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PRIMARY RESEARCH PAPER

The effect of potentially toxic cyanobacteria on ciliates (Ciliophora)

Joanna Kosiba · Elżbieta Wilk-Woźniak · Wojciech Krztoń

Abstract The most frequently observed cyanotoxins are microcystins. They trigger a cascade of events leading to cellular responses. The hypothesis of the study was that cyanobacteria affect ciliates as solitary species and as assemblages. The aim of our study was to determine whether ciliates respond to cyanobacteria because of the presence of cyanotoxins (microcystins—MC). We set up experiments with toxic (*Planktothrix agardhii* and *Microcystis aeruginosa*) and non-toxic (*Aphanizomenon flos-aquae*) cyanobacteria, solitary *Spirostomum* sp. (Ciliophora), and a simple ciliate assemblage. Predicted values showed statistically significant increase during the solitary *Spirostomum* sp. abundance in the presence of toxic *P. agardhii* (MC total concentration in cells 323.9 µg/l) and *M. aeruginosa* (MC total concentration in cells 31.9 µg/l) but a decrease in the presence of non-toxic *A. flos-aquae*. The abundance of *Spirostomum* sp., being a component of ciliate assemblage, decreased significantly in the presence of all the three species of cyanobacteria due to competition from small-sized ciliate species that graze bacteria more effectively compared to large-cell-sized *Spirostomum*. We conclude that toxic cyanobacteria may affect ciliates in various ways, not necessarily because of production of toxins. As a consequence of the presence cyanotoxins, a cascading effect of passing carbon in the food web might be induced.

Keywords Microcystins · *Spirostomum* sp. · Cyanotoxins · Ciliophora

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Introduction

Cyanobacteria are prokaryotic, autotrophic organisms which, developing in mass, create water blooms. One potentially hazardous consequence of cyanobacterial blooms is the production of toxins (e.g. Carmichael, 1994). Various types of cyanotoxins are produced by different species of cyanobacteria, e.g. species from genera: (a) *Anabaena*, *Aphanizomenon* and *Oscillatoria* produce anatoxin-a; (b) *Aphanizomenon*, *Planktothrix*, *Anabaena*, *Cylindrospermopsis* and *Lyngbya* produce saxitoxins; (c) *Microcystis*, *Planktothrix*, *Dolichospermum* and *Aphanizomenon* produce microcystins; (d) *Nodularia* produces nodularins; and (e) *Cylindrospermopsis* and *Aphanizomenon* produce

cylindrospermopsins (Codd et al., 1995, 2005; Sivonen & Jones, 1999; Buratti et al., 2017).

Among toxins, hepatotoxins, and especially microcystins (MCs), which are heptapeptides (Carmichael, 1992), are the most frequently observed and have been well studied (Zurawell et al., 2005; Mantzouki et al., 2018). They are endotoxins (Rapala et al., 1997) and are not released into water until cell lysis (Rohrlack & Hyenstrand, 2007).

Microcystins induce abnormal signalling in multiple pathways mediated by protein phosphatase 2 (PP2A), resulting in increased protein phosphorylation that triggers a cascade of events leading to a series of cellular responses such as: modification of the cytoskeleton and disruption of actin filaments, oxidative stress, induction of apoptosis, and reduced DNA repair or cell proliferation leading to tumour promotion (Codd et al., 1995).

Primary consumers of cyanobacteria (such as protists, rotifers, copepods and cladocerans) can be directly contaminated by consuming cyanobacterial cells (Ferrão-Filho & Koslowsky-Suzuki, 2011). Next, predators on protists and animals can be indirectly contaminated and spread the MCs to other organisms via trophic transfer (Ibelings et al., 2005; Gołdyn et al., 2010; Koslowsky-Suzuki et al., 2012). However, cyanotoxins may be passed into the food web not only because they are cumulated in the invertebrates that consume cyanobacteria cells but also because toxins might be bound to the outside part of cells of protists and animals. This way, cyanotoxins may also be transferred via planktonic animals to higher levels of the food chain.

Although a great deal of research has shown the negative effect of toxic cyanobacteria on metazooplankton (Lampert, 1987; Wilson et al., 2006), much less has been focused on protists (Maršálek & Bláha, 2004; Combes et al., 2013; Tirjaková et al., 2016).

Protists are an important component of the water trophic network, particularly in lakes where cyanobacterial filaments or colonies are too large to be eaten by zooplankton (Havens, 1998). However, more recently, their role has been much appreciated as a very important link in the microbial loop (Sommer et al., 2012). A prominent group of protists constitutes ciliates that are a key component in energy transfer from microbial elements to the higher trophic levels in the food chain (Sherr & Sherr, 1994).

