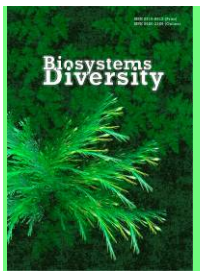

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Virioplankton as an important component of plankton in the Volga Reservoirs

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The distribution of virioplankton, abundance and production, frequency of visibly infected cells of heterotrophic bacteria and autotrophic picocyanobacteria and their virus-induced mortality have been studied in mesotrophic and eutrophic reservoirs of the Upper and Middle Volga (Ivankovo, Uglich, Rybinsk, Gorky, Cheboksary, and Sheksna reservoirs). The abundance of planktonic viruses (VA) is on average by 4.6 ± 1.2 times greater than the abundance of bacterioplankton (BA). The distribution of VA in the Volga reservoirs was largely determined by the distribution of BA and heterotrophic bacterioplankton production (PB). There was a positive correlation between VA and BA and between VA and PB. In addition, BA and VA were both positively correlated with primary production of phytoplankton. Viral particles of 60 to 100 μm in size dominated in the phytoplankton composition. A large number of bacteria and picocyanobacteria with viruses attached to the surface of their cells were found in the reservoirs. Viruses as the most numerous component of plankton make a significant contribution to the formation of the planktonic microbial community biomass. The number of phages inside infected cells of bacteria and picocyanobacteria reached 74–109 phages/cell. Easily digestible organic matter, which entered the aquatic environment as a result of viral lysis of bacteria and picocyanobacteria, could be an additional source of carbon for living bacteria. The results of long-term studies indicate a significant role of viruses in functioning of planktonic microbial communities in the Volga reservoirs.

Keywords: viruses; viral lysis; heterotrophic bacteria; picocyanobacteria; microbial communities; freshwater ecosystems.

Introduction

Research over the past three decades has firmly established that viruses are the most abundant and diverse biological entities (Bratbak & Heldal, 2000; Wommack & Colwell, 2000; Weinbauer, 2004), thereby forming an integral component of microbial food web (viruses, bacteria, picophytoplankton, protozoa) in a great variety of aquatic environments (Bratbak et al., 1994; Clasen et al., 2008; Wilhelm & Matteson, 2008). Viral lysis plays fundamental roles in cycling nutrients and organic matter (Middelboe et al., 2008; Staniewsski & Short, 2014), structuring the microbial food web dynamics and regulating carbon flow (Fuhrman, 1999; Bouvy et al., 2011; Breitbart, 2012), governing microbial diversity (Weinbauer & Rassoulzadegan, 2004; Keshri et al., 2017; Zheng et al., 2020). Bacteriophages prevail in virioplankton in many aquatic ecosystems, including reservoirs (Peduzzi & Schiemer, 2004; Ram et al., 2005; Sime-Ngando, 2014; Hanson et al., 2017). It has been reported that viruses cause the mortality of a significant part of the marine and freshwater bacterioplankton (to 40–60% of the bacterial production) (Wommack & Colwell, 2000; Suttle, 2005, 2007; Wilhelm & Matteson, 2008; Ram et al., 2011) and, at times, can match grazing by bacterivorous protists as a source of bacterial mortality (Fischer & Velimirov, 2002; Peduzzi & Schiemer, 2004). Viruses cause the mortality of a significant part of the marine bacterioplankton (to 40–60% of the total mortality) (Fuhrman, 1999; Wommack & Colwell, 2000; Weinbauer, 2004; Suttle, 2005, 2007). Studies have shown that viruses infecting cyanobacteria (cyanophages) can be major players affecting the mortality and structure of cyanobacterial communities (Mann, 2003; Zong et al., 2018). In pelagic food webs carbon content of bacteria and picocyanobacteria can be either transferred to the upper trophic levels when consumed by protozoa and multicellular filter feeders or, alternatively, contribute to the dissolved organic matter stocks as a result of viral-induced cell lysis (Ram et al., 2015; Vaqué et al., 2017). In addition, viruses mediate the gene transfer within and between species by transduction (Jiang & Paul, 1998; Sime-Ngando, 2014). It has been hypothesized that viral genes and viral activity generate the genetic variability of prokaryotic organisms and are a driving force for ecological functioning and evolutionary development (Weinbauer & Rassoulzadegan, 2004; Zhang & Gui, 2018). Studies on the role of viruses in the structure and functioning of planktonic communities in reservoirs of the Upper and Middle Volga have been carried out since 2005 to the present (Kopylov et al., 2007, 2011a, 2011b, 2013a, 2013b, 2016). In this paper, we analyze the results of long-term studies of the abundance and production of free viruses, the abundance of viruses attached to bacteria, the size of viral particles, frequencies of visibly infected cells, and virus-induced mortality of heterotrophic bacteria and picocyanobacteria in six reservoirs of the Upper and Middle Volga basin. A quantitative assessment of the role of viruses in carbon fluxes in planktonic food webs of the reservoirs has been performed.

Materials and methods

The studies were conducted at the end of July-beginning of September 2005–2010 in six mesotrophic and eutrophic reservoirs of the Volga River and Volga-Baltic waterway (hereinafter referred to as "Volga reservoirs"): Ivankovo (at 11 stations), Uglich (10 stations), Rybinsk (34 stations), Gorky (16 stations), Cheboksary (20 stations), and Sheksna (12 stations). The abundance and production of viruses, bacteria, and picocyanobacteria were determined in integrated water samples.

Planktonic viral particles were counted by epifluorescence microscopy using the SYBR Green I fluorochrome (Thermo Fisher Sci, USA) and aluminum oxide Anodisc filters (Wathman, GE Healthcare, USA) with a pore diameter of 0.02 μm (Noble & Fuhrman, 1998); heterotrophic bacteria were enumerated using DAPI (Thermo Fisher Sci, USA) as a fluorochrome and black nuclear filters with a pore diameter of 0.2 μm (Nucleopore, GE Healthcare, USA) (Porter & Feig, 1980). Picocyanobacteria were enumerated by epifluorescence microscopy according to autofluorescence of phytopigments of picoautotrophic cells (Maclsaac & Stockner, 1993). Filters were examined under an Olympus BX51 epifluorescent microscope (Olympus, Japan) using Cell-F image analysis software at ×1000 magnification. For each water sample, two filters were analyzed; counts yielded a minimum of 600 viruses, 400 bacteria and 400 picocya-

nobacteria. The carbon content in wet bacterial biomass was calculated using the equation describing the relationship between the cell volume (V, μm^3) and carbon content (C, fg C/cells): $C = 120 \times V^{0.72}$ (Norland, 1993).

The carbon content per one viral particle was taken as 10^{-10} μg C (Gonzalez & Suttle, 1993). It was assumed that the carbon content constituted 16.5% of the wet picocyanobacteria biomass (Jochem, 1988).

The production of bacteria was determined by the ^{14}C method (Romanenko & Kuznetsov, 1974) and dilution method (Landry & Hassett, 1982). The production of picocyanobacteria was estimated using the frequency of dividing cells of picocyanobacteria (McDuff & Chisholm, 1982). The primary production of phytoplankton was determined by the ^{14}C method (Romanenko & Kuznetsov, 1974). Grazing rates of heterotrophic nanoflagellates on bacteria were measured using the method of fluorescently labelled bacteria (Simek et al., 2001). Each experiment was replicated twice or thrice.

