DETECTION OF TOXIN PRODUCING CYANOBACTERIA IN WATER RESERVOIRS OF BELARUS BY POLYMERASE CHAIN REACTION

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Effects of HABs include the development of high biomass "red tides", as shown by this *Noctiluca* bloom in New South Wales, Australia.

The last decades have been marked by a new appreciation of the serious impacts of the marine phenomena we now call harmful algal blooms (HABs). These occurrences of toxic or harmful microalgae represent a significant and seemingly expanding threat to human health, fishery resources, and marine ecosystems throughout the world. Many causes, both natural and anthropogenic, may be responsible for this dramatic expansion in HAB effects; it is likely that human activities are making the problems worse through increased nutrient inputs to coastal areas, transportation and discharge of ballast water, and other factors.

Thus, the problem became widely known for marine and costal ecosystems

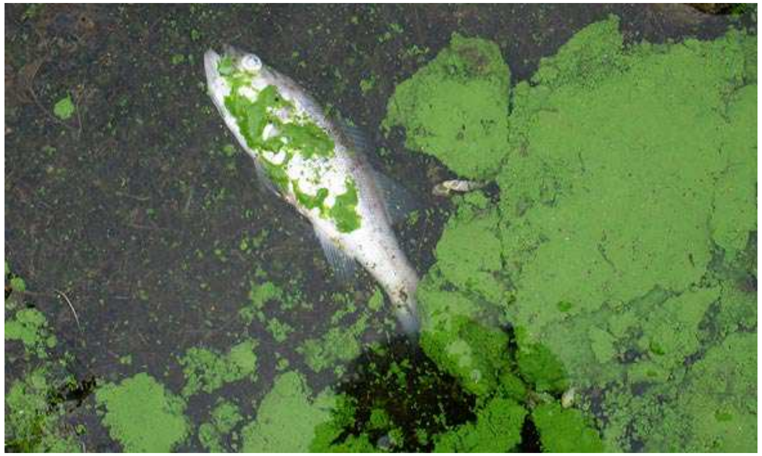
HABs have been linked to numerous fish kill events. In this 1994 fish kill in St. Helena Bay on the west coast of South Africa, the HAB species *Prorocentrum micans* and *Ceratium furca* were the causative organisms. Low oxygen and the subsequent generation of hydrogen sulfide were responsible for the mortality.

Proliferations of algae in marine or brackish waters can cause massive fish kills, contaminate seafood with toxins, and alter ecosystems in ways that humans perceive as harmful.A broad classification of HAB species distinguishes two groups:

the toxin producers, which can contaminate seafood or kill fish,

and the high-biomass producers, which can cause anoxia and indiscriminate mortalities of marine life after reaching dense concentrations.

Some HAB species have characteristics of both groups.



In fresh water this problem are associated with such group of organisms as Cyanobacteria.

Cyanobacteria are organisms with some characteristics of bacteria and some of algae. They are similar to algae in size and, unlike other bacteria, they contain blue-green and green pigments and can perform photosynthesis. Therefore, they are also termed blue-green algae. Human activities (e.g., agricultural runoff, inadequate sewage treatment, runoff from roads) have led to excessive fertilization (eutrophication) of many water bodies. This has led to the excessive proliferation of algae and cyanobacteria in fresh water and thus has had a considerable impact upon recreational water quality. In temperate climates, cyanobacterial dominance is most pronounced during the summer months, which coincides with the period when the demand for recreational water is highest.

