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RESEARCH ARTICLE

Phytoplankton in an ecological status assessment of the vendace-type Lake Dejguny (northeastern Poland)

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Abstract. The aim of the study was to perform an ecological status assessment based on the phytoplankton in the vendace-type Lake Dejguny in accordance with the requirements of the Water Framework Directive. The phytoplankton analyses were conducted during three growth seasons in 2006, 2007, and 2008. The phytoplankton multimetric Phytoplankton Metric for Polish Lakes (PMPL) was used to assess the lake's ecological status. The average total biomass of phytoplankton ranged from 1.6 to 3.9 mg dm^{-3}, while the average biomass of Cyanoprokaryota ranged from 0.4 to 1.4 mg dm^{-3}, with the minimum noted in 2008. The phytoplankton assemblages were dominated mainly by filamentous Cyanoprokaryota and pennate Bacillariophyceae in 2006 and 2008, or exclusively by pennate Bacillariophyceae in 2007. The relatively low total biomass and Cyanoprokaryota biomass, as well as seasonal phytoplankton dynamics with the dominant taxa of *Tabellaria flocculosa*, *Dinobryon sociale*, and *D. divergens* confirmed the lake's mesotrophic state and some of the features of reference conditions in this lake, whereas the predominance of filamentous species *Planktothrix agardhii* and *Planktolyngbya limnetica* was characteristic of eutrophic conditions. However, a clear tendency towards progressive eutrophication stemming from the significant domination of filamentous Cyanoprokaryota was observed, and according to the PMPL the assessment indicated that Lake Dejguny had a good ecological status in 2006 and 2007 and even a high one in 2008.

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Introduction

Phytoplankton is a good biological indicator of water quality, and it is required for the implementation of the Water Framework Directive (WFD) (EC2000/60/WE) in Europe. In accordance with WFD requirements, the total biomass and composition of phytoplankton and chlorophyll *a* concentrations in water are the basis for ecological status assessment, which is expressed as high, good, moderate, poor, or bad, and is applied in many countries (Padisák et al. 2006, Mischke et al. 2008, Kaiblinger et al. 2009, Hutorowicz and Pasztaleniec 2009, Hutorowicz et al. 2011). The official assessment criteria in Poland are currently based only on concentrations of chlorophyll *a*, which is one of the biological components in water, as the measure of phytoplankton biomass (Regulation 2011). Recently, the Phytoplankton Metric for Polish Lakes (PMPL) has been developed (Hutorowicz and Pasztaleniec 2009, Hutorowicz et al. 2011), which comprises both quantitative and qualitative features of algae. Initial test results are positive (Napiórkowska-Krzebietke et al. 2012). Statistically significant responses to anthropogenic factors that express degrees of eutrophication, including total phosphorus, total nitrogen, and Secchi disk visibility, and strong correlations with other metrics in widespread use indicated that the PMPL is a useful tool for lake ecological status assessment. Moreover, positive results of inter calibration confirmed this method adaptable for assessing equivalent water quality levels in many countries of Europe (European Commission 2011). Using the PMPL to assess the ecological status of Polish lakes in scientific works has become more common (Jaworska et al. 2014). The present study focused on the phytoplankton-based assessment of the ecological status of the vendace-type Lake Dejguny, including testing the PMPL assessment method with regard to the requirements of Water Framework Directive.

Study area and methods

The deep, stratified Lake Dejguny is located in the Mazurian Lake Region, which is in the Eastern Unit of northeastern Poland. The area of lake is 765.3 ha, and the volume is 92,617.4 $\times 10^3$ m^3, with maximum and average depths of 45.0 and 12.0 m, respectively (Table 1). The lake is located on post-glacial deposits, and, according to Polish abiotic typology (Kolada et al. 2005), it is a type 6a lake with a Schindler's ratio exceeding two, which is the ratio of the total catchment area to lake volume. Lake Dejguny is classified as a vendace-type lake according to fishing classification as it supports a population of the planktivorous species *Coregonus albula* L. (Fiszer et al. 2012a). The fauna inhabiting the lake, especially the zooplankton *Limnocalanus macrurus* G.O. Sars (Tunowski 2012) and the fish *C. albula*, are very sensitive to unfavorable changes in aquatic ecosystems, thus, they are indicators of very low levels of environmental contamination in the waters of Lake Dejguny.

Quantitative and qualitative phytoplankton analyses were conducted in the growth season from April to November in 2006, 2007, and 2008. Integrated samples were collected at one-meter intervals from the euphotic zone during spring and autumn circulation and from the epilimnion during summer stagnation at the deepest point of the lake (Fig. 1). The phytoplankton analyses were conducted using the Utermöhl method (Utermöhl 1958) and international and European standards for algal-based monitoring (Kelly 2004). Total biomass was estimated with cell volume measurements (Pliński et al. 1984, Kawecka and Eloranta 1994). Some of the data on total biomass and that of particular phytoplankton groups have been published (Napiórkowska-Krzebietke and Hutorowicz 2013, Napiórkowska-Krzebietke et al. 2013). Data on chlorophyll *a* content determined with the Lorenzen method (Lorenzen 1967) were obtained from the literature (Pyka et al. 2007, Napiórkowska-Krzebietke et al. 2013). The seasonal phytoplankton development model was determined using transformed data on total biomass and chlorophyll concentrations from 2006 to 2008, which was obtained by fitting the curve to the data using the Jandel Scientific TableCurve program ver. 3.11.

