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EXPERIMENTAL ARTICLES

Viruses in the Plankton of the Rybinsk Reservoir

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Abstract—The role of autochthonous viruses in the regulation of bacterioplankton abundance and production was studied in the Rybinsk Reservoir. During the ice-free period, the number of virus-like particles varied within the range of $(11.0–57.4) \times 10^6$ particles/ml. The virus to bacterioplankton abundance ratio ranged within 3.0–9.4. From 4 to 25% of bacterioplankton was infected by phages. A single infected cell contained up to 80 mature virus particles. The phage-induced bacterioplankton mortality in different parts of the reservoir constituted 3.7–41.8% (22.5% on average) of bacterioplankton daily production. Heterotrophic flagellates grazed from 7.6 to 68.8% (27.5% on average) of the daily bacterial production. Thus, along with flagellates, viruses are an important factor controlling bacterioplankton development in the reservoir.

Key words: viruses, viral lysis, bacterioplankton, microbial food webs, reservoir.

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Considerable progress in the study of virus ecology has greatly changed our conception of the structural and functional organization of microbial food webs in aquatic ecosystems. Until the 1990s, protozoa were considered to be the main bacterioplankton grazers [1]. However, studies conducted later showed that the abundance of plankton viruses (virioplankton) in seas and lakes is approximately an order of magnitude higher than bacterioplankton abundance and constitutes about 10^7 particles/ml [2, 3]. Bacterial mortality induced by viral lysis may reach 60–100% of the daily bacterial production and exceed the rates of grazing by protozoa [4, 5]. The results of these studies allow us to regard plankton viruses as a significant microbial food web component playing an essential role in regulation of the abundance and species diversity of their hosts: bacteria, cyanobacteria, algae, and protozoa [6].

The bacterium–bacteriophage interactions were mainly studied in seas and lakes [7]. The available evidence of their interactions in such a widespread type of aquatic objects as reservoirs is sporadic [8]. The aim of this work was to study the seasonal dynamics of plankton viruses and their role in the regulation of bacterial production in the Rybinsk Reservoir.

MATERIALS AND METHODS

The studies were conducted at six standard stations situated in the deep-water part of the Rybinsk Reservoir, a shallow large-sized Volga reservoir (Table 1). The the abundance of viruses, bacteria, and heterotrophic flagellates was determined in integral water samples. These samples were obtained by mixing the water sampled throughout the water column depth with a 1-m interval on May 23, June 23, July 20, August 18, September 22, and October 13, 2005. The water samples were fixed adding glutaraldehyde to a concentration of 2% and stored in the dark at 4°C for no more than one month.

Virus particles and bacteria were enumerated using epifluorescence microscopy and the SYBR Green I fluorochrome on the Anodisc Al_2O_3 filters (Whatman) with a pore diameter of 0.02 μm [9]. The number and size of heterotrophic flagellates were determined using epifluorescence microscopy and primulin staining on Nuclepore 0.04-μm black nuclear filters [10]. A minimum of 400 bacteria or viruses or 200 flagellates were counted on each filter. The size of the bacteria and flagellates was assessed with a linear eyepiece micrometer. Their volumes were calculated using the formulas for the sphere, cylinder, and ellipsoid volumes. The organic carbon content in wet bacterial biomass was calculated according to the equation relating the carbon content (C, fg C/cell) to the cell volume (V, μm^3): $C = 120 \times V^{0.72}$ [11].

To determine the frequency of visibly virus-infected bacterial cells (FVIC, % of the total number of bacteria) and the average number of mature phages in infected bacteria (burst size, BS, particles/cell), transmission electron microscopy was used [12]. The reservoir water samples (5 ml) fixed by the addition of glutaraldehyde to a concentration of 2% were placed in 16-mm plastic

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Table 1. Characteristics of water at the sampling stations in the Rybinsk Reservoir in 2005

