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Factors influencing cyanobacteria community structure in *Chara*-lakes

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ABSTRACT

For practical reasons, cyanobacteria attract scientific interest in eutrophicated, often degraded water bodies. Much less attention is paid to blue-greens in less fertilized lakes, including a group of macrophytic lakes with vegetation dominated by charophytes (*Chara*-lakes). In this study, therefore, two small, mid-forest *Chara*-lakes with negligible recreational use were compared with two large *Chara*-lakes subject, along with their drainage basin, to higher human pressure. An attempt was made to find out whether there are any differences in the qualitative and quantitative structure of cyanobacteria and in cyanobacteria functional groups between the studied *Chara*-lakes. In addition, the lakes ecological status was assessed based on their total phytoplankton and cyanobacteria biomass.

Small mid-forest *Chara*-lakes were distinguished by Chroococcales taxa, while Oscillatoriales and Nostocales preferred large recreationally used water bodies with catchment areas changed by human activity. *Aphanothece minutissima*, *Merismopedia tenuissima*, *Aphanizomenon gracile*, *Aphanocapsa holsatica* and *Cyanodictyon planctonicum* differentiated among the studied *Chara*-lakes.

Although cyanobacteria dynamics did not differ *Chara*-lakes from eutrophic water bodies, the dominance of cyanobacteria was only detected in the two large *Chara*-lakes characterised by a worse ecological status compared to the small mid-forest ones.

Alkalinity, water temperature, colour and, to a lesser extent, TP and TN:TP ratio are postulated to be water properties which, in addition to extensive charophyte meadows, control the development of cyanobacteria in the studied *Chara*-lakes.

1. Introduction

Cyanobacteria (Cyanoprokaryota, Cyanophyceae) are a group of ubiquitous, prokaryotic organisms characterised by a high ecological plasticity, thanks to which they occur in varied, often even extreme, habitats (Komárek and Anagnostidis, 1998). Although cyanobacteria are also known from oligotrophic water bodies, most papers are focused on the cyanobacteria significance and indicator value related to the process of eutrophication. Particular attention is paid to eutrophic and hypertrophic lakes where cyanobacterial blooms are caused by potentially toxic, often invasive species (Nixdorf and Deneke, 1997; Wiedner et al., 2002; Kokociński et al., 2010; Kobos et al., 2013; Kokociński et al., 2013). The considerable interest of scientists in this issue is connected with the negative effects of cyanobacterial blooms (Codd et al., 1999; Havens, 2008). As mentioned above, cyanobacteria are also a constant element of the phytoplankton community in lakes of lower trophy, where they usually are of minor importance for the community structure. In these ecosystems, however, cyanobacteria are rarely the main subject of study, although some data available indicate the dominance of small blue-green algae in oligotrophic (Callieri and Stockner, 2000) or oligo-mesotrophic lakes (Napiórkowska-Krzebietke and Hutorowicz, 2013; Dadheech et al., 2014).

In eutrophic lakes and water reservoirs the dominance of cyanobacteria in phytoplankton usually occurs in the summer or autumn (Sommer et al., 1986; Reynolds, 1987; Dembowska 2011). In strongly eutrophic or hypertrophic bodies of water the dominance of cyanobacteria is observed regardless of season (Havens, 2008; Dembowska 2011).

Particular cyanobacteria species demonstrate different optima relative to such environmental parameters as temperature, light or nutrients. Cyanobacteria are very well adapted to conditions of nutrient deficiency and limited availability of underwater light (Reynolds, 1984). The impact of nutrient concentration on the development of blue-green algae is well-documented, especially regarding nitrogen (N), phosphorus (P) and the N to P ratio (TN:TP,

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Smith, 1983; Berman, 2001; Downing et al., 2001). This, however, concerns mainly nutrient rich water bodies.

In oligo- and mesotrophic hard-water lakes with underwater *Chara*-meadows (European Natura 2000 Habitat 3140), specific and extensively developed charophyte vegetation should be regarded as an additional factor for phytoplankton community structure. Charophytes and, particularly, their abundant communities are applied as sensitive indicators of water quality. Nevertheless, it is worth emphasising that charophyte meadows exert a significant influence on ecosystem functioning. They effectively shape water chemistry and contribute to high water transparency (Scheffer et al., 1993; van den Berg et al., 1998; Apolinarska et al., 2011; Blindow et al., 2014). As charophytes produce large amounts of biomass (Pełechaty et al., 2013 and references therein) and decompose it more slowly compared to vascular plants (Kufel and Kufel, 2002), they are regarded as a trap for nutrients (Rodrigo et al., 2007; Kufel et al., 2013). The process of water decalcification, accompanied by the co-precipitation of P from the water column to the sediment and the ability to supply oxygen for the bottom sediment is also connected with charophytes (Kufel and Kufel, 2002; Kufel et al., 2013). Apart from the above-mentioned mechanisms, charophytes affect the qualitative and quantitative structure of phytoplankton by an intensified process of sedimentation of particles from the water column and discharge of allelopathic substances to the water (Wium-Andersen et al., 1982; Jasser, 1995; van Donk and van de Bund, 2002; Gross, 2003). It can, thus, be expected that in *Chara*-lakes, cyanobacteria as a component of phytoplankton will remain under the control of charophytes, being an accessory contributor to the phytoplankton community. The aims of this study, performed in a group of four morphologically varied *Chara*-lakes, were to:

1. recognize the species composition of cyanobacteria of the studied *Chara*-lakes as related to the lake basin morphology, varied recreational use and the size and character of the drainage basin.
2. find out whether there are any differences in the qualitative and quantitative structure of cyanobacteria and in cyanobacteria functional groups between the studied *Chara*-lakes.
3. assess the ecological status of the studied *Chara*-lakes on the basis of total phytoplankton and cyanobacteria biomass
4. determine the key environmental factors that affect the development of cyanobacteria in *Chara*-lakes.

2. Materials and methods

2.1. Studied lakes

The study was carried out in 4 lakes located in the western part of Poland (Fig. 1). Lakes Jasne, Złoty Potok and Niesłysz are located in the Lubuskie Lake District, and Lake Lednica, in the Wielkopolskie Lake District (in the figures and tables of this work, the following abbreviations are used: J, ZP, N and L for Lakes Jasne, Złoty Potok, Niesłysz and Lednica, respectively). All the lakes are macrophytic, with a dominance of charophytes among the submerged vegetation and *Chara tomentosa* L. being among the basic dominant species (Table 1). The features which significantly differentiate the lakes are the depth and surface area, the size and character of the drainage basin and the use of these water bodies. Two lakes, Jasne and Złoty Potok, which are characterised by Schindler's ratio values >2, have a forest drainage basin with a limited human impact and significantly smaller surface areas as well as mean and maximum depths than the other lakes, while water transparencies are higher (Table 1). An agricultural drainage basin and recreational use as well as Schindler's ratio values <2 are characteristic of the other two lakes: Lednica and Niesłysz. Unlike Lake Jasne, the least mineralized among the studied lakes, Lake Lednica is the richest in dissolved substances, especially nitrogen compounds. It is also characterised by the highest water hardness, colour and alkalinity and the lowest clarity. Lake Niesłysz, in turn, is the largest (regarding surface area) and the deepest among the studied lakes. On the basis of the values of Carlson's (1977) trophic state index (TSI), Lakes Jasne, Złoty Potok and Niesłysz are classified as mesotrophic, and Lednica, as slightly eutrophic (Pełechaty et al., 2015).

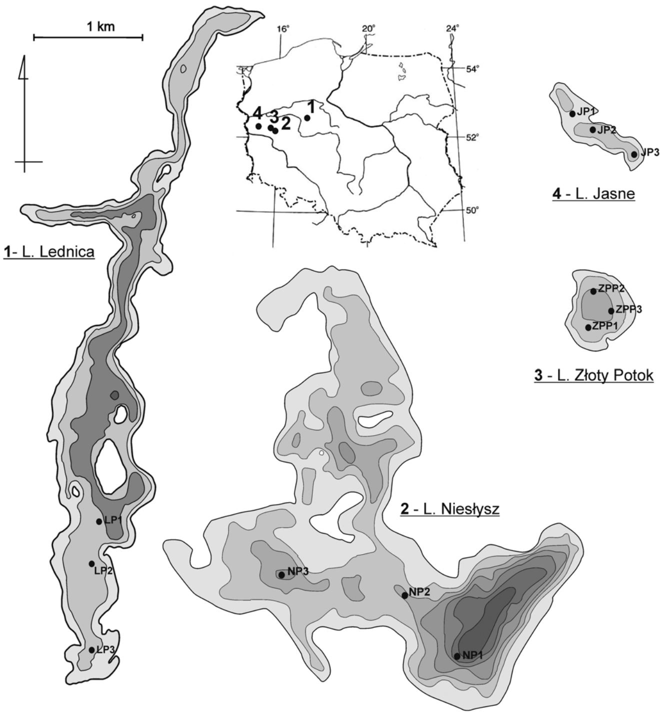

Fig. 1. Lake locations and bathymetry of the studied sites. P1–P3: pelagic sites; L—Lake Lednica, J—Lake Jasne, ZP—Lake Złoty Potok, N—Lake Niesłysz. Each shaded depth contour represents 5 m; the scale bar refers to the lakes.