Figure 2.2C Micrograph of *Microcystis aeruginosa*

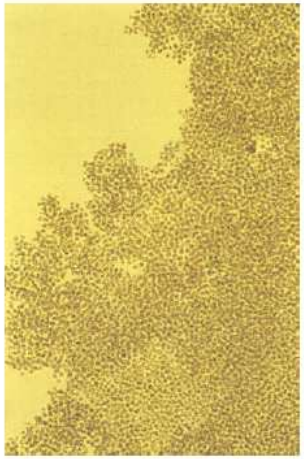


Figure 2.2B Micrograph of *Anabaena lemmermannii*

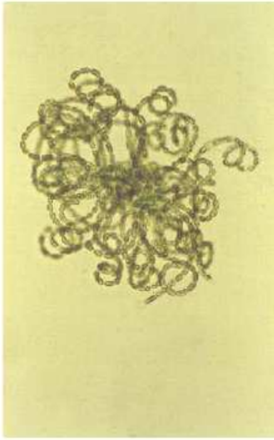
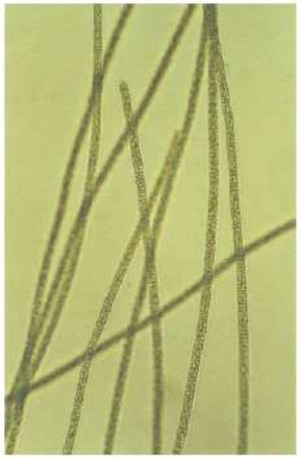


Figure 2.2A Micrograph of *Planktothrix (Oscillatoria) agardhii*



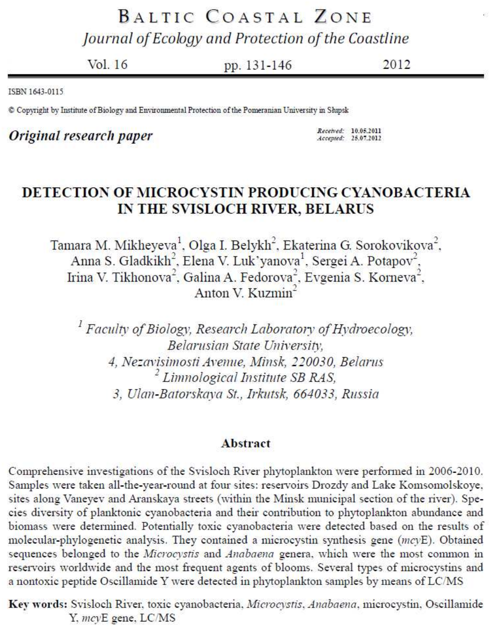
Toxic cyanobacteria are found worldwide in inland and coastal water environments. At least 46 species have been shown to cause toxic effects in vertebrates (Sivonen & Jones, 1999). The most common toxic cyanobacteria in fresh water are *Microcystis* spp., *Cylindrospermopsis raciborskii*, *Planktothrix* (syn. *Oscillatoria*) *rubescens*, *Synechococcus* spp., *Planktothrix* (syn. *Oscillatoria*) *agardhii*, *Gloeotrichia* spp., *Anabaena* spp., *Lyngbya* spp., *Aphanizomenon* spp., *Nostoc* spp., some *Oscillatoria* spp., *Schizothrix* spp. and *Synechocystis* spp. Toxicity cannot be excluded for further species and genera.

CYANOBACTERIAL TOXINS AND THEIR ACUTE TOXICITY

Cyanotoxins	LD_{50} (i.p. mouse)[b] of pure toxin (µg/kg)	Taxa known to produce the toxin(s)	Mechanism of toxicity
Protein phosphatase blockers (cyclic peptides with the amino acid ADDA)			
Microcystins in general (~60 known congeners)	45–>1000	*Microcystis*, *Planktothrix*, *Oscillatoria*, *Nostoc* *Anabaena*, *Anabaenopsis* *Hapalosiphon*	all block protein phosphatases by covalent binding and cause haemorrhaging of the liver; cumulative damage may occur
Microcystin-LR	60 (25–125)		
Microcystin-YR	70		
Microcystin-RR	300–600		
Nodularin	30–50	*Nodularia spumigena*	
Neurotoxins			
Anatoxin-a (alkaloid)	250	*Anabaena*, *Oscillatoria*, *Aphanizomenon*, *Cylindrospermum*	blocks post-synaptic depolarization
Anatoxin-a(s) (unique organophosphate)	40	known only from two species of *Anabaena*	blocks acetylcholinesterase
Saxitoxins (carbamate alkaloids)	10–30	*Aphanizomenon*, *Anabaena*, *Lyngbya*, *Cylindrospermopsis raciborskii*	block sodium channels
Cytotoxin			
Cylindrospermopsin (alkaloid)	2100 in 1 day 200 in 5–6 days	*Cylindrospermopsis raciborskii*	blocks protein synthesis; substantial cumulative toxicity

[a] derived from Turner et al., 1990; Kuiper-Goodman et al., 1999; Sivonen & Jones, 1999.