Station	Coordinates	Depth, m	Temperature, °C	Suspended organic matter content, mg/l	Primary plankton production, mg C/(m^3 day)	Dark CO_2 assimilation, mg C/(m^3 day)
Koprino	58°03′57″N, 38°18′42″E	10.0–13.0 / 12*	12.2–20.5 / 16.5	3.6–6.0 / 5.1	30.0–652.3 / 294.6	2.9–7.3 / 4.6
Mologa	58°13′05″N, 38°27′36″E	12.0–14.8 / 13.0	11.0–21.5 / 16.2	6.5–12.0 / 9.6	63.0–2004.6 / 691.9	2.0–5.0 / 3.3
Navolok	58°22′55″N, 38°23′25″E	6.0–11.0 / 8.0	10.7–21.6 / 15.7	7.0–20.0 / 12.8	25.0–520.4 / 249.0	1.6–5.0 / 3.2
Izmailovo	58°28′N, 38°40′E	6.0–10.0 / 7.8	11.8–22.2 / 16.6	5.0–18.5 / 10.3	29.1–618.3 / 292.8	2.1–6.1 / 3.4
Srednii Dvor	58°32′33″N, 38°19′36″E	8.0–10.0 / 9.2	12.0–22.0 / 16.5	4.5–16.0 / 9.7	26.0–510.0 / 253.4	1.8–6.2 / 3.4
Breitovo	58°19′31″N, 37°56′36″E	8.0–12.0 / 10.3	11.2–19.6 / 15.8	7.0–13.0 / 8.9	10.4–648.1 / 268.1	1.2–6.3 / 3.2

* The numerator shows the parameter fluctuation range; the denominator indicates the mean parameter value.

centrifuge tubes. The tube bottom was made flat by pouring into them 1 ml of Epon 812 epoxy resin, which was then polymerized in a thermostat at 60°C for 48 h. Before centrifugation, diameter-adjusted Vladipor membrane filters with two or three 400-mesh nickel grids fixed to them with a two-sided Scotch tape were placed in the tubes containing water samples. The grids were preliminarily pioloform-coated and carbon-sprayed. The water samples were centrifuged for 3 h on a PC-6 centrifuge with a CH-3/1 rotation-accelerating mechanism at 35000 *g*. After centrifugation, the filter was taken out from the tube and the grids were detached, dried in a closed Petri dish on filter paper, stained with 2% uranyl acetate for 30 s, and examined under a JEM 100C electron microscope (Jeol, Japan) at a 30000–70 000 magnification and an accelerating voltage of 80 kV. No fewer than 400 bacteria and 20 virus-infected bacteria were counted on each grid.

A bacterial cell was considered to be infected if it contained no fewer than five mature phages. Virus particles are known to become visible in bacterial cells under an electron microscope only at the end of the lytic cycle; i.e., the presence of mature phage particles shows the final stage of virus assembly before cell lysis [13]. Therefore, to determine the frequency of infected bacterial cells (FIC, % of the total bacterial number), the formula $FIC = 7.1 \times FVIC - 22.5 \times FVIC^2$ was used [14]. Virus-induced mortality of bacteria expressed as percentage (VMB) was calculated using the formula [14]: $VMB = (FIC + 0.6 \times FIC^2)/(1 - 1.2 \times FIC)$. Virus-induced mortality measured in the number of lysed bacterial cells per milliliter in 1 h (VIM, cells/(ml h)) was found according to the equation: $VIM = VMB \times P_B$, where P_B is bacterial production, cells/(ml h) [15]. Virus production (P_V, particles/(ml h)) was calculated as the average number of mature phages in a bacterial cell multiplied by bacterial production and by the percentage of infected bacteria [8, 15]; it was assumed that the latent period in virus development approximately equals the bacterium generation time [16].

The specific rate of bacterial growth was assessed by the frequency of dividing cells (FDC) according to the formula: $\ln\mu = 0.299 \times FDC - 4.961$, where μ is the specific growth rate, h^{-1} [17]. The doubling time of the number of bacteria (*D*, h) is connected with the specific growth rate by the ratio $D = \ln 2/\mu$. Bacterioplankton production was calculated as the specific growth rate multiplied by the total bacterial number. Bacterial grazing by heterotrophic flagellates (*I*, cells/(ml h)) was calculated according to the formula: $I = N_B V N_F$, where N_B is the bacterioplankton concentration, cells/ml; *V* is the rate of water clarification by flagellates, nl/(cells h); N_F is the heterotrophic flagellate concentration, cells/ml. The values of the rate of water clarification by flagellates were obtained by us in the experiments with fluorescently labeled bacteria [18] at the Koprino station in the Rybinsk Reservoir in 1998. In May, this rate was 5.7; in June, 4.2; in July, 4.2; in August, 3.7; in September, 3.3; and in October, 3.0 nl/(cells h). The primary phytoplankton production was measured using the radiocarbon method [19]. The concentration of suspended organic matter in the water was determined by the standard gravimetric method [20].

