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Original Research

Four-Year Study on Phytoplankton Biodiversity in a Small Hypertrophic Lake Affected by Water Blooms of Toxigenic Cyanobacteria

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Abstract

In a shallow hypertrophic lake, species richness of phytoplankton was very high (174-223 taxa, yearly) but values of Shannon-Weaver index were very low (0.04-1.38). Perennial blooms (2.1×10^7-11.6×10^7 ind.·L^{-1}) of microcystin-producing (up to 123.6 µg intracellular MCs·L^{-1} in 2006, and 43.8-57.3 µg·L^{-1} in 2008-09) cyanobacterium *Planktothrix agardhii* and the time of their appearance affected phytoplankton species richness (34-66% loss) and diversity (4-9 fold decrease in the values of Shannon-Weaver index). Short-term mass development of anatoxin-a-producing cyanobacteria (*Dolichospermum* spp. and/or *Cuspidothrix issatschenkoi*) did not influence algal biodiversity.

Keywords: species richness, *Planktothrix agardhii*, microcystins, anatoxin-a

Introduction

The global decline in biodiversity observed in recent decades, particularly in freshwater ecosystems [1], has resulted in an increased interest in species diversity of freshwater phytoplankton [2]. Planktonic algae, including cyanobacteria, play a key role in functioning of highly eutrophicated water bodies [3]. However, their biology, ecology, and biodiversity are still not fully recognized and understood [4, 5]. Water blooms caused both by prokaryotic (toxin-producing cyanobacteria) and eukaryotic algae are a consequence of eutrophication processes and growing water temperatures due to climate warming [4, 6]. The mass appearance of algae influences physical-chemical features of water by increasing pH and redox potential, worsening light conditions, etc. As a consequence, simplification of aquatic biocenoses occurs [3]. Toxins produced by bloom-forming cyanobacteria may be harmful for living organisms, including algae [7-9]. Hepatotoxic microcystins (MCs), produced by planktonic cyanobacteria such as *Microcystis*, *Planktothrix*, *Dolichospermum* (syn. *Anabaena*), and *Oscillatoria* are common cyanotoxins in fresh water [10-14]. For example, MC-producing cyanobacterium *Microcystis aerugi-*

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nosa and pure MCs inhibited the growth of some planktonic algae under laboratory conditions [8, 9]. Frequent mass appearance of *Dolichospermum*, *Aphanizomenon*, *Cylindrospermum*, and others also can be a source of neurotoxins, including anatoxin-a (ANTX) [15-17]. So far nothing is known about the influence of ANTX on microscopic algae.

The aim of this work was long-term study of changes in species richness and diversity of phytoplankton in a shallow lake with recurrent blooms of toxigenic cyanobacteria.

Materials and Methods

Study Area, Sampling, and Physical-Chemical Analysis of the Lake Water

Water samples for chemical and phytoplankton analyses, as well as detection of cyanotoxins, were collected once a month (March-November) in 2006-08 and (April to November) in 2009, from the surface (0-0.5m) water layer in the pelagic zone of a small (6 ha), shallow (2.9 m) flow-through and hypertrophic Lake Syczyńskie in eastern Poland (N 51º17', E 23º14').

Basic physical parameters of water, including temperature, pH, conductivity, and oxygen saturation were measured 'in situ' by means of a 556 MPS probe (Envag). Water transparency was determined with Secchi disk (SD). $N\text{-}NH_4$, $N\text{-}NO_3$, $P\text{-}PO_4$, P_{tot}. [18] and chlorophyll-a [19] concentration in water were also determined under laboratory conditions. The Carlson Trophic State Index (based on transparency, TP, and chl-*a*) was calculated [20].

Cyanotoxin Extraction

For determination of intra- and extracellular MCs and ANTX, 0.5-1.0 L of lake water was filtered through Whatman GF/C filters, and extracts of the filtered off phytoplankton were prepared using ultrasonication (3 times for 5 min., 50 W, ultrasonic homogenizer from Sonoplus, Bandelin) in 75% (v/v) methanol (Merck, pure p.a.) containing 0.002 M HCl. After centrifugation (14,000 rpm for 10 min. 17ºC), supernatants were collected and frozen (-20ºC) until required for cyanotoxin analysis. Filtered lake water was evaporated to dry. The residue was extracted in 75% methanol acidified with 0.01 M HCl, collected and frozen (-20ºC).