[b] LD_{50} = lethal dose$_{50}$ (the dose of a chemical that will, on average, kill 50% of a group of experimental animals); i.p. = intraperitoneal.



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DETECTION OF MICROCYSTIN PRODUCING CYANOBACTERIA IN THE SVISLOCH RIVER, BELARUS

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Abstract

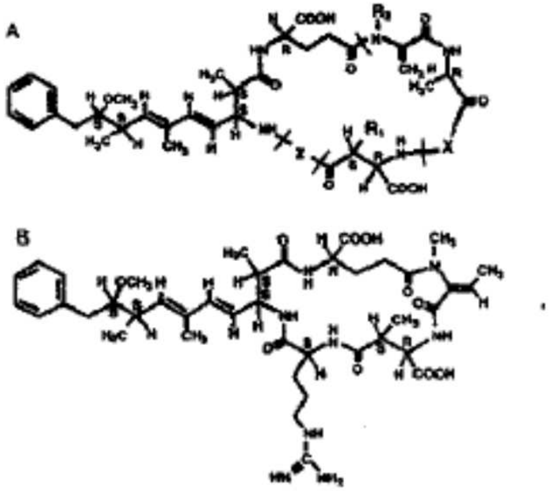
Comprehensive investigations of the Svisloch River phytoplankton were performed in 2006-2010. Samples were taken all-the-year-round at four sites: reservoirs Drozdy and Lake Komsomolskoye, sites along Vaneyev and Aranskaya streets (within the Minsk municipal section of the river). Species diversity of planktonic cyanobacteria and their contribution to phytoplankton abundance and biomass were determined. Potentially toxic cyanobacteria were detected based on the results of molecular-phylogenetic analysis. They contained a microcystin synthesis gene (*mcy*E). Obtained sequences belonged to the *Microcystis* and *Anabaena* genera, which were the most common in reservoirs worldwide and the most frequent agents of blooms. Several types of microcystins and a nontoxic peptide Oscillamide Y were detected in phytoplankton samples by means of LC/MS

Key words: Svisloch River, toxic cyanobacteria, *Microcystis*, *Anabaena*, microcystin, Oscillamide Y, *mcy*E gene, LC/MS

The first investigations of toxic cyanobacteria in Belarus were carried out in 2006 by our laboratory. Potentially toxic cyanobacteria were detected in Svicloch river (within the Minsk municipal section of the river) based on the results of molecular-phylogenetic analysis. They contained a microcystin synthesis gene (*mcy*E). Obtained sequences belonged to the *Microcystis* and *Anabaena* genera, which were the most common in reservoirs worldwide and the most frequent agents of blooms.

The first detection of potentially toxic cyanobacteria in fish farm was conducted in pond of farm "Beloye" in 2012.

The aim of present work was to perform PCR screening of water samples to identify the aminotransferase domain sequence of mcyE gene which is involved in the non-ribosomal synthesis of microcystin and nodularine in cyanobacteria of *Microcystis*, *Anabaena*, *Planktothrix, Nodularia* and *Nostoc* genera



