Vertical distribution of cyanobacteria biomass and cyanotoxin production in the polymictic Siemianówka Dam Reservoir (eastern Poland)

Magdalena Grabowska, Hanna Mazur-Marzec

Received – 31 August 2012/Accepted – 27 March 2013. Published online: 25 March 2014; ©Inland Fisheries Institute in Olsztyn, Poland Citation: Grabowska M., Mazur-Marzec H. 2014 – Vertical distribution of cyanobacteria biomass and cyanotoxin production in the polymictic Siemianówka Dam Reservoir (eastern Poland) – Arch. Pol. Fish. 22: 41-51.

Abstract. The summer-autumn dominance of the toxic cyanobacterium Planktothrix agardhii was described in the lowland polymictic Siemianówka Dam Reservoir (SDR) in 2010 and 2011. The study was conducted at a station located in the deepest part of the reservoir. The species composition of phytoplankton was very similar at the four depths analyzed. Microcystins were continually present in the cyanobacterial biomass. Demethylated microcystin-RR (dmMC-RR) and microcystin-RR (MC-RR) were identified as the major microcystin variants in most samples, while demethylated microcystin-LR (dmMC-LR) and microcystin-LR (MC-LR) were each recorded only once. The concentration of microcystin-RR correlated strongly with the biomass of P. agardhii. The effect of environmental factors on cyanobacterial biomass the production of microcystins by cyanobacteria was minor, but increased water temperatures and pH favored the production of microcystins. Phytoplankton biomass was also influenced by how water outflow from the reservoir was regulated; the biomas increased with depth when the upper flaps were opened, but it was very similar throughout the water column when they were closed. According to the Polish phytoplankton-based index (IFPL), the ecological potential of the reservoir was determined to be poor.

Keywords: cyanobacteria, *Planktothrix agardhii*, microcystins, outflow, ecological potential

M. Grabowska [🖃] Department of Hydrobiology University of Białystok Świerkowa 20B, 15-950 Białystok, Poland e-mail: magra@uwb.edu.pl

H. Mazur-Marzec Department of Marine Biology and Ecology University of Gdańsk, Poland

Introduction

Strong thermal stratification in deep lakes and dam reservoirs results in vertical variation in phytoplankton composition (Reynolds et al. 2002, Halstvedt et al. 2007, Koreiviené and Karosiené 2012). In shallow water bodies, stratification is rarely observed (Rücker et al. 1997). Toxic cyanobacteria species occur in eutrophic waters in both strongly stratified (Barco et al. 2004, Halstvedt et al. 2007, Rohrlack et al. 2008, Karadžić et al. 2010, Koreiviené and Karosiené 2012) and polymictic water bodies (Rücker et al. 1997, Grabowska and Pawlik-Skowrońska 2008, Toporowska et al. 2010, Grabowska and Mazur-Marzec 2012, Solis et al. 2012).

The mass occurrence of Р. agardhii (Oscillatoriales) was commonly observed in well-mixed hypertrophic or eutrophic lakes and dam reservoirs at most latitudes (Köhler and Hoeg 2000, Reynolds et al. 2002, Yéprémian et al. 2007, Pawlik-Skowrońska et al. 2008, Mazur-Marzec et al. 2008, Solis et al. 2012). This species can persist in lakes throughout the year (Rücker et al 1997, Halstvedt et al. 2007, Toporowska et al. 2010), and sometimes develops into monospecific populations (Reynolds et al. 2002). The success of P. agardhii in shallow, polytrophic lakes could be supported by the turbulent mixing regimes and low water transparency (Reynolds et al. 2002, Mischke 2003, Karadžić et al. 2010). In stratified lakes, the mass development of *P. agardhii* is limited to the metalimnion.

During cyanobacterial blooms, hepatotoxic cyclic peptides called microcystins (MCs) frequently occur in aquatic ecosystems. The production of these cyanobacterial metabolites is a strain-specific feature (Kurmayer and Christiansen, 2008, Christiansen et al. 2006, Rohrlack et al. 2008, Kurmayer et al. 2011). Moreover, the environmental conditions optimal for the growth of cyanobacteria might not be optimal for cyanotoxin production (Kotak et al. 2000). *Planktothrix* tends to produce demethylated forms of microcystins (Fastner et al. 1999, Kurmayer et al. 2004, Welker et al. 2004); however, the production of other MC variants by this cyanobacterium has also been reported (Rohrlack et al. 2008, Solis et al. 2012).