An understanding of the functionality of different group of species and changes in the food web network of water ecosystems is important because a reduction or alteration of the planktonic population can cause a deficit or an unbalance in food availability for higher levels of the trophic web (Zaccaroni & Scaravelli, 2008). Based on the assumption that a decrease in the number of ciliates in water bodies containing toxic cyanobacteria is a result of cyanobacteria producing toxic compounds that induce harmful effects to these organisms (Martins et al., 2011), we hypothesised that cyanobacteria affect ciliates as solitary species and as assemblages. Our previous field studies showed that cyanobacterial blooms significantly lowered diversity, density and biomass of ciliate communities (Kosiba et al., 2018). The aim of our study was to determine whether ciliates respond to cyanobacteria because of the presence of cyanotoxins (microcystins).

Materials and methods

To determine whether potentially toxic cyanobacteria affect ciliates, we set up four experiments (Fig. 1). For the experiments, we used the biomass of species as follows: (1) three species of potentially toxic cyanobacteria (*Aphanizomenon flos-aquae* Ralfs ex Bornet & Flahault, *Microcystis aeruginosa* Kütz. and *Planktothrix agardhii* (Gomont) Anagnostidis & Komárek); (2) solitary species of ciliates (*Spirostomum* sp.); and (3) a simple ciliate assemblage consisting of four species (*Spirostomum* sp., *Euplotes patella* (Müller), *Strobilidium* sp. and *Paramecium aurelia*-complex). Among the ciliates, we chose *Spirostomum* sp. because the species from this genus were found to be sensitive to cyanotoxins (Tarczyńska et al., 2001). Cyanobacteria biomasses used for experiments were obtained as (a) biomass of the strain from culture (*P. agardhii*—strain SAG 6.89), and (b) biomass of the species collected from their natural habitats. *A. flos-aquae* and *M. aeruginosa* were taken during the peak of blooms. *A. flos-aquae* was collected from a shallow, eutrophic pond (Podkamycze 1), located close to Kraków (southern Poland; 50°05′11″N, 19°50′01.6″E), and *M. aeruginosa* was collected from a shallow, eutrophic oxbow lake (Tyniec 1), located in Kraków (50°01′47″N, 19°49′39.8″E).
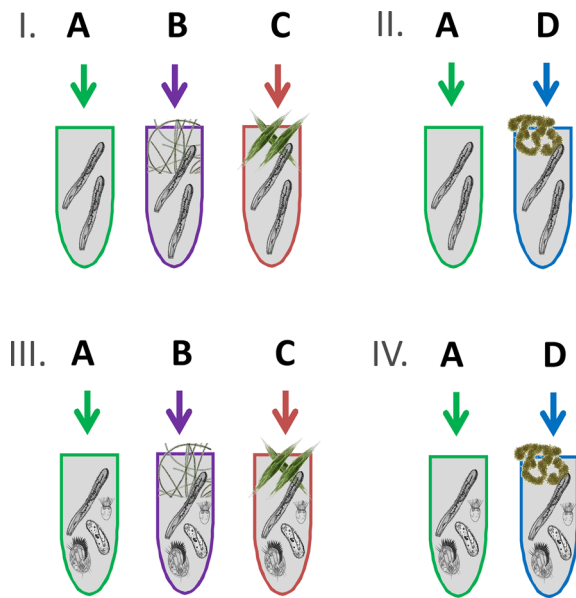
Fig. 1 Design of the experiments. I. Experiment 1. Solitary *Spirostomum* versus filamentous cyanobacteria—A: control sample: *Spirostomum* sp. + Żywiec brand mineral water; B: *Spirostomum* sp. + *P. agardhii*; C: *Spirostomum* sp. + *A. flos-aquae*; II. Experiment 2. Solitary *Spirostomum* versus chroococcal cyanobacteria: A: control sample: *Spirostomum* + Żywiec brand mineral water; D: *Spirostomum* sp. + *M. aeruginosa*; III. Experiment 3. *Spirostomum* in the ciliates assemblage versus filamentous cyanobacteria: A: control sample: *Spirostomum* in the ciliates assemblages + Żywiec brand mineral water; B: *Spirostomum* as a component of assemblage + *P. agardhii*; C: *Spirostomum* as a component of assemblage + *A. flos-aquae*; IV. Experiment 4. *Spirostomum* in the ciliates assemblage versus chroococcal cyanobacteria: A: control sample: *Spirostomum* in the ciliates assemblages + Żywiec brand mineral water; D: *Spirostomum* as a component of assemblage + *M. aeruginosa*

The control sample contained in Experiments 1 and 2: solitary *Spirostomum* cells + Żywiec brand mineral water, and in Experiments 3 and 4: ciliates assemblage + Żywiec brand mineral water.