HPLC-DAD Analysis of Microcystins

The HPLC-photodiode array detection system (Shimadzu) was used for MC detection and identification. The detection range was 200-300 nm. MC-LR, -RR, -YR, -LA, -LY, - LW, -LF, -WR (Alexis) were used as standards. Extracts were separated using acetonitrile (Merck) acidified with 0.05% trifluoroacetic acid (gradient 30-100%) at a flow rate of 0.7 ml/min in a RP-18 Purosphere column (125×3 mm, Merck).

HPLC-FLD Analysis of Anatoxin-a

ANTX in extracts was determined using liquid chromatography (HPLC, Beckman) with fluorescence detection (Shimadzu) according to James et al. [21]. For ANTX derivatization, 10% NBD-F (4-fluoro-7-nitrobenzofuran; Fluka) was used. The detector parameters were as follow: excitation wavelength 470 nm and emission wavelength 530 nm. Extract separation was obtained using an RP-18 Purosphere column (125×3 mm, Merck) and TFA (0.05 %) acidified acetonitrile at a flow rate of 0.6 ml/min. For identification and quantitative determinations, standard ANTX (Tocris, Bioscence) was used.

Laboratory Methods of Identification and Enumeration of Phytoplankton

Taxonomical identification of live phytoplankton was carried out using a light microscope, whereas the quantitative structure (abundance) of the algae community was analyzed (in fixed material) by means of the Uterműl method. The algal systematics were based on Van den Hoek et al. [22]. 100 μm for all algae with straight filaments, one curve of coiled and one colony of coccoid taxa were recognized as individuals. Taxa with contributions in the total algal abundance higher than 50% were considered dominants. Shannon-Weaver index of species diversity [23] and Duffy dominance index [24] were calculated. The comparison of phytoplankton community structure in particular years was made using Jaccard's index [25]. Correlations between *P. agardhii* abundance and number of algal taxa were evaluated for periods of *P. agardhii* blooming.

Results

A four-year study revealed that in the shallow, hypertrophic (TSI=65-79) lake (Table 1), species richness of phytoplankton was very high and comprised 174-193-223-189 taxa in particular years (Fig. 1). Chlorophyta (44-51%), Bacillariophyceae (14-18%), and Cyanobacteria (12-17%) dominated in the total number of taxa (Fig. 1). Low values (0.43-0.49) of Jaccard's similarity index, evaluated over four-years, indicated high variability of the phytoplankton community from year to year. Physical-chemical conditions (Table 1) such as high concentrations of dissolved inorganic nitrogen, phosphorus, and their ratio, as well as high summer temperatures, supported mass development of cyanobacteria and some eukaryotes (Fig. 2A, Table 2) that worsened light climate in the lake. Perennial water blooms caused mainly by the cyanobacterium *Planktothrix agardhii* (Gom.) Anagn & Kom. (max. abundance: 2.1×10^7-11.6×10^7 ind.$\cdot L^{-1}$) with an admixture of *Limnothrix redekei* (Van Goor) Meffert, *Planktolyngbya limnetica* (Lemm.) Kom.-Legn. & Cronberg, *Aphanizomenon gracile* (Lemm.) Lemm. and spring-early summer mass development of *Dolichospermum* spp., were observed in 2006, 2008, and 2009, but not in 2007. High variability in the time of the appearance of *P. agardhii* blooms was noted (Fig. 2B).

Table. 1. Physical-chemical characteristics of Lake Syczyńskie water in 2006-09 (mean values and range).