The toxin-producing species of cyanobacteria are of special concern as they can threaten aquatic oranimals. ganisms, terrestrial and humans (Kuiper-Goodman et al. 1999). Microcystins, the most frequently occurring cyanobacterial toxins, are strong inhibitors of protein phosphatases and are thought to contribute to the formation of liver tumors. They accumulate in aquatic organisms, including mussels, fish, and birds. In some geographical regions, they can also be found in drinking water sources. Numerous incidents of human and animal intoxications were described by Kuiper-Goodman et al. (1999). MC-RR accumulation was detected in the muscles and livers of the fish species Silurus glanis L., Cyprinus carpio L., and Carassius auratus (L.) from the temperate eutrophic Lake Suwa (Japan), with the highest contents in C. auratus (79.4 mg kg⁻¹ BW; Xie et al. 2007). Permanent presence of microcystin at levels exceeding those safe for consumption was noted in the muscles of fish from the hypertrophic, flow-through Syczyńskie Lake (Pawlik-Skowrońska et al. 2012). Hydraulic management through outlet selection has been proposed as a valid approach for cyanobacteria reduction in water bodies (Rigosi and Rueda 2012). Outlets at different depths can control phytoplankton composition efficiently by changing the thermal structure of a stratified water body (Cahşkan and Elçi 2009, Rigosi and Rueda 2012).

Microorganisms, including cyanobacteria, with short generation times react rapidly to environmental changes and are considered to be good indicators of trophic status (Reynolds et al. 2002). Generally, water bodies with high shares of cyanobacteria are classified to ecosystems of poor ecological status (Napiórkowska-Krzebietke et al. 2009, Hutorowicz et al. 2011).

The aim of the present work was to test the hypothesis that environmental factors have a minor effect on the production of microcystin. Additionally, the impact of different environmental factors and water management strategy on the biomass and structure of phytoplankton was examined. For the purpose of the study, the Siemianówka Dam Reservoir, which is dominated by *Planktothrix agardhii*, was selected for the study and the Polish phytoplankton-based index IFPL was determined.

Study area

The Siemianówka Dam Reservoir (SDR) (52°55'N, 23°50'E) is a shallow, polymictic reservoir that was constructed in the upper sector of the Narew River in 1990. The morphometric parameters of the reservoir (max. area -32.5 km²; max. capacity -78.5 Mm³; mean depth - 2.5 m; mean retention time - 4 to 6 months) are typically lowland. The physical and chemical parameters of the SDR indicate that it is a highly eutrophic water body (Grabowska et al. 2003). In the SDR the potentially toxic cyanobacteria have permanently dominated the summer and autumn phytoplankton community since the reservoir was constructed cyanobacterial toxins have been detected there many times (Grabowska and Pawlik-Skowrońska 2008. Kabziński et al. 2008, Grabowska and Mazur-Marzec 2011).

Two methods are used to drain water from the SDR into the Narew River. In the first method, water is discharged through two deep outlets located 3 m

above the bottom. In second method, which is used less frequently, the bottom drainage is supplemented with surface water discharge by opening the upper flaps. The SDR is also used for fish farming, fishing, and recreation.

Methods

The phytoplankton structure, microcystin concentrations, and water quality in the Siemianówka Dam Reservoir were assayed three times in 2010 from July to October and once in July 2011. Water samples were collected at four depths (0.5 m, 2 m, 4 m, and 6 m) from the deepest station, near the dam. Field measurements included: Secchi disc visibility, temperature, pH, oxygen saturation, and dissolved oxygen concentration (HachLange Sonde, USA). The position of upper flap was also observed in the field.

The reservoir's ecological potential was determined using the Polish phytoplankton-based index (IFPL; Błachuta and Picińska-Fałtynowicz 2010, Regulation 2011). The IFPL has two metrics: phytoplankton composition and chlorophyll *a* concentration. The Metric Phytoplankton is calculated for 75 trophic indicator species belonging to different algae groups, and the final IFPL value is the averaged value of these two metrics.

Laboratory analyses of nitrogen, phosphorus, and iron concentrations were performed according to methods described in Hermanowicz et al. (1976). Water samples (500 ml) for phytoplankton studies were immediately fixed in Utermöhl solution. Phytoplankton biomass was analyzed quantitatively with an inverted Olympus CX40 microscope. The algal biomass was determined based on the phytoplankton cell volumes measured by the authors of this study. Chlorophyll *a* concentration was determined with spectrophotometry. The samples were filtered on GF/C filters and extracted with boiling 90% ethanol (Lorenzen 1965, Nusch 1980).

Statistical analyses were performed with STATGRAPHICS 1.4 PL software.

Analysis of microcystins (MCs)

Water samples (500 ml - 1000 ml) collected from the reservoir were filtered through 47 mm fiberglass filter discs (Whatman GF/C), which had been stored at -20°C prior to extraction and analyses. Methanol (90%) extracts from the material were prepared with a 15-min bath sonication (Sonorex, Bandelin, Berlin, Germany) followed by 1-min probe sonication with an HD 2070 Sonopuls ultrasonic disrupter equipped with a MS 72 probe (Bandelin, Berlin, Germany; 20 kHz, 25% duty cycle). After centrifugation at 10,000 g for 15 min, the supernatants were transferred to a chromatographic vial. The microcystins (MCs) were analyzed using the HPLC-DAD and LC-MS/MS systems, the detailed methodology of which was described previously (Grabowska and Mazur-Marzec 2011).