2.2. Sampling and data analysis

The study was carried out once a month between June and October 2008. The basic physicochemical analyses of the lakes' surface water (water temperature, oxygen concentration, conductivity, pH) were conducted with the use of portable measuring equipment (Elmetron CX-401—Elmetron Sp. j., Zabrze, Poland, CyberScan 200 and CyberScan 20—Eutech Instruments Europe BV, Nijkerk, The Netherlands). Three sites were established in each lake in the pelagic zone (P1, P2, P3; Fig. 1). Field measurements and sampling were carried out in the surface layer of each site, and the obtained results were then averaged. Water transparency (SD) was measured with the use of a Secchi disk.

Water samples for further hydrochemical and algological analyses were taken with a 3 L water sampler (Uwitec, Mondsee, Austria) and poured into 1 L bottles. Samples for hydrochemical analyses were fixed with chloroform, and those for algological analyses with Lugol's solution. In addition to taxa identification in fixed samples, live samples of phytoplankton were collected each time with the use of a plankton net 10 μm mesh in diameter.

Hydrochemical analysis involved anions (Cl^-, NO_3^-, NO_2^-, SO_4^{2-}, PO_4^{3-}), cations (NH_4^+, Na^+), water colour, total hardness and alkalinity, total nitrogen (TN) and total phosphorus (TP). The applied equipment and standard analytical methods are presented in detail in Pełechaty et al. (2010).

Water samples for algological analyses were subject to sedimentation and concentrated up to a volume of 15 mL, and then

Table 1
Morphometry, land use, mean values (June-October) of the physicochemical properties of water and dominant *Chara* species in the studied lakes (basic morphometric data after Pełechaty et al., 2015 and references therein).

Characteristic	Unit	Lake Jasne	Lake Złoty Potok	Lake Niesłysz	Lake Lednica
Surface area	ha	15.1	32.8	486.2	341.4
Mean depth	m	4.3	5.9	7.8	7.0
Maximum depth	m	9.5	13.7	34.7	15.1
Stratification	–	incomplete	complete	complete	complete
Trophic state index	TSI	44.1	45.2	44.3	49.3
Lake type	–	natural, closed	natural, closed	natural, outflow	natural, outflow
Catchment area	km^2	3.44	3.8	56.24	38.0
Main land use	–	forests, recreational use limited	forests, recreational use limited	forests, agricultural and recreational	agricultural in 75% and recreational
Secchi depth visibility	m	4.99	4.79	4.08	3.57
Water temperature	°C	18.05	17.77	17.31	17.37
Oxygen	$mg\,L^{-1}$	9.26	9.41	10.25	10.43
pH	–	8.39	8.34	8.43	8.49
Electrolytic conductivity	–	248.0	315.3	282.4	771.8
Water colour	$mg\,L^{-1}$ Pt	9.9	10.5	8.9	12.7
TP	$mg\,L^{-1}$	0.047	0.182	0.071	0.062
$NH_4{}^+$	$mg\,L^{-1}$	0.157	0.124	0.172	0.396
NO_{2^-}	$mg\,L^{-1}$	0.010	0.040	0.010	0.231
$NO_3{}^-$	$mg\,L^{-1}$	0.043	0.222	0.057	5.856
TN	$mg\,L^{-1}$	1.151	2.343	1.239	13.489
TN:TP	–	28	19	19	214
$SO_4{}^2$	$mg\,L^{-1}$	54.4	45.1	43.2	172.7
Na^+	$mg\,L^{-1}$	4.54	6.59	7.87	20.66
Cl^-	$mg\,L^{-1}$	11.7	13.9	16.8	77.9
K^+	$mg\,L^{-1}$	0.62	0.73	1.07	8.55
Mg^{2+}	$mg\,L^{-1}$	2.58	4.68	5.73	23.04
Ca^{2+}	$mg\,L^{-1}$	45.15	56.61	48.38	111.59
Total hardness	°dH	6.92	9.02	8.14	20.97
Alkalinity	$mval\,L^{-1}$	1.3	2.2	1.9	2.8
Dominat species in *Chara* meadows	–	*Chara tomentosa* *C. rudis* *C. polyacantha*	*Chara tomentosa* *Nitellopsis obtusa* *C. aspera*	*Nitellopsis obtusa Chara tomentosa*	*Chara contraria* *C. tomentosa Nitellopsis obtusa*

additionally fixed with formalin. Qualitative identifications were carried out using a light microscope (at 200×, 400×, 1000× magnification). Cyanobacteria determination was done according to the following authors: Komárek and Anagnostidis (1998, 2005), Komárek and Zapomělová (2007). Phytoplankton individuals (single cells, colonies, or filaments with a length of 100 μm) were counted in 100 fields of a Fuchs-Rosenthal counting chamber (height: 0.2 mm, area: 0.0625 mm^2). To calculate the biomass the species were approximated to simple geometric or combined forms (Wetzel and Likens, 1991). The measurements necessary to calculate the biomass were carried out separately for each sample by measuring 30 individuals and determining the mean size for the species. Cyanobacteria richness was expressed as the number of taxa, whereas their diversity was determined as the Shannon diversity index (H') calculated from the taxa abundance and biomass (Shannon, 1948).

Identified cyanobacteria were assigned to functional groups (groups of species with similar morphology, ecology and physiology named using alphanumerical codes), defined according to Reynolds et al. (2002) and Padisák et al. (2009). Species that contributed with at least 2% of relative abundance or biomass in at least one sample were considered.

In addition, the ecological status of the lakes was assessed with a modified Phytoplankton Metric for Polish Lakes ($PMPL_{MOD}$; Napiórkowska-Krzebietke, 2015). $PMPL_{MOD}$ consists of two metrics: “total biomass” (MTB) and “biomass of Cyanobacteria” (*MBC*). The following equations were used:

A.) For lakes with Schindler's ratio <2 (Lakes Niesłysz, Lednica)

$$MTB = 0.8727 + 1.2900 \times \ln(TB)$$

Table 2
Boundaries of $PMPL_{MOD}$ for ecological status classes.

$PMPL_{MOD}$	Ecological status
0–1	High
1–2	Good
2–3	Moderate
3–4	Poor
4–5	Bad

$$MBC = 1.4113 \times \ln\left[\left(BC + \left(BC \times \frac{BC}{TB}\right)\right)/2\right] + 1.8112$$

- For lakes with Schindler's ratio >2 (Lakes Jasne, Złoty Potok)

$$MTB = 0.8135 + 1.0325 \times \ln(TB)$$

$$MBC = 0.8135 \times \ln\left[\left(BC + \left(BC \times \frac{BC}{TB}\right)\right)/2\right] + 1.2835$$

$$PMPL_{MOD} = MTB + MBC/2$$

Where: TB—total biomass, BC—biomass of cyanobacteria.

Metric values (MTB, MCB) smaller than 0 were set to 0, and values larger than 5 were set to 5 for further $PMPL_{MOD}$ calculations. $PMPL_{MOD}$ values corresponding to particular ecological status classes are presented in Table 2.

2.3. Statistical data analyses

In order to determine the relationships between the biomass or abundance of the dominant cyanobacteria taxa and the chem-

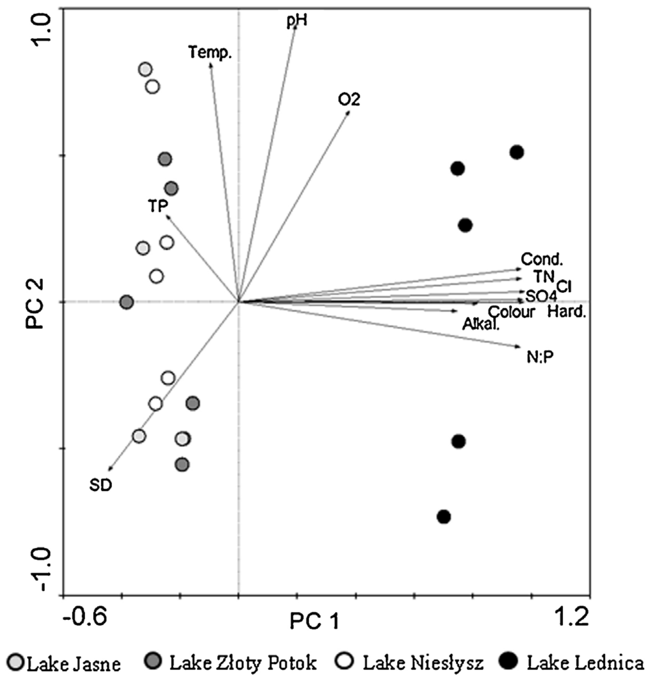

Fig. 2. PCA output for the physical-chemical properties of waters. For each of lake N = 5 (one site, five sampling months). Explanations: TP—total phosphorus concentration, hard.—total hardness, TN—total nitrogen concentration, Cond.—conductivity, Temp.—temperature, Alkal.—alkalinity, SD—Secchi depth visibility. PC1 explained 81% and PC2 explained 11% of the variance observed.

ical parameters Pearson's correlation coefficient was calculated. In addition, multivariate ordination techniques were applied to identify the key factors of physical-chemical variation within the studied *Chara*-lakes and the most important environmental predictors for the abundance and biomass of cyanobacteria species, which contributed with at least 2% of relative abundance or biomass in at least one sample (Ter Braak and Šmilauer, 2002; Wu et al., 2014). Principal Component Analysis (PCA) was applied to determine physicochemical properties, which explained most of the variance observed within the studied group of lakes. Redundancy Analysis (RDA) was applied in order to analyse the relationships between the abundance and biomass of cyanobacteria species and water physical-chemical properties during the whole study period. RDA was based on the results of the Detrended Correspondance Analysis (DCA) of the aforementioned abundance and biomass of cyanobacteria species which evidenced gradients shorter than 3 standard deviations (Ter Braak and Šmilauer, 2002). Prior to the analyses, blue-green algae abundance, biomass and environmental variables were log-transformed so as to minimize the inconsistencies between the empirical distribution and the theoretical normal one. For all the statistics, $P < 0.05$ was used to determine significance. CANOCO 4.5 for Windows (Wageningen UR, Netherlands) and STATISTICA 10 (StatSoft Inc., Tusla, OK, USA) software packages were used.