Transmission electron microscopy was used to estimate the frequency of visibly infected cells (FVIC, % of the total bacterial abundance) and the mean number of fully matured phages in infected bacteria (i.e., burst size (BS), particles/cell). For electron microscopy, viruses and bacteria were centrifuged at 100,000 g for 1 hour using an OPTIMA L-90k ultracentrifuge with a 45 Ti rotor (Beckman Coulter, USA) on Pioloform/carbon-coated 400-mesh nickel grids (SPI, USA). The grids were analyzed under a JEM 1011 electron microscope (Jeol, Japan) at ×50,000–150,000 magnification. At least 800 bacterial cells and 600 viruses were analyzed for each grid. Mature phages become clearly visible in the host cell only at the end of the latent period immediately prior to cell lysis. The fraction of all infected cells in bacterioplankton (FIC, % of the total abundance of bacteria) and viral-mediated mortality of bacteria (VMB, % of bacterioplankton production) were calculated according to the equations (Binder, 1999).

The fraction of all infected picocyanobacterial cells (FIC, of the total abundance of bacteria) and fraction of picocyanobacterial mortality due to viral lysis (FMVL, % of the daily production of picocyanobacteria) were calculated according to the equation (Mann, 2003). The rate of virus-induced mortality of bacteria or picocyanobacteria (VIM) expressed as cells/(mL × day), or as mg C/(m^3 × day) was calculated using the equation: VIM = VMB × PBAC, where PBAC is bacterioplankton production, or VIM = FMVL × PPC, where PPC is picocyanobacterial production. The virioplankton production (PV) was determined as the product of BS by VIM (cells/(mL × day)) (Noble & Steward, 2001). The viral turnover time (VTT) was determined by dividing the abundance of free viruses by their production.

The rate of easily oxidizable organic matter release from the lysed bacterial and picocyanobacterial cells into an aquatic environment was calculated as the difference between VIM (mg C/(m^3 × day)) and PV (mg C/(m^3 × day)). The values that were obtained are overestimates, since values of energy consumption of viruses for the synthesis of capsid proteins and replication of nucleic acids were not taken into account in our calculations because these data are not available in the literature.

Results

Characteristics of abiotic factors, abundance, biomass of bacteria and picocyanobacteria, phytoplankton primary production and bacterioplankton production. In 2005–2007, the average water temperature for the study period ranged within 19.9–21.4 ºC in August and 16.6–17.2 ºC at the beginning of September (Table 1). In July 2010, the water temperature in the Gorky and Cheboksary reservoirs increased to record values that were higher than the water heating under the normal thermal regime by 8.0–8.5 ºC. The main reason for the anomalously high air temperature in the central regions of European Russia, and, as a result, the high water temperature in the Volga reservoirs was the blocking anticyclone of an unusual intensity and duration of the existence (55 days).

Table 1

Depth (D), transparency (Tr) and temperature (T) of water, abundance of bacteria (N_B) and biomass (B_B), phytoplankton primary production (P_{PHY}) and bacterioplankton production (P_B) of reservoirs (on average for a reservoir)

Reservoir	Parameters	D, m	Tr, cm	T, ºC	N_B, 10^6 cells/mL	B_B, mg C/m^3	P_{PHY}, mg C/(m^3 × day)	P_B, mg C/(m^3 × day)
Ivankovo	x ± SD	8.8 ± 1.4	90 ± 8	20.6 ± 0.8	12.0 ± 2.2	288 ± 60	1775 ± 925	160 ± 28
(August 24–26, 2005)	Limits	3.0–16.0	40–120	18.3–27.8	6.2–31.0	132–818	482–5414	81–320
Uglich	x ± SD	10.8 ± 1.4	113 ± 6	20.5 ± 0.3	10.2 ± 1.1	220 ± 30	620 ± 98	107 ± 14
(August 22–24, 2005)	Limits	4.0–19.0	100–160	19.9–23.0	5.4–15.3	98–397	367–931	29–166
Rybinsk	x ± SD	11.2 ± 0.8	122 ± 14	19.9 ± 0.2	6.2 ± 0.6	110 ± 14	488 ± 95	55 ± 21
(August 19–20, 2005)	Limits	5.5–16.5	100–170	19.2–20.5	4.1–8.4	76–171	290–919	33–92
Rybinsk	x ± SD	9.4 ± 3.4	148 ± 32	21.4 ± 0.6	7.1 ± 1.6	129 ± 26	499 ± 81	124 ± 24
(August 08–16, 2007)	Limits	2.0–13.0	100–200	22.2–24.1	5.0–10.4	95–173	213–1162	100–182
Gorky	x ± SD	8.6 ± 0.5	114 ± 4	16.6 ± 0.2	9.9 ± 0.6	203 ± 13	522 ± 88	83 ± 7
(September 02–04, 2005)	Limits	6.7–13.0	90–150	15.4–17.4	4.4–13.6	89–259	173–989	38–132
Gorky	x ± SD	9.3 ± 4.3	98 ± 2	28.0 ± 0.2	11.6 ± 1.2	306 ± 24	945 ± 211	169 ± 32
(July 21–24, 2010)	Limits	4.5–16.0	60–120	25.5–33.0	6.3–18.5	145–355	152–2295	70–349
Cheboksary	x ± SD	5.1 ± 1.1	112 ± 11	17.2 ± 0.1	8.8 ± 0.4	181 ± 15	358 ± 69	109 ± 17
(September 07–08, 2005)	Limits	3.0–8.0	90–140	17.0–17.4	7.7–9.8	146–220	253–489	74–144
Cheboksary	x ± SD	7.2 ± 3.7	97 ± 2	27.4 ± 0.1	15.4 ± 0.9	300 ± 80	1466 ± 284	258 ± 27
(July 25–28, 2010)	Limits	2.5–16.0	40–180	25.0–29.0	7.6–20.9	277–475	196–4746	119–574
Sheksna	x ± SD	6.4 ± 0.9	106 ± 9	21.1 ± 0.1	6.2 ± 0.4	125 ± 15	332 ± 54	50 ± 5
(August 03–09, 2005)	Limits	4.0–13.0	60–160	20.5–21.7	3.5–8.0	59–240	104–739	29–81
Sheksna	x ± SD	4.9 ± 2.0	108 ± 2	21.3 ± 0.1	7.9±0.1	142 ±32	352 ± 44	136 ± 3
(August 08–12, 2007)	Limits	1.3–9.0	50–180	20.5–22.7	4.9–10.8	70–188	100–744	72–260

Among the studied reservoirs, the lowest water transparency and the highest primary production of phytoplankton were recorded in the Ivankovo reservoir and in the Gorky and Cheboksary reservoirs in the anomalously hot summer of 2010 (Table 1). The maximum abundance, biomass and production of bacterioplankton were also recorded in the Ivankovo reservoir and in the Gorky and Cheboksary reservoirs in July 2010 (Table 1). A strong positive correlation was found between the phytoplankton primary production and NB ($r = 0.68$, $n = 10$, $P = 0.05$). A large number of bacteria with viruses attached to the surface of their cells were found in the reservoirs (Fig. 2). There were from 1 to 39 viruses on the surface of a cell. In July–September, the abundance of bacteria with viruses attached to their cells (NBV) varied from 12.5 (Sheksna Reservoir, 2007) to 45.0% (Cheboksary Reservoir) of their total abundance. The minimum and maximum values (NBV/NB), calculated on average for a reservoir, differed by 1.4 times. The average NBV/NB for the reservoirs during all years of study was 23.0 ± 2.5% (Fig. 1d).