Results

During the study period, cyanobacteria dominated in the reservoir and constituted at an average 90.2% of phytoplankton biomass. The lowest cyanobacteria contribution to the phytoplankton community (78%) was noted in July 2010 in the deepest layers of the water column at 4 m and 6 m (Fig. 1a).

Although five other groups of phytoplankton (Bacillariophyceae, Chlorophyceae, Cryptophyta, Dinophyta, Euglenophyta) were always present, their maximum biomass did not exceed 5.1 mg l^{-1} . Zygnematophyceae and Chrysophyceae were observed occasionally (max. 0.84 mg l^{-1} and 0.02 mg l^{-1} , respectively).

The highest total phytoplankton biomass (PB) (90.0 mg 1^{-1}) with a high contribution of Oscillatoriales was recorded in September 2010 at a depth of 2 m. The subsurface maximum of cyanobacteria biomass was noted quite often at a depth of 2 m, but as depth increased, it decreased (e.g., 12 July 2010).

Throughout the study period, the cyanobacteria were dominated by four species belonging to the



Figure 1. Total phytoplankton biomass with Cyanobacteria contribution (white bars) (a), water temperature, microcystin, and oxygen concentration (b), and N-NH₄, dissolved phosphorous (DP), and total phosphorous (TP) (c) in different layers of water in SDR.

Table 1

Range of microcystin concentrations and water parameters measured during cyanobacteria dominance in the SDR in 2010-2011. MC-LR – microcystin LR, dmMC-LR – demethylated microcystin LR, MC-RR – microcystin RR, dmMC-RR – demethylated microcystin RR, DP – dissolved phosphorous, TP – total phosphorous

Depth (m)	0.5	2.0	4.0	6.0
MC-LR (µg l ⁻¹)				1.67
dmMC-LR ($\mu g l^{-1}$)			1.68	
MC-RR ($\mu g l^{-1}$)	6.31-8.27	5.53-13.0	5.33-12.5	5.09-15.1
dmMC-RR ($\mu g l^{-1}$)	7.25-16.2	8.82-14.2	8.33-14.2	4.43-14.2
pН	8.6-9.3	8.3-9.6	8.5-9.6	7.8-9.6
oxygen (mg l ⁻¹)	9.4-12.8	9.1-13.2	2.4-10.8	0.3-10.7
$N-NH_4^+ (\mu g l^{-1})$	72-236	69-196	29-161	59-349
N-NO ₃ ($\mu g l^{-1}$)	60-91	19-55	30-75	8-96
DP (μg l ⁻¹)	84-187	64-164	65-156	72-206
TP ($\mu g l^{-1}$)	246-273	200-286	254-552	216-379
$P-PO_4^{-} (\mu g l^{-1})$	6-18	7-18	10-15	6-15
$\frac{\text{Fe}^{2+}/\text{Fe}^{3+}}{(\mu g l^{-1})}$	45-183	47-183	73-104	54-140

order Oscillatoriales. Planktothrix agardhii (Gom.) Anagn. & Kom was the main representative of Oscillatoriales in all samples, and its contribution to the total cyanobacterial biomass (CB) fluctuated from 77.1% to 99.0%. Thin filaments of Limnothrix redekei (Van Goor) Meffert, Pseudanabaena limnetica (Lemn.) Kom., and Planktolyngbya spp. made significantly lower contributions within the range of 0.93-4.48% CB. The cyanobacteria from Nostocales were always represented by species from the genus Aphanizomenon: A. gracile (Lemm.) Lemm., A. flos-aquae (L.) Ralfs ex Bornet et Flahault, and Cuspidothrix issatschenkoi (Usač.) Rajan. et al. (former A. issatschenkoi), but their contribution to the CB did not exceed 15.4%. Species of the genus Dolichospermum: D. circinalis (Rabenh. ex Bornet et Flahaut.) P. Wacklin L. Hoffmann & J. Komárek, D. planctonicum (Brunth.) P. Wacklin L. Hoffmann & J. Komárek and D. flos-aquae (Bréb. Ex Bornet et Flahault) P. Wacklin L. Hoffmann & J. Komárek occurred in July and only sporadically in September (max. 2.2% CB).

On one sampling day, the temperature in the whole water column was similar, with the exception of 12 July 2010 when the highest values of water temperature (26.4°C) were recorded. On that day,

the difference between the surface and the deepest layers exceeded 5°C (Fig. 1b). The development of thermal stratification at the dam in July 2010 resulted in rapid decreases in oxygen concentrations at depths of 4 m and 6 m to 2.4 and 0.3 mg I^{-1} , respectively (Fig. 1b, Table 1). Throughout the study period, no significant differences in either chemical or physical parameters were observed. Statistically significant differences were only noted for nitrate concentrations (N-NO₃) in water samples collected at 0.5 m and 2 m. Secchi disc visibility fluctuated within a narrow range from 0.4 m (September 2010) to 0.5 m (July 2001 and July 2011), except in October 2010, when the value increased to 0.9 m.