Planktothrix agardhii (strain SAG 6.89) was obtained from the laboratory of the Department of Hydrobiology Adam Mickiewicz University in Poznań. This strain was bought from the SAG collection in Goettingen. *P. agardhii* was cultured using the method: 'batch culture' WC medium in the cell culture flasks Greiner BioOne, at the temperature of 21°C, and light intensity of 25 μmol m^{-2} s^{-1}. The biomass of cyanobacteria (*A. flos-aquae* and *M. aeruginosa*) was concentrated from 10 L of water from each water body using plankton net (mesh size

50 μm) during the blooms. The clonal strains of *Aphanizomenon flos-aquae* and *Microcystis aeruginosa* were isolated from the biomass of cyanobacteria. *A. flos-aquae* strains were maintained in modified AF-6 medium (Andersen, 2001) without the addition of nitrogen compounds, and *M. aeruginosa* strains were grown in a modified MWC medium with the addition of selenium (MWC + Se; Johansson et al., 2016) under a 12:12-h light–dark cycle and at a light intensity of approximately 30 μmol m^{-2} s^{-1} using cool, white fluorescent illumination (Philips TL-D 36W/840) at 20°C. The strains were deposited in the culture collection of algae and cyanobacteria at the Nature Research Centre in Vilnius (Lithuania) (Koreivienė et al., 2016). Those strains will be used for the further experiments.

In the present experiments, we wanted to imitate natural conditions; therefore, cyanobacteria were not purified from bacteria. Trichomes (*A. flos-aquae*) and colonies (*M. aeruginosa*) were picked up from samples and conditioned in the 1-l glass beakers in water from its natural habitat under the following conditions: daylight, regime 12:12, temperature 21°C, during one day. For this purpose, 5 l of water from the pond for *A. flos-aquae* and 5 l of water from the oxbow lake for *M. aeruginosa* were filtered using Whatman filters GF/C in order to remove all organisms. The remaining filtered water was kept in the refrigerator in sterilised glass flasks for the experiments. Before adding it to the experiments, it was kept in the laboratory atmosphere until warming up to 21°C. Trichomes and colonies of cyanobacteria were taxonomically identified, checked for conditions (healthy/not healthy/lysis of the cells) and measured under a Nikon Eclipse 80i light microscope at a magnification of × 40. The number of trichomes and colonies were counted in the 1-ml planktonic chamber with glass cover, using the same microscope equipped with a Nikon DS-Fi1 camera and the program NIS-Elements BR v. 3.22.12. The trichomes and colonies were healthy and in good condition. No lysis of cells were observed. They did not gather into clumps.

Spirostomum sp. was obtained from culture maintained in the Department of Hydrobiology of Jagiellonian University in Kraków, and was cultured in spring water (Żywiec Zdrój brand) and fed with buckwheat. Prior to the experiment, *Spirostomum* sp. cultures were maintained under the conditions: the

daylight, regime 12:12, and temperature 21°C in a Pol-Eko ILN 53/115/240 incubator. A simple assemblage of ciliates containing *Spirostomum* sp. was maintained in the laboratory of the Institute of Nature Conservation, cultured in spring water (Żywiec Zdrój brand) and fed with straw.

Because the blooms of *A. flos-aquae* and *M. aeruginosa* were created at different times, we set up two experiments as follows: Experiments 1 and 3 tested the effect only of the filamentous species, *P. agardhii* and *A. flos-aquae* (Fig. 1; I and III), and Experiments 2 and 4 tested the effect of the chroococcal species—*M. aeruginosa* (Fig. 1; II and IV).

The experiment was performed in Corning® Cell Wells containing 10 ml of medium. The medium was used as follow: (a) for control samples—spring water (Żywiec Zdrój brand); (b) for samples with *P. agardhii*—filtered water from culture containing *P. agardhii*; (c) for samples with *A. flos-aquae*—filtered water from the pond (natural habitat); and (d) for samples with *M. aeruginosa*—filtered water from the oxbow lake (natural habitat).

Every well was fed the similar number of ciliates (150 individuals of *Spirostomum* in the first and second experiments and 10–40 individuals of *Spirostomum* in the third and fourth experiments), and cyanobacteria trichomes or colonies (*P. agardhii* 50,000 trichomes ml^{-1}; *A. flos-aquae* 660,000 trichomes ml^{-1}; *M. aeruginosa* 40,000 colonies ml^{-1}), which were filled with drops of the incubated culture using pipets.