During the study period, the abundance (NPC) and biomass (BPC) of picocyanobacteria in the reservoirs varied widely from 2×10^3 to 432×10^3 cells/mL and from 0.3 to 115.0 mgC/m^3, respectively. The minimum and maximum NPC and BPC, calculated on average for a reservoir, differed by 3.5 and 4.6 times, respectively (Fig. 1a, b). The abundance of picocyanobacteria with viruses attached to their cells (NPCV) varied from 8.0 (Sheksna Reservoir, 2007) to 59.0% (Gorky Reservoir, 2010) of their total abundance. The minimum and maximum values (NPCV/NPC), calculated on average for a reservoir, differed by 2.3 times (Fig. 1c). The average NPCV/NPC value for all reservoirs during all study periods was 18.2 ± 4.2%. There were from 1 to 32 phages attached to the surface of one cell.

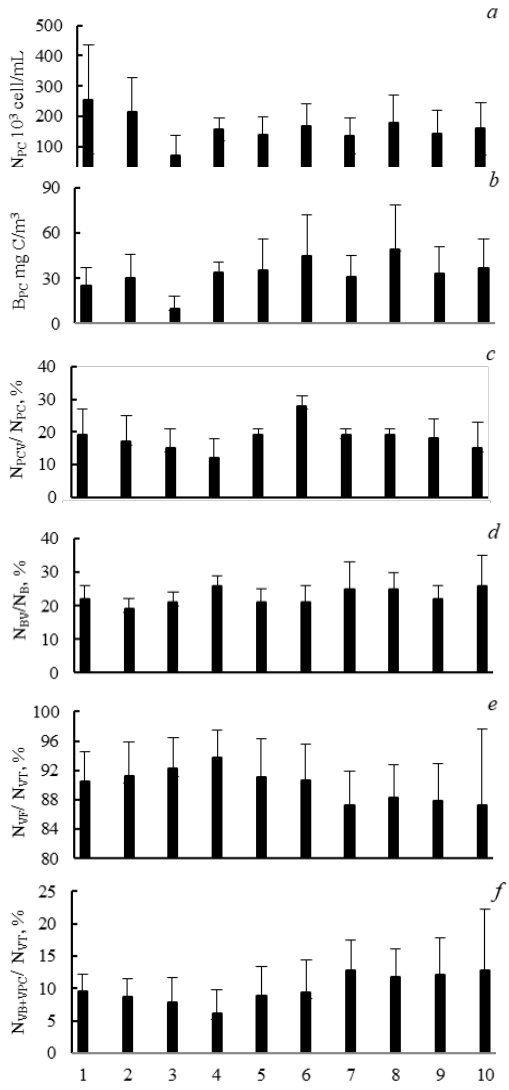

Fig. 1. Distribution (on average for a reservoir, x ± SD) of the concentration of picocyanobacteria, proportion (% of the total abundance) of picocyanobacteria and bacteria with attached viruses, proportion (% of the total abundance) of free and attached viruses: *a* – abundance of picocyanobacteria (NPC, 10^3 cells/mL); *b* – biomass of picocyanobacteria (BPC, mgC/m^3), *c* – proportion of picocyanobacteria with attached viruses of the total abundance of (NPCV/ NPC, %), *d* – proportion of bacteria with attached viruses of the total abundance of bacteria (NBV/NB, %), *e* – proportion (%) of free viruses (NVF/NVT) of the total abundance of virioplankton, *f* – proportion (%) of viruses attached to bacterial and picocyanobacterial cells (NVB+VPC/NVT) of the total abundance of virioplankton in reservoirs; *1* – Ivankovo (August 24–26, 2005), *2* – Uglich (August 22–24, 2005), *3* – Rybinsk (August 19–20, 2005), *4* – Rybinsk (August 08–16, 2007), *5* – Gorky (September 02–04, 2005), *6* – Gorky (July 21–24, 2010), *7* – Cheboksary (September 07, 2005), *8* – Cheboksary (July 25–28, 2010), *9* – Sheksna (August 03–09, 2005), *10* – Sheksna (August 08–16, 2007)

Total abundance and structure of virioplankton. At the end of summer and in early autumn, the abundance of free viral particles (NVF) in plankton of the reservoirs varied widely; the minimum and maximum values of this parameter differed by 8.5 times (Fig. 1, 2, Table 2). The average NVF was 41.6 ± 13.4 × 10^6 viruses/mL for all reservoirs in the period July to early September during all years of studies. There was a positive correlation between the average NB and NVF for the reservoirs (r = 0.66, n = 10, P = 0.05). A positive correlation was also found between NVF and bacterioplankton production (r = 0.63, n = 10, P = 0.05). A positive correlation was found between NPC and NVF (r = 0.52, n = 10, P = 0.05). These facts indicate that virioplankton in the reservoirs is to a large extent represented by prokaryotic viruses. The abundance of virioplankton exceeded the abundance of bacterioplankton by 1.1–11.9 (on average 4.6 ± 1.2) times. A strong positive correlation was found between the phytoplankton primary production and NVF (r = 0.69, n = 10, P = 0.05).

The size of planktonic free viruses determined in the Sheksna Reservoir in August 2007 showed that virioplankton contained viral particles with a capsid diameter of 19 to 435 nm. The average value of this parameter was 80 ± 2 nm for all water samples. The average proportions of different size groups in the total viral abundance were as follows: less than 40 nm, 10.8 ± 1.4% (limits: 0.0–23.5%), 40–60 nm, 26.4 ± 2.4% (0.0–6.8%), 60–100 nm, 39.9 ± 2.0 (34.3–52.6%), 100–150 nm, 16.5 ± 1.9% (2.6–34.2%), 150–200 nm, 4.9 ± 0.8% (0.0–10.7%) and more than 200 nm, 1.5 ± 0.5% (0.0–7.1%). Thus, viruses with a capsid diameter of 40–100 nm made the main contribution to the total abundance of free viruses. Viral particles with a capsid diameter from 26 to 254 nm were found in the virioplankton composition in the Rybinsk Reservoir in August 2007. The average value of this parameter for the studied stations was 80 ± 4 nm. The average proportions of different size groups in the total viral abundance were as follows: less than 40 nm, 9.5 ± 2.6% (range 0.0–28.9%), 40–60 nm, 24.2 ± 5.0% (0.0–49.0%), 60–100 nm, 41.5 ± 3.7 (24.3–64.0%), 100–150 nm, 20.0 ± 5.8% (0.0–66.7%), 150–200 nm 4.2 ± 1.5% (0.0–13.6%) and more than 200 nm, 0.6 ± 0.4% (0.0–5.1%). The total abundance of virioplankton (NVT) in the Volga reservoirs varied widely, averaging 46.1 ± 14.3 × 10^6 viruses/mL in all reservoirs in the period between July and early September during all years of studies (Table 2). At the same time, the proportion of NVF in NVT varied within 59.9–97.6% (on average 90.4 ± 2.6%), and the proportion of viruses attached to bacteria and picocyanobacteria in NVT varied within 3.0–40.1% (on average 9.6 ± 2.2%, Fig. 1e). The minimum and maximum values NVF/NVT and NVB+VPC /NVT calculated on average for a reservoir, differed by 1.1 and 2.1 times (Fig. 1c). The average NVF/NVT and NVB+VPC /NVT value for all reservoirs during all study periods were 90.0 ± 2.2% and 10.0 ± 2.2%. The minimum and maximum values of the ratio of the total biomass of virioplankton to biomass of their hosts (bacteria+picocyanobacteria, BVT/BVB+VPC) differed by 5.6 times, averaging 2.6 ± 0.8% (Table 2).