The phytoplankton assemblage diversity was determined using the Shannon-Weaver diversity index (Shannon and Weaver 1949) and evenness (Pielou 1969), and was calculated from the taxa biomass. The TWINSPAN two-way indicator species analysis program (Hill 1979, Hill and Šmilauer 2005) was

Table 1
Morphometric parameters of Lake Dejguny (PL30129)

Parameters	Lake Dejguny
Surface area (ha)	765.3
Maximum depth(m)	45.0
Mean depth (m)	12.0
Volume (103 m^3)	92617.4
Maximum length (m)	6500
Maximum width (m)	2400
Length of shoreline (m)	23000
Shoreline development	2.42
Type of stratification	stratified
Abiotic type	6a
Type of fishing classification	vendace

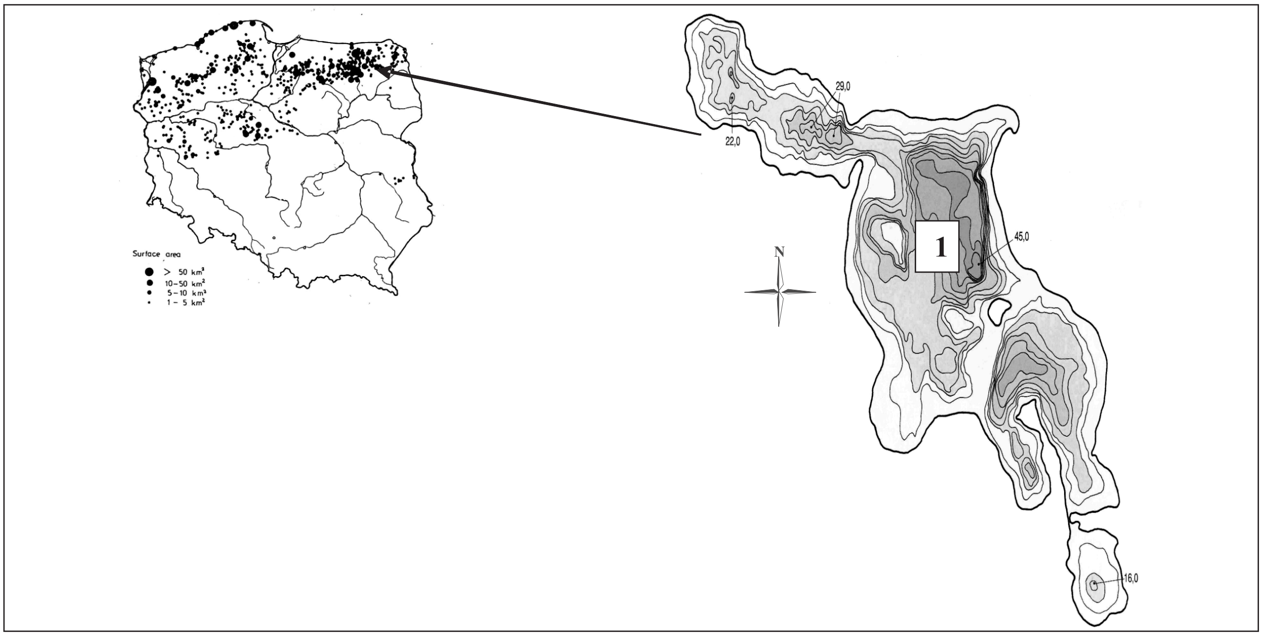

Figure 1. Location of sampling site 1 in Lake Dejguny at the deepest point.

used to rank species according to biomass, which created an ordered, two-way table of their occurrence. Classification was performed using the dichotomized separation method with discriminating species. The multimetric PMPL based on total biomass (Metric Total Biomass, MTB), Cyanoprokaryota biomass (Metric Biomass of Cyanoprokaryota, MBC), and chlorophyll *a* content (Metric Chlorophyll *a*, MC) (Hutorowicz et al. 2011) was used to assess the ecological status of Lake Dejguny.

Results

Seasonal phytoplankton development comprised two comparably high peaks of total biomass that occurred during the spring and the autumn (Fig. 2). Certain differences were observed in the occurrence of the minimal biomass in the summer months of August 2006 and 2008, and June 2007. The maximum total biomass values were 5.6, 6.0, and 2.8 mg dm^{-3} in 2006, 2007, and 2008, respectively. Analogously, the average seasonal total biomass values were 3.2, 3.9, and 1.6 mg dm^{-3}, with the lowest mean value noted in 2008. The model of seasonal changes based on transformed total biomass and chlorophyll concentration data from 2006 to 2008 confirmed the occurrence of two maximums in phytoplankton development during the water mixing periods at the beginning of April and in late October and early November in this lake (Fig. 3).