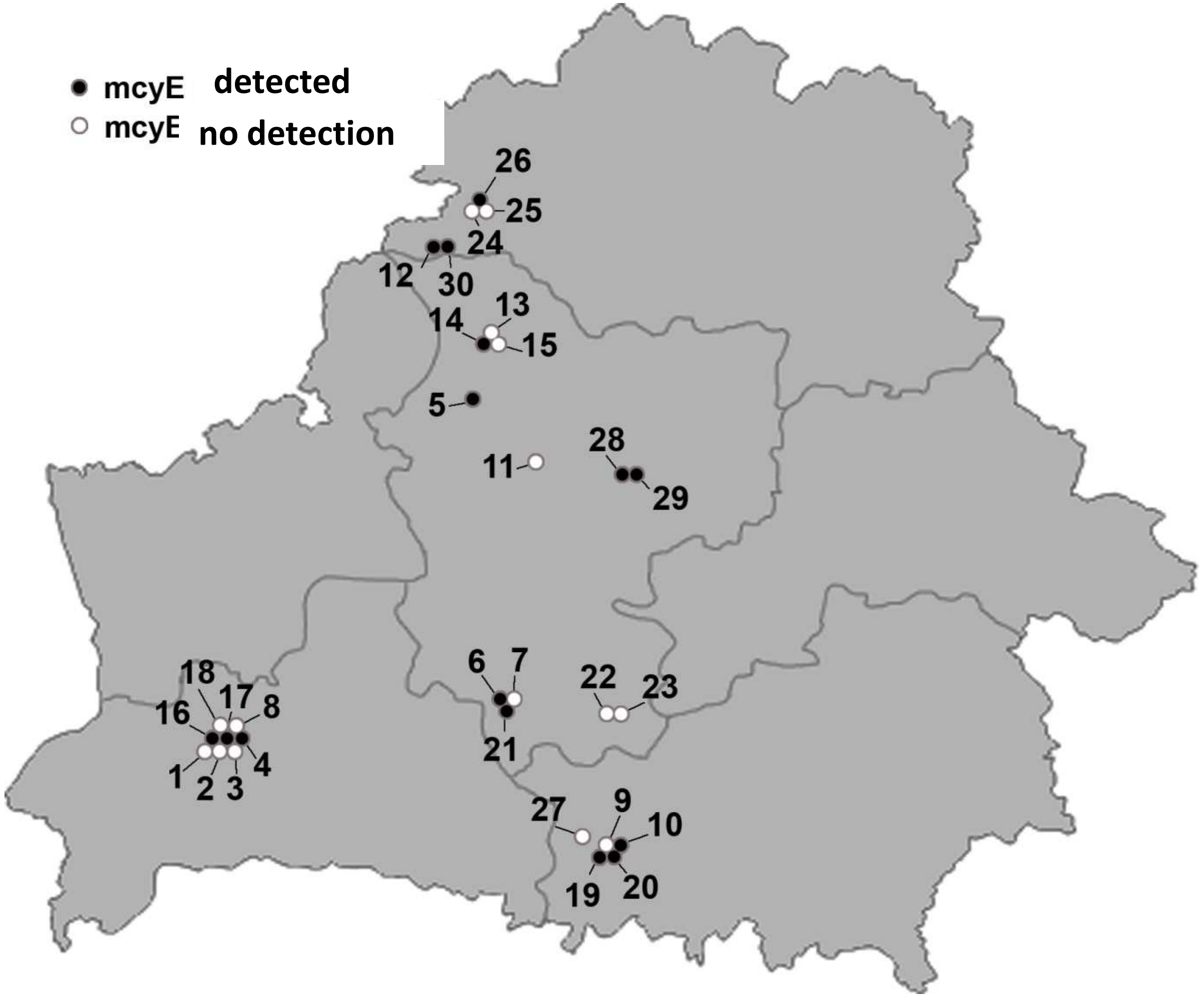
. Общая структура микроцистина (А) и нодулярина (В) (Цит. по: Sivonen, 1996; Carmichael, 1995)

Microcystins are cyclic heptapeptides which are synthesized nonribosomally by a large multienzyme complex (Nishizawa et al. 1999). At present, over 90 variants of MCs are known (Welker et al. 2004).