Parameters	2006	2007	2008	2009
Water temperature (°C)	15.5 0.3-26.8	14.8 7.1-24.9	14.8 5.2-22.6	15.8 5.6-25.4
pH	7.8 7.2-8.6	7.7 7.1-8.3	7.9 7.7-8.4	8.2 7.3-8.9
Conductivity ($\mu S \cdot cm^{-1}$)	446 272-610	491 394-606	458 330-560	466 411-633
Transparency (m)	0.56 0.17-1.70	1.20 0.62-1.89	0.48 0.20-0.90	0.47 0.25-1.10
Oxygen saturation (%)	109 4-226	106 91-134	122 114-153	140 101-234
$N\text{-}NH_4$ ($mg \cdot L^{-1}$)	0.455 0.230-1.201	0.238 0.025-0.814	0.345 0.125-0.775	0.469 0.167-0.800
$N\text{-}NO_3$ ($mg \cdot L^{-1}$)	0.166 0.035-0.499	0.096 0.038-0.246	0.077 0.030-0.196	0.231 0.024-0.884
$P\text{-}PO_4$ ($mg \cdot L^{-1}$)	0.090 0.017-0.307	0.120 0.016-0.276	0.054 0.003-0.240	0.075 0.012-0.147
$P_{tot.}$ ($mg \cdot L^{-1}$)	0.220 0.121-0.409	0.193 0.058-0.406	0.282 0.195-0.403	0.456 0.199-0.657
DIN/DIP ratio	9.3 3.7-17.8	3.4a 0.7-8.8 (55.3b)	28.3 4.0-91.5	18.2 4.5-57.2
Chlorophyll-*a* ($\mu g \cdot L^{-1}$)	105.3 15.3-325.9	34.9 8.2-51.2	143.1 36.7-277.9	71.0 11.1-116.9
TSI	73 52-86	65 63-76	78 69-87	79 65-90

DIN/DIP – dissolved inorganic nitrogen and phosphorus ratio, TSI – Trophic State Index; a – average value for March-October period; b – DIN/DIP ratio in November

In 2006 the most intense *P. agardhii* bloom lasted from June to November, in 2008 from August to November, and in 2009 from May to September. A strong decrease in phytoplankton species richness was noted during *P. agardhii* blooms. In 2006 and 2008, the taxa number decreased from 76 and 84 to 44 and 55, respectively, whereas in 2009 (when the bloom started earlier) it increased from 30 in spring to 89 in summer, soon after breakout of *P. agardhii* bloom. *P. agardhii* abundance and the number of algal taxa correlated negatively in particular years:

- in 2006: $y=-6.329\ln(x)+157.66$, $R^2=0.22$
- in 2008: $y=-1.971\ln(x)+91.095$, $R^2=0.50$
- in 2009: $y=-38.83\ln(x)+727.86$, $R^2=0.61$.

In 2007, when DIN/DIP ratio was low (0.7-8.8 from March to October) in comparison with other years (3.7-91.5), cyanobacterial bloom was replaced by successive mass development of diatoms, cryptophytes, and green-algae (Fig. 2A, Table 2), and species richness of phytoplankton changed considerably from month to month (over the range of 23-96 taxa).

Bloom-forming cyanobacteria produced cyanotoxins (Fig. 3). Very high concentrations (up to 123.6 $\mu g \cdot L^{-1}$ in 2006, and 43.8-57.3 $\mu g \cdot L^{-1}$ in 2008-09) of intracellular microcystins (Fig. 3A) were noted and they correlated strongly ($R^2=0.80$-0.98) with *P. agardhii* abundance. Extracellular MCs (Fig. 3B) also were present in the lake water, but in much lower concentrations (up to 2.3 $\mu g \cdot L^{-1}$). Besides MC-producing *P. agardhii*, mass development of Nostocales cyanobacteria *Dolichospermum* spp. and *Aphanizomenon* (including *Cuspidothrix issatschenkoi*), that were able to produce ANTX, was observed in spring-early summer seasons (max. abundance: $12.16\times10^6 \cdot L^{-1}$, $1.61\times10^6 \cdot L^{-1}$, and $1.74\times10^6 \cdot L^{-1}$ in 2006, 2008 and 2009, respectively). Concentrations of intracellular ANTX (Fig. 3C) were about 30-times lower than MCs (Fig. 3A) and the extracellular ANTX was detected only in 2008 (Fig. 3C), at

the highest ANTX production by cyanobacteria. Generally, during mass appearance of Nostocales and high ANTX concentrations there was no decrease in phytoplankton species richness observed, in contrast to periods with *P. agardhii* blooms.

