.6 ± 1.4	0.2–7.5 / 1.6 ± 0.4	2.6–26.3 / 9.0 ± 0.6
Gorky	4.0–47.5 / 17.3 ± 2.7	0.7–9.6 / 3.4 ± 0.7	3.0–38.8 / 13.9 ± 2.2
Cheboksary	20.3–30.8 / 24.0 ± 2.4	2.8–6.1 / 4.4 ± 0.9	15.7–24.7 / 19.6 ± 2.0
Sheksna	2.9–19.2 / 8.8 ± 2.0	0.2–3.6 / 1.1 ± 0.4	2.6–18.3 / 7.7 ± 1.8
Novinki	7.2	0.8	6.4

a significant share of the bacterioplankton. At the same time, bacterial lysis results in the release of organic substances and compounds of biogenic elements stimulating the development of heterotrophic bacterioplankton and phytoplankton. Qualitative analysis of the processes evidences the importance of viruses in the carbon flow in the trophic nets of the reservoirs under study. Therefore, it is extremely important to take viruses into account when analyzing the structure and functioning of aquatic communities.

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REFERENCES

1. Bergh, O., Borsheim, K.Y., Bratbak, G., and Heldal, M., High Abundance of Viruses Found in Aquatic Environments, *Nature*, 1989, vol. 340, pp. 467–468.

2. Fuhrman, J.A., Marine Viruses and Their Biogeochemical and Ecological Effects, *Nature*, 1999, vol. 399, pp. 541–548.

3. Wilhelm, S.W. and Suttle, S.A., Viruses and Nutrient Cycling in the Sea, *BioScience*, 1999, vol. 49, pp. 781–788.

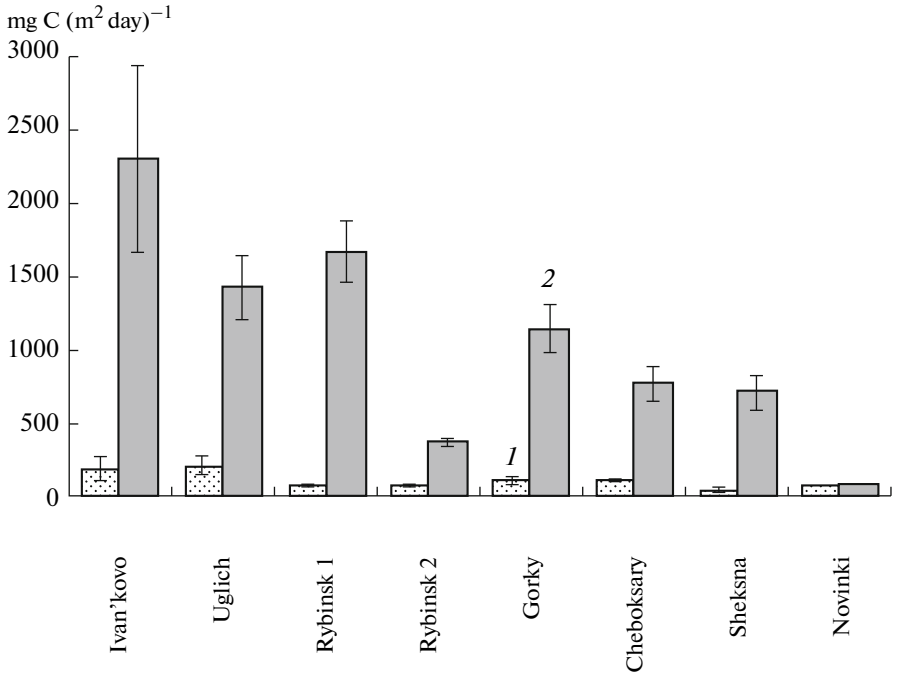

Fig. 3. Rate of organic substance release from lysed bacterial cells (*1*) and primary phytoplankton production (*2*).

4. Wommack, K.E. and Colwell, R.R., Virioplankton: Viruses in Aquatic Ecosystems, *Microbiol. Mol. Biol. Rev.*, 2000, vol. 64, pp. 69–114.

5. Weinbauer, M.G., Ecology of Prokaryotic Viruses, *FEMS Microbiol. Rev.,* 2004, vol. 28, pp. 127–181.

6. Suttle, C.A., Marine Viruses-Major Players in the Global Ecosystem, *Nat. Rev. Microbiol.*, 2007, vol. 5, pp. 801–812.

7. Wilhelm, S.W. and Matteson, A.R., Freshwater and Marine Virioplankton: a Brief Overview of Commonalities and Differences, *Freshwater Biol.*, 2008, vol. 53, pp. 1076–1089.

8. Sommaruga, R., Krossbacher, M., Salvenmoser, W., Catalan, J., and Psenner, R., Presence of Large Virus-Like Particles in a Eutrophic Reservoir, *Aquat. Microb. Ecol.*, 1995, vol. 9, pp. 305–308.

9. Simek, K., Pernthaler, J., Weinbauer, M.G., Hornak, K., Dolan, J.R., Nedoma, J., Masin, M., and Amann, R., Changes in Bacterial Community Composition and Dynamics and Viral Mortality Rates Associated with Enhanced Flagellate Grazing in a Mesoeutrophic Reservoir, *Appl. Environ. Microbiol.*, 2001, vol. 67, no. 6, pp. 2723–2733.

