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Viral Infection of Picocyanobacteria in the Rybinsk Reservoir During the Freezing Period

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Abstract—The abundances of virioplankton and planktonic picocyanobacteria in deep and shallow water sites of Rybinsk Reservoir during the freezing period (water temperature 0.3–0.9°C) varied from (37.1 to 84.1) × 10^6 ((57.3 ± 2.1) × 10^6, on average) particles/ml and from 13.5 to 75.0 × 10^3 ((48.7 ± 3.4) × 10^3, on average) cells/ml, respectively. The fraction of picocyanobacteria with viruses attached to their cell surface was 6.5–29.0% (12.0 ± 0.8%, on average). The proportion of visible infected cells was 0.7–7.6% (2.2 ± 0.3%, on average) of the numbers of picocyanobacteria. It is likely that viruses play an important role in the regulation of picocyanobacteria abundance during the freezing period.

Keywords: picocyanobacteria, virioplankton, virus-induced cyanobacteria mortality, freeze-up period, Rybinsk Reservoir

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INTRODUCTION

Autotrophic picoplankton (solitary cyanobacteria and algae less than 2 μm in size) are present in all types of freshwater ecosystems (Stockner, 1991). In the majority of these ecosystems, the concentrations of picocyanobacteria are an order higher than of picoalgae and the former are the main component of the autotrophic picoplankton (Mikheeva, 1998).

Picocyanobacteria (PC) are an important component of the planktonic food webs, and heterotrophic nanoflagellates, infusoria, and multicellular fine filter-feeders actively consume them. In the Upper Volga reservoirs, PC play an important role for plankton. In summer, a considerable part of them dies out due to viral infection and lysis (Kopylov et al., 2010). Although the Boreal waterbodies are covered by ice for half the year, these processes during the freezing period have not yet been studied.

The goal of the present paper is to determine the level of quantitative development of virioplankton and planktonic picocyanobacteria, as well as to assess the intensity of viral infection in picocyanobacteria during the freezing period at low water temperatures.

MATERIALS AND METHODS

The studies were carried out at the Volzhskii reach of Rybinsk Reservoir at one deep-water (site 1, 58°05.61′ N, 38°18.04′ E) and two shallow-water (site 2, 58°05.77′ N, 38°17.53′ E and site 3, 58°08.83′ N, 38°22.75′ E) sampling sites. At each site the water was sampled on February 2, 15, and 26 and March 11 and 27, 2008, using a 0.5 L Plexiglas Ruttner bathometer. The water was sampled at 2–3 layers of the water column including the surface (about 20 cm under ice) and near-bottom (about 50 cm above the bottom) layers.

Planktonic virus particles were analyzed by the epifluorescence microscopy technique using SYBR Green I stain and Anodisc (Wathman, United States) aluminum oxide filter with a 0.02 μm pore size (Noble and Fuhrman, 1998). The number of PC was determined using epifluorescence microscopy by the autofluorescence of their cell pigments (McIsaac and Stockner, 1993) on a black nuclear filter with 0.2 μm pores (JINR, Dubna, Russia). The viruses and PC were counted using Olympus BZ51 (Olympus, Japan) epifluorescence microscope equipped with the system for analysis of the images.

To determine the frequency of clearly visible cells of picoautotrophs infected with the viruses (*FVIC*, % of the total number of PC) and the mean number of mature phages in the infected cells (Burst size (*BS*), particles/cell), we used transmission electron microscopy. The viruses and PC were precipitated on nickel Pioloform/carbon coated grids for electron microscopy (400 mesh density) with by centrifugation at 100000 g (35000 rpm) for 1 h at 4°C on an OPTIMA L-90k ultracentrifuge (Beckman Coulter, USA) equipped with a 45 Ti rotor. The grids were analyzed at 50000–150000 magnification on a JEM 1011 electron microscope (Jeol, Japan). The cells of picoautotrophs

were considered infected if they contained four or more mature phages.

Two stages in the cycle of virus lytic infection of bacterial cells are defined: latent period and lysis. The latent period is the time from the beginning of cell infection to the beginning of lysis. Mature phages appear in the cell directly before the lysis. This is why the viruses are invisible in the cells during most of the latent period. The share (%) of infected cells containing clearly visible mature cyanophage viruses in the total number of PC (*FVIC*) is an important parameter in the studies of viruses and cyanobacteria. The time from the beginning of infection to the first appearance of visible virus particles is called the "eclipse-period." Such particles are present in the infected bacterial cells from the end of the eclipse-period to the lysis. Thus, the share of all infected cells (the frequency of infected cells, *FIC*) in the PC population is much higher than *FVIC*. It was proposed to assess *FIC* as a product of *FVIC* and the ratio of the duration of the eclipse-period to the duration of the latent period (ε) (Proctor and Fuhrman, 1990). In our calculations the ε value was taken as 0.75 (Suttle, 2000; Mann, 2003).