In 2006, Cyanoprokaryota dominated and represented up to 74% of the total biomass in summer (Fig. 4). However, while the highest percentage share of total biomass occurred in summer, the greatest biomass of Cyanoprokaryota of approximately 3.0 mg dm^{-3} (52%) was observed during the spring peak in seasonal phytoplankton development. In spring and autumn 2006, the other large biomass contributors were Bacillariophyceae (max. 69%), whereas in early summer Chrysophyceae comprised about 47% of the total biomass. The next year, Bacillariophyceae dominated the phytoplankton with an average share of 76%. Cyanoprokaryota was the co-dominant group only in August. The contribution of other groups, namely Cryptophyta and Chlorophyta, was 10% at most in June. Generally, in 2008 the major taxa were again Bacillariophyceae (12-55%) and Cyanoprokaryota (8-58%). In April Bacillariophyceae co-dominated with Cryptophyta,

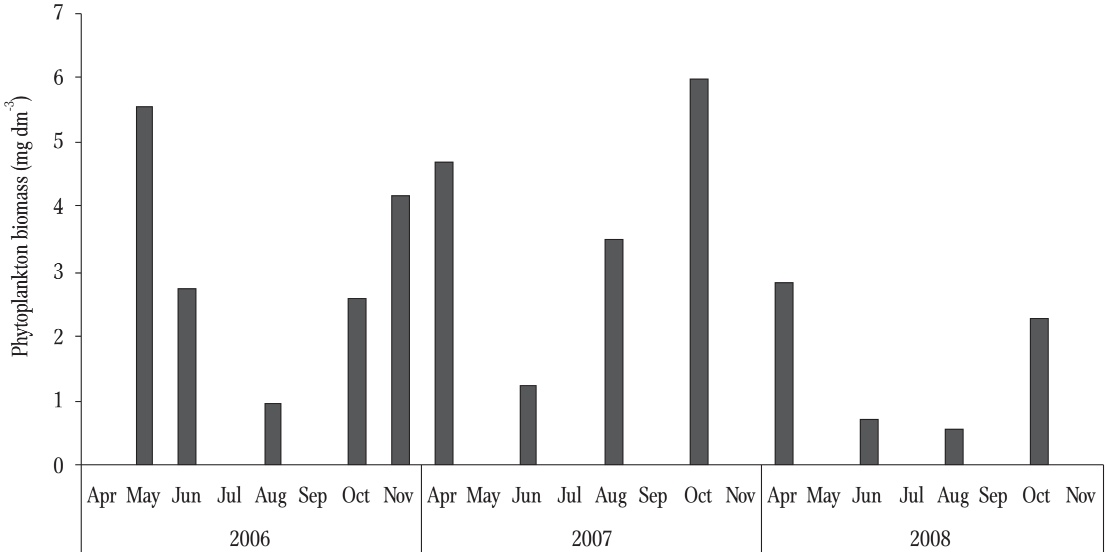

Figure 2. Seasonal dynamics of phytoplankton in 2006-2008 in Lake Dejguny; data from June and August 2006-2008 according to Napiórkowska-Krzebietke and Hutorowicz (2013), adapted.