10. Peduzzi, P. and Schiemer, F., Bacteria and Viruses in the Water Column of Tropical Freshwater Reservoirs, *Environ. Microbiol.*, 2004, vol. 6, no. 7, pp. 707–715.

11. Pradeep Ram, A.S., Boucher, D., Sime-Ngando, T., Debroas, D., and Romagoux, J.C., Phage Bacteriolysis, Protistan Bacterivory Potential, and Bacterial Production in a Freshwater Reservoir: Coupling with Temperature, *Microbiol. Ecol.*, 2005, vol. 50, no. 1, pp. 64–72.

12. Kopylov, A.I., Kosolapov, D.B., and Zabotkina, E.A., Viruses in the Plankton of the Rybinsk Reservoir, *Mikrobiologiya*, 2007, vol. 76, no. 6, pp. 879–887 [*Microbiology* (Engl. Transl), vol. 76, no. 6, pp. 782–790].

13. Edel'shtein, K.K., *Vodokhranilishcha Rossii: Ekologicheskie problemy i puti ikh resheniya* (Water Reservoirs of Russia: Environmental Problems and Solutions), Moscow: GEOS, 1998.

14. Noble, R.T. and Fuhrman, J.A., Use of SYBR Green for Rapid Epifluorescence Count of Marine Viruses and Bacteria, *Aquat. Microb. Ecol.*, 1998, vol. 14, pp. 113–118.

15. Porter, K.G. and Feig, Y.S., The Use of DAPI for Identifying and Counting of Aquatic Microflora, *Limnol. Oceanogr.*, 1980, vol. 25, no. 5, pp. 943–948.

16. Norland, S., The Relationship between Biomass and Volume of Bacteria, in *Handbook of Methods in Aquatic Microbial Ecology*, Kemp, P., Sherr, B., Sherr, E., and Cole, J., Eds., Boca Raton, FL: Lewis, 1993, pp. 303–308.

17. Gonzalez, J.M. and Suttle, C.A., Grazing by Marine Nanoflagellates on Viruses and Viral-Sized Particles Ingestion and Digestion, *Mar. Ecol. Prog. Ser.*, 1993, vol. 94, pp. 1–10.

18. Newell, S.Y. and Christian, R.R., Frequency of Dividing Cells as an Estimator of Bacterial Productivity, *Appl. Environ. Microbiol.,* 1981, vol. 42, no. 1, pp. 23–31.

19. Romanenko, V.I. and Kuznetsov, S.I., *Ekologiya mikroorganizmov presnykh vodoemov. Laboratornoe rukovodstvo* (Ecology of Freshwater Microorganisms. A Laboratory Manual), Leningrad: Nauka, 1974.

20. Binder, B., Reconsidering the Relationship between Virally Induced Bacterial Mortality and Frequency of Infected Cells, *Aquat. Microbiol. Ecol.*, 1999, vol. 18, pp. 207–215.

21. Proctor, L.M., Okubo, A., and Fuhrman, J.A., Calibrating Estimates of Phage-Induced Mortality in Marine Bacteria: Ultrastructural Studies of Marine Bacteriophage Development from One-Step Growth Experiments, *Microbiol. Ecol.*, 1993, vol. 25, pp. 161–182.

22. Noble, R.T and Steward, G, Estimating Viral Proliferation in Aquatic Samples, in *Methods in Microbiology*, Paul, J., Ed., San Diego: Academic, 2001, pp. 67–84.

23. Maranger, R. and Bird, D.F., Viral Abundance in Aquatic Systems: a Comparison Between Marine and Fresh Waters, *Mar. Ecol. Prog. Ser.*, 1995, vol. 121, pp. 217–226.

24. Fischer, U. and Velimirov, B., High Control of Bacterial Production by Viruses in a Eutrophic Oxbow Lake, *Aquat. Microbiol. Ecol.*, 2002, vol. 27, pp. 1–12.

25. Clasen, J.L., Brigden, S.M., Payet, J.P., and Suttle, C.A., Evidence That Viral Abundance across Oceans and Lakes Is Driven by Different Biological Factors, *Freshwater Biol.*, 2008, vol. 53, pp. 1090–1100.

26. Weinbauer, M.G. and Hofle, M.G., Significance of Viral Lysis and Flagellate Grazing as Factors Controlling Bacterioplankton Production in a Eutrophic Lake, *Appl. Environ. Microbiol.*, 1998, vol. 64, pp. 431–438.

27. Vrede, K., Stensdotter, U., and Lindstrom, E.S., Viral and Bacterioplankton Dynamics in Two Lakes with Different Humic Contents, *Microbiol. Ecol.*, 2003, vol. 46, pp. 406–415.

28. Hennes, K.P. and Simon, M., Significance of Bacteriophages for Controlling Bacterioplankton Growth in a Mesotrophic Lake, *Appl. Environ. Microbiol.*, 1995, vol. 61, pp. 333–340.

29. Prada, V., Herndl, G., and Weinbauer, M.G., Viral Burst Size of Heterotrophic Prokaryotes in Aquatic Systems, *J. Mar. Biol. Assoc. UK*, 2006, vol. 86, pp. 613–621.

30. Middelboe, M., Jorgensen, N.O.G., and Croer, N., Effects of Viruses on Nutrient Turnover and Growth Efficiency of Noninfected Marine Bacterioplankton, *Appl. Environ. Microbiol.*, 1996, vol. 62, pp. 1991–1997.