RESULTS AND DISCUSSION

At the deep-water sites of the reservoir, the depth varied from 9.5 to 10.6 m; at the shallow-water sites, 3.5–4.5 m (Table 1). Water transparency was 2.8–3.5 m; the thickness of the ice was 40–60 cm; the thickness of the snow cover above the ice reached up to 10 cm. The values of pH were weakly alkaline: 7.31–7.88 (7.60, on average); electric conductivity was 374–400 (385, on average) μSm/cm. The water temperature fluctuated from 0.3 to 0.9°C and was higher at the deep-water site. In March the water temperature was higher (0.67°C, on average) than in February (0.54°C, on average). The concentration of water borne oxygen varied from 7.3 to 11.3 (9.1, on average) mg/L. No clear stratification of the water column during the freeze-up period was observed at the sites studied.

During the study period, the number of virioplankton (N_V) was high (Table 2). At the deep-water sites in the water column, the minimal and maximal values of N_V differed by 1.1–2.0 times. In February the mean for the number of viruses ($(57.2 \pm 3.6) \times 10^6$ particles/mL) in the water column differed slightly from the number in March ($(66.8 \pm 2.4) \times 10^6$ particles/mL). At the shallow-water sites 2 and 3, the mean for the water column values of N_V in February ($(56.1 \pm 2.2) \times 10^6$ and $(50.5 \pm 6.5) \times 10^6$ particles/mL, respectively) differed from the March values insignificantly ($(56.5 \pm 2.3) \times 10^6$ and $(53.9 \pm 4.9) \times 10^6$ particles/mL, respectively).

The number of PC (N_{PC}) at the studied sites of the reservoir was low, being three orders of magnitude lower than the number of planktonic viruses (Table 2). In February the values of N_{PC} at all sites at various depths differed by a factor of not more than 1.6 while in March the minimal values of this parameter in the near-bottom water layer were 1.5–4.8 times lower than the maximal values. In February at the deep-water site 1, the mean for the whole water column N_{PC} value (50728 ± 3430 cells/mL) was lower than in March (34883 ± 9488 cells/mL). At sites 2 and 3, the mean for the water column values of N_{PC} in February (69450 ± 1974 and 61900 ± 2654 cells/mL, respectively) were also considerably higher than in March (37500 ± 10818 and 39781 ± 10109 cells/mL, respectively). Virus particles were attached to the surfaces of a considerable part of PC (Table 2). At the deep-water site 1, the mean for the whole span of the observation period share of PC with attached viruses in N_{PC} (12.7 ± 1.5%) was slightly higher than for the shallow-water sites (site 2, 10.2 ± 1.1%; site 3, 10.0 ± 1.7%).

The number of viruses attached to one picocyanobacterium reached 11, and on average for the sample ranged from 1.2 to 3.0 viruses. The number of cyanophage viruses attached to cyanobacterial cells varied from 3831 to 34742 (on average, 9426 ± 1189), which accounted for 0.01–0.06 (on average, 0.02 ± 0.10)% *NV*.

At the deep-water site, the *FVIC* values changed from 1.0 to 7.6 N_{PC}, averaging 2.9 ± 0.5% (Table 3). The *FIC* values varied from 3.0 to 22.8% (8.8 ± 1.4%, on average) of N_{PC}. In February the values of *FVIC* and *FIC* (4.0 ± 0.5 and 12.0 ± 1.6% of N_{PC}, respectively) were higher than in March (1.4 ± 0.2 and 4.2 ± 0.3% of N_{PC}), respectively.

At the shallow-water sites 1 and 2, the values of *FVIC* and *FIC* fluctuated from 0 to 4.7 (1.7 ± 0.3%, on average) and from 0 to 1.41% (5.1 ± 0.8%, on average) N_{PC}, respectively. In March the intensity of infection by viruses (*FVIC* = 0.86 ± 0.20% and *FIC* = 2.58 ± 0.61% of N_{PC}) was also lower than in February ((*FVIC* = 2.31 ± 0.37% and *FIC* = 6.93 ± 1.10% of N_{PC}). A positive correlation ($R = 0.55$; $p = 0.05$) between the number of visibly infected PC and the number of viruses attached to the cells of cyanobacteria was observed.