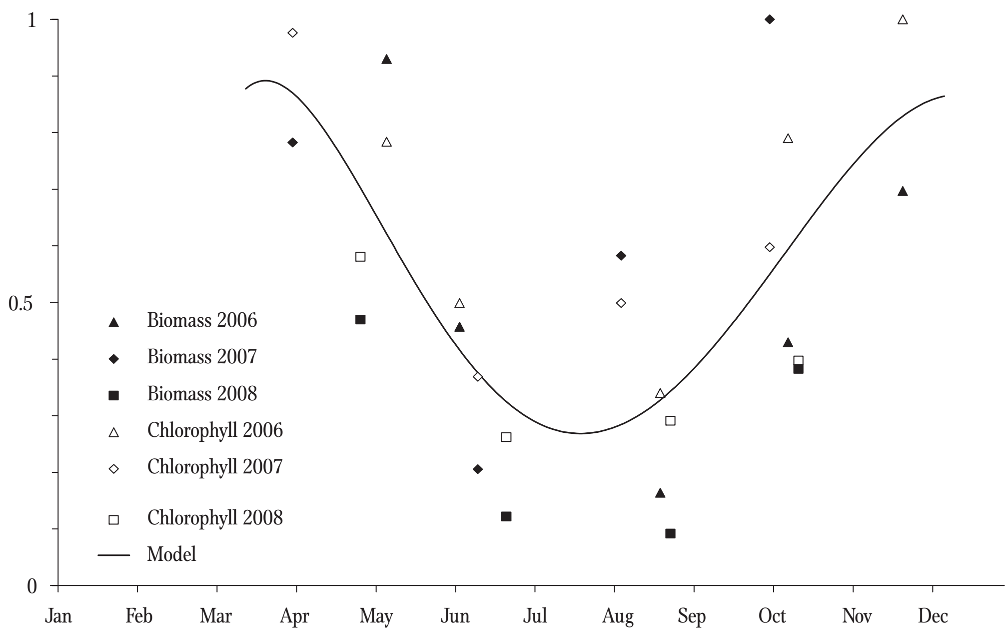

Figure 3. Model of seasonal phytoplankton development in Lake Dejguny in 2006-2008, according to Napiórkowska-Krzebietke et al. (2013), adapted.

whereas in June it co-dominated with Chlorophyta. Additionally, Chrysophyceae comprised about 11% of the total biomass in spring, whereas Dinophyta contributed approximately 13% in summer.

A total of 138 taxa, representing Chlorophyta, Cryptophyta, Cyanoprokaryota, Dinophyta, Euglenophyta, and Heterokontophyta, which includes Bacillariophyceae and Chrysophyceae, were identified in the 2006-2008 period in Lake Dejguny. The highest number of taxa was observed within Chlorophyta and Bacillariophyceae, whereas the lowest was noted within Euglenophyta, Cryptophyta, and Chrysophyceae. In general, the Shannon-Weaver diversity index ranged from 0.6 to 2.5 (Fig. 5), while the average seasonal values were 1.91, 1.33, and 2.17 in 2006, 2007, and 2008,

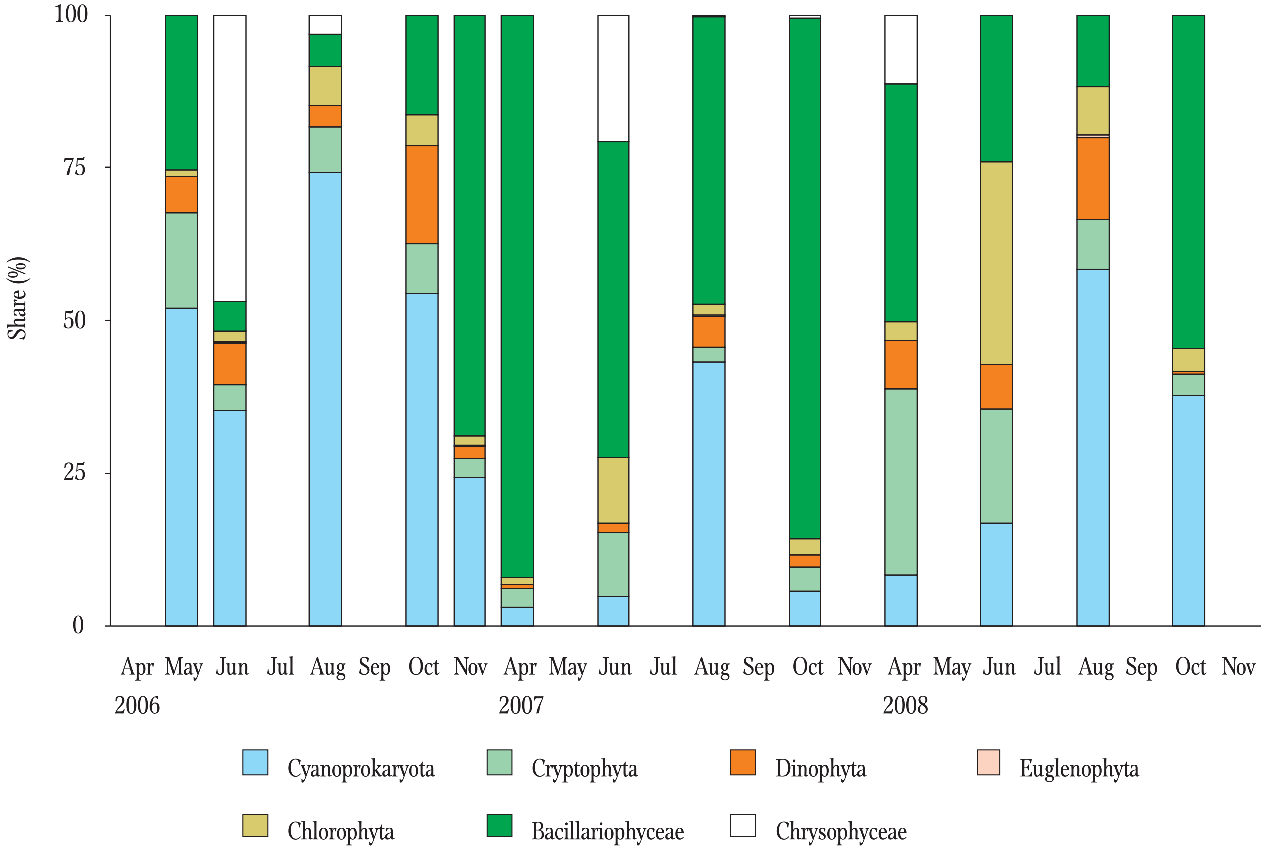

Figure 4. Phytoplankton biomass structure from April to November in 2006-2008 in Lake Dejguny; data from June and August 2006-2008 according to Napiórkowska-Krzebietke and Hutorowicz (2013), adapted.