3. Results

3.1. Water properties

Mean values of the studied physicochemical parameters of water are presented in Table 1. The PCA analysis clearly distinguished Lake Lednica from the rest of the studied lakes, which were similar to each other (Fig. 2). 92% of the variance was explained by the first and second PCA axes and the most differentiating parameters related to solute content and water hardness. In addition, the TN:TP ratio values were also highly diversified in the studied *Chara*-lakes (Table 1). The highest TN:TP values were calculated for Lake Lednica together with the mean value that exceeded 200 in this lake compared to other lakes in which TN:TP mean ratio values did not exceed 30.

3.2. Phytoplankton characteristics

The lowest total phytoplankton abundances were found in Lakes Jasne (from 1.4 to 3.2×10^3 ind mL^{-1}, mean 2.2×10^3 ind mL^{-1}) and Złoty Potok (1.8–3.8 × 10^3 ind mL^{-1}, 2,7 × 10^3 ind mL^{-1}) with the dominance of chrysophytes, cryptophytes and green algae (Table 3; Fig. 3a). Despite a similarity in total phytoplankton abundance, the greater share of cyanobacteria, especially in the autumn months, clearly distinguished Lake Niesłysz phytoplankton (1.8–3.8 × 10^3 ind mL^{-1}, 3.2×10^3 ind mL^{-1}) from that determined in Lake Złoty Potok. The most abundant and variable phytoplankton was found in Lake Lednica where it was dominated by chrysophytes, cryptophytes and green algae in June, September and October or by cyanobacteria and chrysophytes in July and August.

In the case of biomass, Lakes Jasne (0.4–1.2 mg L^{-1}, 0.88 mg L^{-1}), Złoty Potok (0.2–0.9 mg L^{-1}, 0.5 mg L^{-1}) and Niesłysz (0.6–1.6 mg L^{-1}, 1.1 mg L^{-1}) were characterised by low values of biomass, formed mainly by dinophytes, chrysophytes, cryptophytes and cyanobacteria (especially in the case of Lake Niesłysz). The highest phytoplankton biomasses were recorded in Lake Lednica (0.2–26.8 mg L^{-1}, 8.4 mg L^{-1}) where the biomass values were associated with *Peridinium gatunense* (Dinophyta) blooms in July and September. In the remaining months chrysophytes, cyanobacteria and cryptophytes dominated in June, August and October, respectively.

3.3. Characteristics of the cyanobacteria structure

Among 239 phytoplankton taxa in the four investigated ecosystems, 51 cyanobacteria taxa were determined, representing three orders: Chroococcales, Oscillatoriales and Nostocales (24, 21 and 6 taxa, respectively). The greatest cyanobacteria species richness was found in Lake Niesłysz (29 taxa), and the lowest in Lake Jasne (20). In each of the lakes, the Chroococcales order was represented by the highest number of taxa, while the lowest number was each time observed for Nostocales (Table 3). The share of cyanobacteria in the total number of taxa in Lakes Jasne and Złoty Potok did not exceed 20% whereas in Lakes Niesłysz and Lednica it was respectively 25% and 21%.

Cyanobacteria species determined in this study are presented in Table 4. The greatest frequencies were found for *Dolichospermum lemmermannii* present in all the studied samples, as well as for *Radiocystis geminata* and *Planktothrix agardhii*, observed in 75% and 70% of the studied samples, respectively (Table 4).

In Lakes Jasne and Złoty Potok, the abundances of cyanobacteria were low but demonstrated temporal variability (Fig. 3a). The highest cyanobacteria abundances were found in these lakes in July and August respectively, and their share in the total number of phytoplankton specimens did not exceed 20% in Lake Jasne and 21% in Lake Złoty Potok. In these lakes, cyanobacteria were dominated by representatives of Chroococcales, whose share in the total abundance of cyanobacteria was the highest in Lake Jasne (always above 90%; Fig. 3b). Similarly to Lake Jasne, the most numerous among cyanobacteria of Lake Złoty Potok were representatives of Chroococcales, although a distinct increase of the share of Oscillatoriales and Nostocales was observed in October.

Unlike the small, mid-forest *Chara*-lakes, higher numbers of cyanobacteria were observed in lakes Niesłysz and Lednica, even with the dominance of this group in the phytoplankton community (Fig. 3a). In Lake Niesłysz, the peak of cyanobacteria develop-

Table 3
Cyanobacteria richness, diversity, abundance and biomass on the background of phytoplankton assemblage.

		Lake Jasne	Lake Złoty Potok	Lake Niesłysz	Lake Lednica
Total number of phytoplankton taxa	No. of taxa	112	118	138	105
Total number of cyanobacteria taxa		20	22	29	22
Chroococcales		12	11	14	12
Oscillatoriales		7	8	10	8
Nostocales		1	3	5	2
Cyanobacteria share in the total number of taxa	%	18	19	25	21
Cyanobacteria	Mean abundance No. of individuals mL^{-1}	346.7	339.4	929.1	843.7
Chlorophyta		468.3	335.4	93.6	647.4
Bacillariophyceae		189.2	89.6	52.4	85.9
Chrysophyceae		752.2	1394.6	1163.8	1652.9
Xanthophyceae		16.4	25.7	9.2	2.2
Dinophyta		15.1	7.6	12.9	180.8
Euglenophyta		0	1.5	1.0	0
Cryptophyta		387.0	512.0	927.4	397.3
Total abundance		2174.9	2705.9	3189.4	3810.2
Cyanobacteria	Mean biomass mg L^{-1}	0.115	0.074	0.569	0.583
Chlorophyta		0.128	0.083	0.038	0.339
Bacillariophyceae		0.100	0.027	0.031	0.052
Chrysophyceae		0.052	0.140	0.088	0.262
Xanthophyceae		0.004	0.005	0.002	0.002
Dinophyta		0.290	0.087	0.249	6.806
Euglenophyta		0.000	0.003	0.000	0.000
Cryptophyta		0.110	0.074	0.120	0.329
Total biomass		0.798	0.493	1.098	8.374
Dominant taxa in the cyanobacterial biomass		*Aphanothece clathrata* *Aphanocapsa holsatica*	*Dolichospermum lemmermannii* *Merismopedia tenuissima* *Cuspidothrix issatschenkoi*	*Dolichospermum lemmermannii* *Aphanizomenon gracile* *Cuspidothrix issatschenkoi* *Dolichospermum mendotae*	*Aphanocapsa holsatica* *Aphanothece clathrata* *Aphanizomenon gracile* *Planktothrix agardhii*
Shannon diversity index (H') for Cyanobacteria abundance	–	1.57	1.37	1.37	1.01
Shannon diversity index (H') for Cyanobacteria biomass	–	1.23	0.98	0.98	0.74

ment was observed at the end of the summer and in autumn (September–October), when the share of cyanobacteria in the phytoplankton community was respectively 55% and 63%. The quantitative structure of the cyanobacteria community was different in this lake being primarily made up of representatives of Nostocales and Oscillatoriales (Fig. 3b). In Lake Lednica, the highest abundance of cyanobacteria, almost exclusively including representatives of Chroococcales, was noted in the height of summer (July–August; Fig. 3a). In June and October the structure of cyanobacteria differed to the summer due to the occurrence of the Oscillatoriales and Nostocales representatives (Fig. 3b).

Biomass of cyanobacteria in mid-forest *Chara*-lakes (Jasne and Złoty Potok) was very low (not exceeding 0.3 mg L^{-1}) and accounted at the maximum for 30% of the total biomass in Lake Jasne (October) and 28% in Lake Złoty Potok (June; Fig. 4a). In Lake Jasne, cyanobacteria biomass was dominated by representatives of Chroococcales over the whole study period (Fig. 4b). The dominance of Chroococcales in Lake Złoty Potok was only observed in July; in the other months the blue-green algae biomass consisted of Nostocales, and in October also of Oscillatoriales. The highest share of cyanobacteria in the total phytoplankton biomass was found in Lake Niesłysz (in September and October over 75%; Fig. 4a). In this ecosystem, the cyanobacteria biomass was almost exclusively made up of filamentous forms of the Nostocales and Oscillatoriales orders (Fig. 4a, b) in the whole study period. In the case of Lake Lednica, characterised by the highest phytoplankton biomass, cyanobacteria dominated the phytoplankton community in August (55% of the total biomass). In the other months their share did not exceed 5% (Fig. 4a). In this ecosystem, the groups with the greatest importance in forming the cyanobacteria biomass in the summer were Chroococcales, and in June and October, Nostocales and Oscillatoriales (Fig. 4b).

The analysis of the Shannon diversity index (H') calculated for the abundance and biomass of cyanobacteria in both cases showed the highest mean values of H' in Lake Jasne, and the lowest, in Lake Lednica (Table 3). In Lakes Złoty Potok and Niesłysz, the mean H' values were at the same level.