The study revealed that the intensity of infection of PC by viruses decreased during the observation period from the beginning of February to the end of March. It is likely that the high intensity of viral infection observed in PC in February was the reason for the high mortality of the latter during this period. As a result, and presumably owing to the lack of gain in the PC number in March, their number decreased considerably and the intensity of viral infection dropped sharply. At the same time, lysis of cyanobacteria and a relatively high number of mature phages inside their cells lead to the release of free viruses into the environment. The latter fact may be one of the reasons for the high number of free viruses under the ice.

In Rybinsk Reservoir the numbers of virioplankton in February, March, and July differed inconsiderably

Table 1. Characteristics of the sampling sites in Rybinsk Reservoir in February–March 2008

Date	Ice thickness, cm	Depth, m	*T**, °C	O_2**, mg/L	Electric conductivity, μSm/cm	pH
1	2	3	4	5	6	7
Sampling site 1						
02.02.2008	40	10.6	0.8	11.3 / 8.8	374	7.31
15.02.2008	42	10	0.8	10.6 / 9.6	382	7.74
26.02.2008	57	9	0.8	8.9	377	7.63
11.03.2008	48	9	0.8	8.7 / 8.8	382	7.61
27.03.2008	45	9	0.9	7.3 / 8.0	392	7.61
Sampling site 2						
02.02.2008	55	4.1	0.4	11.3 / 7.8	388	7.59
15.02.2008	55	4	0.3	9.7 / 8.6	388	7.59
26.02.2008	60	3.5	0.7	9.4 / 8.6	380	7.56
11.03.2008	51	3.5	0.5	8.8	383	7.56
27.03.2008	50	3.5	0.6	8.3 / 7.9	399	7.51
Sampling site 3						
02.02.2008	55	4.4	0.3	11.0 / 7.9	382	7.70
15.02.2008	55	4	0.3	9.4 / 9.2	388	7.89
26.02.2008	57	3.7	0.5	9.3 / 8.9	379	7.63
11.03.2008	52	3.7	0.7	8.7 / 8.7	382	7.58
27.03.2008	54	4	0.5	8.7 / 7.4	400	7.55

* Under ice; ** top: under ice (ui); bottom: above bottom (ab).

Table 2. Total number of planktonic viruses (N_V, 10^6 particles/mL), number of picocyanobacteria (N_{PC}, 10^3 cells/mL), number of picocyanobacteria with viruses attached to their cells (N_{APC}, % N_{PC}) and number of viruses and number of viruses attached to picocyanobacteria (N_{VPC}, particles/ml) during the freezing period in Rybinsk Reservoir (February–March 2008)

Date	Water layer	N_V	N_{PC}	N_{APC}	N_{VPC}	N_{VPC}/N_V
1	2	3	4	5	6	7
Sampling site 1						
02.02.2008	ui	53.55	61.7	7.4	11415	0.02
	5	51.40	58.0	8.3	14442	0.03
	10	61.9	59.9	29.0	34742	0.06
15.02.2008	ui	65.45	57.8	10.4	12012	0.02
	5	59.50	42.5	16.0	21080	0.04
	10	76.89	38.0	10.5	12369	0.02
26.02.2008	ui	47.60	58.1	9.8	10231	0.02
	5	39.52	44.7	7.5	5364	0.01
	10	59.79	35.9	12.7	6839	0.01
11.03.2008	ui	37.08	47.7	9.1	6511	0.02
	5	62.77	75.0	7.3	6570	0.01
	10	65.45	15.5	17.7	5487	0.01
27.03.2008	ui	73.78	34.5	15.9	6583	0.01
	5	77.35	17.9	10.7	3831	0.01
	10	84.07	18.7	18.4	5505	0.01
Sampling site 2						
02.02.2008	ui	46.41	65.0	10.1	9848	0.02
15.02.2008	ui	66.33	72.2	8.0	8670	0.01
	ab	71.40	74.7	11.7	13110	0.02
26.02.2008	ui	48.81	70.5	6.5	9165	0.02
	ab	47.60	64.8	12.5	8910	0.03
11.03.2008	ui	58.23	63.0	7.5	5059	0.01
	ab	60.69	27.5	17.7	5534	0.01
27.03.2008	ui	49.98	13.5	9.3	3967	0.01
	ab	57.12	23.0	9.0	3105	0.01
Sampling site 3						
02.02.2008	ui	39.27	63.4	7.0	8873	0.02
15.02.2008	ui	60.9	71.7	21.4	18413	0.03
	ab	67.83	57.8	12.3	12086	0.02
26.02.2008	ui	41.65	58.5	9.0	6318	0.02
	ab	42.69	58.1	10.5	7324	0.02
11.03.2008	ui	50.08	68.1	9.3	7603	0.02
	ab	42.69	38.5	7.7	4447	0.02
27.03.2008	ui	57.20	31.5	20.2	11453	0.02
	ab	65.68	21.0	14.3	4204	0.01

ui, under ice; ab, above bottom.