Viral infection and virus-induced mortality of heterotrophic bacteria. The frequency of visibly infected bacterial cells (FVIC) by viruses, i.e. the proportion of bacteria containing mature phage particles inside cells in NB (Fig. 2) varied from 0.5% of NB in the Sheksna Reservoir to 5.0% of NB in the Gorky and Cheboksary reservoirs averaging 2.3 ± 0.5% of NB (Table 3). Based on these data, it was calculated that from 3.5% to 22.9% (on average 14.8 ± 2.8%) of all bacteria in the studied reservoirs were infected by phages. Viruses infected heterotrophic bacteria of different morphology. Viruses-bacteriophage more often infected rods and vibrios (Fig. 3a).

Bacteria inside cells contained up to 860 phages/cell. The average number of phages per a water sample varied from 7 to 123 (on average 32 ± 14 phages/cell for all water samples, Table 3). Virus-induced mortality of bacterioplankton (VMB) in the Volga reservoirs varied widely (Table 3), averaging 20.7 ± 4.9% of the daily bacterioplankton production in the studied reservoirs (Table 3). In the second half of the summer, the abundance (VIMNB) and biomass (VIMBB) of bacteria that died as a result of viral lysis significantly differed both between water samples ((0.1–3.1) × 10^6 cells/(mL × day) and (2.2–102.4) mg C/(m^3 × day), respectively) and between average values for the reservoirs (Fig. 3b, c), averaging 1.3 ± 0.8 × 10^6 cells/(mL × day) and 25.2 ± 16.4 mg C/(m^3 × day), respectively for all reservoirs. The organic matter (VIMBB – PVB), from 2.0 to 101.6 mg C/(m^3 × day), on average 21.5 ± 14.6 mg C/(m^3 × day) for all water samples, was released due to viral lysis into the aquatic environment in different parts of the reservoirs. Among the studied reservoirs, the highest values (VIMBB – PVB) were recorded in the Gorky and Cheboksary reservoirs during the hot summer of 2010 (Fig. 3d). In the second half of the summer, the production of bacteriophages (PVB) varied widely in the area of the reservoirs (Table 3). The minimum and maximum values of this parameter differed by 259 times, averaging (39.5 ± 23.9) × 10^6 particles/(mL × day). The viral turnover time (VTT) fluctuated significantly from 0.2 to 12.8 days but the average values for the reservoirs differed to a lesser extent (Fig. 3e). VTT was on average 2.6 ± 1.3 day for all water samples.

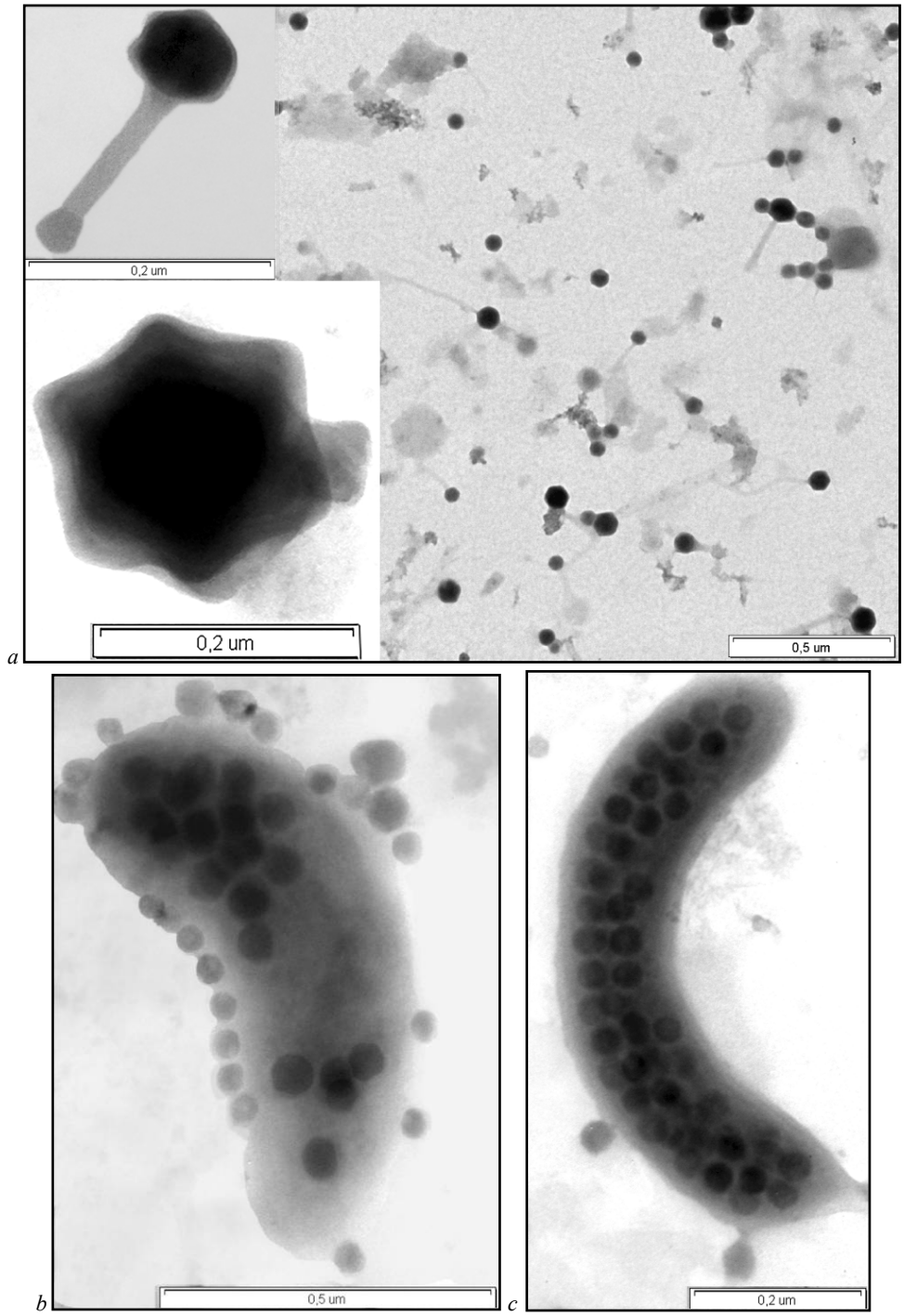

Fig. 2. Viruses in Volga reservoirs: *a* – free viral particles, *b* – viruses attached to a bacterial cell, *c* – viruses inside a bacterial cell

Table 2

The abundance of free viruses (N_{VF}), abundance of viruses attached to bacteria and picocyanobacteria, (N_{VB+VPC}), total abundance of virioplankton (N_{VT}), total biomass of virioplankton (B_{VT}), ratio of virioplankton biomass to biomass of bacteria + picocyanobacteria (B_{VT}/B_{B+PC}) in the reservoirs (on average for a reservoir)