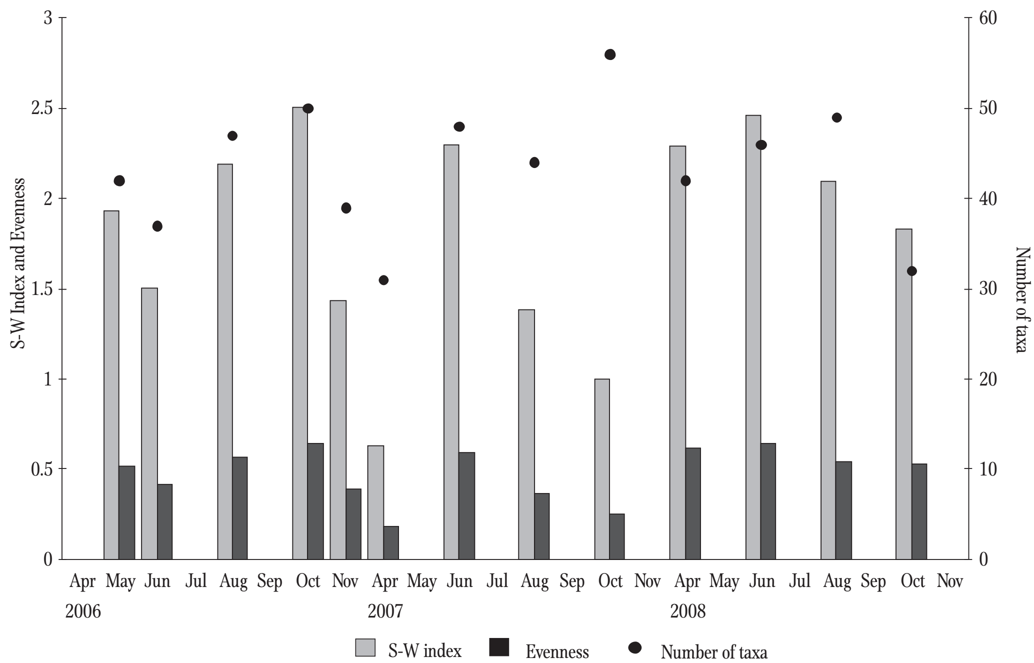

Figure 5. Number of taxa and diversity according to the Shannon-Weaver index and evenness based on the phytoplankton biomass of Lake Dejguny in 2006-2008; data from June and August 2006-2008 according to Napiórkowska-Krzebietke and Hutorowicz (2013), adapted.

Table 2

Phytoplankton biomass and percentage share of total biomass (seasonal averages) in relation to the PMPL-based ecological status assessment of Lake Dejguny in 2006-2008 (according to Napiórkowska-Krzebietke et al. 2013, adapted). *SD – standard deviation

	2006		2007		2008	
Phytoplankton group	mg dm^{-3}	%	mg dm^{-3}	%	mg dm^{-3}	%
Cyanoprokaryota:						
Chroococcales	0.03	1.0	0.03	0.8	0.03	2.0
Nostocales	0.15	4.6	0.02	0.5	0.01	0.4
Oscillatoriales	1.22	38.1	0.46	11.9	0.35	21.5
Heterokontophyta:						
Chrysophyceae	0.26	8.2	0.08	2.0	0.08	4.9
Bacillariophyceae:						
Centrales	0.27	8.3	0.04	1.1	0.07	4.5
Pennales	0.71	22.3	2.88	74.5	0.57	35.4
Euglenophyta	<0.01	<0.1	<0.01	<0.1	<0.01	0.1
Dinophyta	0.21	6.7	0.09	2.2	0.09	5.5
Cryptophyta	0.28	8.7	0.15	3.9	0.28	17.3
Chlorophyta	0.07	2.1	0.12	3.1	0.13	8.2
Total (SD)*	3.21 (0.11)	100	3.87 (0.24)	100	1.62 (0.04)	100
Ecological status	good		good		high	

respectively. The evenness index fluctuated from 0.2 to 0.6 at averages of 0.51, 0.35, and 0.58, respectively. The lowest species diversity was observed in 2007, with the minimum in April, whereas the highest was noted in 2008, especially in June. Species richness fluctuated within a very narrow range, and it was relatively constant throughout the period studied.