The obtained results indicate that the time of appearance of the toxigenic *P. agardhii* bloom determined the species structure and richness of phytoplankton, including the bloom-forming species (Table 2) in particular seasons. In 2006, when the *P. agardhii* bloom began in May, eight other species of algae (including five cyanobacteria, one diatom and two small chorophytes) developed in mass. In the next year, free of cyanobacteria bloom, a two-fold higher number of algal taxa (19) reached high abundance, and succession of eukaryotic microalgae was observed. In early spring, centric diatoms *Stephanodiscus minutulus* and *S. hantzschii* dominated, then pennate diatoms *Nitzschia paleacea*, *Staurosira construens*, *Aulacoseira granulata* developed abundantly. In July, cryptophytes (with the dominant *Chroomonas acuta*) and two coccoid chlorophytes dominated, whereas in August and September seven other taxa of chlorophytes (with the dominant *Coelastrum microporum*) reached high densities. In autumn, cryptophytes and centric diatoms developed in mass, again. In 2008, 28 taxa developed abundantly. The species richness of the most abundant taxa was high, particularly in spring and early summer (six taxa of diatoms and 12 coccoid chlorophytes) before the *P. agardhii* bloom. In July, only the cyanobacterium *Planktolyngbya limnetica* reached over 50% of the total phytoplankton abundance. Intense *P. agardhii* bloom in the second half of 2008 and first half of 2009 caused a decrease in species richness (to 12 of the most abundant taxa). In 2009 the contribution of cyanobacteria taxa was similar to 2006 and 2008 (Table 2).

The values of Shannon-Weaver index (Fig. 4) were generally very low (0.04-1.38) during all the study. Phytoplankton biodiversity decreased considerably in periods with *P. agardhii* blooms (the values of Shannon-Weaver index were 4-9 fold lower). Changes of the values of the Duffy index (Fig. 4) were opposite to the values of Shannon-Weaver index and reflected well the dominant structure of phytoplankton (Table 2) in particular years and seasons.

Discussion

Cyanobacterial blooms may affect aquatic ecosystems in various ways [3]. Our four-year study revealed that in the hypertrophic polymictic lake with recurrent blooms of the filamentous microcystin-producing *P. agardhii* (Oscillatoriales), phytoplankton species richness was generally very high (174-223 taxa). It was higher than e.g. in the eutrophic lake in Germany, where 119 taxa were found [26], in the dam reservoir (132 taxa) in eastern Poland [27], and in the hypertrophic reservoir in South Korea with 66 phytoplankton taxa [28]. In the periods free of cyanobacterial blooms in Lake Syczyńskie, higher number of phytoplankton taxa was noted than during mass development of cyanobacteria. The values of the diversity (Shannon-Weaver) index also were higher.

The obtained results revealed that long-lasting *P. agardhii* blooms and the time of their appearance affected essentially the phytoplankton species richness and diversity.