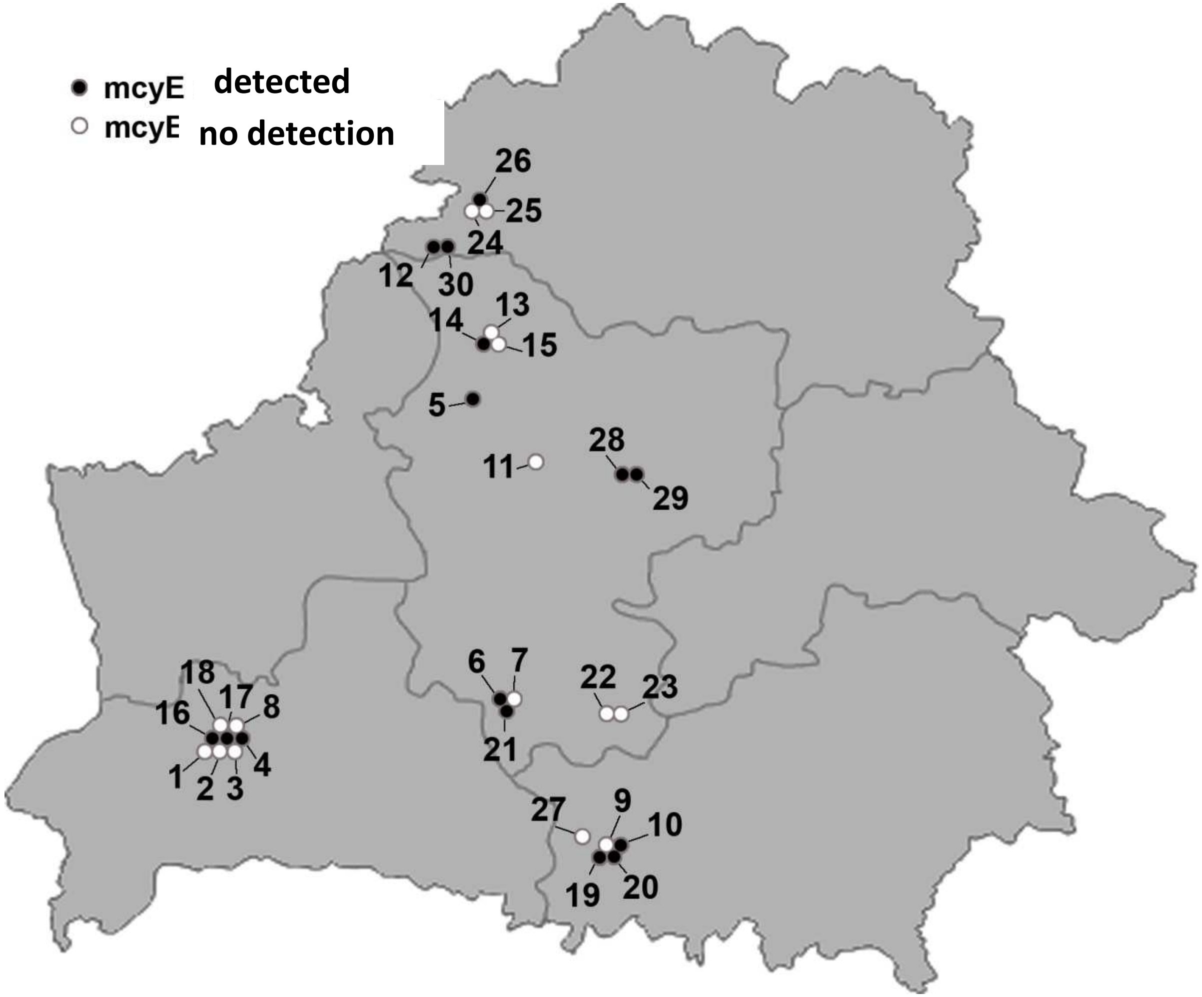
According to the standards specified by WHO, concentration of MC-LR in drinking water should not exceed 1 Dg/L, the abundance of cyanobacteria 20×106 cells/L and concentration of microcystins 2-4 Dg/L in recreational waters are considered dangerous (Guidelines for safe... 2003, Guidelines for drinking-water... 2004).