In the light of the RDA analysis of abundance the species responsible for the variety of cyanobacteria of the studied lakes were: *Aphanothece minutissima*, *Merismopedia tenuissima*, *Aphanizomenon gracile*. Further key species identified by the RDA were *Aphanocapsa holsatica*, a species correlated with the first axis, *Cyanodictyon planctonicum* correlated with the first and second axes, as well as other species which to a similar extent accounted for the variability observed (Fig. 5a). Alkalinity, water temperature, colour and, to a lesser extent, also TP and TN:TP ratio values as well as O_2 concentrations turned out to be the most significant environmental factors for the variety of cyanobacteria dominants. In addition, the RDA output clearly revealed differences among the studied lakes, especially between Jasne, Złoty Potok and Niesłysz which

Table 4
List of cyanobacteria species identified in four *Chara*-lakes.

Lakes	Lake Jasne					Lake Złoty Potok					Lake Niesłysz					Lake Lednica						
Samples Taxa	J/VI	J/VII	J/VIII	J/IX	J/X	Z/VI	Z/VII	Z/VIII	Z/IX	Z/X	N/VI	N/VII	N/VIII	N/IX	N/X	L/VI	L/VII	L/VIII	L/IX	L/X	Frequency[a]	Functional group
Chroococcales																						
Aphanocapsa delicatissima W. et G.S. West						+		+	+	+			+				+	+			II	**K**
Aphanocapsa holsatica (Lemm.) Cronberg et Kom.		+	+	+	+												+	+			II	**K**
Aphanothece clathrata W. et G. S. West	+	+	+	+	+								+	+							II	**K**
Aphanothece minutissima (W.West) Komárková–Legnerová et Cronberg						+	+	+	+	+	+	+	+	+	+						III	**K**
Aphanothece smithii Komárková–Legnerová et Cronberg						+	+		+	+			+	+							II	**K**
Chroococcus limneticus Lemm.						+	+						+				+	+	+	+	II	**L$_O$**
Chroococcus microscopicus Komárková–Legnerová et Cronberg		+	+		+					+							+	+			II	**L$_O$**
Chroococcus minimus (Keissler) Lemm.	+	+	+	+	+	+			+	+	+										III	**L$_O$**
Chroococcus obliteratus Richter	+					+	+						+	+			+	+			II	**L$_O$**
Cyanocatena planctonica Hindák		+	+	+	+		+	+	+	+							+	+	+		III	**K**
Cyanodictyon planctonicum Meyer	+	+	+	+	+								+				+				II	**K**
Merismopedia tenuissima Lemm.		+	+	+		+	+	+	+								+	+	+		III	**L$_O$**
Radiocystis geminata Skuja	+	+	+	+	+				+	+		+	+	+	+	+	+	+	+		IV	**L$_O$**
Oscillatoriales																						
Limnothrix lauterbornii (Schmidle) Anagnostidis		+	+	+							+	+				+				+	II	**S1**
Planktothrix agardhii (Gom.) Anagn. et Kom.			+		+	+	+	+	+		+			+	+	+	+	+	+	+	IV	**S1**
Pseudanabaena limnetica (Lemm.) Kom.								+		+		+	+	+	+		+	+	+		III	**S1**
Nostocales																						
Aphanizomenon gracile (Lemmerm.) Lemmerm.											+	+	+	+	+	+			+	+	II	**H1**
Cuspidothrix issatschenkoi (Usachev) Rajaniemi et al.						+	+	+	+	+			+	+	+						II	**H1**
Dolichospermum lemmermannii (Richter) Wacklin, Hoffmann et Komárek	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	V	**H1**
Dolichospermum mendotae (Trelease) Wacklin, Hoffmann et Komárek											+	+	+	+	+						II	**H1**

Species with frequency I:**Chroococcales**: *Aphanocapsa incerta* (Lemm.) Cronberg et Kom. [J/VII, J/IX, N/VII, N/IX; **K**], *Chroococcus cf. turgidus* (Kützing) Nägeli [N/VI; **L**$_O$], *Chroococcus minutus* (Kützing) Nägeli [Z/VI, Z/IX-X, N/VII; **L**$_O$], *Chroococcus* sp. [J/VI-VII; **L**$_O$], *Coelomoron pusillum* (Van Goor) Kom. [N/VII; **L**$_M$], *Microcystis wesenbergii* (Kom.) Kom. in Kondr. [L/VIII-X; **M**], *cf. Pannus microcystiformis* Hindák [N/IX; **L**$_O$**/K**], *Rhabdoderma lineare* Schmidle et Lauterborn [N/IX; **K**], *Snowella litoralis* (Häyrén) Komárek et Hindák [J/VII; **L**$_O$**]**.
Oscillatoriales: *Geitlerinema amphibium* (Agardh ex Gomont) Anagn. [J/IX, N/X, L/VIII; **S1**], *Jaaginema subtilissimum* (Kützing ex De Toni) Anagnostidis et Komárek [Z/VIII, N/IX;**S1**], *Komvophoron constrictum* (Szafer) Anagn. et Kom. [L/VII-VIII; S1], *Komvophoron minutum* (Skuja) Anagn. et Kom. [Z/X, N/VII;**S1**], *Leptolyngbya amplivaginata* (van Goor) Anagnostidis et Komárek [N/X; **S1**], *Leptolyngbya* sp. [J/IX; **S1**], *Limnothrix redekei* (van Goor) Meffert [Z/X; **S1**], *Oscillatoria ornata* Kützing ex Gomont [Z/VII; **S1**], *Oscillatoria sancta* Kützing ex Gomont [N/VII; **MP**], *Oscillatoria tenuis* Agardh ex Gomont [L/IX; **S1**], *Phormidium cf. pavlovskoense* Elenkin [N/VI; **S1**], *Phormidium hamelii* (Frémy) Anagn. et Kom. [J/VII; **S1**], *Phormidium* sp. [J/VI-IX; **S1**], *Phormidium splendidum* (Grev. ex Gom.) Anagn. et Kom. [L/IX; **S1**], *Planktolyngbya limnetica* (Lemmermann) Komárková-Legnerová et Cronberg [N/VIII-X; **S1**], *Pseudanabaena catenata* Lauterborn [J/VIII-X; MP], *Pseudanabaena galeata* Böcher [Z/X, N/IX, L/VII-VIII; **MP**], *Trichodesmium lacustre* Kleb. [Z/VI, Z/X, N/VIII-IX; **S1**].
Nostocales: *Dolichospermum circinale* (Rabenhorst ex Bornet et Flahault) Wacklin, Hoffmann et Komárek [N/VIII-X; **H1**], *Dolichospermum viguieri* (Denis et Frémy) Wacklin, Hoffmann et Komárek [Z/VIII-X, N/X; **H1**].

[a] Frequency of occurrence of the species according to Starmach (1989): I (1–20% samples), II (20–40%), III (40–60%), IV (60–80%), V (80–100%).

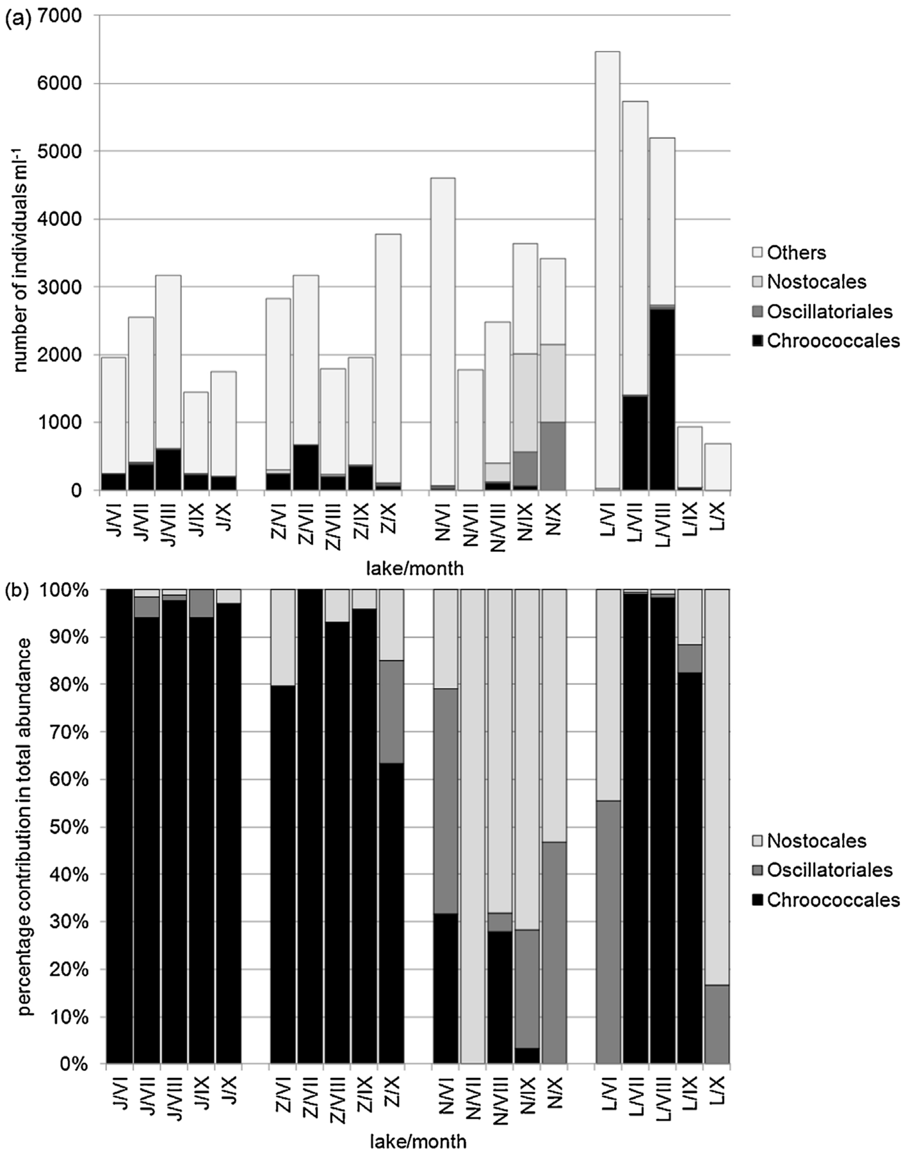

Fig. 3. Monthly variations of: (a) Phytoplankton total abundance with the share of main cyanobacteria groups; (b) Relative abundance of main cyanobacteria groups.