During the growth season in 2006, the highest average biomass of approximately 44% of the total biomass was formed by Cyanoprokaryota, especially from the order Oscillatoriales, and Lake Dejguny was assessed as having a good ecological status (Table 2). The prominent taxa were filamentous *Planktothrix agardhii* (Gom.) Anagn. & Kom. and *Planktolyngbya limnetica* (Lemm.) Kom.-Leg. & Cronb., and *Aphanizomenon gracile* Lemm. from the order Nostocales. At this time, Bacillariophyceae co-dominated the phytoplankton at about 30% with major representatives of the order Pennales, with the the dominant species of *Tabellaria flocculosa* (Roth) Kütz. The centric Bacillariophyceae *Cyclotella* sp. div., Chrysophyceae *Dinobryon sociale* Ehr., a major biomass contributor in June, and the nanoplanktonic Cryptophyta *Rhodomonas* sp. were also relatively high biomass contributors.

The distinct predominance of the pennate Bacillariophyceae in excess of 70% occurred in the next year when the ecological status of Lake Dejguny was good, and this included mostly *T. flocculosa*. Cyanoprokaryota represented only 13%, with *P. agardhii* as the dominant taxon. In 2008, the phytoplankton was again dominated by Bacillariophyceae and Cyanoprokaryota, and the ecological status of lake was graded upwards from good to high. Although the percentage shares of both these groups were analogous to that in 2006, theirs biomass were significantly lower. The phytoplankton assemblages were also dominated by the same species. Other important taxa, representing approximately 5-10% of the total biomass, included *Fragilaria crotonensis* Kitt., *Cryptomonas* sp., and *Dinobryon divergens* Imh.

Significant diversification in phytoplankton development was detected with divisive classification performed with two-way indicator species analysis

Table 3
Ecological status assessment according to the Phytoplankton Metric for Polish Lakes (PMPL) and the partial metrics of MTB – Metric Total Biomass, MBC – Metric Biomass of Cyanoprokaryota, MC – Metric Chlorophyll *a*. Ecological status scale: 0-1.00 (high), 1.01-2.00 (good), 2.01-3.00 (moderate), 3.01-4.00 (poor), 4.01-5.00 (bad)

Year	MTB	MBC	MC	PMPL	Ecological status
2006	2.02	0.79	1.21	1.34	Good
2007	2.21	1.37	1.03	1.53	Good
2008	1.30	0	0.31	0.54	High

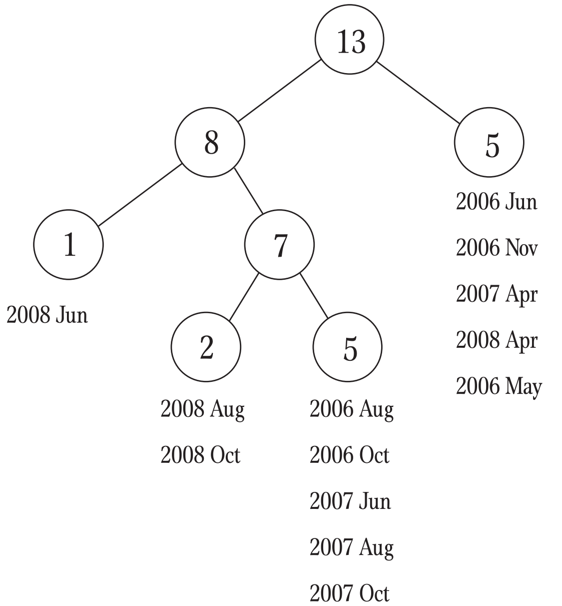

Figure 6. Classification dendogram for phytoplankton samples from Lake Dejguny in 2006-2008 determined with TWINSPAN software.

(Fig. 6). TWINSPAN classified samples into two basic groups on the first hierarchical level. The first group comprised spring to early summer and autumn samples that were all linked with cold periods and water circulation in 2006 and 2007 and, exceptionally, April 2008. Baccilariophyceae from the genus *Cyclotella* and *Fragilaria*, and Cryptophyta *Cryptomonas erosa* Ehr. and *C. marssonii* Skuja were in this group. The second group included the rest of samples, which were subdivided in subsequent divisions. First the sample collected in June 2008 was separated, followed by those from August and October 2008. The summer and early autumn samples collected in 2006 and 2007 formed a coherent subgroup linked with more stable conditions during the summer water stagnation. The entire branch of the dendrogram comprised mainly Chlorophyta from the order Chlorococcales with *Oocystis* noted often and abundantly, while *Scenedesmus*, *Coelastrum*, and *Tetraedron*, and Desmidiales, mainly *Cosmarium*, and also *Staurastrum*, were noted sporadically. These taxa were not noted in samples from the first branch except in two cases

The highest values exceeding 2.01 of partial phytoplankton metrics noted in MTB indicated the that Lake Dejguny was of a moderate ecological status in 2006 and 2007 (Table 3). The values of MBC, MC, and even the multimetric PMPL ranged from 0 to 1.53. Despite this, according to the PMPL the ecological status of this lake was assessed as good in 2006 and 2007, and as high in 2008.