Discovery of a gene locus responsible for MC synthesis in *Microcystis aeruginosa* (*mcyA–J-genes) allowed the development of genus- and species-specific markers* which are widely used for detection of toxigenic cyanobacteria (Nishizawa et al. 1999).

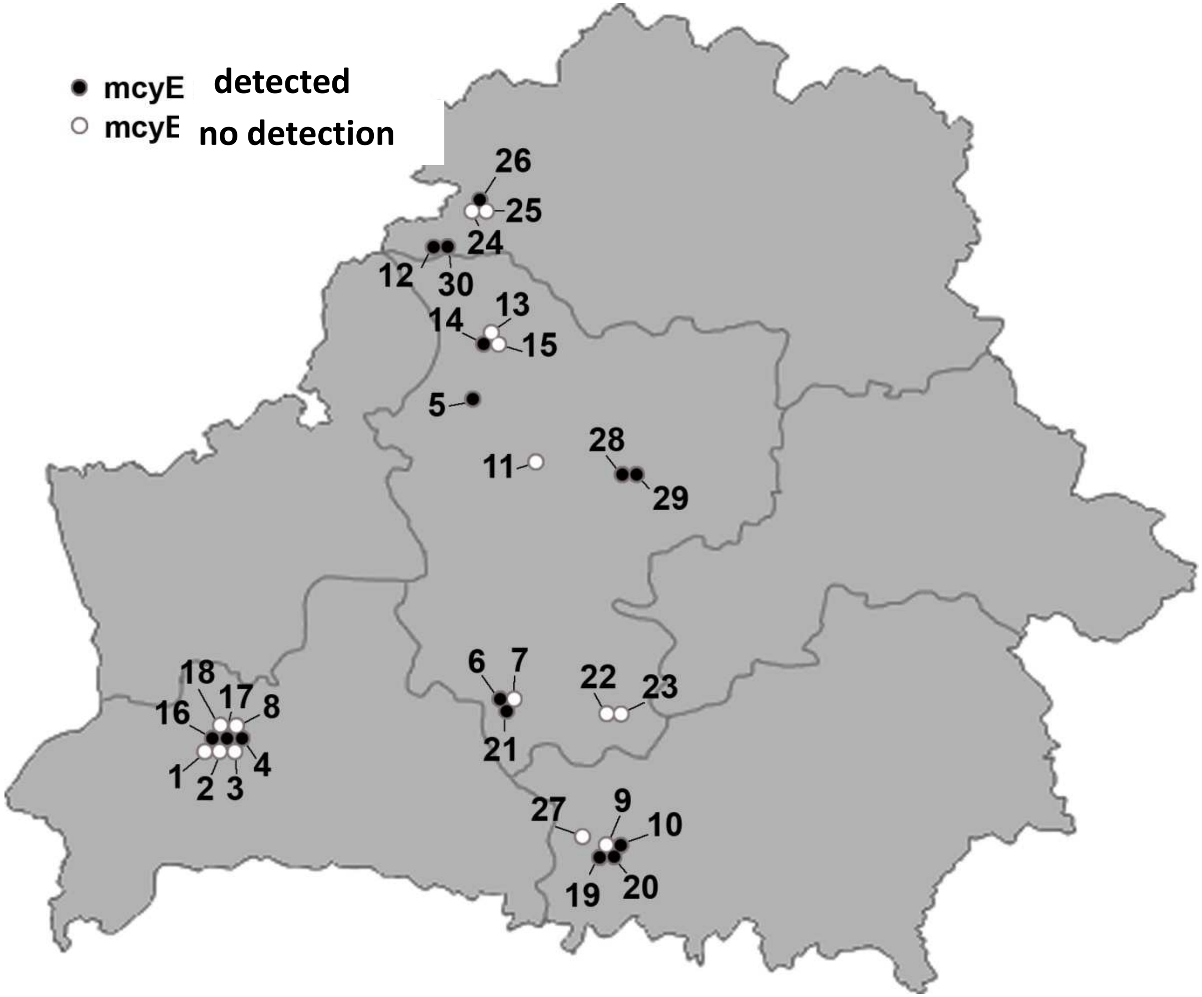
Universal primers HepF and HepR (Jungblut and Neilan 2006) were used to reveal genes involved in the synthesis of microcystins. Isolation of DNA, PCR, cloning and sequencing were performed as described (Belykh et al. 2011)