differed in the abundance of dominating cyanobacteria species. Lake Jasne had the greatest abundance of *Cyanodictyon planctonicum*, *Aphanocapsa holsatica*, as well as *Aphanothece clathrata* and *Chroococcus microscopicus*. For Lake Złoty Potok the distinctive species were *Aphanothece minutissima*, *Aphanothece smithii* and *Cuspidothrix issatschenkoi*, and Lake Niesłysz differed from the others in the quantitative dominance of *Planktolyngbya limnetica*, *Pseudanabaena limnetica* and *Aphanizomenon gracile*. Cyanobacteria of Lake Lednica were more diverse in comparison with the other lakes, so they do not make a distinct group, yet a greater similarity of Lake Lednica to Lake Niesłysz than to the other two lakes (particularly to Lake Złoty Potok) is visible. The first two axes presented in this figure jointly accounted for slightly more than 60% of the observed variability of cyanobacteria.

In the case of the dominants biomass, the first and second axes accounted for a much higher percentage (90%) of the species—environment relations (Fig. 5b). The biomass of *Aphanocapsa holsatica* was correlated with the first axis; it was also positively related to most of the environmental variables. The biomass of several species, particularly *Aphanizomenon gracile* and *Pseudanabaena limnetica*, was correlated with the second axis; these species did not demonstrate any clear relation to physicochemical properties of water. Unlike in the case of cyanobacteria abundance, the RDA analysis for blue-green algae biomass did not show any differences between the lakes but it showed the clear distinctiveness of two samples from Lake Niesłysz dominated by *Aphanizomenon gracile*, *Pseudanabaena limnetica* and others, and two samples from Lake Lednica dominated by *Aphanocapsa holsatica*. These four samples are also distinctive in Fig. 4 where they are named N/IX, N/X and L/VII, L/VIII.

3.4. Correlations

In addition to the RDA results (Fig. 5a,b), Chroococcales abundances revealed the greatest number of Pearson's correlations with the physicochemical properties of water (Table 5). The abundance of *Aphanothece clathrata* demonstrated negative correlations with conductivity, total hardness and Na^+ concentration ($P < 0.01$) as well as the concentrations of $N\text{-}NO_2^-$, $N\text{-}NO_3^-$, TN and Cl^- ($P < 0.05$). Alkalinity as a parameter with the highest correlations was very highly ($P < 0.001$) or highly ($P < 0.01$) significantly negatively correlated with species such as: *Aphanothece*

Table 5

The Pearson's correlations between the main physicochemical parameters and the abundance or biomass of cyanobacteria dominant species and orders in all studied lakes (*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$). Only species for which at least one significant correlation was found are presented.

		Oxygen	SD	Conductivity	Alkalinity	Colour	P-PO$_4$	TP	N-NH$_4$	N-NO$_3$	N-NO$_2$	TN	TN: TP	SO$_4$$^{2-}$	Cl$^-$	Total hardness	Na$^+$
Abundance	*Aphanothece clathrata*	n.s.	n.s.	−0.57**	−0.83***	−0.53*	n.s.	n.s.	n.s.	−0.47*	−0.44*	−0.54*	n.s.	n.s.	−0.50*	−0.59**	−0.60**
	Aphanothece minutissima	n.s.	n.s.	n.s.	n.s.	−0.47*	0.56*	0.54*	−0.46*	−0.50*	n.s.	n.s.	−0.65**	−0.67**	−0.46*	n.s.	n.s.
	Aphanothece smithii	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	−0.44*	n.s.	n.s.	n.s.
	Chroococcus minimus	n.s.	n.s.	−0.54*	−0.65**	n.s.	n.s.	n.s.	n.s.	−0.49*	n.s.	−0.52*	n.s.	n.s.	−0.60**	−0.57**	−0.69*
	Cyanocatena planctonica	−0.54*	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
	Cyanodictyon planctonicum	n.s.	n.s.	n.s.	−0.60**	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
	Merismopedia tenuissima	−0.54*	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
	Cuspidothrix issatschenkoi	n.s.	n.s.	n.s.	n.s.	−0.52*	n.s.	n.s.	−0.44*	n.s.	n.s.	n.s.	−0.47*	−0.56*	n.s.	n.s.	n.s.
	Planktolyngbya limnetica	n.s.	n.s.	n.s.	n.s.	−0.64**	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Biomass	*Aphanocapsa holsatica*	n.s.	n.s.	0.50*	n.s.	n.s.	n.s.	n.s.	n.s.	0.48*	0.60**	0.46*	0.45*	0.51*	0.50*	0.50*	0.47*
	Aphanothece clathrata	n.s.	n.s.	−0.44*	−0.79***	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	−0.48*	−0.58**
	Dolichospermum mendotae	n.s.	n.s.	n.s.	n.s.	−0.54*	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
	Aphanizomenon gracile	n.s.	n.s.	n.s.	n.s.	−0.53*	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
	Cuspidothrix issatschenkoi	n.s.	n.s.	n.s.	n.s.	−0.65**	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
	Planktothrix agardhii	n.s.	−0.48*	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
	Pseudanabaena limnetica	n.s.	n.s.	n.s.	n.s.	−0.50*	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
	Chroococcales	n.s.	n.s.	0.49*	n.s.	n.s.	n.s.	n.s.	n.s.	0.47*	0.62**	0.44*	0.45*	0.53*	0.49*	0.48*	n.s.
	Oscillatoriales	n.s.	n.s.	n.s.	n.s.	−0.47*	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
	Nostocales	n.s.	n.s.	n.s.	n.s.	−0.57**	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.

n.s.—not significant.

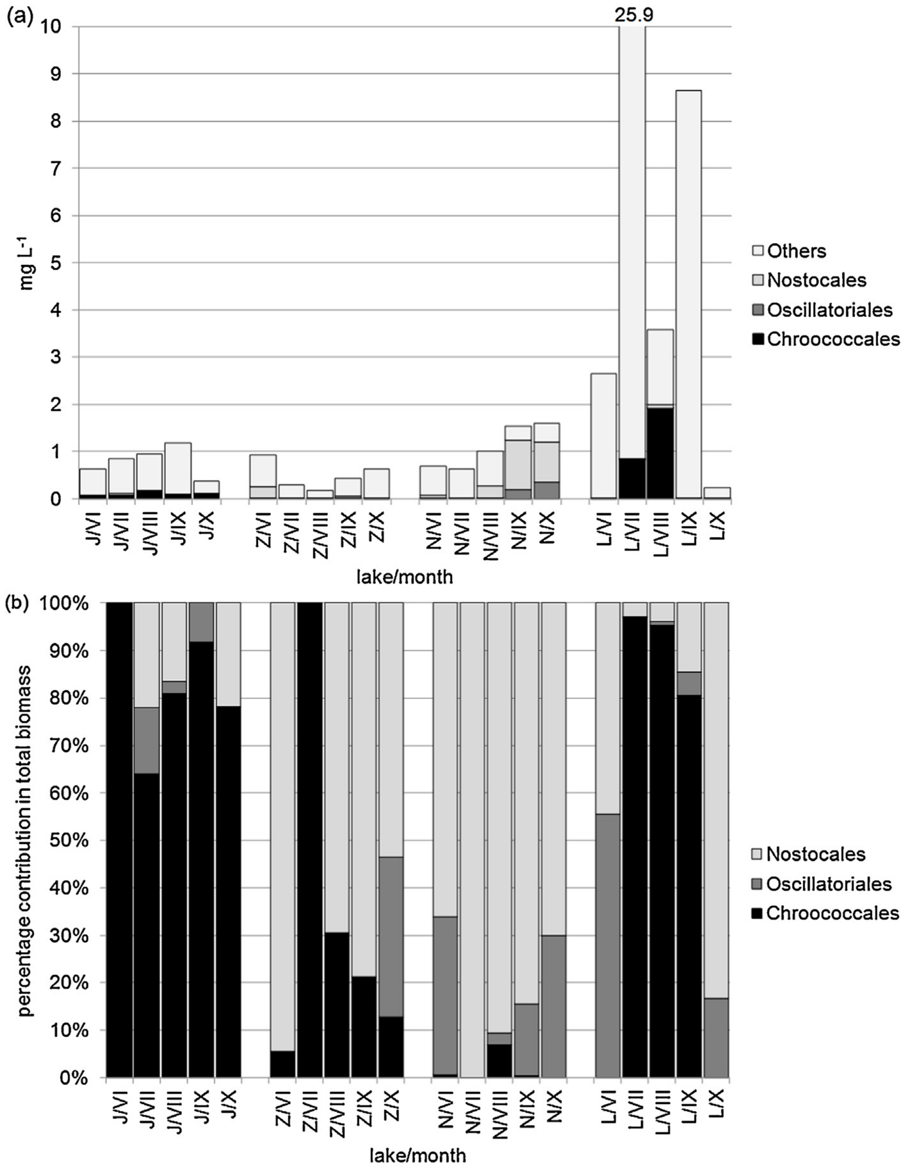

Fig. 4. Monthly variations of: (a) Phytoplankton biomass with the share of main cyanobacteria groups; (b) Relative biomass of main cyanobacteria groups.

clathrata, Chroococcus minimus and *Cyanodictyon planctonicum.* Negative correlations were also found for the water colour and the abundance of *Aphanothece minutissima, A. clathrata, Cuspidothrix issatschenkoi* and *Planktolyngbya limnetica.* This parameter was also correlated with the biomass of *Dolichospermum mendotae, Aphanizomenon gracile, C. issatschenkoi* and *Pseudanabaena limnetica.* The Chroococcales biomass demonstrated a highly statistically significant ($P < 0.01$) positive correlation with N-NO_2^- and statistically significant ($P < 0.05$) positive correlations with conductivity, N-NO_3^-, TN, SO_4^{2-}, Cl^- concentrations, TN:TP ratio and total hardness. Statistically significant correlations were also found between the Nostocales and Oscillatoriales biomasses and the colour of water (Table 5).