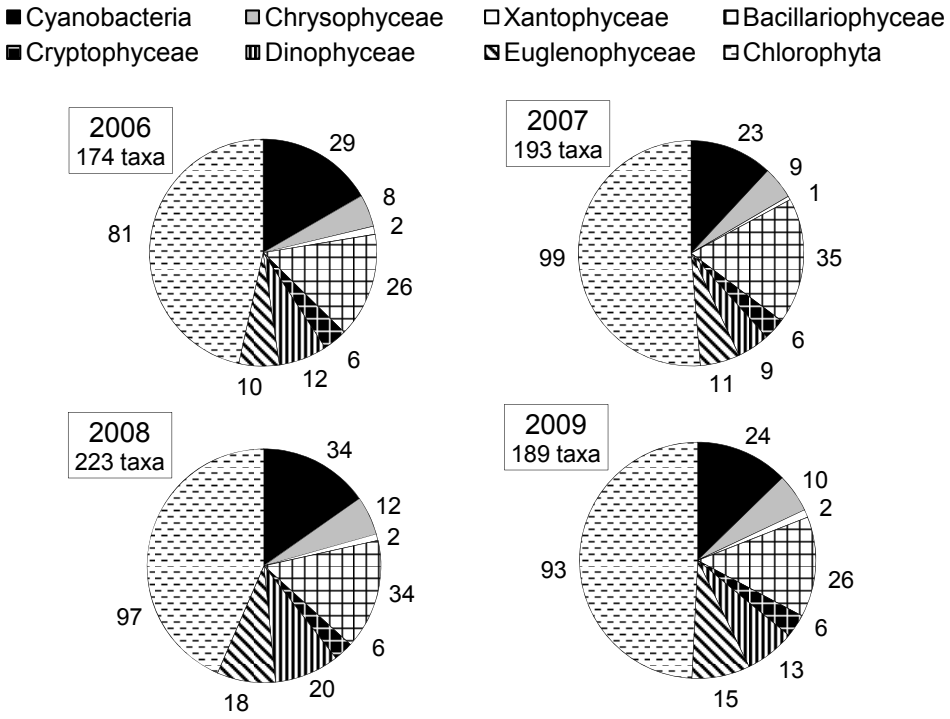

Fig. 1. Yearly changes in species richness of particular taxonomic groups in the total taxa number of phytoplankton in Lake Syczyńskie in 2006-09.

Table 2. Seasonal and yearly changes in the structure of the most abundant (>1.0×10^6 ind.·L^{-1}) and dominant* (contribution in abundance > 50%) planktonic algae in 2006-09.

Algal taxa	2006	2007	2008	2009
Cyanobacteria				
Aphanizomenon gracile	J		J, Ag	O
Aphanocapsa holsatica	Ag		O	Ag
Dolichospermum lemmermannii	My			
D. cf. *heterosporum*			J	
D. planctonicum				Jy
Limnothrix redekei	J-Jy, S-N	M		
Limnothrix sp.			J	
Planktolyngbya limnetica	Ag-N		My-Jy*-O	S-O
Planktothrix agardhii	My-Jy*-O*-N		Ag*-N*	My*-Ag*, O
Snowella atomus			O	O
Bacillariophyceae				
Asterionella formosa			A	
Aulacoseira granulata		J		
Nitzschia paleacea		My		
Nitzschia sp.			Jy	
Staurosira construens		My		
Stephanodiscus hantzschii	J	M, N*	M*	S-O*-N
S. minutulus		M*		A, O-N*
Synedra ulna			Jy	
S. ulna var. *acus*		S	M-A	N
S. ulna var. *oxyrhynchus*			Jy-Ag	
Cryptophyceae				
Chroomonas acuta		M, Jy*	J	
Cryptomonas erosa		Jy		
Cryptomonas cf. compressa		O*	J	
Chlorophyta				
Actinastrum hantzschii		S	My-J	
Chlamydomonas globosa			A	
C. cf. monadina			My	
Chlorella vulgaris				Ag-S
Chlorella sp.			My	S
Chloromonas teilinglii			My	
Coelastrum astroideum			Jy-Ag	
C. microporum		Ag*	J-Jy	
Gloeotila subtilis		S		
Dictyosphaerium sp.		S		
Kirchneriella contorta			My	
Micractinium pusillum		S		
Monoraphidium komarkovae	A	Jy		

Table 2. Continued.

Algal taxa	2006	2007	2008	2009
M. minutum			My-J	
Oocystis lacustris	J	Ag	My	
Scenedesmus cf. *ecornis*		Ag		
Sphaerocystis planctonica			J	
Tetraëdron minimum			J-Jy	
Chlorophyceae gen non determ		Jy	A-My, S	S
Total number of taxa	9	19	28	12

M – March, A – April, My – May, J – June, Jy – July, Ag – August, S – September, O – October, N – November