Chemical analyses revealed the presence of microcystins (MCs) in all 16 samples that were collected and analyzed over the two-year period (Fig. 1b). Four MC analogues were identified, but only demethylated MC-RR and MC-RR were detected at all depths (Table 1). Demethylated MC-RR was detected in July 2010 and July 2011, while MC-RR was detected in September and October 2010. The concentrations of dmMC-LR and MC-LR in individual samples were below 2 μ g l⁻¹ (Table 1). In 1 mg wet mass of cyanobacteria, MCs concentrations ranged from 0.15 μ g in September 2010 at a depth of 2 m to

Spearman correlations between microcystin, cyanobacteria, *P. agardhii* biomass, and water parameters in the SDR in 2010-2011. MCs – total concentrations of microcystins, dmMC-RR – concentrations of demethylated microcystin RR, MC-RR – concentrations

of microcystin RR, DP – dissolved phosphorous, TP – total phosphorous									
	MCs	dmMC-RR	MC-RR	Cyanobacteria	P. agardhii				
pН	0.518*	0.743*	-0.429	-0.135	-0.212				
Temperature	0.537*	0.216	0.883*	0.201	0.087				
Oxygen	-0.177	0.883*	-0.905*	0.035	-0.029				
Oxygen saturation	0.629*	0.299	0.667	0.277	0.185				
N-NH ₄	0.347	0.395	0.167	-0.232	-0.253				
N-NO ₃	-0.437	-0.216	-0.322	-0.261	-0.619				
SRP	-0.134	0.205	-0.805*	-0.695*	-0.716*				
DP	0.091	0.479	0.429	0.124	0.209				
TP	0.160	0.048	-0.575	-0.244	-0.287				
Fe ²⁺ /Fe ³⁺	0.278	0.428	0.667	0.321	0.418				
Cyanobacteria	0.303	-0.156	0.952*	-	0.985*				
P. agardhii	0.297	-0.156	0.952*	0.985*	-				

Table 2

*statistically significant (P < 0.05)

a maximum of 0.91 μg in July 2011 at a depth of 0.5 m.

The highest total concentrations of MCs (from 14.2 μ g l⁻¹ at 2 m to 16.2 μ g l⁻¹ at 0.5 m) were recorded in all four layers of the SDR on 18 July 2011 (Fig. 1b), when the most uniform vertical distribution of cyanobacteria and the highest concentration of N-NH₄ (except depth 2 m) were noted (Fig. 1). The lowest total concentration of MCs was detected on 12 July 2010 at 6 m (4.43 μ g l⁻¹) when oxygen deficits were recorded on the reservoir bottom (Fig. 1b). MCs were present in both insolated (to about 2 m when SEC = 0.9 m and to 1 m when SEC = 0.4-0.5 m) and shaded water layers. At similar concentrations of total phosphorous (TP) in the collected water samples (mainly ranging from 200 to 300 μ g l⁻¹), the differences in toxin concentrations were as high as four Table 3

fold. The two highest concentrations of TP (379 and $552 \ \mu g \ l^{-1}$) were noted on 18 July 2011 (at 4 and 6 m, respectively) and coincided with the highest concentrations of microcystins (Fig. 1).

Our results revealed that the strongest correlation was between *P. agardhii* biomass and MC-RR concentrations, while there was a weaker one between MC concentrations and physical and chemical water parameters (Table 2).

According to the IFPL classification, the reservoir is of poor ecological potential (Table 3). However, very diverse values were recorded for the various metric measures. The metric phytoplankton (MP) values from all the terms were less than 0.2, which suggests that the ecological potential of the reservoir is bad. Similar MP values arose from the very similar phytoplankton structure that was dominated by

Ecological potential of the SDR according to metric values of the IFPL; class boundaries of the ecological potential of dam reservoirs: <0.2 bad; ≥ 0.2 poor; ≥ 0.4 moderate; ≥ 0.6 good; ≥ 0.8 high

Date	Chlorophyll a (µg l ⁻¹)	Metric Phytoplankton	Metric Chlorophyll <i>a</i>	IFP	Ecological potential
12 July 2010	47.7	0.110	0.50	0.31	poor
27 September 2010	36.8	0.063	0.50	0.28	poor
19 October 2010	22.0	0.038	0.75	0.39	poor
18 July 2011	64.4	0.073	0	0.04	bad
Final evaluation		0.071	0.44	0.25	poor

filamentous cyanobacteria. In October 2010, the lowest MP stemmed from the predominance of *P. agardhii* (96.4% PB) over other species. Metric Chlorophyll *a* (MCH) was more varied with values ranging from 0 to 0.75, and which correspond to the three levels of ecological potential: poor, good, moderate. These two metrics complied only in July 2011; in other cases, the values of MCH were two or even three classes higher than MP, which increased the final index value substantially (Table 3).