The statistical processing of data was carried out using the Statistica 6.0 program. The correlation coefficients were calculated for a significance level of $P < 0.05$.

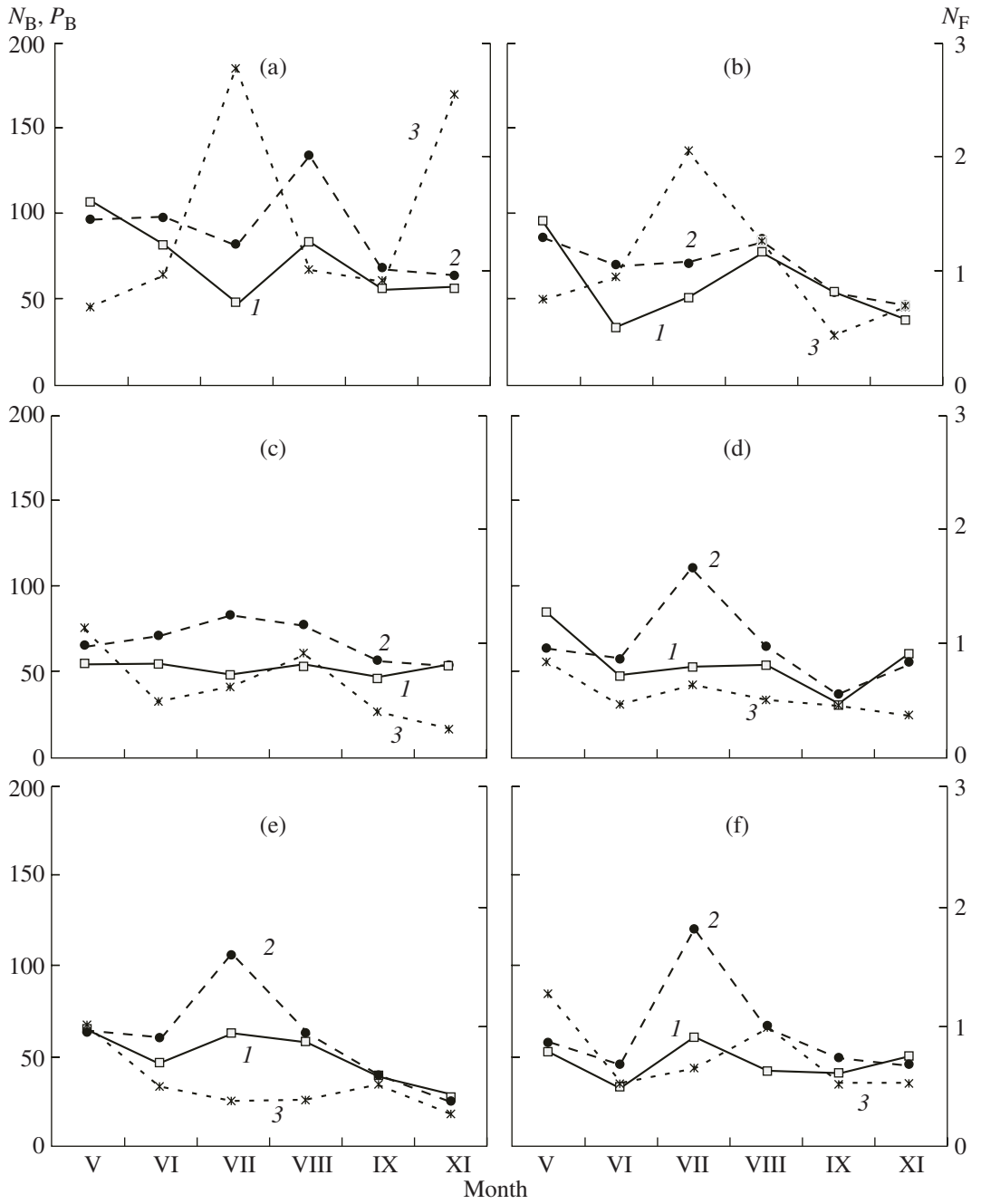

Fig. 1. Seasonal dynamics of bacterioplankton cell number ((*1*), N_B, 10 cells/ml) and production ((*2*), P_B, 10^3 cells/(ml h)) and the number of heterotrophic flagellates ((*3*), N_F, 10^3 cells/ml) at six standard stations of the Rybinsk Reservoir: (a) Koprino, (b) Mologa, (c) Navolok, (d) Izmailovo, (e) Srednii Dvor, and (f) Breitovo.