3.5. Functional groups

In all the studied lakes, 7 Reynolds functional groups were identified among cyanobacteria: K, L_O, S1, H1, L_M, M and MP. Representatives of each group are presented in Table 4. The most important were groups K and L_O in Lakes Jasne, Złoty Potok and Lednica, H1 in Lakes Złoty Potok, Niesłysz and Lednica, and S1 in Lakes Niesłysz and Lednica. A clear seasonal variability of functional groups within the blue-green algae was found in Lake Lednica. Groups H1 and S1 prevailed in June and October, while K and L_O were prevalent from July to September (Fig. 6a,b).

A cluster analysis of the abundance and biomass of the cyanobacteria functional groups (Fig. 7a,b respectively), confirmed a strong distinction between small lakes with forest catchment area and recreationally used big lakes with agricultural and built-up surrounding areas. Based on the cyanobacteria abundance, Lakes Jasne and Złoty Potok were clustered in one group, while Niesłysz and Lednica constituted the other group (Fig. 7a). The analysis of cyanobacteria biomass revealed the highest similarity in the case of Lakes Jasne and Złoty Potok, whereas the other lakes, especially Lake Lednica, were distinguished from the small mid-forest lakes (Fig. 7b).

3.6. Phytoplankton index of the ecological status

The $PMPL_{MOD}$ index significantly differentiated the studied lakes (Fig. 8). The mean values of <1 were accounted for Lakes Złoty Potok and Jasne and indicated a high ecological status. In Lake Led-

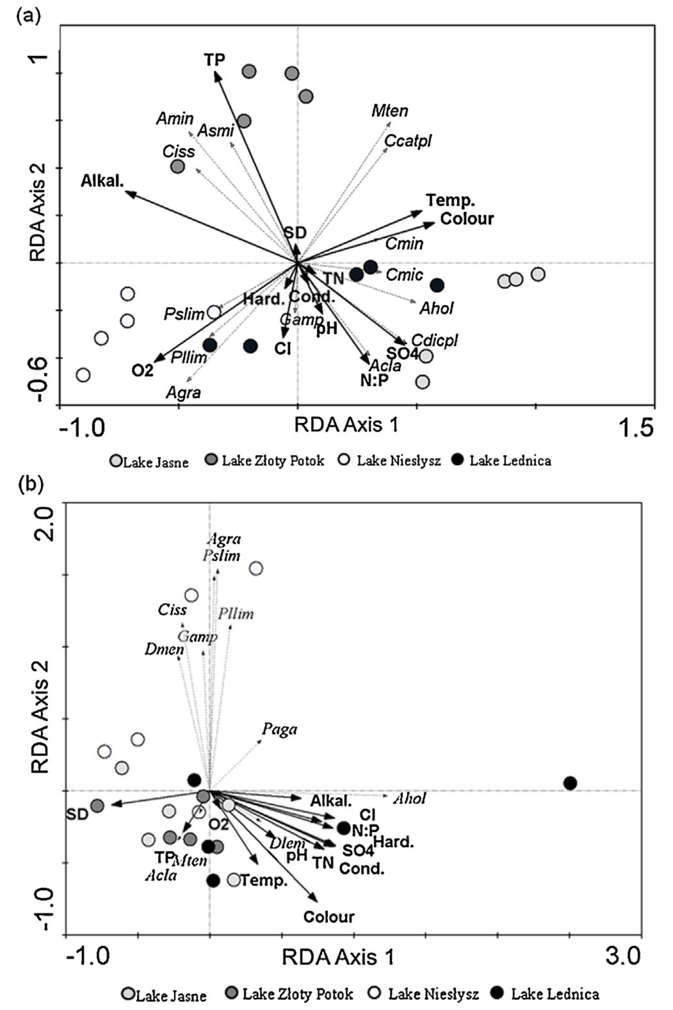

Fig. 5. The results of the redundancy analysis in the ordination space of the first and second RDA axes, describing:
(a) the abundance of cyanobacteria dominants (species that contributed with at least 2% of relative abundance) and the selected physical-chemical properties and their relation to the location of the studied lakes. RDA Axis 1 explained 40.5% and RDA Axis 2 explained 20.2% of the variance observed. Explanations: *Ahol—Aphanocapsa holsatica, Acla—Aphanothece clathrata, Amin—Aphanothece minutissima, Asmi —Aphanothece smithii, Cmic—Chroococcus microscopicus, Cmin—Chroococcus minimus, Ccatpl—Cyanocatena planctonica, Cdicpl—Cyanodictyon planctonicum, Mten—Merismopedia tenuissima, Agra—Aphanizomenon gracile, Ciss—Cuspidothrix issatschenkoi, Gamp—Geitlerinema amphibium, Pllim—Planktolyngbya limnetica, Pslim—Pseudanabaena limnetica*, TP—total phosphorus concentration, hard.—total hardness, TN—total nitrogen concentration, Cond.—conductivity, Temp.—temperature, Alkal.—alkalinity, SD—Secchi depth visibility.
(b) the biomass of cyanobacteria dominants (species that contributed with at least 2% of relative abundance or biomass) and the selected physical-chemical properties and their relation to the location of the studied lakes. RDA Axis 1 explained 58.9% and RDA Axis 2 explained 37.4% of the variance observed. Explanations: *Ahol—Aphanocapsa holsatica,Acla—Aphanothece clathrata, Mten—Merismopedia tenuissima, Dmen—Dolichospermum mendotae, Agra—Aphanizomenon gracile, Ciss—Cuspidothrix issatschenkoi, Dlem—Dolichospermum lemmermannii, Pllim—Planktolyngbya limnetica, Pslim—Pseudanabaena limnetica, Paga—Planktothrix agardhii*, TP—total phosphorus concentration, hard.—total hardness, TN—total nitrogen concentration, Cond.—conductivity, Temp.—temperature, Alkal.—alkalinity, SD—Secchi depth visibility.

nica, the $PMPL_{MOD}$ values were >2 but <3 and indicated a moderate ecological status. In Lake Niesłysz, mean $PMPL_{MOD}$ value, <1, evidenced high ecological status except in September, when it was only good ($PMPL_{MOD} = 1.7$).

4. Discussion

In this study, a total of 51 cyanobacteria taxa were determined altogether in four *Chara*-lakes diversified in terms of the lake depth and surface area, the size and character of the drainage basin and the use of these water bodies. The highest total number of phytoplankton taxa and cyanobacteria taxa were found in Lake Niesłysz, the largest among the studied bodies of water, characterised by relatively higher habitat variability. In other lakes cyanobacteria species richness was lower and did not differ. The numbers of cyanobacteria taxa were proportionate to the total phytoplankton taxa numbers in the studied *Chara*-lakes (Table 3). The majority of the determined species are common in the temperate zone, mainly in eutrophic waters (Komárek and Anagnostidis, 1998, 2005; Komárek, 2013). The greatest variety was found in the Chroococcales order, represented by small cell-sized colonial species. These species rarely form blooms and often occur in great numbers in the phytoplankton of not only eutrophic but also mesotrophic lakes (Komárek and Anagnostidis, 1998).

Dolichospermum lemmermannii, an N-fixing representative of Nostocales, turned out to be the most frequent cyanobacterium in the studied *Chara*-lakes (Table 4). It is a potentially toxic, blooming species associated with *meso*-eutrophic waters, common in the temperate zone (Reynolds, 1998; Sivonen and Jones, 1999; Komárek and Zapomělová, 2007; Komárek, 2013). As reported by Callieri et al. (2014), blooms of *D. lemmermannii* are found in Alpine eutrophic and even oligotrophic waters. As a response to global warming, this species is spreading in Europe, occupying new, less fertile environments. Due to Soares et al. (2013) *D. lemmermannii* often coexists with *Aphanocapsa* and *Radiocystis* representatives, which corroborates our findings in *Chara*-lakes. In the reported study, *D. lemmermannii* co-occurred with *Radiocystis geminata* and *Aphanocapsa holsatica*, associated with mesotrophic or slightly eutrophic waters (Komárek and Anagnostidis, 1998). This co-existence was particularly evident in Lake Jasne, the least fertilized body of water characterised by the greatest share of charophyte meadows. Another frequently noted taxon, *Plaktothrix agardhii,* revealed the highest quantities in lakes Lednica and Niesłysz. This species, very common in western Poland (Pełechata et al., 2006; Kobos et al., 2013), is adapted to eutrophic, turbid water of shallow lakes, where it blooms (Dokulil and Teubner, 2000; Komárek and Anagnostidis, 2005; Napiórkowska-Krzebietke and Hutorowicz, 2006; Kobos et al., 2013). In addition, it has the ability to produce microcystins (eg. Sivonen and Jones, 1999; Grabowska and Mazur-Marzec, 2011; Kobos et al., 2013).

