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PHYTOPLANKTON, PHYTOBENTHOS, AND PHYTOPERIPHYTON

Molecular Genetic Identification and Seasonal Succession of Toxigenic Cyanobacteria in Phytoplankton of the Rybinsk Reservoir (Russia)

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Abstract—Data on the hepato- and neurotoxigenic cyanobacteria in phytoplankton of the Rybinsk Reservoir have been obtained for the first time. Different methods for revealing toxigenic cyanobacteria (light microscopy, PCR analysis, and enzyme-linked immunosorbent assay) demonstrate the same results. Hepatotoxins microcystins and for the first time neurotoxins saxitoxins were detected in the reservoir, whereas cylindrospermopsin and anatoxin-a were not revealed. The presence of *mcyE* and *stxA* genes responsible for microcystin and saxitoxin biosynthesis in total phytoplanktonic DNA is demonstrated. The following three genera of cyanobacteria containing *mcyE* gene are identified: *Microcystis* (*M. aeruginosa*, *M. viridis*), *Planktothrix* (*P. agardhii*), and *Dolichospermum* (*Anabaena*). It is hypothesized that saxitoxin-producing cyanobacteria *Dolichospermum (Anabaena)* inhabit the Rybinsk Reservoir.

Keywords: microcystins, saxitoxins, cylindrospermopsin, anatoxin-a, *mcyE*, *stxA*, *aoaA*, *anaC* genes, phytoplankton, cyanobacteria, Rybinsk Reservoir

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INTRODUCTION

The massive development of cyanobacteria (an obsolete term for blue-green algae) in reservoirs causes a broad range of negative effects, the most dangerous of which is the poisoning of animals and humans with cyanotoxins. This group includes secondary metabolites differing in chemical structure (peptides and alkaloids) and mechanisms of toxic action (inhibitors of phosphatases and biosynthesis of proteins, acetylcholinesterase, and sodium channel blockers).

Hepatotoxins (microcystins and cylindrospermopsin) and neurotoxins (anatoxin-a and saxitoxins) have been found in reservoirs around the world [13]. Hepatotoxins cause mostly necrotic liver injury, whereas neurotoxins cause neurologic effects such as seizures, convulsions, and paralysis of the respiratory muscles [8]. Many cyanobacterial toxins possess carcinogenic and mutagenic effects. The main representatives of toxigenic cyanobacteria in the waterbodies of tepid latitudes are the species of the genera *Microcystis*, *Planktothrix*, *Dolichospermum* (*Anabaena*), and *Aphanizomenon* [8, 13].

Studies of cyanobacterial toxins are focused on microcystin-producing cyanobacteria widespread in various waterbodies [18, 21]. Studies on the detection of hepato- and neurotoxin-producing cyanobacteria during the seasonal succession of phytoplankton are performed comparatively rarely [15]. Several structural variants of microcystins have been detected in the Rybinsk Reservoir [4].

The goals of the present work were the molecular genetic identification of hepato- and neurotoxin-producing cyanobacteria, the immunochemical detection of hepato- and neurotoxins, an analysis of the dynamic of cyanotoxins content, and the presence of toxins biosynthesis genes in cyanobacterial populations during the seasonal succession of phytoplankton in the Rybinsk Reservoir.

MATERIALS AND METHODS

The samples were collected in the Volzhsky stream pool of the Rybinsk reservoir at the station situated above the former town of Mologa (standard station Mologa, 58°13′ N, 38°27′ E) from May to October 2013. In total, ten samples were studied to determine composition, algal cell number, and biomass of phytoplankton, and seven of them were analyzed using molecular biological methods. The samples were collected from the surface 2-m water layer (the euphotic zone of the reservoir). Phytoplankton was concentrated by sequential filtration through 5 and 1.2 μm membrane filters and fixed with a Lugol solution sup-

plemented with formaldehyde and glacial acetic acid. Phytoplankton cell number was determined using 0.02 mL Uchinskaya-2 counting chamber; biomass was measured using the volumetric counting method [1, 5].

To extract DNA and perform polymerase chain reaction (PCR), phytoplankton was concentrated using MFAS-M-3 membrane filters (Vladipor, Russia). DNA was extracted using Diatom DNA Prep 200 reagents kit (Isogen Laboratory, Russia) according to the manufacturer's recommendation. To detect one of cyanobacterial genes *mcyE* responsible for microcystins biosynthesis, PCRs were performed using genus-specific primers mcyE-F2/MicmcyE-R8 (*Microcystis*), mcyE-F2/AnamcyE-12R (*Dolichospermum* (*Anabaena*)), and mcyE-F2/mcyE-plaR3 (*Planktothrix*), which allow one to identify microcystin producers in mixed cyanobacterial populations at the genera level [18, 21]. To detect *aoaA*, *anaC*, and *stxA* genes responsible for the synthesis of cylindrospermopsin, anatoxin-a, and saxitoxins, respectively, specific primers CatF1/CatR1, anaCgenF/anaCgenR, and sxtaf/sxtar were used [10, 11, 19]. Specific fragments of the genes responsible for cyanotoxin biosynthesis were amplified using a GenPak PCR Core reagents kit (Isogen Laboratory, Russia) as described previously [10, 11, 18, 19, 21].