Discussion

The most common fish in the vendace-type Lake Dejguny is the planktivorous *C. albula*, which is susceptible to deteriorating environmental conditions that occur in lakes with waters of relatively low trophic level and with high dissolved oxygen concentrations (Fiszer et al. 2012b). Furthermore, the glacial relict crustacean *L. macrurus*, which is very sensitive to water quality degradation and to fish predation, was also noted in this lake (Tunowski 2012), and has also been identified in many oligotrophic inland lakes (Gannon et al. 1978). These two species can be used successfully as bioindicators of high water quality in Lake Dejguny. The assessment of Carlson's trophic state based on environmental variables indicated that conditions in this lake were meso-eutrophic (Pyka et al. 2007, Napiórkowska-Krzebietke et al. 2013) and that the lake exhibits some tendency towards progressive eutrophication because of internal enrichment (Dunalska et al. 2006) and human pressure (Domska et al. 2010). During the growth seasons in 2006 and 2007, the chlorophyll *a* content in Lake Dejguny was as high as 11.8 µg dm^{-3}, whereas it was significantly lower with values of up to 6.8 µg dm^{-3} in 2008 (Pyka et al. 2007, Napiórkowska-Krzebietke et al. 2013). The assessment based on its average content indicated the ecological status of this lake was good in 200-2007 and high in 2008 (Regulation 2011). Furthermore, the classification of ecological status according to chosen physicochemical parameters such as Secchi disk visibility and total phosphorus and total nitrogen concentrations that support the biological elements, confirmed at least a good status. In relation to reference, or undisturbed conditions, the relatively low values of parameters expressing the trophic state of Lake Dejguny (chlorophyll *a*, total phosphorus, Secchi disk visibility) were partially comparable to reference values for Polish lakes, especially in 2008 (Table 4). On the other hand, the concentrations of chlorophyll *a* and total phosphorus were higher than the values noted in European reference lakes in the Central-Baltic region (Cardoso et al. 2007, Carvalho et al. 2008).

The values of total biomass in Lake Dejguny were quite low and typical of mesotrophic lowland harmonic lakes in temperate climatic zones (Hillbricht-Ilkowska and Wiśniewski 1994). The maximum value of approximately 6.0 mg dm^{-3} was also noted in mesotrophic Alpine lakes (Tolotti 2001). According to Oleksowicz (1988), the seasonal phytoplankton development with two maximums of total biomass occurring in spring and autumn is characteristic for lakes with medium-sized nutrient pools. Differences in the shapes of curves describing seasonal dynamics might have resulted from fluctuating weather conditions in the three years studied. In general, the structure of the phytoplankton assemblage dominants were as follows: filamentous Cyanoprokaryota and pennate Bacillariophyceae (2006), pennate Bacillariophyceae (2007), pennate Bacillariophyceae and filamentous Cyanoprokaryota (2008). Cyanoprokaryota, especially filamentous taxa, dominate very often in more eutrophic lakes (Dokulil and Teubner 2000, Napiórkowska--Krzebietke and Hutorowicz 2006, Wiśniewska et al. 2007). Bacillariophyceae from the order Pennales are most abundant at moderate trophic levels, whereas the distinct dominance of Chrysophyceae in June 2006 refered to the least eutrophic lake conditions (Lyche Solheim et al. 2008, Ptacnik et al. 2008). The values of the Shannon-Weaver index usually ranged between 1 and 3, and, according to Wilhm (1975), were typical of moderately-contaminated conditions. Exceptionally, values below 1, which are characteristic of heavily contaminated conditions, were observed in April and October 2007. According to Yap (1997), the mean Shannon-Weaver index values in 2006 and 2007 were characteristic of slightly and moderately contaminated waters, while in 2008 they indicated good quality waters. The lowest species diversity in the phytoplankton assemblages was

Table 4
Values (range and median) of eutrophication parameters in Lake Dejguny in comparison to reference lakes

Lakes	Year	Parameter: Chlorophyll *a* (µg dm^{-3})	Total phosphorus (mg dm^{-3})	Secchi disk visibility (m)
Lake Dejguny[1]	2006	4.0-11.8 (9.22)	0.062-0.122 (0.088)	2.5-4.0 (3.2)
	2007	4.3-11.5 (6.46)	0.034-0.082 (0.052)	2.9-4.5 (4.0)
	2008	3.1-6.8 (4.05)	0.061-0.078 (0.068)	3.5-4.4 (4.3)
Polish reference stratified lakes[2]	-	0.5-9.4 (3.75)	0.014-0.079 (0.033)	2.4-6.9 (3.6)
Central-Baltic region L-CB1[3]	-	2.8[4]	0.010-0.034 (0.019)[5]	-

[1]acc. to Pyka et al. (2007), Napiórkowska-Krzebietke et al. (2013); [2]acc. to Soszka et al. (2008); [3]L-CB1 – lowland shallow lakes (mean depth 3-15 m) with high alkalinity; [4]acc. to Carvalho et al. (2008), median value; [5]acc. to Cardoso et al. (2007)

observed in 2007 simultaneously with the highest biomass, whereas the highest values of the Shannon-Weaver index were noted in 2008, which coincided with the lowest algae biomass.