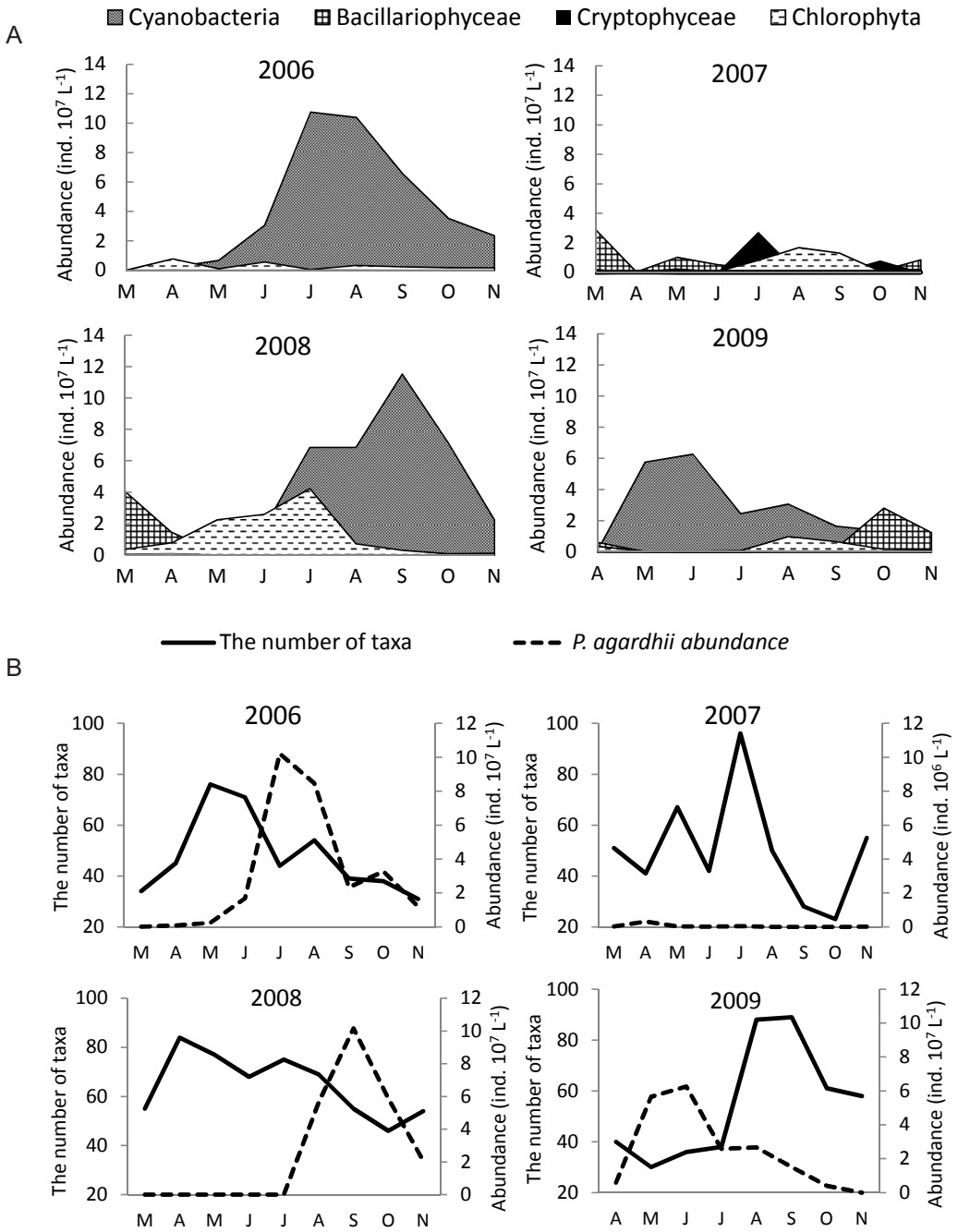

Fig. 2. Monthly changes in the abundance of dominant algal groups (A), phytoplankton species richness, and abundance of *P. agardhii* (B) in Lake Syczyńskie in 2006-09.