Water samples were collected from natural water reservoirs and fish farm ponds during 2012-2016 years. We analyzed samples from 24 different water reservoirs: ponds of fish farms "Selets", "Izobelino", "Krasnaya Sloboda", "Beloe", "Vilejka", "Luban", "Novinki", "Krasnaya Zorka", "Shemetovo", lakes Bolshie Shvakshty, Svir, reservoirs Drozdi, Zaslavskoe and some other.



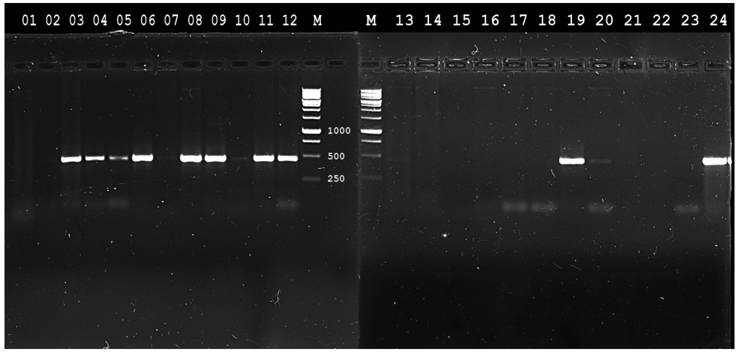
It allow to detect potentially toxic cyanobacteria in water samples. Positive samples have been revealed in several fish farm ponds and natural reservoirs. 50% of ponds were positive in 2012-2013. 31% and 61% in 2014-2015 and 2016, respectively.



No correlation between geographical distribution of positive ponds and climatic conditions of the region was revealed. Also no year to year tendency was shown. It should be mentioned that using of PCR gives us qualitative results and allow to reveal the presence of potentially toxic cyanobacteria.