RESULTS

During the ice-free period, the primary phytoplankton production in the Rybinsk Reservoir varied within a wide range, between 25 and 2005 mg C/(m^3 day) (Table 1). In different reservoir zones, the minimal and maximal values of this parameter differed 20- to 62-fold. The maximal values were recorded in July and the minimal values were noted in October. The plankton primary production values allow the Rybinsk Reservoir to be assigned to a mesotrophic-type reservoirs [21].

Compared to variations in the primary production, the seasonal fluctuations in bacterioplankton abundance, biomass, and production were less significant (Fig. 1). The maximal bacterial abundance values were, as a rule, recorded in May, and those of production in the summer months. The average bacterioplankton cell volume varied between 0.052 and 0.104 μm^3; the biom-

Table 2. Seasonal variations in the frequency of visibly infected bacterial cells (FVIC, %), rate of virally induced lysis of bacteria (VIM, 10^3 cells/(ml h)), and rate of heterotrophic flagellate grazing of bacteria (FG, 10^3 cells/(ml h))

Station	Parameters	May	June	July	Aug.	Sept.	Oct.	Average
Koprino	FVIC	0.9	–*	2.9	2.3	3.7	3.2	2.6
	VIM	6.6	–	21.9	27.0	24.8	19.1	19.9
	FG	40.9	32.7	56.0	31.1	16.6	43.4	36.8
Mologa	FVIC	2.8	3.8	2.9	3.8	2.4	2.0	3.0
	VIM	21.9	25.9	19.0	32.0	11.4	7.8	19.7
	FG	40.4	13.0	44.1	35.9	7.6	7.8	24.8
Navolok	FVIC	0.5	1.1	1.7	1.5	3.7	4.0	2.1
	VIM	2.4	6.2	10.2	9.5	20.7	21.8	11.8
	FG	35.7	11.6	12.3	18.3	6.2	4.1	14.7
Izmailovo	FVIC	0.8	0.8	1.0	4.1	4.0	3.0	2.3
	VIM	4.7	3.6	8.7	27.1	14.7	15.1	12.3
	FG	40.7	10.3	14.0	10.2	5.0	6.8	14.5
Srednii Dvor	FVIC	3.3	1.1	1.2	1.0	3.1	1.4	1.9
	VIM	20.4	5.2	11.1	5.0	11.0	2.8	6.3
	FG	37.8	9.7	9.6	8.2	6.4	2.1	12.3
Breitovo	FVIC	2.1	2.3	1.2	3.9	3.5	3.8	2.8
	VIM	10.4	9.1	11.4	25.9	16.3	16.7	14.9
	FG	37.3	6.9	15.9	14.7	6.7	7.5	14.8

* Hereafter, a dash denotes the absence of data.

ass values varied between 54 and 252 mg C/m^3. The values of the specific growth rate of bacteria were between 0.009 and 0.020 h^{-1}, while the doubling time of bacterial number varied between 35 and 77 h.

The number of heterotrophic flagellates varied from 250 and 2780 cells/ml (Fig. 1). The maximal values were recorded at the Koprino and Mologa stations in the Volga stretch of the Rybinsk Reservoir in July, whereas at the other stations, situated in the Central stretch, this parameter peaked in May. Heterotrophic flagellates grazed bacteria at a rate of (2.1–56.0) × 10^3 cells/(ml h), which constituted 7.6–68.8% of the bacterial production in one hour (Table 2).