Reservoirs	N_{VF}, 10^6 viruses/mL		N_{VB+VPC}, 10^6 viruses/mL		N_{VT}, 10^6 viruses/mL		B_{VT}, mg C/m^3		B_{VT}/B_{B+PC}, %	
	x±SD	Limits	x±SD	Limits	x±SD	Limits	x±SD	Limits	x±SD	Limits
Ivankovo (August 24–26, 2005)	55.2 ± 9.9	15.7–120.3	5.8 ± 1.6	2.2–17.4	61.0 ± 37.0	17.9–137.7	6.1 ± 3.7	1.8–13.8	2.2 ± 0.7	1.2–3.8
Uglich (August 22–24, 2005)	42.9 ± 5.3	21.7–73.8	4.1 ± 1.7	1.8–7.6	47.0 ± 18.4	23.5–81.4	4.7 ± 1.8	2.4–8.1	2.2 ± 0.6	1.5–3.1
Rybinsk (August 19–20, 2005)	30.8 ± 15.0	17.4–57.4	2.6 ± 0.8	1.8–3.8	33.4 ± 15.7	19.2–60.8	3.3 ± 6.1	1.9–6.1	3.0 ± 0.6	2.0–3.6
Rybinsk (August 08–16, 2007)	57.0 ± 6.5	21.1–90.5	3.4 ± 1.5	0.6–6.1	60.4 ± 24.6	25.1–93.6	6.0 ± 2.4	2.5–9.4	4.6 ± 1.4	1.8–6.6
Gorky (02–04.09.2005)	49.1 ± 18.8	21.2–85.6	4.8 ± 1.8	2.2–8.6	53.9 ± 19.4	23.6–88.8	5.4 ± 1.9	2.4–8.9	2.7 ± 0.6	1.7–3.5
Gorky (July 21–24, 2010)	47.4 ± 30.0	24.2–134.0	4.3 ± 1.8	2.2–8.6	51.7 ± 29.9	28.0–138.2	5.2 ± 3.0	2.8–13.8	2.6 ± 1.4	1.4–5.7
Cheboksary (September 07, 2005)	30.6 ± 2.3	26.3–36.6	4.5 ± 1.1	3.0–5.2	35.1 ± 5.0	30.8–41.8	3.5 ± 0.5	3.1–4.2	2.0 ± 0.2	1.7–2.2
Cheboksary (July 25-28, 2010)	55.9 ± 10.2	38.2–77.1	7.3 ± 2.6	2.0–5.8	63.2 ± 11.2	44.8–85.9	6.3 ± 1.1	4.5–8.6	2.0 ± 0.6	1.3–3.4
Sheksna (August 03–09, 2005)	20.4 ± 4.5	9.4–24.8	2.8 ± 0.8	1.6–3.6	23.2 ± 4.8	11.4–28.1	2.3 ± 0.5	1.2–2.8	2.3 ± 0.5	1.2–3.8
Sheksna (August 08–12, 2007)	27.2 ± 12.8	12.4–55.7	4.0 ± 2.6	1.4–10.8	31.2 ± 13.2	19.5–65.1	3.1 ± 1.3	2.0–6.5	2.4 ± 1.6	1.2–6.7

Table 3

Frequency of visibly infected bacterial cells (FVIC), frequency of all infected bacterial cells (FIC), virus-induced bacterial mortality (VMB), number of mature phages inside bacterial cells (BS),viral production (P_V) in the reservoirs (on average for a reservoir)

Reservoirs	FVIC, % of N_B		FIC, % of N_B		VMB, % of the bacterio-plankton production		BS, phages/cell		P_V, 10^6 viruses/(mL × day)	
	x±SD	Limits	x±SD	Limits	x±SD	Limits	x±SD	Limits	x±SD	Limits
Ivankovo (August 24–26, 2005)	2.1 ± 0.8	1.2–3.6	14.0 ± 4.8	8.3–17.5	19.1 ± 8.3	10.5–34.8	58 ± 22	17–83	72.8 ± 39.5	11.3–131.5
Uglich (August 22–24, 2005)	2.7 ± 1.4	1.4–4.9	17.0 ± 7.5	9.4–33.5	22.6 ± 10.0	10.8–40.2	45 ± 28	27–109	47.3 ± 30.2	12.2–111.4
Rybinsk (August 19–20, 2005)	2.8 ± 1.3	1.0–4.1	17.6 ± 8.0	6.9–24.3	26.0 ± 16.5	7.8–39.3	12 ± 4	7–20	12.8 ± 8.6	3.0–26.0
Rybinsk (August 08–16, 2007)	2.0 ± 0.7	1.0–3.5	13.0 ± 4.3	6.9–22.1	17.2 ± 7.6	7.8–34.1	22 ± 8	11–39	26.9 ± 19.4	10.4–81.1
Gorky (September 02–04, 2005)	2.3 ± 0.9	0.8–3.8	15.0 ± 5.4	5.5–23.7	20.5 ± 9.3	6.1–37.8	41 ± 18	17–72	34.4 ± 27.6	6.4–95.9
Gorky (July 21–24, 2010)	2.8 ± 1.3	1.3–5.0	17.8 ± 7.3	8.9–29.9	26.9 ± 15.3.	10.5–55.0	23 ± 19	7–61	74.4 ± 112.0	2.7–389.5
Cheboksary (September 07, 2005)	2.6 ± 1.1	1.8–4.0	16.8 ± 6.4	11.4–24.8	24.8 ± 12.0.	14.1–40.6	38 ± 14	24–58	39.4 ± 13.3	28.0–57.3
Cheboksary (July 25–28, 2010)	2.5 ± 0.9	0.8–5.0	16.1 ± 5.3	5.5–29.9	22.8 ± 10.4.	6.1–55.0	31 ± 35	5–123	59.3 ± 69.6	3.4–300.6
Sheksna (August 03–09, 2005)	1.8 ± 0.9	0.8–3.8	11.7 ± 5.4	5.5–23.7	15.0 ± 8.7.	6.1–37.8	22 ± 11	12–47	11.3 ± 14.5	2.3–47.3
Sheksna (August 08–12, 2007)	1.4 ± 0.8	0.5–3.5	9.2 ± 5.4	3.5–22.1	11.8 ± 8.5.	3.7–34.1	26 ± 12	8–47	22.8 ± 23.5	2.8–106.0