DNA isolated from axenic cultures of *Microcystis aeruginosa* (strain PCC 7806) and *Planktothrix agardhii* (strain NIVA-CYA 126) producing microcystins (provided by Elke Dittmann, University of Potsdam, Germany), as well as from cylindrospermopsin-producing strain *Aphanizomenon* sp. 10E9 and saxitoxin-producing strain *Aphanizomenon* sp. AB59 (provided by Jutta Fastner, Federal Environmental Agency, Berlin, Germany), were used as a positive control. Control microcystins-producing stains of the genus *Dolichospermum* (*Anabaena*) and anatoxin-a-producing cyanobacteria were not used in the study. In these cases, we used data on the size of specific PCR products. DNA from non-axenic culture of *Gloeocapsa decorticans* (A. Braun) Richter in Wille unable to produce the studied cyanotoxins (provided by the Department of Botany and Microbiology of Yaroslav State University, Yaroslavl, Russia) was used as a negative control. PCR products were separated in 1.5% agarose, stained with ethidium bromide, and analyzed under UV light.

To detect toxigenic species of cyanobacteria, DNA was isolated from single cyanobacterial colonies [7, 22]. To exclude false-positive and false-negative results, a modified method proposed in our previous work was used [7]. Colonies of *Microcystis* and *Aphanizomenon flos-aquae* (L.) Ralfs were collected from concentrated planktonic samples using a sterile dissecting needle. Each colony was studied using a light microscope to exclude the presence of other potentially toxigenic cyanobacteria, then washed with sterile water three times and placed in Eppendorf tubes. Then DNA was extracted and PCR was performed. To detect *mcyE* gene responsible for microcystin synthesis, genus-specific primers mcyE-F2/MicmcyE-R8 were used. This allowed us to exclude false-positive results caused by the presence of extraneous microcystin-producing cyanobacteria. The same DNAs were examined for the presence of representatives of the genus *Microcystis* using 209F/409R primers designed for the amplification of *Microcystis*-specific fragments of cyanobacterial 16S rRNA genes [16]. When amplicons were not obtained using both primer systems (mcyE-F2/MicmcyE-R8 and 209F/409R), results of PCR-analysis were considered false-negative (for example, due to PCR inhibition and the absence or low concentration of DNA) and excluded from subsequent processing. Colonies of *Aphanizomenon flos-aquae* were studied using the same methods, but in this case primers sxtaf/sxtar specific for *stxA* gene responsible for saxitoxin production were used for the analysis. To control the presence of *Aphanizomenon flos-aquae* colonies in Eppendorf tube, PCβF/PCαR primers specific to intergenic spacer of cyanobacterial *cpc*A and *cpc*B genes (*cpc*BA-IGS) involved in phycocyanin biosynthesis were used [17].

Concentrations of cyanotoxins (microcystins, cylindrospermopsin, saxitoxins, and anatoxin-a) were measured using enzyme-linked immunosorbent assay (ELISA) using appropriate reagents kits (Abraxis LLS, United States) according to the manufacturer's recommendation. To determine the content of dissolved cyanotoxins in water, it was measured in filtered natural water. To reveal intracellular microcystins, the toxins were extracted from the collected phytoplankton biomass with distilled water using ultrasonic treatment.

The results were statistically processed. To estimate the correlation between the parameters, Spearman's rank correlation coefficient (r_S) was calculated.

RESULTS AND DISCUSSION

During the study, the seasonal dynamic of the biomass was determined mainly by the development of diatoms and cyanobacteria (Fig. 1). Average biomass of phytoplankton and cyanobacteria for the vegetation season were 6.1 ± 0.7 and 2.5 ± 0.6 mg/L (or 41% of the total biomass), respectively. Diatom (*Stephanodiscus hantzschii* Grun., *Aulacoseira subarctica* (O. Müll.) Haworth, *Cyclotella meneghiniana* Kütz.) spring peak and long summer maximum with two peaks caused by the growth of cyanobacteria in early July (*Aphanizomenon flos-aquae*, *Dolichospermum planctonicum* (Brunnth.) Wacklin et al. (=Syn.: *Anabaena schereme-tievi* Elenk.), *Dolichospermum* (*Anabaena*) sp., and *Microcystis aeruginosa*) and diatoms with cyanobacteria in early August (*Actinocyclus normanii* (Greg.) Hust., *Aulacoseira granulata* (Ehr.) Sim., and *Aphanizomenon flos-aquae*) were observed in the seasonal dynamic. After the diatom spring peak, the develop-