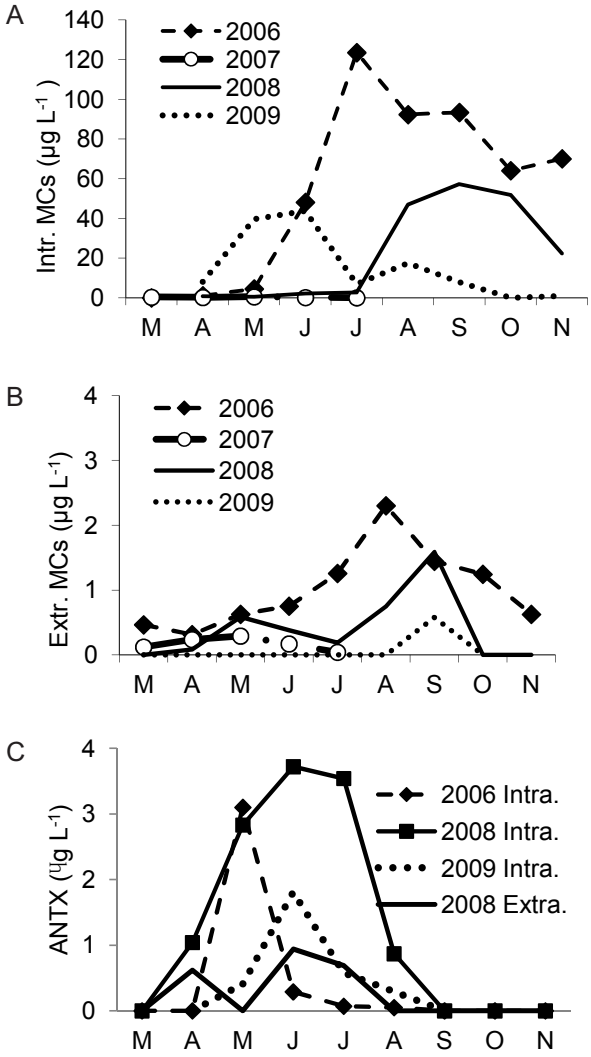

Fig. 3. Monthly changes in concentrations of intracellular (A), extracellular MCs (B), and ANTX (C) in Lake Syczyńskie in 2006-09.

Similarly, during blooms of other cyanobacteria *Cylindrospermopsis raciborski* and *Microcystis aeruginosa* in a shallow tropical reservoir, phytoplankton species richness decreased [4]. However, we did not observe biodiversity loss during short-term mass development of anatoxin-a-producing *Dolichospermum* spp. In Lake Syczyńskie, phytoplankton diversity, evaluated by Shannon-Weaver and Duffy indexes, varied both seasonally and yearly; however, it was generally lower than in other hypertrophic or eutrophic water bodies. For example, Shannon-Weaver index estimated for Lake Sempach (Switzerland) ranged 0.1-4.4 [29], Lake Swarzędzkie (Poland) 2.3-5.6 [30], fish pond 0.0-4.2 [31], and for a hypertrophic reservoir in South Korea 0.4-2.0 [28]. Low taxa number and the dominance of one or co-dominance of a few algal species accounted for the decrease of the values of Shannon-Weaver index observed in the studied lake, as well as in other highly eutrophic water bodies [29-31]. The phytoplankton diversity loss observed in Lake Syczyńskie during *P. agardhii* blooms might also be a consequence of a few other co-varying factors. The most important seemed to be light limitation caused by intense proliferation of cyanobacteria, which grow faster than eukaryotic algae and may develop in high mass in a shorter time, even during several days [3]. *P. agardhii* prefers low-light conditions and turbid waters [5, 26, 30]. Changes in water temperature, total concentrations of nutrients [28, 29], and, especially, a decrease in the ratio of easy available dissolved inorganic nitrogen to dissolved inorganic phosphorus (DIN/DIP) may inhibit development of cyanobacteria and support the mass appearance of eukaryotic algae, which was observed in the studied lake in 2007 and in cyanobacteria bloom-free time periods. Cyanobacterial metabolites might also have negative effects on development of algae [8, 9, 32] and other hydrobionts [7, 33, 34]. In Lake Syczyńskie, *P. agardhii* produced high amounts of microcystins, although there can