It is worth mentioning that in none of the studied *Chara*-lakes were cyanobacteria blooms observed and the maximum cyanobacteria biomass, found in Lake Lednica, was only 2 mg L^{-1} (Fig. 4a). In addition, cyanobacteria dominated in the phytoplankton community in two lakes only, in Lakes Niesłysz and Lednica. Although, in accordance with the common seasonal dynamics in the temperate zone (Reynolds and Petersen, 2000), the peak of cyanobacteria development in the studied lakes was observed in summer in Lakes Jasne, Złoty Potok and Lednica and in the autumn months in Lake Niesłysz (Figs. 3a, 4a).

The analysis of physicochemical parameters of the studied *Chara*-lakes proved the highest fertility of Lake Lednica, reflected not only in the greatest cyanobacteria biomass but also in the $PMPL_{MOD}$ index indicative of the lake's moderate ecological status, the worst among the studied lakes (Table 1; Figs. 2, 8). What seems important is that the $PMPL_{MOD}$ index values differentiated between Lakes Złoty Potok and Jasne, the two smaller mid-forest lakes of greater charophyte share, and the larger Lakes Niesłysz and the above-mentioned Lake Lednica. The $PMPL_{MOD}$ index values were not only lower in Lakes Złoty Potok and Jasne, indicating a high ecological status, but the values were also less diversified compared to

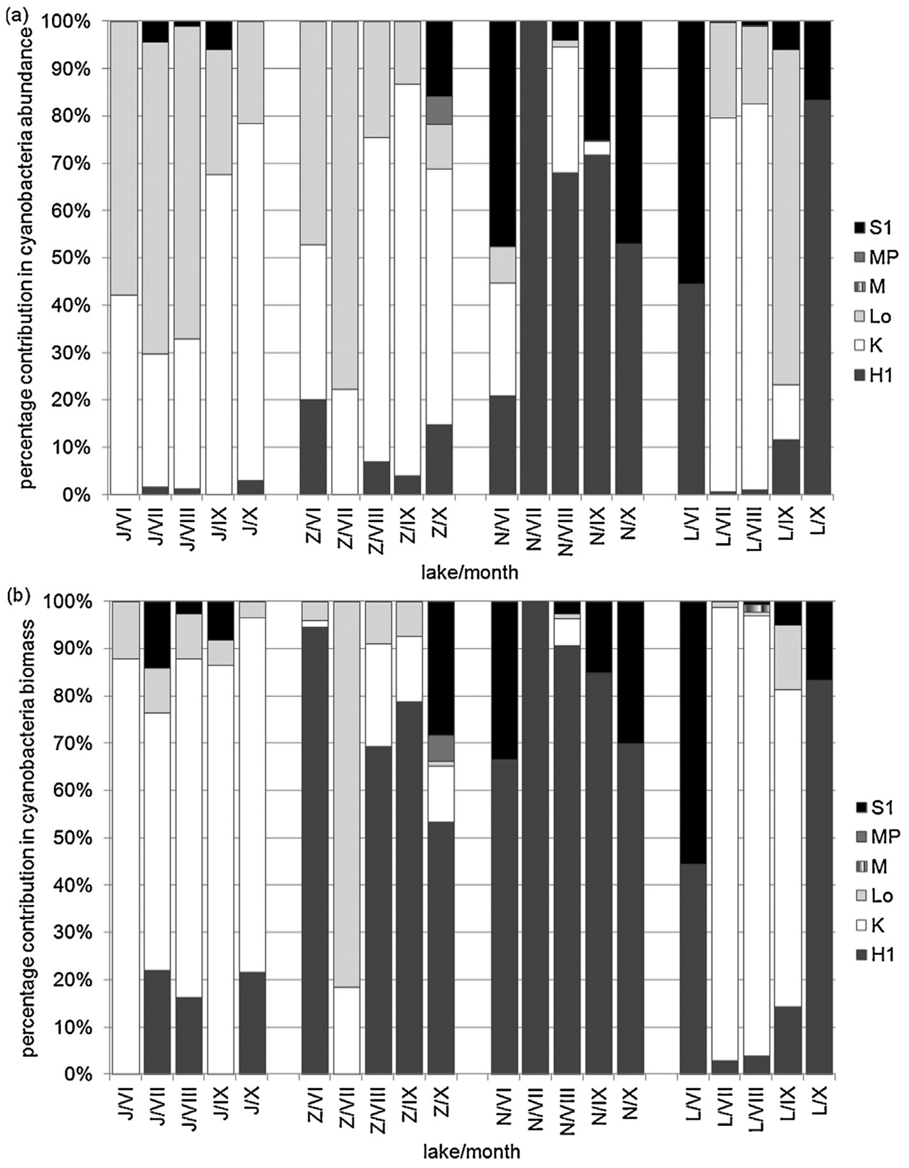

Fig. 6. Participation of each functional group in the total number (a) and biomass (b) of cyanobacteria.

Lake Niesłysz, characterised by PMPL_{MOD} index values indicative of a high and a good ecological status, and Lake Lednica, representing a moderate ecological status. This may result from the recreational use of the two latter lakes and the agricultural character of their larger drainage basin, particularly in the case of Lake Lednica, compared to Lakes Złoty Potok and Jasne (Table 1), in which extensive charophyte meadows, in addition to a limited human impact, contribute to their high ecological status. As mentioned above, Lake Lednica also had the highest mean value of total phytoplankton biomass resulting from the mass development of dinoflagellate *Peridinium gatunense* in July and September (Table 3).The highest trophy of Lake Lednica was accompanied by the highest values of water colour, alkalinity, total hardness, conductivity and concentration of oxygen dissolved in the surface layer. The TN:TP ratio values, considered among key factors of cyanobacteria development, were far higher compared to the rest of lakes studied. This seems to be related to secondary P limitation by extensive external enrichment of the lake with N compounds from the drainage basin, which often occurs in eutrophic lakes (Reynolds, 1984), rather than too low P concentrations (Table 1).

Lake Jasne, a small, mid-forest shallow lake, was the opposite of Lake Lednica. The highest water transparency in this lake can be attributed both to the low trophy and to the presence of dense meadows of *Chara rudis, C. tomentosa* and *C. polyacantha* which reduce the re-suspension of sediments and contribute to lowered nutrient availability for phytoplankton. As a result of both the aforementioned and other positive feedbacks known from literature (van den Berg et al., 1998; van Donk and van de Bund, 2002; Pełechaty et al., 2015), Lake Jasne revealed the lowest fertility in this study. Lakes Złoty Potok and Niesłysz were more similar to Lake Jasne than to Lake Lednica as regards water properties. The only exception was TN:TP ratio whose values were higher in Lake Złoty Potok compared to Lakes Jasne and Niesłysz.

As confirmed by many studies, cyanobacteria become the dominant group with low values of the TN:TP ratio (Forsberg and Ryding, 1980; Smith and Bennet, 1999), conditions favourable for heterocytous cyanobacteria. Non-heterocytous cyanobacteria and other algal groups prefer high TN:TP values. In Lakes Złoty Potok and Niesłysz, the mean TN:TP ratios were found to be 19 (ranging from 6 to 29 in Lake Złoty Potok and, in Lake Niesłysz, from 11 to 25), thus due to Forsberg and Ryding (1980) either N or P lim-

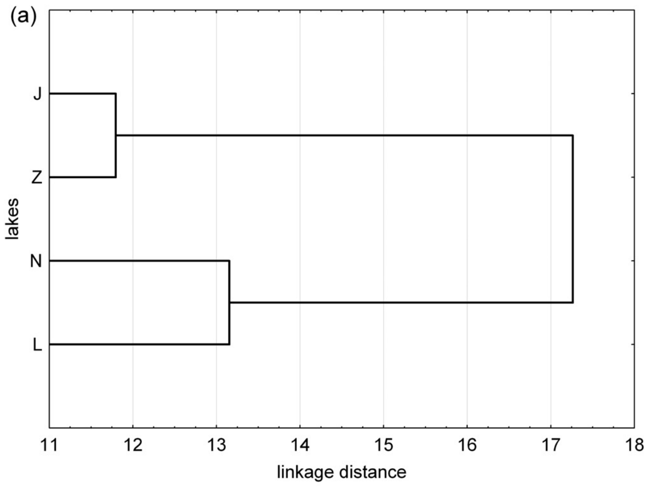

Fig. 7. Hierarchical cluster analysis based on abundance (a) and biomass (b) of cyanobacteria functional groups of four *Chara*-lakes.