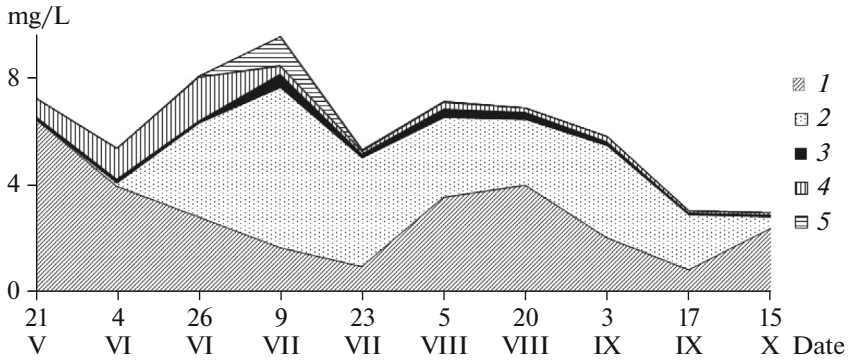

Fig. 1. Seasonal dynamic (mg/L) of different groups of the phytoplankton in 2-m water layer at Mologa station in 2013: (*1*) diatoms, (*2*) cyanobacteria, (*3*) green algae, (*4*) cryptomonads, and (*5*) dinoflagellates.

ment of cryptomonads and dinoflagellates was observed (Fig. 1). The dynamic of the phytoplanktonic cell number was determined by the growth of cyanobacteria. A long maximum caused mainly by the development of *Aphanizomenon flos-aquae* was observed from the beginning of July to the beginning of September. The number of *Aphanizomenon flos-aquae* was 47 to 76% of the total phytoplankton cell number. Average number of cyanobacteria was 3.86×10^7 cells/L (75%). Cyanobacterial vegetation started in late May to early June due to the development of *Planktothrix agardhii* (Table 1). In June, cyanobacteria were represented by the species of the genera *Microcystis* (*M. wesenbergii* (Kom.) Kom., *M. aeruginosa*, *M. flos-aquae* (Wittr.) Kirch.), *Aphanocapsa* (*A. holsatica* (Lemm.) Cronb. et Kom., *A. incerta* (Lemm.) Cronb. et Kom.), *Dolichospermum lemmermannii* (Richter) Wacklin et al. (=Syn.*: Anabaena lemmermannii* P. Richter), *Pseudanabaena mucicola* (Naumann et Hub.-Pest.) Schwabe, and *Snowella*. In early July, the species diversity of cyanobacteria was maximal (18 taxa) due to the development of *Microcystis*

Table 1. Biomass of potentially toxigenic species, concentration of cyanobacterial toxins, and presence of the genes involved in cyanotoxins biosynthesis in the Rybinsk Reservoir in 2013

Parameter	Date						
	May 21	June 4	June 24	July 9	August 5	August 20	September 17
	Biomass, mg/L						
Microcystis spp.	–	0.005	0.40	1.20	0.70	1.90	1.00
Planktothrix agardhii	0.004	0.007	–	–	–	–	–
Dolichospermum (*Anabaena*) spp.	–	0.10	2.60	2.10	0.10	0.05	0.02
Aphanizomenon flos-aquae	–	0.02	0.50	2.60	2.10	0.45	1.00
	Toxins concentration, μg/L						
Dissolved microcystins	0.05	0.14	0.36	0.62	0.38	1.99	0.63
Intracellular microcystins	0.04	0.01	1.34	3.00	3.00	2.77	5.20
Saxitoxins	*	*	0.05	0.02	*	*	*
Cylindrospermopsin	*	*	–	–	–	–	–
Anatoxin-a	*	*	–	–	–	–	*
	Presence of the genes of cyanotoxins						
mcyE (Microcystis)	+	+	+	+	+	+	+
mcyE (Anabaena)	–	–	+	+	–	–	–
mcyE (Planktothrix)	+	+	–	–	–	–	–
stxA	–	–	+	+	–	–	–
aoaA	–	–	–	–	–	–	–
anaC	–	–	–	–	–	–	–

"–" Parameter was not detected, "+"gene was amplified, and "*" no data.