The assemblage of ciliates contained four species (*Spirostomum* sp., *Euplotes patella*, *Strobilidium* sp. and *Paramecium aurelia*-complex). The ciliates were fed at the beginning of the experiment (0 day): each well was inserted with 1 grain of buckwheat (experiments with solitary *Spirostomum*) or a blade of straw (1 cm; experiments with simple assemblages of ciliates).

Because microcystins are stable in the dark (Chorus & Bartram, 1999), in order to keep the cyanotoxins stable for as long as possible, we decided to perform the experiments in the dark. The experiments were kept at a constant temperature of 21°C. Each experiment was performed in triplicate. The experiments were conducted over 14 days. The plates with cells were constantly gently shaken. Individuals of ciliates were counted on the following days: 0, 3rd, 7th, 10th and 14th. Samples for ciliates counting were taken by micropipets (1 ml). The cells were fixed by adding Lugol's iodine solution and were not returned to the wells. The number of cells was counted under the Nikon Eclipse 80i light microscope at a magnification of $\times$ 20 in the 1-ml planktonic chamber with glass cover.

In order to obtain knowledge about the concentration of microcystins, an analysis of toxins in the cyanobacteria cells and dissolved in the water was performed. Because *A. flos-aquae* are able to produce anatoxin-a, we also checked *A. flos-aquae* for its presence. For the analysis of toxins (microcystins and anatoxin-a) in the cyanobacterial cells, HPLC analyses were conducted. Immediately after sampling, 1-l samples of water containing cyanobacteria (samplings were done at the same places and time as was done while gathering the cyanobacteria biomass), and 1-l samples of water from the *Planktothrix* culture were filtered through Whatman filters GF/C. For a detailed description of the method and equipment used for the analysis, see Kaczkowski et al. (2017).

For the microcystins dissolved in the water, we used 1-l samples of water from the *P. agardhii* culture and water collected from the pond and oxbow lake. 1-l water samples were filtered using Whatman GF/C filter papers to separate cyanobacterial cells from the water. Extracellular microcystins were concentrated in Baker C_{18} solid-phase extraction (SPE) cartridges (Deventer, Netherlands; sorbent mass: 500 mg) and eluted using 90% MeOH containing 0.1% trifluoroacetic acid (TFA) according to the methods of Meriluoto & Codd (2005).

The microcystins were analysed using an Agilent 1100 apparatus with a diode matrix (DAD) (Quinn & Keough, 2002). The concentrations of microcystins dissolved in the water were determined in the Central Laboratory of the Municipal Water and Sewage Company in Kraków, Poland. In both cases, the microcystins were identified using the microcystin standards MC-LR, MC-RR and MC-YR based on their characteristic absorption spectra and retention times.

Intra- and extracellular toxins were measured in biomass and in the same water used for the experiments.

Statistics We used a generalized linear model (GLM) to test the relationship and differences among the relationships between *Spirostomum* sp. and different species of potentially toxic cyanobacteria. The

generalized linear model (GLM) is an extension of the simple linear regression model for a continuous response variable given one or more continuous and/or categorical predictors. It includes multiple linear regression, as well as analysis of variance and analysis of covariance (Quinn & Keough, 2002). We calculated the GLM using the Poisson distribution; the dependent variables were a) cyanobacteria species and b) cyanobacteria species + day of experiment. Plots of the predicted values were created using the 'ggeffects' package (Lüdecke, 2017). Data were considered statistically significant at $P < 0.05$. All statistical analyses were performed by means of R v. 3.4.2 (R Core Team, 2017).

Results

The biomass of cyanobacteria used for the experiments showed the presence of microcystins for *P. agardhii* and *M. aeruginosa*, but not for *A. flos-aquae*. We did not find demethylated variants of microcystins in the *P. aghardii* biomass and also did not find anatoxin-a in the biomass of *A. flos-aquae* (Table 1). Microcystins were present as three analogues: microcystin-LR (MC-LR), microcystin-RR (MC-RR) and microcystin-YR (MC-YR). In the *Microcystis aeruginosa* cells, MC-LR and MC-RR were having a similar concentration, but in the *Planktothrix agardhii* cells, the highest concentration was found for MC-RR. The strain of *P. agardhii* was highly toxic.

Microcystins were also found as dissolved in the water from the *P. agardhii* culture and in the water from the oxbow lakes where *M. aeruginosa* bloomed, but not in the water from the pond where *A. flos-aquae* bloomed (Table 1).

The *Spirostomum* sp. population versus biomasses of different species of cyanobacteria

In the experiments, we examined the effects of toxic and non-toxic cyanobacteria biomasses on the population of *Spirostomum* as solitary species (Experiments 1—filamentous cyanobacteria and 2—chroococcal cyanobacteria; for the experiment description, see "Materials and methods" and Fig. 1).