Discussion

The mass occurrence of P. agardhii is a common phenomenon in both shallow and deep water bodies at most latitudes. Comparable contributions of the species to those found in the SDR were recorded in highly eutrophic shallow waters in Germany (Rücker et al. 1997, Nixdorf et al. 2003, Mischke 2003) and Poland (Mazur-Marzec et al. 2008, Toporowska et al. 2010, Solis et. al 2012). P. agardhii domination is restricted to the thermocline in stratified lakes and reservoirs. It belonged to the most numerous species in the metalimnion of the Garaśli and Bukulja water supply reservoirs in Serbia (Karadžić et al. 2010). P. agardhii with Ceratium hirundinella and Fragilaria crotonensis dominated only in the metalimnion in the dimictic Aukśtadvaris Reservoir in Lithuania. (Koreiviené and Karosiené 2012). In the stratified Lake Steinsfjorden in southeastern Norway, P. agardhii and P. rubescens dominated the phytoplankton community for several decades and regularly formed blooms in the thermocline in summer. The cyanobacteria also occurred under the ice cover during winter months (Halstvedt et al. 2007). During periods of total water mixing, P. agardhii contribute more than 95% to the phytoplankton biomass both in the upper and deeper water layers of Lake Steinsfjorden (Halstvedt et al. 2007). Such a high prevalence of *Planktothrix* over other cyanobacteria in the lake was limited to short mixing periods, while in the polymictic SDR, it was observed for most of the vegetation season. The mass occurrence of P. agardhii throughout the year in eutrophic water

bodies was also described by Rücker et al. (1997) and Toporowska et al. (2010).

In stratified reservoirs, vertical changes in the dominant groups of phytoplankton communities result from strong variations in factors such as ligh and temperature conditions, and nutrients concentration. In the polymictic SDR, thermal and nutrient condition were very similar throughout the study period even in the deepest part of the reservoir, and while light conditions deteriorate with depth, this does not inhibit the growth of *P. agardhii* because this species tolerates shade well (Reynolds et al. 2002).

In the SDR, the permanent and strong predominance of the toxic P. agardhii over other cyanobacteria impacts water quality negatively. Our results showed strong correlation between P. agardhii biomass and MC-RR concentrations (Table 2). This indicates that the microcystins detected in the collected phytoplankton samples most likely derived from this cyanobacterium. A similar correlation was described by Rohrlack et al. (2008) in Lake Steinsfjorden. The correlation between P. agardhii biomass and MC-RR concentration (r Spearm. >0.900) was also noted by Solis et al. (2012) in the highly eutrophic reservoirs of Tomaszne in eastern Poland. Several authors suggested that demethylated MC-RR is the major microcystin variant in "Planktothrix lakes" (Fastner et al. 1999, Barco et al. 2004, Kurmayer et al. 2004, Welker et al. 2004).

The results obtained in this work concur with our previous studies in the SDR. Grabowska and Pawlik-Skowrońska (2008) reported a strong increase in P. agardhii biomass that overlapped with increases in MC concentrations, but which was concomitant with a decrease in the total biomass of cvanobacteria of the orders Chroococcales and Nostocales. The correlation between MC concentrations in seston and cyanobacteria abundance or biomass is not a common phenomenon (Messineo et al. 2009). The co-occurrence of microcystin- and non-microcystin-producing strains of cyanobacteria complicates this relationship (Kurmayer and Christiansen 2009). In addition, high variability in MC concentrations among individual cyanobacterial strains was noted. It was shown in most studies that different environmental factors, e.g., temperature, nutrients, and pH, can indirectly induce changes in cyanotoxin content in individual strains, but usually by a factor of no more than three to four (Kurmayer and Christiansen 2009). In the SDR, changes in MC content per 1 mg of cyanobacteria biomass measured in different seasons and under different environmental conditions were, in most cases, within this limit; however, a maximum factor of six was also recorded. This finding indicates the MC-producing cyanobacterial community in the SDR is stable.

The biomass of cyanobacteria and the concentrations of MCs in different lakes dominated by Planktothrix can vary significantly from below 0 mg l⁻¹ to over 600 mg l⁻¹ (Köhler and Hoeg 2000, Mischke 2003, Yéprémian et al. 2007) and from undetectable levels to above $170 \ \mu g \ l^{-1}$ (Yéprémian et al. 2007, Grabowska and Pawlik-Skowrońska 2008, Kabziński et al. 2008, Mazur-Marzec et al. 2008, Pawlik-Skowrońska et al. 2008, Grabowska and Mazur-Marzec 2011). Kurmayer et al. (2011) showed that in given lakes the proportion of genes encoding the synthesis of cyanobacterial peptides, non-ribosomal microcvstin (mcvB), aeruginoside (aerB), and anabaenopeptin (apnC) is stable and independent of seasonal fluctuations in Planktothrix density. This finding, based on studies carried out in 23 lakes located in five European countries, indicates the stable composition of Planktothrix community. For example, the same four sub-populations of Planktothrix were recorded for over 33 years in Lake Steinsfjorden in Norway (Rohrlack et al. 2008).