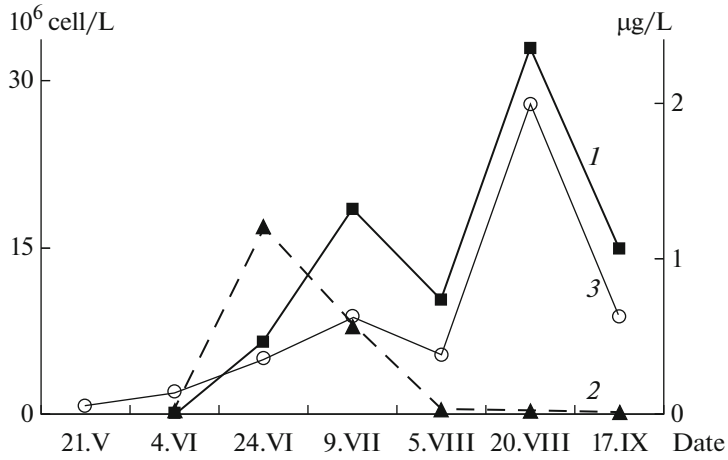

Fig. 2. Seasonal variations of cell number (10^6 cells/L) of *Microcystis* (*1*) and *Dolichospermum* (*Anabaena*) (*2*), as well as concentration of dissolved microcystins (*3*).

viridis, *Aphanothece clathrata* W. et G.S. West f. *brevis* (Bachm.) Elenk., *Aphanocapsa planctonica* (G.M. Smith) Kom. et Anagn., *Planktolyngbya limnetica* (Lemm.) Kom.-Leg. et Cronb., and *Merismopedia minima* G. Beck. Later, to October cyanobacterial diversity was enriched by the representatives of the genera *Chroococcus*, *Aphanocapsa, Pseudanabaena acicularis* (Nyg.) Anag. et Kom., *Limnothrix planctonica* (Wołos.) Meffert, and *L. redekei* (Van Goor) Meffert and included 12 to 14 taxa.

In the water of the Rybinsk Reservoir, microcystins and saxitoxins were detected, whereas the presence of cylindrospermopsin and anatoxin-a was not observed (Table 1). Dissolved and intracellular microcystins were detected during the whole period of observation and their total concentration fluctuated between 0.09 and 5.8 μg/L. Traces of saxitoxins were detected in the middle of summer (June 24 and July 9) (Table 1). The concentration of microcystins was minimal in spring and in early summer and dramatically increased in the summer–autumn period (Table 1). The presence of cyanobacteria of the genera *Microsystis* and *Dolichospermum* (*Anabaena*), potential microcystins producers, were constantly observed in summer and autumn. The seasonal dynamic of dissolved microcystins corresponded to fluctuations of *Microcystis* cell number. A close and significant correlation between these parameters was revealed ($r_S = 0.96$, $p < 0.05$, $n = 6$). This correlation was not revealed for the genus *Dolichospermum* (*Anabaena*) (Fig. 2).

In addition to the representatives of the genera *Microcystis* and *Dolichospermum* (*Anabaena*), potentially toxigenic species *Planktothrix agardhii* and *Aphanizomenon flos-aquae* were detected in the phytoplankton of the Rybinsk Reservoir (Table 1). Genes *mcyE* and *sxtA* were detected in total phytoplankton DNA by PCR amplification. This indicated the ability of cyanobacteria of the Reservoir to synthesize microcystins and saxitoxins, respectively (Figs. 3a–3d). Genes involved in anatoxin-a and cylindrospermopsin synthesis were not detected (Table 1).

In late May to early June, a small number of potentially toxigenic cyanobacterium *Planktothrix agardhii* was detected in phytoplankton. A PCR analysis of phytoplanktonic DNA detected the presence of the *mcyE* gene in the cells of this species (Fig. 3c). From the middle of summer, the species was not detected in the samples under light microscope and according to molecular biological analysis, fragment of *mcyE* gene specific to the genus *Planktothrix* was not recognized in the total phytoplanktonic DNA until the end of vegetation period (Fig. 3c, Table 1).

From May to September, *mcyE* gene-containing populations of *Microcystis* was observed (Fig. 3a). Microscopy did not reveal the presence of representatives of the genus in May, but they were constantly detected in the phytoplankton from June to September (Table 1). A PCR analysis of the DNA isolated from single colonies of *Microcystis* revealed a high share of *M. aeruginosa* and *M. viridis* containing *mcyE* gene coding for microcystin synthetase (Table 2). This gene was absent in *M. flos-aquae* and *M. wesenbergii* (Table 2).