The GLM showed an increase in the number of *Spirostomum* individuals in the control sample (no cyanobacteria, no toxins) and in the sample with highly toxic *P. agardhii*, but a decrease in the sample with non-toxic *A. flos-aquae* (Experiment 1, Table 2, Fig. 2A). In the second experiment, the GLM analysis showed stable populations of *Spirostomum* (Table 2) in the control sample (no cyanobacteria, no toxins), and an increase in the number of *Spirostomum* individuals in the sample with toxic *M. aeruginosa* (Table 2, Fig. 2B).

The number of *Spirostomum* individuals changed in the samples with the presence of particular cyanobacteria species (Table 2). All the differences were statistically significant except for the experiment with *A. flos-aquae* (Table 2). We also found strong statistical differences in the number of *Spirostomum* individuals in the presence of cyanobacteria species over time (Table 2). The number of *Spirostomum* individuals increased over time in the samples with *P. agardhii* and *M. aeruginosa*, but decreased in the sample with *A. flos-aquae* (Fig. 2A and 2B).

Spirostomum sp. as a component of ciliate assemblages versus cyanobacteria

In these experiments, we examined the effect of cyanobacteria biomass on *Spirostomum* as a component of a simple ciliate assemblage consisting of four species (Experiments 3—filamentous cyanobacteria and 4—chroococcal cyanobacteria).

Table 1 Concentrations of microcystins (µg/l) in the cells of cyanobacteria and dissolved in the water used for the experiments

Number of experiment	Sample	MC-RR (in cells)	MC-YR (in cells)	MC-LR (in cells)	MC-tot	
					In cells	Dissolved in water
1, 3	*P. agardhii*	282.6	11.9	29.4	323.9	29.9
1, 3	*A. flos-aquae*	0.0	0.0	0.0	0.0	0.0
2, 4	*M. aeruginosa*	14.3	2.8	14.8	31.9	0.4

Table 2 Results of the generalized linear model (GLM) for solitary *Spirostomum* sp.

Treatment	Estimate	SE	Z	P
Intercept (Control 1) Experiment 1	5.030	0.031	159.654	**< 0.001**
Planktothrix 1	− 0.427	0.045	− 9.343	**< 0.001**
Aphanizomenon 1	− 0.164	0.058	− 2.798	**< 0.01**
Control 1: day	0.087	0.003	28.057	**< 0.001**
Planktothrix 1: day	0.088	0.004	20.802	**< 0.001**
Aphanizomenon 1: day	− 0.612	0.028	− 21.360	**< 0.001**
Intercept (Control 2) Experiment 2	5.600	0.027	205.899	**< 0.001**
Microcystis 2	− 0.161	0.034	− 4.676	**< 0.001**
Control 2: day	− 0.018	0.003	− 5.525	**< 0.001**
Microcystis 2: day	0.207	0.003	53.680	**< 0.001**