Table 3. Frequency of visible infected picocyanobacteria cells (*FVIC*, % N_{PC}), frequency of infected picocyanobacteria (*FIC*, % N_{PC}) and number of viral particles inside the picocyanobacteria cells (*BS*, particles/cell)

Date	Water layer	*FVIC*	*FIC*	*BS*	
				max	average
Sampling site 1					
02.02.2008	ui	7.6	22.8	28	14 ± 2
	5	4.3	12.9	298	132 ± 58
	10	5.0	15.0	287	57 ± 28
15.02.2008	ui	3.5	10.5	11	7 ± 2
	5	3.0	9.0	8	7 ± 0.6
	10	3.3	9.9	5	5
26.02.2008	ui	4.1	12.3	4	4
	5	2.7	8.1	163	39 ± 31
	10	2.5	7.5	8	6 ± 2
11.03.2008	ui	1.5	4.5	6	5 ± 0.4
	5	1.3	3.9	134	44 ± 24
	10	1.4	4.2	5	4 ± 0.3
27.03.2008	ui	1.9	5.7	4	4
	5	1.0	3.0	4	4
	10	1.0	3.0	21	10 ± 4
Sampling site 2					
02.02.2008	ui	2.7	8.1	167	138 ± 15
15.02.2008	ui	1.4	4.2	4	4
	ab	2.4	7.2	20	8 ± 4
26.02.2008	ui	2.2	6.6	4	4
11.03.2008	ui	0.7	2.1	5	4 ± 1
	ab	1.2	3.6	16	12 ± 4
27.03.2008	ui	1.0	3.0	5	4 ± 1
	ab	0	0	0	0
Sampling site 3					
02.02.2008	ui	2.7	8.1	167	138 ± 7
15.02.2008	ui	4.7	14.1	11	6 ± 2
	ab	2.5	7.5	35	8 ± 5
26.02.2008	ui	1.2	3.6	6	4 ± 2
	ab	1.0	3.0	31	17 ± 8
11.03.2008	ui	1.4	4.2	56	16 ± 12
	ab	1.2	3.6	5	5
27.03.2008	ui	1.4	4.2	8	6 ± 2
	ab	0	0	0	0

ui, under ice; ab, above bottom.

Table 4. Number of virioplankton (N_V, 10^6 particles/mL) and picocyanobacteria (N_{PC}, 10^3 cells/mL), frequency of visible infected picocyanobacteria cells (*FVIC*, % N_{PC}), and number of mature cyanophages inside infected picocyanobacteria cells (*BS*, particles/cell) in July 2007 and February–March 2008

Parameter	2008		2007
	February	March	July*
T, °C	0.3–0.8	0.5–0.9	22.2–22.3
N_V	55.18 ± 2.61 39.27 – 76.89	60.16 ± 3.46 37.08 – 84.07	51.48 ± 8.02 21.10 – 90.54
N_{PC}	58.59 ± 2.57 35.90 – 74.70	35.39 ± 5.48 13.50 – 75.00	161.57 ± 15.52 100.53 – 236.62
FVIC	3.2 ± 0.4 1.0 – 7.6	1.1 ± 0.1 0 – 2.4	2.4 ± 0.4 1.0 – 4.5
BS	33 ± 12 4 – 138	10 ± 3 4 – 44	20 ± 6 5 – 58

the top is the mean ± error of mean; the bottom is min–max; * according to Kopylov et al., 2010.

but the abundance of PC during the freezing period was, on average, 3.2 times lower than in summer (Table 4). The share of visibly infected cells in N_{PC} and the number of mature viruses inside the cells in February were considerably higher than in summer; in March, they were lower.

CONCLUSIONS

In Rybinsk Reservoir during the freezing period, the number of virioplankton was high, being comparable to its number during the vegetation period. From February to March, the share of the infected picocyanobacteria cells decreased considerably, but, on average, for the observation period it was approximately the same as in summer.

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