In addition to toxigenic species of the genus *Microcystis*, a short peak of the growth of *Dolichospermum* (*Anabaena*) containing microcystin synthetase gene *mcyE* was observed at the beginning of summer (June 24 to July 9) (Fig. 3b, Table 1). Microscopy demonstrated the appearance of *Dolichospermum lemmermannii* and *D. planktonicum*, as well as several unidentified species of the genus *Dolichospermum* (*Anabaena*) during this period. During the same period, the appearance of saxitoxin-producing cyanobacteria was revealed by detection of the *sxtA* gene involved in saxitoxin biosynthesis in total phytoplanktonic DNA (Fig. 3d). It coincided with development of the species of the genus *Dolichospermum*

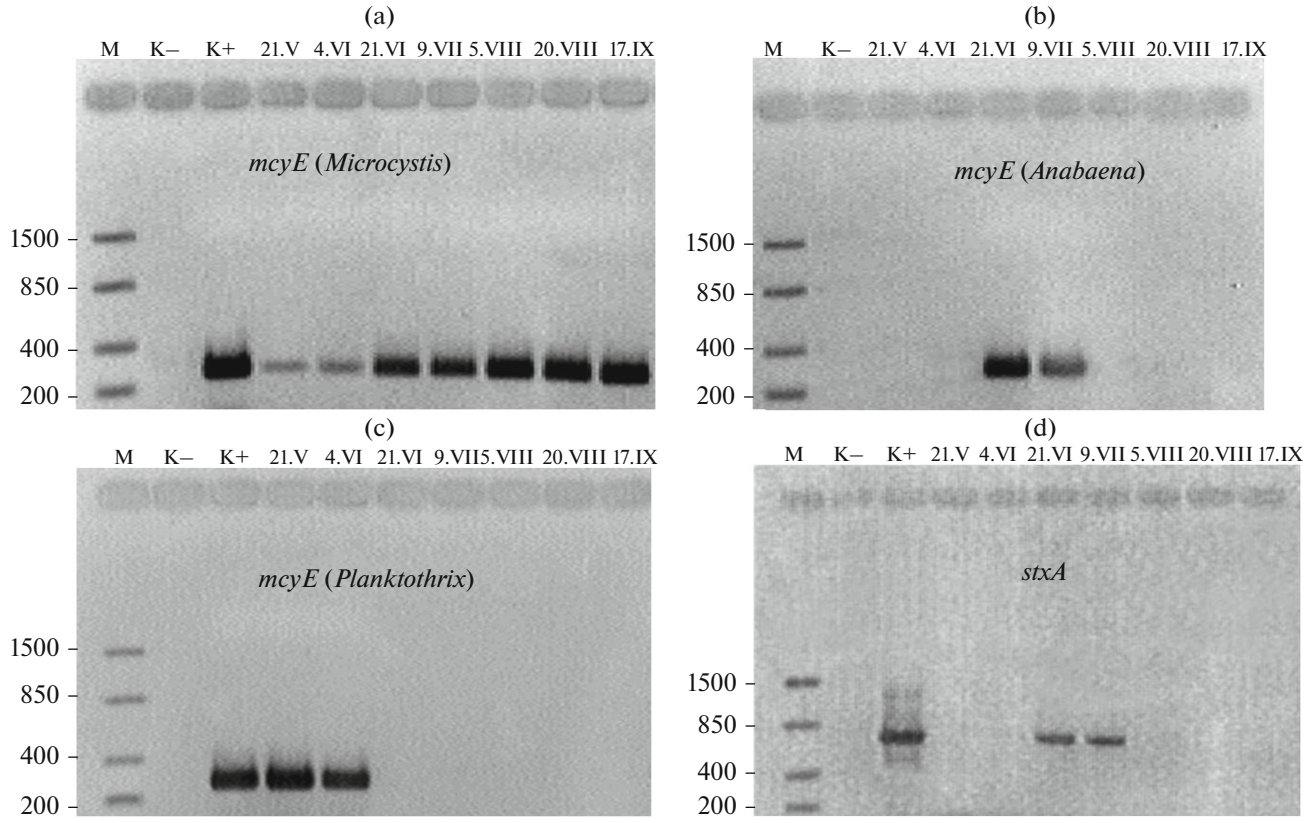

Fig. 3. Electrophoregram of amplified fragments of phytoplanktonic DNA from the Rybinsk Reservoir samples collected on different dates (May to September): (a) products obtained with mcyE-F2/MicmcyE-R8 primers for the detection of *mcyE* gene in *Microcystis* (250 bp), (b) products obtained with mcyE-F2/AnamcyE-12R primers for the detection of *mcyE* gene in *Dolichospermum (Anabaena)* (250 bp), (c) products obtained with mcyE-F2/mcyE-plaR3 primers for the detection of *mcyE* gene in *Planktothrix* (250 bp), and (d) products obtained with sxtaf/sxtar primers for the detection of *stxA* gene (600 bp); (M) molecular weight marker (bp), (K–) negative control (DNA of cyanobacteria without target genes), and (K+) positive control (DNA of cyanobacteria containing target genes).