Estimates of the GLM coefficients and their standard errors (SE) are presented. Z—GLM test statistic, P—statistical significance are emboldened
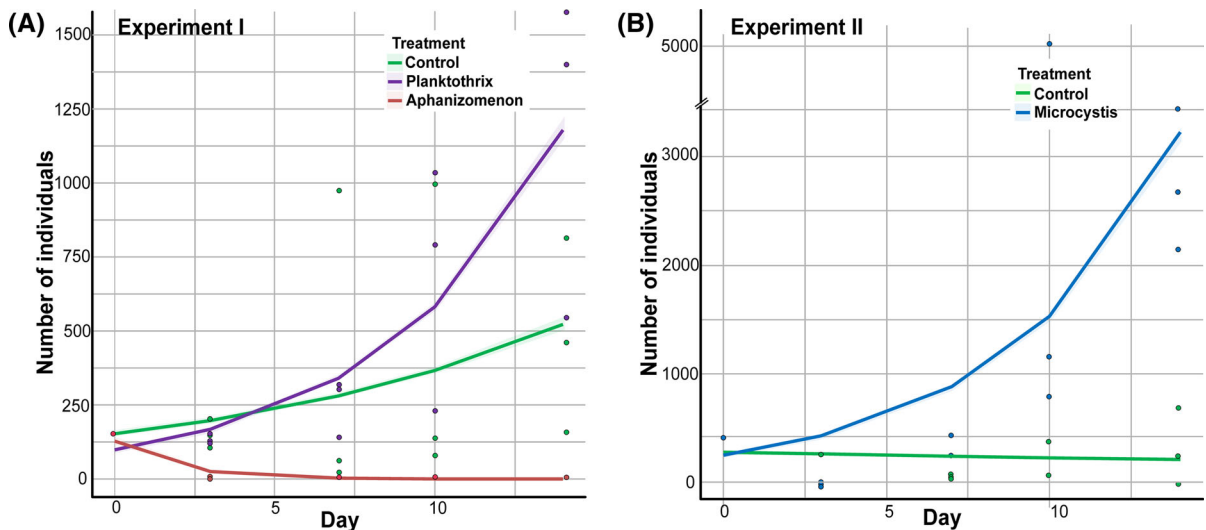
Fig. 2 The predicted number of individuals for *Spirostomum* sp. (solitary specimen): (**A**) Experiment 1: green line and dots—control sample, violet line and dots—sample with the addition of *P. agardhii*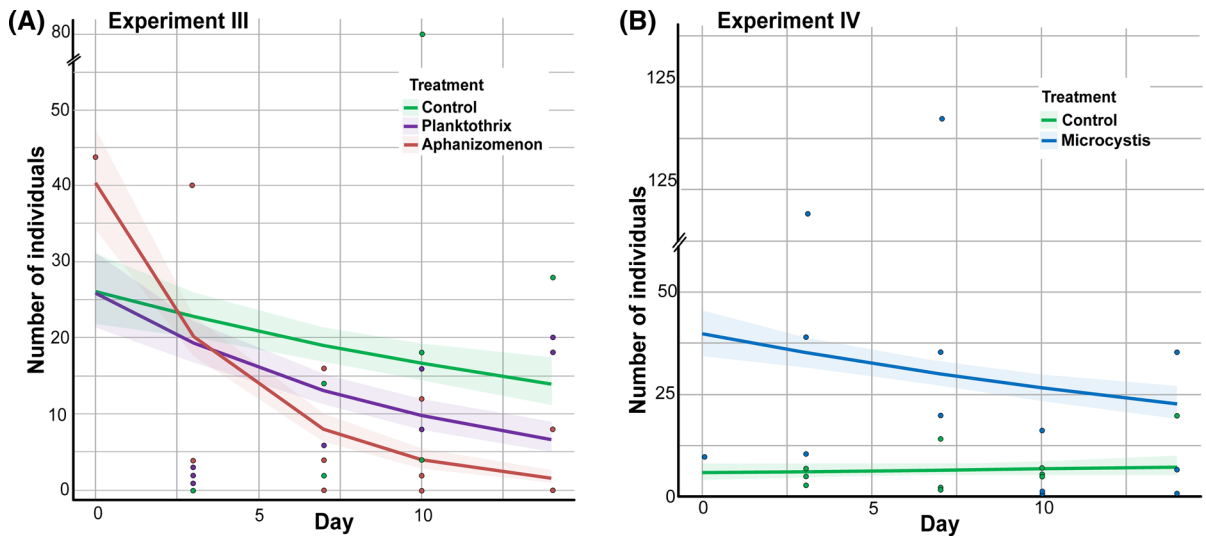, red line and dots—sample with the addition of *A.* *flos-aquae*; (**B**) Experiment 2: green line and dots—control sample, blue line and dots—sample with the addition of *M. aeruginosa*

The GLM analysis showed a decrease of the *Spirostomum* population being a component of ciliate assemblages in the presence of cyanobacteria, for both toxic and non-toxic types (Fig. 3A, B).

The statistically significant differences were found for the number of *Spirostomum* individuals being a component of ciliate assemblage in the presence of *A. flos-aquae* (non-toxic) and *M. aeruginosa* (toxic), but not in the presence of *P. agardhii* (highly toxic, Table 3). A statistically significant decrease of the number of *Spirostomum* individuals in time was observed in the presence of all three species of cyanobacteria. The strongest decrease was found for

A. flos-aquae, a weaker one for *P. agardhii*, and the weakest for *M. aeruginosa* (Table 3).

We observed weak but significant decrease of *Euplotes patella* (Table 4, Fig. 4, Supplementary material) over time in the presence of *P. agardhii* and weak and also significant increase of *E. patella* in the presence of *A. flos-aquae* and *M. aeruginosa*. For *Strobilidium* sp. (Table 5, Fig. 5, Supplementary material), we observed weak and significant decrease in the presence of *A. flos-aquae* and *P. agardhii*, but increase in the presence of *M. aeruginosa*. However, for *Paramecium aurelia*-complex (Table 6, Fig. 6, Supplementary material), we found statistically significant increase in the presence of *A. flos-aquae* and

Fig. 3 The predicted number of individuals of *Spirostomum* sp. (as a component of ciliate assemblage): (A) Experiment 3: green line and dots—control sample, violet line and dots—sample with the addition of *P. agardhii*, red line and dots—sample with the addition of *A. flos-aquae*; (B) Experiment 4: green line and dots—control sample, blue line and dots—sample with the addition of *M. aeruginosa*

Table 3 Results of the generalized linear model (GLM) for *Spirostomum* sp.