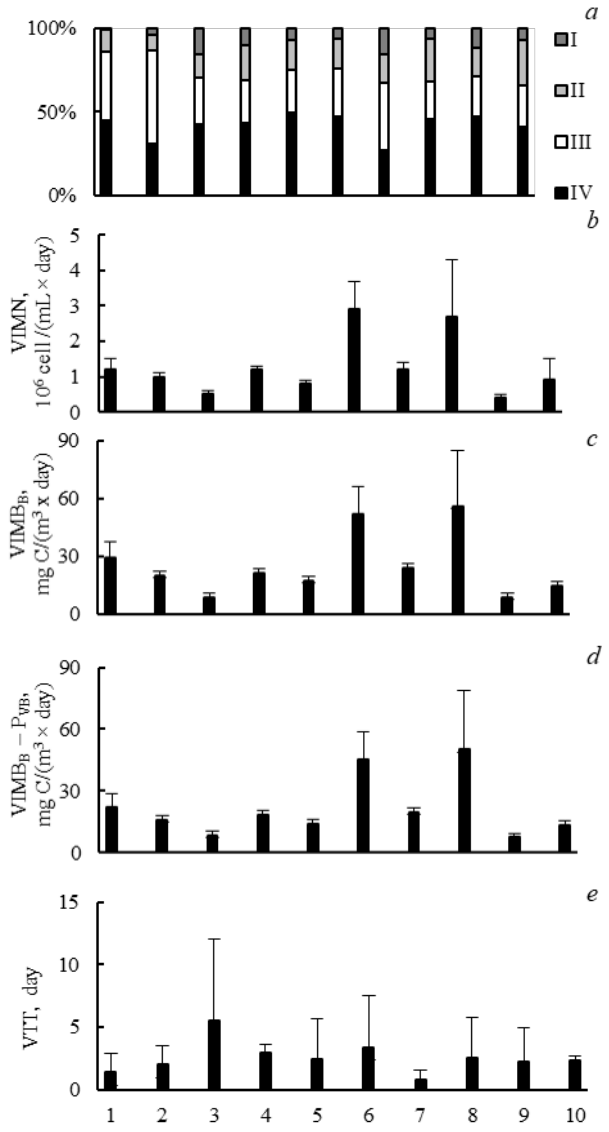

Fig. 3. Distribution (on average for a reservoir, x ± SD) of the proportion of bacteria of different morphology of the total abundance of lysed bacteria, abundance and biomass of lysed bacteria, the amount of organic matter released to the aquatic environment as a result of viral lysis of bacteria and turnover time of the viral abundance in the Volga reservoirs: *a* – proportion (%) of rods (IV), vibrios (III), cocci (II), filaments (I) in the total abundance of infected bacteria (NBFVIC) in the reservoirs, *b* – abundance of bacteria lysed by viruses per day (VIMNB, 10^6 cells/(mL × day)), *c* – biomass of bacteria lysed by viruses per day (VIMBB, mg C/(m^3 × day)), *d* – amount of organic matter released to the aquatic environment as a result of viral lysis of bacteria, (VIMBB – PVB, mg C/(m^3 × day)), *e* – turnover time of the viral abundance (VTT, day)

Viral infection and virus-induced mortality of picocyanobacteria. The frequency of visibly infected picocyanobacterial cells (FVIC) varied from 0.6% of NPC in the Sheksna Reservoir to 6.0% of NB in the Cheboksary Reservoir, averaging 2.2 ± 0.8% of Nв (Table 4). Based on these data, it was calculated that from 2.4% to 24.0% (on average 9.0 ± 3.2%) of all bacteria in the studied reservoirs were infected by viruses. Picocyanobacteria contained up to 874 phages/cell. The average abundance of phages in the cell per water sample varied from 6 to 236 (on average 27 ± 15) phages/cell (Table 4).

Virus-induced mortality of picocyanobacteria (FMVL) varied widely (Table 4), averaging 14.2 ± 5.2% of the daily picocyanobacterial production for the studied reservoirs (Table 4, Fig. 4a). In its turn, the lysis of picocyanobacteria by cyanophages reduced the total primary production of phytoplankton on average by 1.9 ± 1.5% (range 0.4–6.0%) in the Rybinsk Reservoir, by 1.1 ± 0.9% (range 0.2–3.5%) in the Gorky Reservoir, by 1.8 ± 1.5% (range 0.3–4.3%) in the Sheksna Reservoir. The abundance of picocyanobacteria that died due to viral lysis (VIMPC) significantly differed both between water samples from 6.2 до 140.2 × 10^3 cells/(mL × day) and between average values for the reservoirs (Fig. 4b), averaging 34.9 ± 13.8 × 10^3 cells/(mL × day) for all reservoirs. As a result of the viral lysis of picocyanobacteria, the organic matter (VIMBPC – PVPC) from 1.6 to 23.5 mg C/(m^3 × day) was released into the aquatic environment in different parts of the reservoirs, averaging 6.9 ± 2.0 mg C/(m^3 × day) for all water samples (Fig. 4c). The production of picocyanophages (PVPC) also varied widely in the area of the reservoirs (Table 4). The minimum and maximum values of the production differed by 182 times, averaging (0.83 ± 0.86) × 10^6 particles/(mL × day). Thus, the production of picocyanophages, on average for a reservoir, in these reservoirs was 28–219 times lower than the production of bacteriophages.

Discussion

Across the six studied reservoirs the viral abundance (VA) was the highest in the eutrophic reservoirs (15.7–134.0 × 10^6 viruses/mL), intermediate in meso-eutrophic reservoirs (17.4–90.5 × 10^6 viruses/mL) and the lowest in a mesotrophic reservoir (9.4–55.7 × 10^6 viruses/mL). The average virus/bacteria ratio (VBR) for a reservoir varied between 3.4 and 7.6. We found a correlation between viral and bacterial abundance (BA) and BA and VA were both positively correlated with primary production of phytoplankton. The strong positive correlation between viral and bacterial dynamics are tightly linked and support the hypothesis that the majority of virioplankton of the lakes and reservoirs are bacteriophages (Peduzzi & Schiemer, 2004; Ram et al., 2005; Hardbower et al., 2012). In various freshwater bodies, the abundance of free planktonic viruses enumerated by epifluorescence microscopy varied widely from 0.02 × 10^6 particles/mL in an oligotrophic lake (Hofer & Sommaruga, 2001) to 379 × 10^6 particles/mL in a hypertrophic lake (Wilhelm & Smith, 2000). In fresh waters the VBR varies from 0.03 to 41 (up to 141 in Antarctic lakes), but the VBR value is usually within 3–10 (Wommack & Colwell, 2000). In Lake Michigan, the eutrophic site was distinct with one-to two fold higher virus abundance than mesotrophic and oligotrophic sites (accordingly, 1.5–563.0 × 10^6, 0.5–68.9 × 10^6 and 0.3–29.5 × 10^6 particle/mL)

(Hanson et al., 2017). The authors found strong correlations between the abundances of viruses and prokaryotes and chlorophyll concentration across the trophic gradient. European mesotrophic lakes Bourget and Constance and eutrophic Lake Plußsee have appropriately high VA ($34–82 \times 10^6$, $10–40 \times 10^6$ and $11–88 \times 10^6$ viruses/mL, respectively) and show clear trends in VA in relation to the trophic status (Hennes & Simon, 1995; Weinbauer & Hofle, 1998; Tomas et al., 2011). A strong positive correlation between the viral abundance and the trophic status of a water body was found for 24 Quebec lakes (Maranger & Bird, 1995).

The analysis of the literature data shows that in most freshwater bodies, the proportion of bacteria containing mature phages inside cells (FVIC) varied from 0.7% to 4.3% of NB, i.e. from 4.9% to 26.5% of all bacteria were infected with bacteriophages, which suggests that virus-induced mortality of bacteria was from 5.4% to 45.0% of the bacterioplankton production (Weinbauer & Hofle, 1998; Wommack & Colwell, 2000; Simek et al., 2001; Vrede et al., 2003; Ram et al., 2005; Wilhelm & Matteson, 2008). During particular periods, the virus-induced mortality of bacterioplankton in some lakes reached 60–97% of its daily production (Hennes & Simon, 1995; Wilhelm & Matteson, 2008; Fischer & Velimirov, 2002).