The number of plankton viruses ranged within (11.0–57.4) × 10^6 particles/ml and constituted, for the reservoir, an average of 31.1 × 10^6 particles/ml (Fig. 2). The highest virioplankton concentration was characteristic of the fluvial zone of the Volga stretch (station Koprino). Here, the maximal number of virus particles was recorded in August; at the other stations, it was recorded in July. The virus to bacterial number ratio varied between 3.0 and 9.4 (6.0 on average). Correlation analysis revealed a tight correlation between the number of viruses and bacterial production (r = 0.77) and bacterial activity (dark CO_2 assimilation, r = 0.78). A weaker correlation was revealed with the total bacterioplankton abundance (r = 0.49) and the primary phytoplankton production (r = 0.42). Correlations were also established between the number of plankton viruses and temperature (r = 0.51) and water clarity (r = 0.32). The concentration of virus particles correlated negatively with the suspended matter content (r = –0.42). Virus production ranged within (36–542) × 10^3 particles/(ml h), peaking in August, September, or May (Fig. 2). The doubling time of virioplankton abundance constituted 1.2–21.1 days (7.6 days on average).

The frequency of visibly virus-infected bacteria, i.e., the share of the bacteria containing mature phage particles in the cells, constituted 0.9–4.1% of the total bacterioplankton abundance (Table 2, Fig. 3). Based on this, it was calculated that from 4 to 25 % (16% on average) of all the bacteria in the reservoir water column