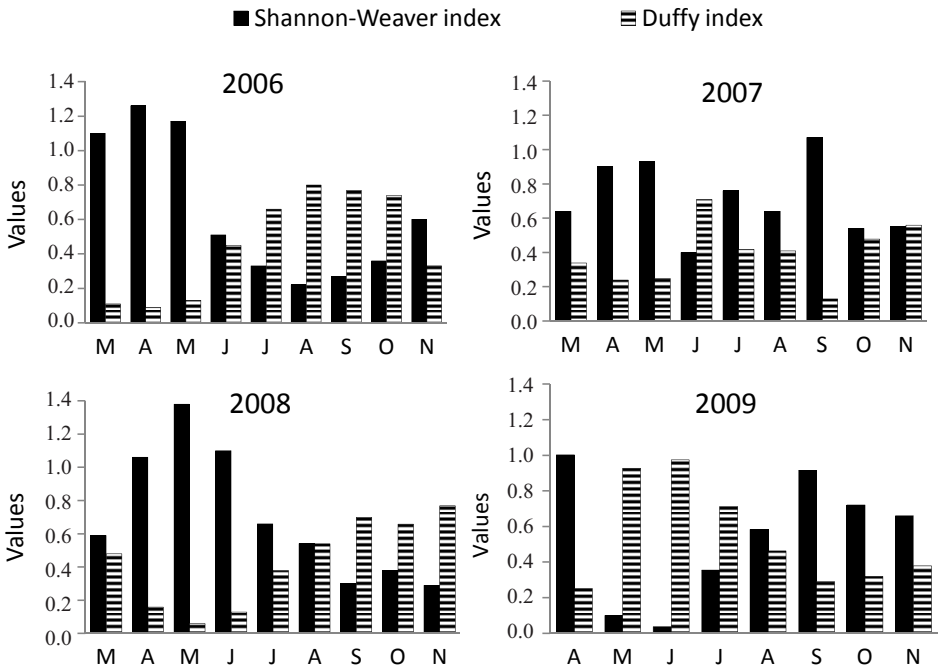

Fig. 4. Monthly changes in phytoplankton diversity in Lake Syczyńskie in 2006-09.

also exist some non-producing strains [11]. *P. agardhii* may also produce many other biologically active metabolites like aeruginosins 205a, -B, and microviridins D-F [35]. Short-term experiments [8] showed that extracellular MC-LR and MC-RR (produced also *by P. agardhii*) inhibited growth of some planktonic Chlorococcales and one cyanobacterium, but at environmentally irrelevant concentrations of 100-5000 $\mu g \cdot L^{-1}$. In Lake Syczyńskie concentrations of extracellular MCs were much below that level, but periodically they reached up to 11 $\mu g \cdot L^{-1}$ [12]. A recent report of Béres et al. [9] showed that the presence of living *M. aeruginosa* cells had more inhibitory effects on the growth of cryptophyte *C. ovata* than the crude extract of the cyanobacterium *M. aeruginosa* or the purified MC-LR (conc. 20-1,100 $\mu g \cdot L^{-1}$). Possibly, the long-term presence of *P. agardhii* may also inhibit cryptophytes, because we did not observe their simultaneous development. This is in agreement with the report [5] indicating an increase in biomass of Cryptophyceae when *P. agardhii* or *C. raciborskii* biomass decreased. MCs may also be produced by *Dolichospermum* spp. [17]. However, in the lake studied these cyanobacteria produced mostly anatoxin-a, which may also be harmful for aquatic organisms [32, 34, 36, 37] such as macrophytes, insect larvae, and fish. Some planktonic *Dolichospermum* spp. (beside ANTX) [11] produce other neurotoxins like anatoxin-a(S), as well as cytotoxic lipopeptides (e.g. anabaenolysins; [38]). However, nothing is known about the influence of ANTX and other cyanobacterial metabolites on phytoplankton.

Conclusions

Long-lasting blooms of MC-producing *P. agardhii* and time of their appearance influenced essentially species richness and diversity of phytoplankton in the small polymictic lake. High total species richness and high variability of qualitative structure of phytoplankton indicate that the algal community may be very dynamic even in a hypertrophic lake affected by blooms of toxin-producing cyanobacteria. Very low species diversity was a characteristic feature of the phytoplankton, independent of the algal group and/or species that form water blooms.

Acknowledgements

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