The similarity in phytoplankton composition in SDR waters at the four depths studied stemmed from this reservoir's susceptibility to wind mixing. With the exception of samples collected on 18 June 2010, there was no thermal stratification in the reservoir, and similar environmental conditions for phytoplankton development prevailed throughout the vertical profile of the polymictic SDR. Light and oxygen conditions deteriorated with depth, but the values of the other water parameters measured were similar. The way in which water outflow from the reservoir is regulated could play a significant role in the vertical distribution of phytoplankton in the deepest part of the SDR. The biomass of phytoplankton measured at the four depths was most similar when the upper flaps were closed (19 October 2010 and 18 July 2011). When one of the three upper flaps was opened, algal biomass in the surface layer was lower in relation to the deeper parts of water column, particularly at 2 m. This could have resulted from the removal of significant cyanobacteria biomass that had accumulated in the surface layer near the dam. In the case of the SDR, which lacks permanent thermal water stratification, this additional water discharge did not impact the phytoplankton vertical structure, and cyanobacteria continued to dominate all other algae groups in all water layers. Surface decreases in phytoplankton biomass were short-lived since losses of cyanobacteria from the surface layer were quickly compensated for. As demonstrated by Rigosi and Rueda (2012), only in deeply stratified reservoirs can water outflow from a selected depth stratum permanently alter phytoplankton composition. Warm water masses with gas vesicle-containing cyanobacteria occur close to the surface, while cold waters with few or without cyanobacteria occur in the lower water depths. Discharging warm surface water causes the risk of cyanobacterial contamination downstream in rivers (Ingleton et al. 2008). Discharging water from both surface and bottom layers of the SDR increases contamination of Narew River waters with large loads of microcystincyanobacteria (Grabowska -producing and Mazur-Marzec 2011).

Phytoplankton biomass and species composition or assemblages are used widely as important indicaecological status tors of the of lakes (Napiórkowska-Krzebietke et al. 2009, Hutorowicz et al. 2011) and the ecological potential of dam reservoirs (Błachuta and Picińska-Fałtynowicz 2010). When the total phytoplankton biomass of lakes is predominated by cyanobacterial taxa, this usually results in categorizing the lakes as having poor or bad ecological status (Napiórkowska-Krzebietke et al. 2009, Hutorowicz et al. 2011). In the polymictic SDR, cyanobacteria were the main component of the total

phytoplankton biomass in 2010 and 2011. Among them, the highest biomass was for Planktothrix agardhii at an average of 91.9% PB for all of the samples analyzed. The strong, permanent predominance of cyanobacteria over other species had a negative effect on the water quality in SDR, which lowered its final assessment. According to the IFPL classification criteria, the reservoir was of poor ecological potential. The SDR assessment based on Metric Chlorophyll *a* gave better results, and suggested that the basin was in the moderate class. The assessment based on Metric Phytoplankton was definitely worse and the SDR was designated as being in the bad class. This was also supported by the presence of microcystins. P. agardhii blooms in the SDR are always associated with the presence of microcystins. The risk that fish farmed in the reservoir contain toxins dangerous to human health is high. Therefore, it is advisable to introduce an additional indicator for ecological assessment of water ecosystem based on cyanotoxin concentrations. As it was dokumented by other authors, the toxin--producing cyanobacteria can pose a threat to aquatic organisms and also to humans and terrestrial animals (Kuiper-Goodman et al. 1999, Xie et al. 2007, Pawlik-Skowrońska et al. 2012).

The distinction between the bad potential of the phytoplankton component and moderate potential for chlorophyll *a* was somewhat problematic. The same differences between the partial metrics of the Phytoplankton Metric for Polish lakes (PMPL) were also noted by Napiórkowska-Krzebietke et al. (2009) and Hutorowicz et al. (2011). According to Hutorowicz et al. (2011), the highest assessment certainty occurs when a metric has a value close to the mid-range of the class. Most of the metrics for the SDR met this requirement.

Conclusions

The results obtained in the current study showed that *P. agardhii* was the main component of the cyanobacteria in the deepest part of the SDR. This species was also the main producer of intracellular

microcystin at all depths. MCs, mainly the dmMC-RR and MC-RR variants, were detected within a broad range of physical and chemical water parameters such as water temperature, light regime, oxygen levels, and nutrient concentrations. In the SDR, the intensity of MC production, expressed as MC concentration per cyanobacterial biomass, was stable and independent of environmental conditions. This result confirmed the hypothesis that MC production by cyanobacteria was only marginally affected by environmental conditions. The vertical distribution of phytoplankton biomass was influenced by the way in which the water outflow from the reservoir was regulated. The permanent predominance of cyanobacteria is an indicator of poor ecological potential of the SDR.

Acknowledgments. In 2010, the study was supported by the Ministry of Science and Higher Education under project number N N305 156136.