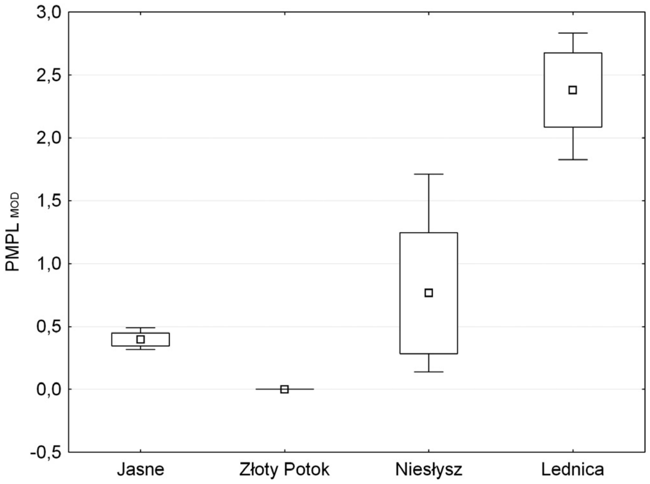

Fig. 8. Minimum, maximum, and mean values ± standard error of $\mathrm{PMPL}_{\mathrm{MOD}}$ (modified Phytoplankton Metric for Polish Lakes) calculated for the four studied *Chara*-lakes. For each lake N = 3.

ited the occurrence of phytoplankton, including cyanobacteria, in these lakes. In Lake Niesłysz, heterocytous Nostocales were the dominant cyanobacteria group. In Lake Złoty Potok, a change in dominance from non-heterocytous Chroococclaes to heterocytous Nostocales occurred in August, at the lowest TN:TP value (TN:TP = 6, Fig. 4b). The high values of the TN:TP ratio obtained for Lake Jasne and, particularly, for Lake Lednica, indicate P–limitation. In these lakes, the dominant group among cyanobacteria (both regarding abundance and biomass) were Chroococcales (Figs. 3b, 4b), for the whole study period in Lake Jasne, and, in Lake Lednica, from July to September.

The RDA analyses and Pearson's correlations selected a number of environmental variables of prime importance for the development of cyanobacteria in the studied *Chara*-lakes (Fig. 5). In addition to the concentration of TP, water temperature, oxygen content and the TN:TP ratio, the analyses highlighted the significance of water colour and alkalinity with which many cyanobacteria species correlated negatively with high significance (Table 5). Alkalinity revealed the strongest correlations in this study, which seems worth emphasising as it is one of the most important ecological factors for *Chara*-lakes, related to the richness in HCO_3^- ions being the source of CO_2 for charophytes (Kufel and Kufel, 2002 and references therein). In accordance with our findings, the study of phytoplankton structure in European lakes has also led to a conclusion that, in addition to TP concentrations, water colour and alkalinity are the most significant factors determining the structure of phytoplankton dominance (Riera et al., 1992; Maileht et al., 2013). Similarly to our study, Maileht et al. (2013) evidenced a negative correlation between *Aphanothece clathrata* and alkalinity. In the studied *Chara*-lakes, however, this correlation was very strong and highly statistically significant. As for the correlation between *A. clathrata* and water colour, in *Chara*-lakes it was negative and statistically significant while in the study of Maileht et al. (2013) it was positive. The negative, highly statistically significant correlation of *Planktolyngbya limnetica* and water colour in *Chara*-lakes was compliant with the results obtained by Maileht et al. (2013) for a number of European lakes.

According to Reynolds (1996), the species composition and quantitative share of particular cyanobacteria species change with increasing water trophy towards the dominance of filamentous forms in eutrophic or hypertrophic lakes, while in mesotrophic and oligotrophic waters they occur in lower concentrations. In the group of studied *Chara*-lakes, the lowest share of filamentous cyanobacteria (Nostocales and Oscillatoriales) was observed in Lake Jasne, the least fertilized of the water bodies, characterised by the most extensive charophyte meadows and a high ecological status. This was evidenced in both abundance and biomass throughout the entire study period (Figs. 3b, 4b). Given the abundance, the contribution of filamentous cyanobacteria clearly differentiated between small mid-forest Lakes Jasne and Złoty Potok from the large Lakes Niesłysz and Lednica, subject to higher human impact. This clear division into two groups was not mirrored in the biomass analysis which evidenced a high percentage share of filamentous cyanobacteria in Lakes Niesłysz and Złoty Potok (Fig. 4). Interestingly, in Lake Lednica the contribution of filamentous forms was restricted to June and October (Fig. 4b). In this lake the dominance of coccoid, non-nitrogen fixing cyanobacteria was noticeable from July to September, whereas in June and October cyanobacteria only included filamentous forms (Figs. 3b, 4b), which is in accordance with the PEG-model (Sommer et al., 1986).

The highest share of filamentous cyanobacteria was observed in Lake Niesłysz. In the light of the RDA analysis this body of water was clearly distinct from the other lakes regarding cyanobacteria structure (Fig. 5a). *Aphanizomenon gracile, Pseudanabaena limnetica, Cuspidothrix issatschenkoi* and, to a lesser degree, *Planktolyngbya limnetica, Geitlerinema amphibium* and *Dolichospermum mendotae* were a group of species characteristic of this ecosystem. The species which especially differentiated the cyanobacteria biomass of Lake Niesłysz from the other lakes was the N-fixing cyanobacteria, *A.*

gracile, that, due to the cylindrospermopsin production in the lakes of western Poland (Kokociński et al., 2013), seems to be a potentially harmful alga for this lake.

The dominant functional groups of cyanobacteria, which in the studied *Chara*-lakes included K, Lo, H1 and S1, demonstrated a close relation of cyanobacteria structure with the stability of the water column in the lake or the water mixing. It emphasizes the significant influence of physical processes on the seasonal dynamics of cyanobacteria functional groups with varied morphometry. This was also confirmed by the cluster analysis, which on the basis of the abundance and biomass of cyanobacteria functional groups showed the difference between the smaller mid-forest lakes with negligible human impact and the larger, deeper lakes with a higher human impact on the drainage basin and the lakes themselves.

The K group, involving non-nitrogen fixing cyanobacteria with small colonial cells, is connected with shallow lakes, rich in nutrients (Padisák et al., 2009). The species of this group occurred both in the most and least fertile ecosystem. Thus, they seem to be less dependent on high nutrient concentrations, which is consistent with the opinion of Blomqvist et al. (1994).

In our study, species of the Lo group were primarily connected with mesotrophic lakes with small surface areas, forest drainage basis and lower trophy. The species of this group are referred to as typical for the summer epilimnion of mesotrophic lakes, sensitive to prolonged and deep mixing (Reynolds et al., 2002).

Representatives of the H1 group were mostly identified in this study in the larger lakes Niesłysz and Lednica. According to Padisák et al. (2009), species belonging to this group prefer eutrophic lakes, both stratified and shallow with low nitrogen content, which was only partly consistent with these lakes' characteristics, particularly in the case of Lake Lednica, where this group dominated among cyanobacteria in June and October at very high nitrogen concentrations. As mentioned by Reynolds et al. (2002), the H1 group tolerates low nitrogen concentrations and low light and is sensitive to the mixing regime. This was also contrary to the environmental conditions of Lake Lednica but was in line with those in Lake Niesłysz. In this lake, the H1 group representatives dominated cyanobacteria abundance and biomass during the entire study period and revealed the highest share in the summer, while the lowest contribution was evidenced during the mixing period.

For the S1 group, an increase in the contribution among cyanobacteria was observed in June and October, which was particularly evident in the large Lakes Niesłysz and Lednica. The representatives of this group, namely Oscillatoriales species (*Planktothrix agardhii, Pseudanabaena limnetica, Geitlerinema amphibium*) prefer turbid, mixed layers of waters with limited light accessibility (Reynolds et al., 2002) and are often observed in eutrophic lakes of Europe, regardless of their morphometry or mictic type (Nixdorf et al., 2003), which is not consistent with the conditions in lakes Niesłysz and Lednica. Even though the two lakes are more eutrophicated compared to the other *Chara*-lakes studied, they are clear-water bodies of water with charophyte meadows indicative of generally good ecological conditions.

In the light of the presented study, apart from the known impact of trophy and in addition to the abundant underwater meadows of charophytes, water alkalinity, colour, and mixing regime seem to play the key role in the development of cyanobacteria in *Chara*-lakes. The lake morphology and their recreational use along with the drainage basin subject to human activity enhance the risk of an increase in the share of cyanobacteria in the phytoplankton community of *Chara*-lakes, reflected in their ecological status which for the entire study period in Lake Lednica or periodically in Lake Niesłysz was worse compared to the high ecological status of the small mid-forest *Chara*-lakes.

5. Conclusions

Irrespective of the lakes' morphometric features and catchment areas characteristics the peaks of cyanobacteria development in the studied *Chara*-lakes were observed during the summer and autumn months. Therefore, the pattern of cyanobacteria dynamics was not different from that known for eutrophic lakes with phytoplankton dominance. However, out of the studied *Chara*-lakes the dominance of cyanobacteria was only detected in large water bodies with catchment areas changed by human activity. These lakes were also characterised by a worse assessment of ecological status compared to the small mid-forest *Chara*-lakes.

Chroococcales representatives were typical of small mid-forest *Chara*-lakes, while Oscilatoriales and Nostocales taxa tended to occur in large lakes surrounded by areas subject to intense human pressure.

Aphanothece minutissima, *Merismopedia tenuissima*, *Aphanizomenon gracile, Aphanocapsa holsatica* and *Cyanodictyon planctonicum* turned out to be species which differentiated among the studied *Chara*-lakes.

Alkalinity, water temperature, colour and, to a lesser extent, TP and TN:TP ratio are postulated to be the water properties which, in addition to extensive charophyte meadows, control the development of cyanobacteria in the studied *Chara*-lakes.

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