Sample	Estimate	SE	Z	P
Intercept (Control 1) Experiment 3	3.259	0.090	36.187	< **0.001**
Planktothrix 1	− 0.006	0.130	− 0.047	0.962
Aphanizomenon 1	0.436	0.122	3.577	< **0.001**
Control 1: day	− 0.044	0.012	− 3.728	< **0.001**
Planktothrix 1: day	− 0.052	0.018	− 2.767	< **0.01**
Aphanizomenon 1: day	− 0.186	0.023	− 8.046	< **0.001**
Intercept (Control 2) Experiment 4	1.760	0.178	9.854	< **0.001**
Microcystis 2	1.923	0.192	9.977	< **0.001**
Control 2: day	0.015	0.020	0.745	0.456
Microcystis 2: day	0.0554	0.022	− 2.454	< **0.05**

Spirostomum sp. being a component of ciliate assemblage. Estimates of the GLM coefficients and their standard errors (SE) are presented. Z—GLM test statistic, P—statistical significance are emboldened

P. agardhii but no significant changes in the presence of *M. aeruginosa*.

Discussion

The toxic effect of cyanotoxins on vertebrates is well known, but they are also harmful for invertebrates, e.g. planktonic animals, and may be responsible for the appearance of clonal subpopulations of invertebrates such as *Daphnia* sp. (e.g. Schwarzenberger et al., 2013). Although planktonic animals are subjected to cyanotoxins because of the uptake of toxic cells, toxins dissolved in the water may also have a negative effect (Reinikainen et al., 2002), because some microcystin congeners may cross cell membranes by other mechanisms, including diffusion (Chorus & Bartram, 1999).

There are no clear responses of zooplankton to cyanotoxins, and especially of ciliates. Information in

the literature is contradictory: for example, the toxicities of *Fischerella epiphytica*, *Gleotrichia echinulata* and *Nostoc linckia* were demonstrated to *Paramecium caudatum* (Ransom et al., 1978). On the other hand, a different response of *Tetrahymena pyriformis* to microcystins (Ward & Codd, 1999) was shown, with no lethal effect of microcystins to *Nassula* sp. that grazed on toxic *Planktothrix agardhii* (Combes et al., 2013). *Nassula* sp. is also considered as a species grazing on *Aphanizomenon* and *Anabaena* and may reduce cyanobacterial blooms (Canter et al., 1990). Also, *Paramecium* cf. *caudatum* was found to be a successful grazer of toxin-producing *Cylindropsermopsis* (Fabbro et al. 2001). However, cyanobacteria–ciliates relationships might be modyfied because of differences in cyanobacteria cells, shape and size, which determine the capability of defending cyanobacteria themselves against protozoan grazers (Pajdak-Stós et al., 2001; Fiałkowska & Pajdak-Stós, 2014).

Our studies indicate that microcystins in cells and dissolved in water do not harm ciliates, even when concentrations of toxins are high. On the other hand, some studies reported that the high toxicity of extract containing anatoxin-a was harmful to *Tetrahymena thermophila* (Sierosławska et al., 2010). It illustrates that cyanotoxin–ciliate relationships should be studied as species-specific phenomena. Our studies also showed that the abundance of *Spirostomum* sp. increased in the presence of toxic cyanobacteria with a high to extremely high concentration of microcystins in the cells and also with high concentrations of microcystins dissolved in the water. On the other hand, the abundance of *Spirostomum* decreased in the presence of *A. flos-aquae*, strain which did not produce either microcystins or anatoxin-a. These results indicate that the factor that negatively affected *Spirostomum* was neither the presence nor the high concentration of toxins (microcystins, anatoxin-a). In such a case, it might be another direct factor such as the presence of different kinds of metabolites produced by *A. flos-aquae* (Řezanka & Dembitsky, 2006) that affected *Spirostomum* abundance, or some other indirect factor. In our opinion, a better explanation of this phenomenon is the indirect relation between cyanobacteria and ciliates. There are known ciliates–bacteria relationships but they are not well understood (Boscaro et al., 2018). Cyanobacterial bloom may significantly induce an increase in bacterial production (Kuosa & Kivi, 1989), which could be a food source

for bacterivorous ciliates, e.g. *Spirostomum*. Bacteria growing up on different cyanobacteria species might be specific, and may inhibit the growth of specific ciliates species. Pearman et al. (2016) observed that the bacterial and protists community changed because of cyanobacterial bloom. At least, some species of ciliates appear to selectively feed upon other bacteria if offered a choice (Caron et al., 1991).