Virus-induced mortality of autotrophic picoplankton in marine habitats varies from 1% to 21% of their daily production (Fuhrman, 1999; Ortmann et al., 2002; Mann, 2003; Baudoux et al., 2007). The studies in freshwater bodies were largely aimed at the study of the role of viruses in the control of abundance of large colonial cyanobacteria but little is known about the effect of viral lysis on mortality of natural populations of autotrophic picoplankton (Honjo et al., 2006; Tijdens et al., 2008). A close correlation between the abundances of viruses and picocyanobacteria was found in some lakes, which evidences the presence of a significant amount of cyanophages in the virioplankton composition (Colombet et al., 2009). At the same time, studies using dilution techniques and flow cytometry show that approximately 10% of picocyanobacterial loss in lakes could be attributed to viral lysis (Personnic et al., 2009).

Viruses as the most numerous component of planktonic communities in the Volga reservoirs constituted (mgC/m^3) only 1.5–2.4% (on average 2.0 ± 0.3%) of the total biomass of the microbial community (viruses, bacteria, protozoa) and 0.4–1.0% (on average 0.7 ± 0.2%) of the total planktonic biomass (Table 5). The viral biomass in marine pelagic communities constitutes about 6% of the biomass of heterotrophic bacterioplankton (Steward et al., 2007).

As a result of viral lysis of host cells (in our work, bacteria and picocyanobacteria), easily digestible organic compounds are released into the aquatic environment together with viruses. Thus, carbon and other nutrients in the composition of the suspended organic matter of cells were transformed into a soluble form. These soluble compounds are actively used by heterotrophic bacteria and, thus remain within the planktonic microbial community and are not channeled to higher trophic levels of food webs; it is the so-called viral shunt (Wommack & Colwell, 2000; Weinbauer, 2004). The total rate of release of organic matter of lysed bacterial and picocyanobacterial cells into the Volga reservoirs (ΣVIM – PV) varied from 21 to 1296 (on average 272 ± 134) mg $C/(m^2 \times day)$ or from 6.8 to 132.2 (on average 40.7 ± 16.8) mg $C/(m^3 \times day)$ (Table 6). The lysed bacteria constituted the main fraction in ΣVIM – PV (Fig. 4d).

The viral shunt was an important source of the easily digestible organic matter input: the ΣVIM – PV values were 6.3–52.9% (on average 18.4 ± 5.2%) of the phytoplankton primary production for m^2 and could provide 3.4–31.6% (on average 7.0 ± 1.4%) daily organic carbon demand for heterotrophic bacterioplankton (Table 6). Fischer & Velimirov (2002) estimated that in a eutrophic lake, on average 15.2 μg C/(L × day) was released into the aquatic environment due to viral lysis of bacterial cells, which was 46% of the daily bacterial production in the water column or about 14% of daily demands of bacteria for organic matter.

The abundance of planktonic viruses in natural waters depends both on their production and decay or extraction of viral particles from the water column (Bratbak et al., 1994). The abundance of viruses in the water column may decrease due to the following reasons: grazing of heterotrophic nanoflagellates (HNF) on free viruses (Suttle & Chen, 1992; Gonzalez & Suttle, 1993; Bettarel et al., 2005), sedimentation of large suspended particles with attached viruses; decay by bioactive molecules such as exoenzymes, proteases, or nucleases, which extract nucleic acids from viral capsids (Bratbak et al., 1994; Noble & Furman, 1997, 1999), mortality caused by viriophages (La Scola et al., 2008; Fischer & Suttle, 2011; Yau et al., 2011). The experimental studies have demonstrated that the grazing rate of HNFs on free viruses is low, from 1.9 to 3.3 viruses/(ind. × h) (Suttle & Chen, 1992; Bettarel et al., 2005). HNF can, apparently, extract only large viral particles from water.

During the period of study, the abundance of free viruses with a capsid diameter more than 0.2 μm (the size of the smallest bacteria about 0.2 μm) varied from 0 to 2.2×10^6 viruses/mL in the Rybinsk and Sheksna reservoirs. Using the clearance rates we found that in the reservoirs, HNF grazing rates on large viral particles could be 1.1–12.0 (on average 5.0 ± 3.8 viruses/(flagellate × h)), i.e. the number of free viruses grazed by the HNF community was low (Table 7).

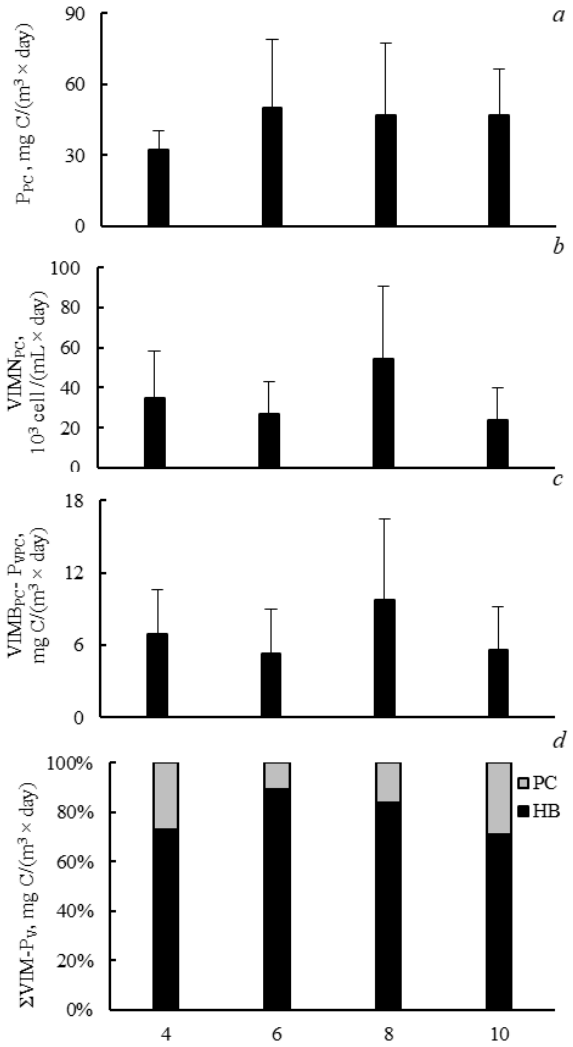

Fig. 4. Distribution (on average for a reservoir, x ± SD) of the production of picocyanobacteria, abundance of lysed picocyanobacteria, amount of organic matter released to the aquatic environment as a result of viral lysis of picocyanobacteria and proportion of heterotrophic bacteria and autotrophic picocyanobacteria of the total amount of organic matter released to the aquatic environment as a result of viral lysis in the Volga reservoirs: *a* – picocyanobacterial production (PPC, mg $C/(m^3 \times day)$), *b* – abundance of picocyanobacteria lysed by viruses per day (VIMNPC, 10^3 cells / (mL × day)), *c* – amount of organic matter released to the aquatic environment as a result of viral lysis of picocyanobacteria, (VIMBPC–PVPC, mg $C/(m^3 \times day)$), *d* – proportion of heterotrophic bacteria (HB) and picocyanobacteria (PC) in the total amount of organic matter released into aquatic environment as a result of viral lysis of heterotrophic bacteria and picocyanobacteria (ΣVIM–PV, mg $C/(m^3 \times day)$) in reservoirs: *4* – Rybinsk (August 08–16, 2007), *6* – Gorky (July 21–24, 2010), *8* – Cheboksary (July 25–28, 2010); *10* – Sheksna August 08–16, 2007)