Author contributions. M.G. developed the research concept, analyzed phytoplankton materials, and water quality, and wrote the text; H. M.-M. analyzed the microcystins and contributed to the discussion.

References

- Barco M., Flores C., Rivera J., Caixach J. 2004 Determination of microcystin variants and related peptides present in a water bloom of *Planktothrix (Oscillatoria) rubescens* in Spanish drinking water reservoir by LC/ESI-MS – Toxicon 44: 881-886.
- Błachuta J., Picińska-Fałtynowicz J. 2010 Methodological guidance for carrying out monitoring and assessment of the ecological potential of dam reservoirs in Poland – Wrocław, GIOŚ, p. 44 (in Polish).
- Cahşkan A., Elçi Ş. 2009 Effects of selective withdrawal on hydrodynamics of a stratified reservoir – Water Resour. Manag. 23: 1257-1273.
- Fastner J., Erhard M., Carmichael W.W., Sun F., Reinhart K.L., Chorus I. 1999 – Characterization and diversity of microcystin in natural blooms and strains of the genera of *Microcystis* and *Planktothrix* from German freshwaters – Arch. Hydrobiol. 145: 147-163.
- Grabowska M., Górniak A., Jekatierynczuk-Rudczyk E., Zieliński P. 2003 – The influence of hydrology and water quality on phytoplankton community composition and

biomass in a humoeutrophic reservoir, Siemianówka reservoir (Poland) – Ecohydrol. Hydrobiol. 3: 185-196.

- Grabowska M., Pawlik-Skowrońska B. 2008 Replacement Chroococcales and Nostocales by Oscillatoriales caused a significant increase in microcystin concentration in a dam reservoir – Oceanol. Hydrobiol. Stud. 37: 23-33.
- Grabowska M., Mazur-Marzec H. 2011 The effect of Cyanobacterial blooms in the Siemianówka Dam Reservoir on the phytoplankton structure in the Narew River – Oceanol. Hydrobiol. Stud. 40: 19-26.
- Halstvedt C.B., Rohlack T., Andersent T., Skulberg O., Edvardsen B. 2007 – Seasonal dynamics and depth distribution of *Planktothrix* spp. in Lake Steinsfjorden (Norway) related to environmental factors – J. Plankton Res. 29: 471-482.
- Hermanowicz W., Dożańska W., Dojlido J., Koziorowski B. 1976 – Physicochemical analyses of waters and sewage – Arkady, Warszawa, 418 p. (in Polish).
- Hutorowicz A., Napiórkowska-Krzebietke A., Pasztaleniec A., Hutorowicz J., Lyche Solheim A., Skjelbred B. 2011 – Phytoplankton – In: Ecological status assessment of the waters in the Wel River catchment. Guidelines for integrated assessment of ecological status of rivers and lakes to support river basin management plans (Ed.) H. Soszka, Wyd. IRS, Olsztyn: 143-168 (in Polish).
- Ingleton T., Kobayashi T., Sanderson B., Patra R., Macinnis-Ng C.M.O, Hindmarsh B., Bowling L.C. 2008 – Investigation of the temporal variation of Cyanobacterial and other phytoplanktonic cells at the offtake of a large reservoir, and their survival following passage through it – Hydrobiologia 603: 221-240.
- Kabziński A., Grabowska M., Juszczak R., Karkoszka I. 2008
 Determining the influence of environmental factors on the values of Cyanobacterial blooms and hepatotoxin biosynthesis in Siemianówka dam reservoirs in 2007 – In: Ecotoxicology in environmental protection (Eds) B. Kołwzan, K. Grabas, Wrocław University of Technology: 153-158 (in Polish).
- Karadžić V., Subakov-Simić G., Krimzmanić J., Natić D. 2010 – Phytoplankton and eutrophication development in the water supply reservoirs Garaši and Bukulja (Serbia) – Desalination 255: 91-96.
- Köhler J., Hoeg S. 2000 Phytoplankton selection in a river–lake system during two decades of changing nutrient supply – Hydrobiologia 424: 3-24.
- Koreiviené J., Karosiené J. 2012 Temporal and spatial variation of phyto- and zooplankton in the Aukśtadvaris water reservoir during summer stratification – Proc. 31th International Conference of Polish Phycological Society, Olsztyn, 18-20.05.2012, Wyd. IRS, Olsztyn: 47.
- Kotak B.G., Lam A.K.-Y., Prepas E.E., Hrudey S.E. 2000 Role of chemical and physical variables in regulating microcystin-LR concentration in phytoplankton of eutrophic lakes – Can. J. Fish. Aquat. Sci. 57: 1584-93.