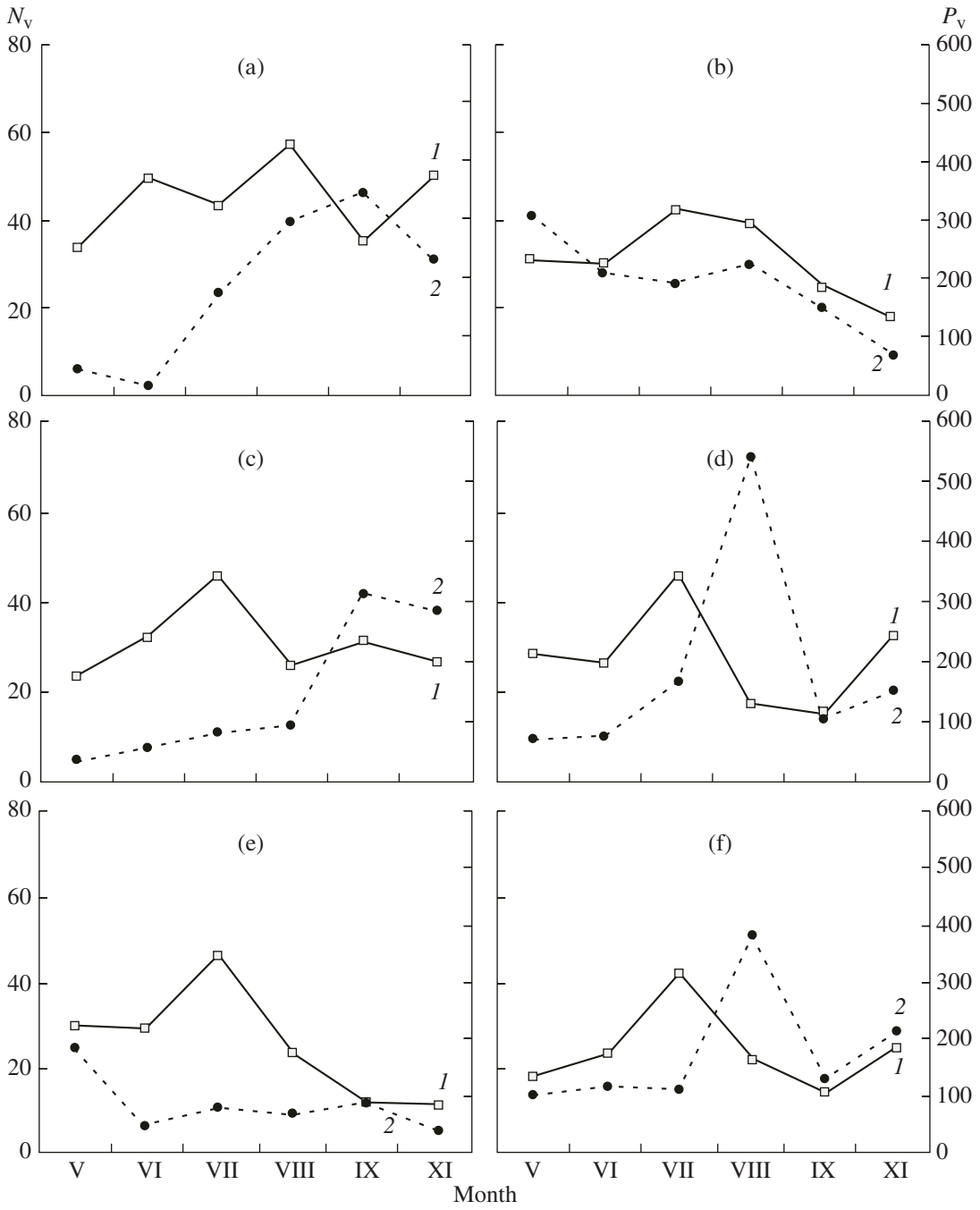

Fig. 2. Seasonal variations in virioplankton abundance ((*1*), N_V, 10^6 particles/ml) and production ((*2*), P_V, 10 particles/(ml h)). See Fig. 1 for designations of the stations.

was infected by phages. Most of the infected bacteria were rod-shaped. As a rule, the share of infected cells in the bacterioplankton was the largest at the end of summer and in autumn. The infected bacteria contained up to 80 mature phage particles. The average phage number constituted 7–22 particles/cell (Table 3).

The rate at which bacteria are lysed by viruses was calculated to be (2.4–32.0) × 10^3 cells/(ml h). Over 24 h, viruses lysed 3.7–41.8% of the bacterioplankton production (Table 2, Fig. 4). As a rule, the highest mortality of bacteria due to virus-induced lysis was observed in the August–September period. In the greater part of the reservoir, the role of viruses in utilization of the bacterial production was comparable to that of heterotrophic flagellates. Only in the fluvial part of the Volga stretch (station Koprino), significantly less

(1.9-fold) bacteria were killed by virus-induced lysis than were grazed by flagellates (Table 4).

DISCUSSION

The abundance of plankton viruses determined by us in the Rybinsk Reservoir is close to the values of this parameter recorded in mesotrophic lakes and a mesoeutrophic reservoir ((1.0–5.0) × 10^7 particles/ml) [8, 22, 23] but lower than those determined in eutrophic lakes ((11.7–25.0) × 10^7 particles/ml) [4, 24]. In our study, no significant correlation between viral and bacterial abundances was found, but a strong positive correlation between viral abundance and bacterial production and dark CO_2 assimilation was established. Scarce data published on the seasonal dynamics of viral and bacterial concentrations in freshwater reservoirs indicate that tight correlation between these parameters is not always observed [25, 26]. However, higher virus particle concentrations are, as a rule, recorded in more productive waters [27]. A close relationship between bacteriophage abundance and bacterioplankton activity is often observed [22].

The average virus to bacterial abundance ratio in the water column of the Rybinsk Reservoir was found to be equal to 6. This value is in the range of values normally recorded in reservoirs (from 3 to 10) [7, 22]. Viruses are an important factor of bacterioplankton control in the Rybinsk Reservoir. In the ice-free period, they lyse an average of 22.5% of bacterial production, which is only1.2 times lower than the amount grazed by heterotrophic flagellates (Table 3). In lakes, viruses were found to utilize from 1.0 to 46.8% of the daily bacterial production [8, 26, 28, 29].

The phenomenon of bacteriophage-stimulated growth and multiplication of the uninfected part of bacterioplankton has been reported. This occurs due to the fact that viral lysis results in the release of a considerable amount of organic substrates and biogenic elements, which are utilized within the microbial community and do not become available at higher trophic levels [7]. In the Rybinsk Reservoir, the high mortality rate of bacterioplankton due to viral lysis also suggests that a large amount of organic substrates, which become available to heterotrophic microorganisms, are released into the aqueous medium. Thus, from May to October, bacteriophages lysed daily from 58 × 10^3 to 768 × 10^3 of bacterial cells in 1 ml of water (an average of 346 × 10^3 cells/ml), which corresponds to from 1.0 to 13.1 μg C/l (an average of 6.2 μg C/l). If we assume that one viral particle contains 10^{-10} μg C [30], then the viruses utilized from 0.1 to 1.7 μg C/(l day) (an average of 0.5 μg C/l day) of the organic matter of infected bacteria for the processes of synthesis of viral nucleic acids and proteins. Thus, the amount of organic carbon released into the water as a result of viral lysis constituted 0.9–12.1 μg C/(l day) (an average of 5.7 μg C/(l day)), or 0.4–50.0% (6.6% on average) of the plankton daily primary production.