(*Anabaena*) and *Aphanizomenon flos-aquae*, potential producers of the saxitoxins (Table 1). However, DNA extracted from 11 colonies of *Aphanizomenon flos-aquae* collected in July did not contain *sxtA* gene involved in saxitoxins synthesis (Table 2).

Thus, the seasonal periodicity of phytoplankton in the Rybinsk Reservoir was characterized by three peaks (spring, summer, and autumn) [2, 3]. Prior to 1981, spring peak was higher than summer, which was caused by the development of diatoms and cyanobacteria. In recent years, summer biomass has exceeded spring biomass. The data that was obtained also indicated that the duration of the summer peak of cyanobacteria increased.

Cryptomonads began to develop in May and June and comprised a significant share of the phytoplank-

Table 2. PCR analysis of single colonies of *Microcystis* and *Aphanizomenon flos-aquae* for detecting the genes involved in the synthesis of microcystins (*mcyE*) and saxitoxins (*stxA*)

Species	Colonies number	Genes		*mcyE* gene-containing colonies, %
		mcyE	*stxA*	
Microcystis aeruginosa	6	+	*	100
Microcystis viridis	11	+	*	91
Microcystis flos-aquae	2	–	*	0
Microcystis wesenbergii	8	–	*	0
Aphanizomenon flos-aquae	11	*	–	*

"–" Gene was not detected, "+" gene was detected, and "*" no data.

ton until October. An increase in the share of cyanobacteria and mixotrophic phytoflagellates is typical for highly productive reservoirs [2].

Molecular genetic identification of toxigenic cyanobacteria during the seasonal succession of phytoplankton in the Rybinsk Reservoir was performed for the first time. Representatives of three cyanobacterial genera containing *mcyE*-genes as well as neurotoxigenic species able to synthesize saxitoxins, were detected. The results of enzyme-linked immunosorbent assay confirmed the results obtained using molecular biological methods, which demonstrated the presence of microcystins and saxitoxins in the same samples.

Results of the present study corresponded to the data on the composition of cyanobacterial toxins in the Rybinsk Reservoir previously obtained using high-performance liquid chromatography–mass spectrometry (HPLC/MC) [4]. In July 2010, three variants of microcystins were detected in the water, whereas anatoxin-a was absent. Concentrations of dissolved microcystins at the Volzhsky stream pool station in 2013 were sufficiently higher (0.05–1.99 μg/L) than those measured in Glavny and Sheksninsky stream pools in 2010 (0.015–0.079 μg/L) [4]. These differences could be caused both by the application of differing methods (ELISA and HPLC/MC) and by the fact that cyanobacterial biomass in July 2013 was higher than in July 2010 (4.1–6.0 and 0.4–1.3 mg/L). The total content of microcystins in summer and autumn 2013 reached of 1.7 to 5.8 μg/L, which exceeded the threshold limit values for these compounds accepted in many countries (of 1 to 1.5 μg/L for drinking water and of 4 to 25 μg/L for recreational zones of reservoirs) [12].

In 2013, microcystins were detected in the water not only in the summer period during the massive development of cyanobacteria, but also throughout the ice-free period, including spring. *McyE* gene-containing populations of *Planktothrix agardhii*, which could be one of the sources of microcystins, were detected in phytoplankton in May. Other species of cyanobacteria were not detected in the sample collected in May using light microscopy. However, fragments of 16S rRNA (data not shown) and *mcyE* genes specific to the genus *Microcystis* were identified in total phytoplanktonic DNA (Fig. 3a). This indicated that not only *Planktothrix agardhii*, but also populations of *Microcystis* potentially able to produce microcystins were present in the samples collected in May. The application of highly sensitive PCR-based methods allowed us to identify *Microcystis* populations.

Positive correlation between the concentration of microcystins and *Microcystis* cell number suggested that they were the main microcystins producers at the station in the Rybinsk Reservoir. This was confirmed using molecular biological analysis, which revealed the permanent presence of *mcyE* gene-containing *Microcystis* from May to September. At least *M. aeruginosa* and *M. viridis* were potentially able to produce microcystins. *M. aeruginosa* is microcystins producer and widespread in reservoirs around the world [8, 13]. Microcystin-producing populations of *M. viridis* were observed in European lakes [22] as well as in the Uglich and the Gorky reservoirs (Russia) [6]. In the cells of *M. flos-aquae* and *M. wesenbergii* from the Rybinsk Reservoir, *mcyE* gene was not detected. This suggested either the absence or insignificant share of the microcystin-producing genotypes in the populations of these species.