The multimetric PMPL and the partial metrics MTB, MBC, and MC were very sensitive to increases in eutrophication levels (Napiórkowska-Krzebietke et al. 2012). Furthermore, in European countries the phytoplankton metrics used for ecological status assessment were also closely related to lake trophic degrees (Ptacnik et al. 2009, Phillips et al. 2013). Some relation to trophic features was also noted in Lake Dejguny. The assessment according to PMPL indicated this lake was of a good ecological status in 2006 and 2007 when the average total biomass of algae was above 3.0 mg dm^{-3}. The phytoplankton assemblages were dominated mainly by Cyanoprokaryota, a group that occurs massively under eutrophic conditions (Ptacnik et al. 2009). The prominent taxa of filamentous *Planktothrix agardhii* and *Planktolyngbya limnetica* are also noted abundantly in the turbid, mixed layers of lakes, and are adapted to shade and low irradiance conditions (Reynolds et al. 2002, Padisák et al. 2009). The mass occurrence of *P. agardhii* in summer was also observed in the deep, metalimnetic layers in Lake Dejguny (Napiórkowska-Krzebietke and Hutorowicz 2013). The co-dominance of *Aphanizomenon gracile* in Cyanoprokaryota assemblages is linked with the eutrophic conditions of the lake (Padisák et al. 2009). The second important group of algae, which co-dominated in 2006 and dominated in 2007, was Bacillariophyceae, especially from the order Pennales *Tabellaria flocculosa*, a typical representative of the mesotrophic epilimnia (Reynolds et al. 2002, Padisák et al. 2009), and dominant in Alpine lakes (Trevisan et al. 2010). In addition, the distinct domination of Chrysophyceae *Dinobryon sociale* in June 2006 and 2007 was a symptom of oligotrophic conditions. In 2008, the ecological status of Lake Dejguny was assessed as high. However, the phytoplankton assemblages were again dominated by the same species of pennate Bacillariophyceae and Cyanoprokaryota, although abundance was significantly lower than in 2006-2007. The important taxa were also *Fragilaria crotonensis* and *Cryptomonas* sp., which are characteristic of more enriched lakes (Padisák et al. 2009) and *D. divergens*. The dominating species of *T. flocculosa*, *D. sociale*, and *D. divergens* were described as reference taxa for lowland European lakes (Järvinen et al. 2013).

The significant differences in the taxonomic structure of the phytoplankton in 2006-2008 was confirmed by two-way indicator species analysis. TWINSPAN permitted identifying the spring and autumn phytoplankton assemblages from the summer ones. This diversification was consistent with seasonal phytoplankton dynamics, i.e., two maximums of total biomass and chlorophyll *a* concentration occurred in both cold periods. Furthermore, distinct separateness was recorded in the samples collected in 2008, in which the minimum of total biomass occurred in phytoplankton development, and filamentous Cyanoprokaryota were exclusively dominants in summer. In this period, the value of PMPL was below 1, which indicated that the ecological status of Lake Dejguny was high.

Conclusions

The average total biomass of phytoplankton in the vendace-type Lake Dejguny was quite low, not higher than 4.0 mg dm^{-3}, and typical of mesotrophic lakes. The seasonal dynamics of two maximums – one in spring and the second in autumn, confirmed the lake had a medium-sized nutrient pool. The phytoplankton assemblages were dominated mainly by filamentous Cyanoprokaryota and pennate Bacillariophyceae in 2006 and 2008, or exclusively by pennate Bacillariophyceae in 2007. The occurrence of the prominent taxa *Planktothrix agardhii*, *Planktolyngbya limnetica*, *Aphanizomenon gracile*, and *Fragilaria crotonensis* indicated that conditions were more eutrophic. The domination of *Tabellaria flocculosa*, *Dinobryon sociale*, and *D. divergens* was linked to the oligo-mesotrophic features of this lake and corresponded to reference conditions in lowland

European lakes. The taxa mentioned above are tolerant of both nutrient and light deficiency, and sensitive to both stratification and unstable conditions that are frequently recorded in deep lakes raging from oligotrophic to eutrophic throughout the world. The partial assessments of the ecological status based on MTB, MBC, and MC were more differentiated than that assessed with the multimetric PMPL. The partial metric MTB classified Lake Dejguny more rigorously than did MBC and MC. However, a clear tendency towards eutrophic conditions was signaled by the significant domination of filamentous Cyanoprokaryota. According to the PMPL assessment, the ecological status of Lake Dejguny was good in 2006 and 2007 and even high in 2008.

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Author contributions. A.N.-K. and A.H. developed the research concept, and analyzed materials; A.N-K. wrote the text.

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