- Kuiper-Goodman T., Falconer J., Fitzgerald J. 1999 Human health aspect – In: Toxic Cyanobacteria in water: a guide to their public health consequences, monitoring and management (Eds) I. Chorus, J. Bartram, World Health Organization, Taylor and Francis, London and New York: 113-153.
- Kurmayer R., Christiansen G., Fastner J., Börner T. 2004 Abundance of active and inactive microcystin genotypes in populations of the toxic cyanobacterium *Planktothrix* spp. – Environ. Microbiol. 6: 831-841.
- Kurmayer R., Christiansen G. 2009 The genetic basis of toxin production in Cyanobacteria – Freshw. Rev. 2: 31-50.
- Kurmayer R., Schober E., Tonk L., Visser P.M., Christiansen G. 2011 – Spatial divergence in the proportions of genes encoding toxic peptide synthesis among populations of the cyanobacterium *Planktothrix* in European lakes – FEMS Microbiol. Lett. 317: 127-137.
- Lorenzen C.J. 1965 A note on the chlorophyll and phaeophytin content of the chlorophyll maximum – Limnol. Oceanogr. 10: 482-483.
- Mazur-Marzec H., Spoof L., Kobos J., Pliński M., Meriluoto J. 2008 – Cyanobacterial hepatotoxins, microcystins and nodularins, in fresh and brackish waters of Pomeranian Province, northern Poland – Oceanol. Hydrobiol. Stud. 37: 3-21.
- Messineo V., Bogialli S., Melchiorre S., Sechi N., Luiglie A., Casidda P., Mariani M.A., Padedda B. M., Di Corcia A., Mazza R., Carloni E., Bruno M. 2009 – Cyanobacterial toxins in Italian freshwaters – Limnologica 39: 95-106.
- Napiórkowska-Krzebietke A., Pasztaleniec A., Hutorowicz A. 2009 – Phytoplankton – an element of ecological status assessment of lakes in the Wel River catchment area – Teka Kom. Ochr. Kszt. Środ. Przyr. OL PAN, 6: 200-205.
- Nusch E.A. 1980. Comparison of different methods for chlorophyll and phaeopigment determination – Arch. Hydrobiol. Beih. Etgebn. Limnol. 14: 14-36.
- Pawlik-Skowrońska B., Pirszel J., Kornijów R. 2008 Spatial and temporal variation in microcystin concentration during perennial bloom of *Planktothrix agardhii* in a hypertrophic lake – Ann. Limnol. Int. J. Limnol. 44: 63-68.
- Pawlik-Skowrońska B., Toporowska M., Rechulicz J. 2012 Simultaneous accumulation of anatoxin-a and microcystins in three fish species indigenous to lakes affected by Cyanobacterial blooms – Ocean. Hydrobiol. Stud. 41: 53-65.
- Regulation 2011 Regulation of the Minister of the Environment of 9 November 2011 on status classification of surface water bodies and environmental quality standards for priority substances – Official Journal of the Laws of 2011, No. 257, Item 1545 (in Polish).
- Reynolds C.S., Huszar V., Naselli-Flores L., Melo S. 2002 Towards a functional classification of the freshwater phytoplankton – J. Plankton Res. 24: 417-428.

- Rohrlack T., Edvardsen B., Skulberg R., Halstvedt C.B., Utkilen H.C., Ptacnik R., Skulberg O.M. 2008 – Oligopeptide chemotypes of the toxic freshwater cyanobacterium *Planktothrix* can form subpopulation with dissimilar ecological traits – Limnol. Oceanogr. 53: 1279-1293.
- Rigosi A., Rueda F.J. 2012 Hydraulic control of short-term successional changes in the phytoplankton assemblage on stratified reservoirs – Ecol. Eng. 44: 216-226.
- Rücker J., Wiedner C., Zippel P. 1997 Factors controlling the dominance of *Planktothrix agardhii* and *Limnothrix redekei* in eutrophic shallow lakes – Hydrobiologia 342/343: 107-115.
- Solis M., Pawlik-Skowrońska B., Kalinowska R. 2012 Composition of potentially toxic Cyanobacteria assemblage and microcystin variants in highly eutrophic reservoirs Tomaszne (E. Poland) – Proc. 31th International

Conference of Polish Phycological Society, Olsztyn, 18-20.05.2012, Wyd. IRS, Olsztyn: 90.

- Toporowska M., Pawlik-Skowrońska B., Krupa D., Kornijów R. 2010 – Winter verus summer blooming of phytoplankton in a shallow Lake effect of hypertrophic conditions – Pol. J. Ecol. 58: 3-12.
- Welker M., Christiansen G., Van Dohren H. 2004 Diversity of coexisting *Planktothrix* (Cyanobacteria) chemotypes deduced by mass spectral analysis of microcystins and other oligopeptydes – Arch. Microbial. 182: 288-298.
- Xie L., Yokoyama A., Nakamura K., Park H. 2007 Accumulation of microcystins in various organs of the freshwater snail *Sinotaia histrica* and three fishes in a temperate lake, the eutrophic Lake Suwa, Japan – Toxicon 49: 646-652.
- Yéprémian C., Gugger M., Briand E., Catherine A., Berger C., Quiblier C., Bernard C. 2007 – Microcystin ecotypes in a perennial *Planktothrix agardhii* bloom – Water Res. 41: 4446-4456.