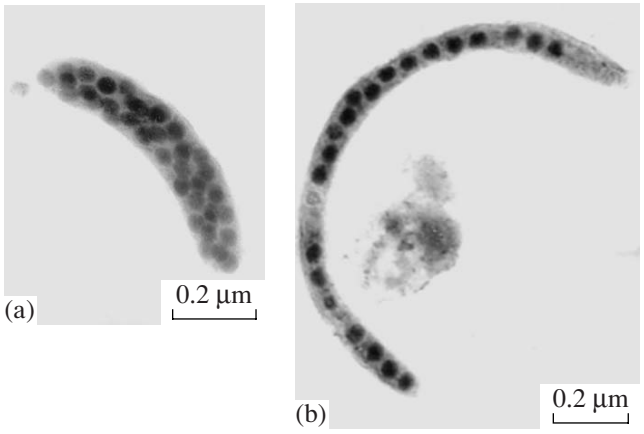

Fig. 3. Electron-microscopic photographs of the virus-infected plankton bacteria of the (a) vibrioid and (b) filamentous form.

Table 3. Seasonal variations in the average number of mature phage particles in bacterial cells (burst size (BS), particles/cell)

Station	May	June	July	Aug.	Sept.	Oct.	Average
Koprino	7 (19)*	–	8 (36)	11 (19)	14 (38)	12 (41)	10.4
Mologa	14 (67)	8 (45)	10 (30)	7 (28)	13 (32)	9 (52)	10.2
Navolok	15 (34)	9 (27)	8 (15)	10 (27)	15 (43)	13 (128)	11.7
Izmailovo	15 (49)	22 (21)	19 (24)	20 (57)	7 (20)	10 (43)	15.5
Srednii Dvor	9 (26)	9 (28)	7 (40)	13 (21)	8 (48)	14 (41)	10.0
Breitovo	10 (34)	13 (47)	10 (40)	15 (46)	8 (30)	13 (40)	11.5

* Tables 3 and 4 show in brackets the coefficients of variation (C_V, %).