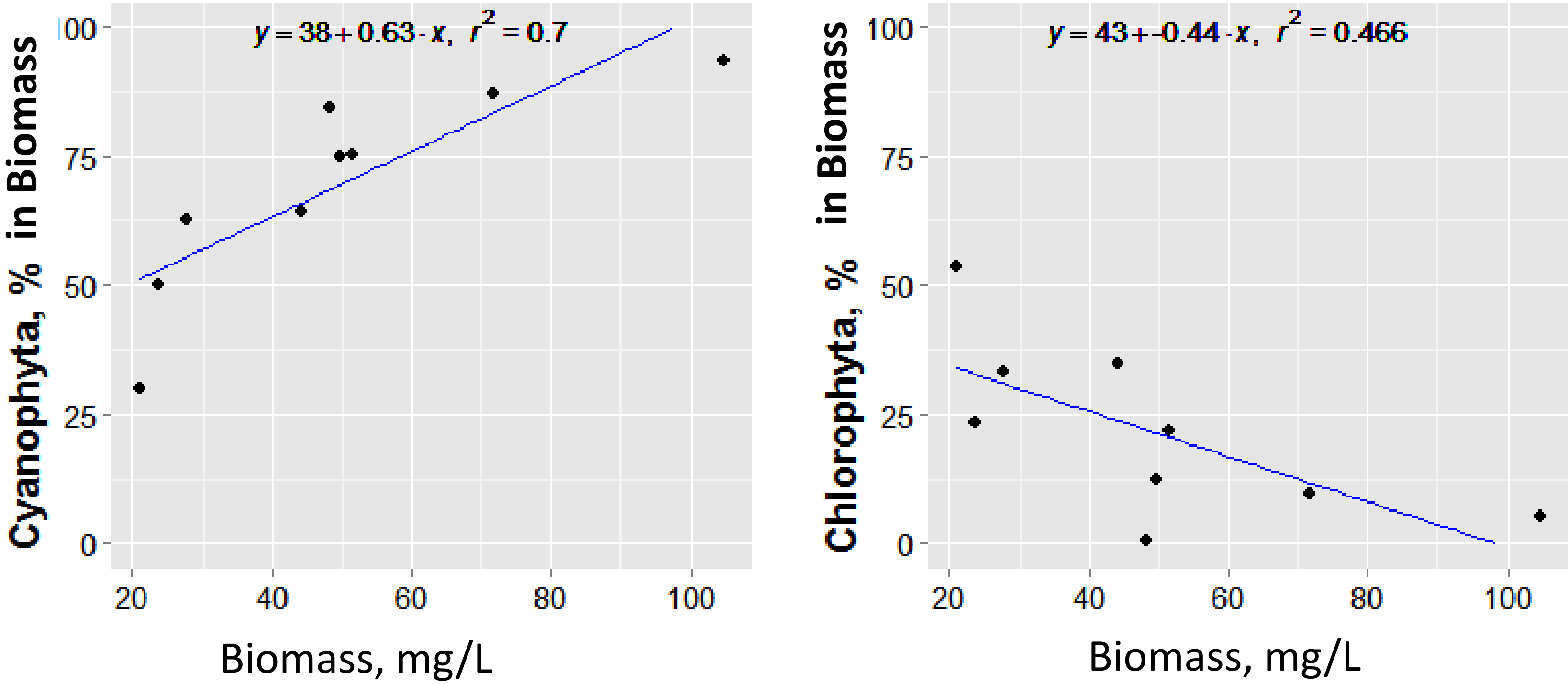
Detection of toxin producing Cyanobacteria in fish ponds in 2016 by polymerase chain reaction

№	Fish farm	Date	Point	Gene
3	Fish farm “Svisloch”	13.07.16	1	*mcy*E
4	Fish farm “Selets”	13.06.16	14	*mcy*E
5	Fish farm “Selets”	13.07.16	17	*mcy*E
6	Fish farm “Selets” (Beloozersk department)	4.08.16	Chanal 1	*mcy*E
8	Fish farm “Selets”	13.07.16	1	*mcy*E
11	Fish farm “Selets” (Beloozersk department)	4.08.16	Chanal 2	*mcy*E
24	Bolshye Svakshty Lake (+ control)	07.06.12.		*mcy*E



Taxonomic structure of phytoplankton in fish pond in the period of algae “bloom” (Biomass 51–100 mg/L)

Parametr	n	Mean	Sd	Median	Min	Max
Density, mln.ind/L	12	148,30	90,14	138,51	29,00	340,00
Density, mln.cells/L	12	2852,66	2450,21	1757,11	552,02	6377,40
Biomass, mg/L	12	69,70	14,44	64,35	51,60	98,17
% in Biomass						
Cyanophyta	**12**	**71,95**	**21,81**	**75,00**	**22,40**	**98,70**
Cryptophyta	12	0,02	0,09	0,00	0,00	0,30
Dynophyta	12	0,00	0,00	0,00	0,00	0,00
Chrysophyta	12	0,24	0,84	0,00	0,00	2,90
Bacillariophyta	12	1,50	1,53	1,10	0,00	4,70
Xantophyta	12	0,00	0,00	0,00	0,00	0,00
Euglenophyta	12	0,58	1,47	0,00	0,00	5,00
Chlorophyta	**12**	**25,71**	**21,11**	**20,70**	**1,30**	**74,30**



Percent of Cyanophyta and Chlorophyta and total phytoplankton Biomass pond in the period of algae “bloom” (Biomass > 20 mg/L) in ponds of fish farm «Vileyka», «Selets» и «Krasnaya Sloboda» in 2014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