The seasonal succession of microcystin-producing cyanobacteria in phytoplankton was clearly defined. Whereas populations of *Microcystis* able to produce microcystins were permanently detected from May to September, the succession of other *mcyE* gene-containing species occurred: a small number of *Planktothrix agardhii* was detected in the spring and early summer and a short peak of toxigenic species of the genus *Dolichospermum* (*Anabaena*) was observed in the middle of summer.

Alongside hepatotoxin-producing species, saxitoxins and neurotoxin-producing cyanobacteria were also found at the scientific station in the Rybinsk Reservoir. To our knowledge, this is the first report about detection of saxitoxin-producing cyanobacteria in the freshwaters of the European part of Russia. The content of saxitoxins measured using ELISA was not high and was similar to the values recently revealed in the freshwater reservoirs, which did not exceed of 0.005 to 0.3 μg/L [14, 23].

Several species producing saxitoxins *Aphanizomenon flos-aquae*, *A. gracile* (Lemm.) Lemm., *Cuspidothrix issatschenkoi* (Usačev) Rajaniemi et al. (=*Aphanizomenon issatschenkoi* (Ussaczev) Pr.-Lavr.), and *Dolichospermum lemmermannii* were reliably detected in European reservoirs [9, 10, 20, 23]. *Aphanizomenon flos-aquae* and species of the genus *Dolichospermum* (*Anabaena*) including *D. lemmermannii* were detected simultaneously with the detection of saxitoxins in water and *sxtA*-containing cyanobacteria in the phytoplankton. Nevertheless, a PCR-analysis of the DNA isolated from single colonies of *Aphanizomenon flos-aquae* did not identify *sxtA* genes. The peak of *Dolichospermum* biomass (June 24 to July 9) coincided with the detection of saxitoxins and *sxtA* gene (Table 1). This confirmed the hypothesis about the revelation of saxitoxin-producing populations of *Dolichospermum* (*Anabaena*).

CONCLUSIONS

Cyanobacteria able to produce hepato- and neurotoxins were identified in phytoplankton of the Rybinsk Reservoir using genetic markers for the first time. The presence of hepatotoxins microcystins was detected from May to September with peak of concentrations in the summer-autumn period. PCR analysis revealed the presence of the *mcyE* gene involved in microcystin biosynthesis in cells of *Microcystis aeruginosa*, *M. viridis*, and *Planktothrix agardhii*, as well as in representatives of the genus *Dolichospermum* (*Anabaena*).

Neurotoxins saxitoxins and *sxtA* gene-containing cyanobacteria were also detected in water and in phytoplankton of the reservoir, respectively. It was proposed that the saxitoxin-producing population of *Dolichospermum* (*Anabaena*) were detected. The genes responsible for cylindrospermopsin and anatoxin-a biosynthesis were not identified in the total phytoplanktonic DNA, and these toxins were not detected in water.