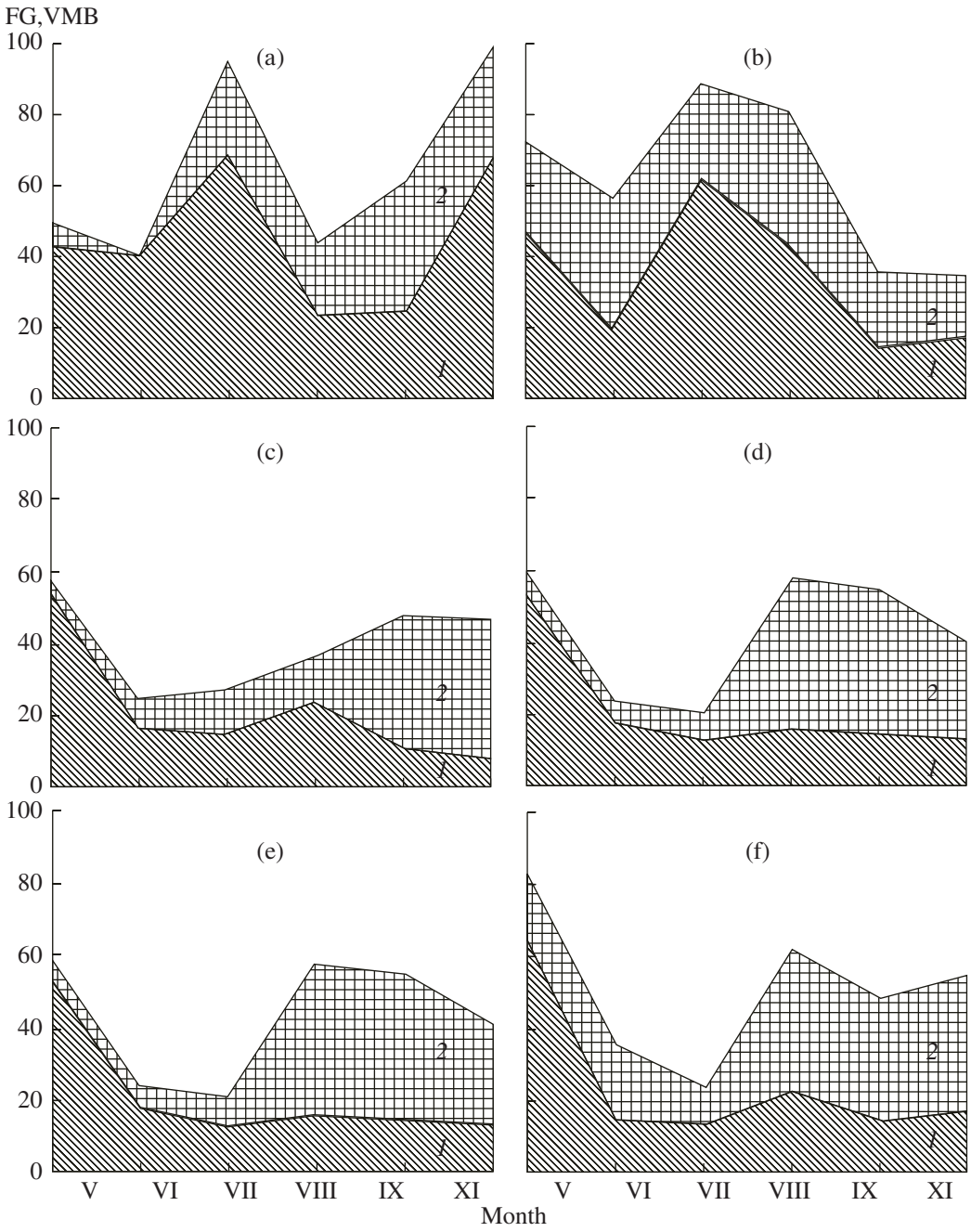

Fig. 4. Seasonal variations in the share of the daily bacterioplankton production (P_B) (*1*) consumed by heterotrophic flagellate grazing (FG, % of P_B) and (*2*) lysed by viruses (VMB, % of P_B). See Fig. 1 for designations of the stations.

Thus, heterotrophic flagellates and viruses are the two most important factors regulating bacterioplankton development in the Rybinsk Reservoir. Together, they utilized from 36.8 to 69.7% of the daily bacterial production in the ice-free period. During the ice-free period, the flagellates grazed an average of 27.5% of bacterioplankton production and bacteriophages lysed 22.5%. The role of these control factors substantially changed during the season. The highest bacterial mortality rate due to virus-induced lysis was observed at the end of summer and in autumn, i.e., in the period of mass development in the reservoir of cladocerans, which decrease the flagellate and infusorium concentration. Which of the two factors of bacterial control predominates is highly important for the functioning of the plankton community. Grazing of bacteria by protozoa with subsequent utilization of the latter by metazoan plankton results in the transfer of carbon and other biogenic elements to the higher levels of food webs, whereas viral lysis leads to element recycling within

Table 4. Shares (%) of the daily bacterial production lysed by viruses (VMB) and consumed by heterotrophic flagellate grazing (FG), averaged over the vegetation season

Station	VMB	FG
Koprino	24.1 (42)	45.6 (41)
Mologa	27.5 (32)	33.4 (53)
Navolok	18.7 (74)	21.3 (74)
Izmailovo	21.7 (72)	20.9 (70)
Srednii Dvor	16.4 (60)	20.2 (81)
Breitovo	26.6 (41)	24.5 (75)
All stations	22.5	27.5

the microbial loop (bacteria ⟶ bacteriophages ⟶ dissolved organic matter ⟶ bacteria). The influence of viruses on bacteria is not unambiguously negative. The biogenic elements released into the aqueous medium as a result of cell lysis stimulate the bacterioplankton activity. In the Rybinsk Reservoir, a positive correlation between virus concentration and bacterial production and dark CO_2 fixation was revealed. All these data give evidence that viruses exert a strong influence on the processes occurring in the plankton food webs and on the carbon and energy flows in the reservoir ecosystem.

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