Table 4
Frequency of visibly infected picocyanobacterial cells (FVIC), frequency of all infected picocyanobacterial cells (FIC), virus-induced mortality of bacteria (FMVL), number of mature phages inside picocyanobacterial cells (BS), virus-picocyanobacteria production (PVPC) in the reservoirs (on average for a reservoir)

Reservoirs	FVIC, % N_{PC}		FIC, % N_{PC}		FMVL, % of picocyanobacterial production		BS, phages/cell		P_{VPC}, 10^6 viruses/(mL × day)	
	x ± SD	Limits	x ± SD	Limits	x ± SD	Limits	x ± SD	Limits	x ± SD	Limits
Rybinsk (August 08–16, 2007)	2.6 ± 1.4	1.0–5.3	10.4 ± 6.8	4.0–21.2	17.2 ± 15.2	7.6–52.0	21 ± 16	6–58	0.72 ± 0.56	0.11–1.62
Gorky (July 21–24, 2010)	1.8 ± 0.8	1.0–3.4	7.2 ± 3.3	4.0–13.6	11.2 ± 5.7.	6.0–22.7	13 ± 4	8–21	0.34 ± 0.23	0.06–0.83
Cheboksary (July 25–28, 2010)	3.2 ± 1.5	0.8–6.0	12.8 ± 5.8	3.2–24.0	19.7 ± 10.4.	4.8–45.6	48 ± 68	6–236	2.08 ± 3.52	0.07–10.95
Sheksna (August 08–12, 2007)	1.4 ± 0.8	0.6–2.9	5.6 ± 4.6	2.4–11.6	8.6 ± 5.5	4.5–24.5	25 ± 29	5–81	0.19 ± 0.15	0.06–0.45

Table 5
Proportion (%) of different components of plankton in total biomass of planktonic community (TBP, mg C/m3) in the Upper and Middle Volga reservoirs

Reservoir	Total biomass of plankton	% of TBP				
		viruses	phytoplankton	bacteria	Protozoa	metazooplankton
Ivankovo (August 24–26, 2005)	871	0.7	37.3	32.1	6.9	23.0
Uglich (August 22–24, 2005)	500	1.0	29.8	40.1	7.6	21.5
Rybinsk (August 19–20, 2005)	567	0.6	59.7	20.6	5.7	13.4
Sheksna (August 03–09, 2005)	603	0.4	54.7	20.3	6.1	18.5
Gorky (September 02–04, 2005)	711	0.8	61.6	29.4	3.5	4.7
Cheboksary (September 07, 2005)	627	0.6	68.8	26.8	1.0	2.8
Cheboksary (July 25–28, 2010)	1531	0.4	63.0	25.0	3.6	8.0

Table 6
The amount of organic matter released to aquatic environment due to viral lysis of bacteria and picocyanobacteria, (VIM-PV), primary production of phytoplankton per m^2 (åPPHY), daily demand of bacterioplankton for organic matter (CB) in Volga reservoirs

Parameters	Rybinsk, 2007		Gorky, 2010		Cheboksary, 2010		Sheksna, 2007	
	x ± SD	Limits	x ± SD	Limits	x ± SD	Limits	x ± SD	Limits
$\Sigma VIM\text{-}P_V$, mg C/(m^2 × day)	208 ± 103	52–418	384 ± 374	28–1039	384 ± 295	83–1296	114 ± 75	21–301
$\sum P_{PHY}$, mg C/(m^2 × day)	1202 ± 775	517–2593	1485 ± 951	246–2922	2776 ± 1960	740–8395	687 ± 350	242–1141
$\Sigma VIM - P_V/\sum P_{PHY}$, %	17.3 ± 10.7	6.3–37.7	25.8 ± 16.5	1.5–52.9	13.8 ± 16.4	3.3–63.4	16.6 ± 10.2	2.7–47.5
$VIM - P_V$, mg C/(m^3 × day)	29.0 ± 10.1	12.9–44.0	49.8 ± 42.6	9.9–132.2	59.7 ± 35.7	16.5–125.1	24.4 ± 10.6	6.8–46.1
C_B, mg C/(m^3 × day)	413 ± 81	333–607	564 ± 350	232–1164	860 ± 396	397–1913	453 ± 163	240–867
$VIM - P_V/C_B$, %	7.0 ± 2.6	3.4–12.1	8.8 ± 4.2	4.3–16.3	6.9 ± 3.3	2.7–17.4	5.4 ± 6.6	1.4–31.6

Table 7
Grazing rates on free viruses of 0.2 µm and more (CVF), grazing rates on bacteria (CB), grazing rates on viruses attached to bacterial cells (CVB) and grazing rates on viruses inside bacterial cells (CBS) by natural populations of heterotrophic nanoflagellates in Volga reservoirs

Parameters	Rybinsk Reservoir		Sheksna Reservoir	
	x ± SD	limits	x ± SD	limits
C_{VF}, 10^6 viruses/(mL × day)	0.04 ± 0.12	0.00–0.43	0.15 ± 0.04	0.04–0.46
C_B, 10^6 cells/(mL × day)	2.2 ± 1.2	0.9–4.7	1.7 ± 0.7	1.0–2.8
C_{VB}, 10^6 viruses/(mL × day)	1.2 ± 0.6	0.1–2.4	0.9 ± 0.8	0.2–2.7
C_{BS}, 10^6 viruses/(mL × day)	6.1 ± 3.8	2.1–12.0	4.4 ± 3.8	0.7–13.9
$C_{VF} + C_{VB} + C_{BS}$, 10^6 viruses/(mL × day)	7.3 ± 4.2	2.2–14.8	5.45 ± 4.3	0.9–17.0
$C_{VF} + C_{VB} + C_{BS}/ P_V$, %	30.6 ± 15.4	13.1–59.8	27.6 ± 13.7	10.0–52.8

A much greater number of viruses reach a higher trophic level when HNF graze on infected bacteria and bacteria with viral particles attached to their cells (Table 7). As a result, a significant amount of viral particles were involved in the food web of the reservoirs, which constitutes a significant percentage of the daily virioplankton production (Table 7). However, the biomass of grazed viruses in the Rybinsk and Sheksna reservoirs (0.73 ± 0.42 mg C/(m^3 × day) and 0.54 ± 0.43 mg C/(m^3 × day), respectively) was 53–58 times lower than the biomass of grazed bacteria (38.8 ± 5.2 mg C/(m^3 × day) and 31.3 ± 2.6 mg C/(m^3 × day), respectively).

Conclusions

The results of the study on ecology of planktonic viruses indicate that virioplankton is an important structural and functional component of the microbial planktonic food web in the Upper and Middle Volga reservoirs. Viruses cause the mortality of a large number of autotrophic and heterotrophic picoplankton. Due to the viral lysis of heterotrophic bacteria and picocyanobacteria cells, a significant portion of their production does not enter a higher trophic level and products of the viral lysis increase the reserves of soluble and insoluble organic matter, i.e. they are retained within the microbial communities. Thus, viruses support their hosts providing them with organic carbon and minerals. Viral particles are involved in the food webs of water bodies when they are directly grazed by protozoa or with host cells. The quantitative analysis of these processes in the studied reservoirs indicates a substantial role of planktonic viruses in carbon fluxes through the planktonic food webs.

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