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REFERENCES

1. Korneva, L.G., Phytoplankton of Rybinsk Reservoir: composition, distribution patterns, and the effects of eutrophication, in *Sovremennoe sostoyanie ekosistemy Rybinskogo vodokhranilishcha (The Current State of the Ecosystem of the Rybinsk Reservoir)*, St. Petersburg: Gidrometeoizdat, 1993, pp. 50–113.
2. Korneva, L.G., Formation of phytoplankton in water bodies of the Volga River basin under the influence of natural and anthropogenic factors, *Extended Abstract of Doctoral (Biol.) Dissertation*, St. Petersburg, 2009.
3. Korneva, L.G., Mineeva, N.M., Elizarova, V.A., et al., *Ekologiya fitoplanktona Rybinskogo vodokhranilishcha (Phytoplankton Ecology in the Rybinsk Reservoir)*, Tolyatti: Izd. Samarsk. Nauch. Tsentra RAN, 1999.
4. Korneva, L.G., Solov'eva, V.V., Russkikh, Ya.V., and Chernova, E.N., Phytoplankton state and cyanotoxin content in Rybinsk, Gorky, and Cheboksary reservoirs during the abnormally hot summer of 2010, *Voda: Khim. Ekol.*, 2014, no. 8, pp. 24–29.
5. *Metodika izucheniya biogeotsenozov vnutrennikh vodoemov (Methods of Study of Ecosystems of Inland Water Bodies)*, Moscow: Nauka, 1975, p. 239.
6. Sidelev, S.I., Babanazarova, O.V., Zubishina, A.A., et al., Toxigenic cyanobacteria and cyanotoxin content in the Upper Volga reservoirs, in */Vodorosli: problemy taksonomii, ekologii i ispol'zovanie v monitoringe: Mater. III Mezhdunar. nauch. konf.* (Algae: Problems of Taxonomy, Ecology, and Application in Monitoring: Proc. III Int. Sci. Conf.), Yaroslavl, 2014, pp. 186–188.
7. Sidelev, S.I., Zubishina, A.A., Babanazarova, O.V., et al., Monitoring of the content of cyanotoxins microcystins in the Upper Volga reservoirs: molecular genetic and analytical approaches, *Voda: Khim. Ekol.*, 2014, no. 8, pp. 88–94.
8. Apeldoorn, M.E., Egmond, H.P., Speijers, G.J.A., and Bakker, G.J.I., Toxins of cyanobacteria, *Mol. Nutr. Food Res.*, 2007, vol. 51, pp. 7–60.
9. Araoz, R. and Molgo, J., Tandeau de Marsac N.Neurotoxic cyanobacterial toxins, *Toxicon*, 2010, vol. 56, pp. 813–828.
10. Fastner, J. and Wiedner, C., Paralytic shellfish poisoning toxin-producing cyanobacterium *Aphanizomenon gracile* in Northeast Germany, *Appl. Environ. Microbiol.*, 2010, vol. 76, pp. 1173–1180.
11. Barón-Sola, Á., Ouahid, Y., and Campo, F., Detection of potentially producing cylindrospermopsin and microcystin strains in mixed populations of cyanobacteria by simultaneous amplification of cylindrospermopsin and microcystin gene regions, *Ecotoxicol. Environ. Safety*, 2012, vol. 75, pp. 102–108.
12. Chorus, I., *Current Approaches to Cyanotoxin Risk Assessment, Risk Management and Regulations in Different Countries. Dessau: Fed. Environ. Agency* (Umweltbundesamt), 2012.
13. Chorus, I. and Bartram, J., *Toxic Cyanobacteria in Water. A Guide to Their Public Health Consequences, Monitoring and Management*, London: E &FN Spon, 1999.
14. Clemente, Z., Busato, R.H., Riverio, C.A., et al., Analyses of paralytic shellfish toxins and biomarkers in a southern Brazilian reservoir, *Toxicon*, 2010, vol. 55, pp. 396–406.
15. Mankiewicz-Boczek, J., Urbaniak, M., Romanowska-Duda, Z., and Izydorczyk, K., Toxic cyanobacteria strains in lowland dam reservoir (Sulejow res, Central Poland): amplification of *mcy* genes for detection and identification, *Pol. J. Ecol.*, 2006, vol. 54, no. 2, pp. 171–180.
16. Neilan, B., Jacobs, D., Del Dot, T., et al., rRNA sequences and evolutionary relationships among toxic and nontoxic cyanobacteria of the genus *Microcystis, Int. J. Syst. Bacteriol.*, 1997, vol. 47, no. 3, pp. 693–697.
17. Neilan, B., Jacobs, D., and Goodman, A.E., Genetic diversity and phylogeny of toxic cyanobacteria determined by DNA polymorphisms within the phycocyanin locus, *Appl. Environ. Microbiol.*, 1995, vol. 61, pp. 3875–3883.
18. Rantala, A., Rajaniemi-Wacklin, P., Lyra, C., et al., Detection of microcystin-producing cyanobacteria in Finnish lakes with genus-specific microcystin synthetase gene *E* (*mcyE*) PCR and associations with environmental factors, *Appl. Environ. Microbiol.*, 2006, vol. 72, pp. 6101–6110.
19. Rantala-Ylinen, A., Känä, S., Wang, H., et al., Anatoxin-a synthetase gene cluster of the cyanobacterium *Anabaena* sp. strain 37 and molecular methods to detect potential producers, *Appl. Environ. Microbiol.*, 2011, vol. 77, pp. 7271–7278.
20. Rapala, J., Robertson, A., Negri, A.P., et al., First report of saxitoxin in Finnish lakes and possible associated effects on human health, *Environ. Toxicol.*, 2005, vol. 20, pp. 331–340.
21. Vaitomaa, J., Rantala, A., Halinen, K., et al., Quantitative real-time PCR for determination of microcystin synthetase gene *E* copy numbers for *Microcystis* and *Anabaena* in lakes, *Appl. Environ. Microbiol.*, 2003, vol. 69, pp. 7289–7297.
22. Via-Ordorika, L., Fastner, J., Kurmayer, R., et al., Distribution of microcystin-producing and non-microcystin-producing *Microcystis* sp. in European freshwater bodies: detection of microcystins and microcystin genes in individual colonies, *Syst. Appl. Microbiol.*, 2004, vol. 27, pp. 592–602.
23. Wörmer, L., Cirés, S., Agha, R., et al., First detection of cyanobacterial PSP (paralytic shellfish poisoning) toxins in Spanish freshwaters, *Toxicon*, 2011, vol. 57, pp. 918–921.

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