Another indirect relation might be an effect of the ability of cyanobacteria to produce biologically active compounds with cyanotoxins showing antibacterial, antiviral, antifungal, and anticancer activities (Bhateja et al., 2006). In such a case, the toxic species are responsible for destroying some adverse microorganisms (viruses, bacteria, pathogens, etc.) that promote the development of some species of ciliates. Antibacterial activity changes according to the cyanobacteria species. These bioactive substances may possibly lead to specific bacterial flora affecting the composition of bacterial communities (Skulberg, 2000) and, as a consequence, promoting the development or decrease of specific species of ciliates.

An increase of ciliates abundance in the presence of toxic cyanobacteria is possible due to some aquatic bacteria having the ability to metabolise high MC concentrations; MCs are a source of dissolved organic carbon for bacteria, which enables an increase of the bacteria's population (Sellner, 1997; Sopanen et al., 2009) and, as a consequence, ciliates. On the contrary, a lack of toxins will cause a decrease of bacteria and, furthermore, a decrease of ciliates.

Bacterial growth can be also limited when nitrogen limitation occurs (Casamatta & Wickstrom, 2000). The presence of *A. flos-aquae* indicates a deficiency of nitrogen in the water and may indicate a scarcity of bacteria, explaining the decrease of *Spirostomum* abundance.

The indirect relation between *Spirostomum* and cyanobacteria is confirmed by the experiment with simple assemblage of ciliates versus toxic/non-toxic cyanobacteria species. In this experiment, *Spirostomum* abundance decreased in the presence of toxic (*M. aeruginosa*, *P. agardhii*) and non-toxic (*A. flos-aquae*) cyanobacteria. This trend indicates an effect of competition between *Spirostomum* and the remaining smaller ciliates species (*Euplotes patella* and *Strobilidium* sp. in the presence of *M. aeruginosa*, and *Paramecium aurelia*-complex and *E. patella* in the presence of *A. flos-aquae*, but *Paramecium aurelia-*

complex in the presence of *P. agardhii*) that feed more effectively (compared with the large *Spirostomum*) on bacteria (Christoffersen et al., 1990). In a shallow lake in Denmark, the importance of small ciliates as transformers of carbon from bacteria growing after *Aphanizomenon* bloom was found (Christoffersen et al., 1990).

In general, we found that the reaction of solitary species of ciliates (*Spirostomum*) to the presence of toxic cyanobacteria is not the same as *Spirostomum* being a component of ciliates assemblage. *Spirostomum* as a single species did not show a negative response to toxic cyanobacteria and toxins dissolved in the water. On the other hand, a decrease of *Spirostomum* abundance being in the ciliate assemblage might be an effect of competition between different species of ciliates. However, our observation is contrary to the one that was shown by Tirjaková et al. (2016) in a small eutrophic pond. In those studies, *Spirostomum* dominated among ciliate communities during cyanobacterial bloom, along with *Euplotes* and *Paramecium* which were also used in our experiments. Therefore, Tirjaková et al. (2016) stated that *Spirostomum* can be considered as a good competitor in natural communities, also possibly because it is microaerophilic and can withstand under a wide range of oxic/anoxic conditions (literature cited in Tirjaková et al., 2016). In the above-mentioned studies, *Spirostomum* abundances were positively correlated with *Euplotes* abundances, which is also opposite to our observation. The differences might be explained by different food preferences and/or by a broader range of food sources in the macrocosm (e.g. the presence of different species of bacteria, etc.), and/or by different structures of ciliates communities (e.g. the presence more species in the pond), and/or by the presence of metazooplankton which may effect ciliates (in pond) or lack of it (in the experiment).

Finally, the results of our studies show that cyanobacterial blooms affected ciliates not necessarily as a direct result of toxins–ciliates relationships, but because cyanobacteria and toxins induce changes in the water food network.

Conclusions

In conclusion, toxic cyanobacteria may positively or negatively affect the *Spirostomum* population (and

other ciliates species) not only because of direct toxic effect but because of indirect interaction as well.

The same species of ciliates may react in a different way to the presence of cyanobacterial blooms, not only because of cyanotoxins but also because of ciliate assemblages composition and the competition between species and the existence of species-specific interactions (e.g. ciliates–ciliates, ciliates–cyanobacteria, ciliates–bacteria).

Compliance with ethical standards

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