Carpathian Journal of Earth and Environmental Sciences, February 2015, Vol. 10, No. 1, p. 41 - 52

PHYTOPLANKTON, CULTURABLE BACTERIA AND THEIR RELATIONSHIPS ALONG ENVIRONMENTAL GRADIENTS IN A STRATIFIED EUTROPHIC LAKE

Beata MESSYASZ[1*], Maciej GABKA[1], Jakub BARYLSKI[2], Grzegorz NOWICKI[2], Łukasz LAMENTOWICZ[1], Anna GOŹDZICKA-JÓZEFIAK[2], Andrzej RYBAK[1], Renata DONDAJEWSKA[3] & Lubomira BURCHARDT[1]

[1]Department of Hydrobiology, Institute of Environmental Biology, Faculty of Biology, Adam Mickiewicz University, Umultowska 89, PL-61-614 Poznań, Poland, messyasz@amu.edu.pl, +48 618 295 761

[2]Department of Molecular Virology, Institute of Experimental Biology, Faculty of Biology, Adam Mickiewicz University, Umultowska 89, PL-61-614 Poznań, Poland

[3]Department of Water Protection, Institute of Environmental Biology, Faculty of Biology, Adam Mickiewicz University, Umultowska 89, PL-61-614 Poznań, Poland

Abstract: Microbial communities living in stratified lakes are known to be early indicators of ecosystem disturbances. Based on information available up to date it was hypothesized that structures of heterotrophic bacteria and phytoplankton assemblages in the eutrophic lake are shaped by different, yet interrelated physicochemical parameters and both groups significantly influence each other. To verify this hypothesis, the species distribution of these groups along the environmental gradients in a eutrophic lake (Lake Góreckie, NW Poland) was investigated. During the study 198 phytoplankton and 26 bacteria taxa were detected in 84 samples. The analysis of their temporal and spatial distribution revealed that phytoplankton and bacteria respond to ecological gradients in a roughly independent manner. Temperature, NH_4^+ and total phosphorus turned out to be the most important environmental gradients for the phytoplankton, while of concentrations oxidized nitrogen forms (NO_3^-, NO_2^-) were more relevant for heterotrophic bacteria. The results obtained in this study allowed us to identify cluster of culturable bacteria connected with high concentrations of chlorophyll a (and thus with the presence of phytoplankton). The group of phytoplankton-associated bacteria includes strains identified as *Bacillus megaterium*, *B. simplex*, *Paenibacillus amylolyticus*, *Enterobacter cancerogenus* and all the detected members of genus *Exiguobacterium*. These results are the next step towards an understanding of the microbial food web functioning in eutrophic lakes; an understanding which is crucial in the context of the protection and management of these ecosystems.

Key words: eutrophic lake, nitrogen cycling, ecological gradients, bacteria, phytoplankton

1. INTRODUCTION

Eutrophication affects now significant portion of European lakes (Kajak, 2001; Lyche Solheim et al., 2012; Pop et al., 2013) and often leads to the loss of biodiversity and ecosystem productivity (Reynolds, 1987; Yang et al., 2008; Conley et al., 2009). Moreover, the phytoplankton blooms that may cause further depletion of nutrients and oxygen or a deterioration of light conditions are commonly associated with eutrophication (Harris, 1978; Reynolds, 1980; Shaw et al., 2003; Gołdyn et al., 2013). This problem is certainly connected with the human activity (Wetzel, 2001; Yang et al., 2008; Conley et al., 2009) and increased supply of nutrients (mainly phosphorous and nitrogen) is considered as its most important driving factor (Caron, 1994; Conley et al., 2009). Nevertheless, the microflora is not without an impact on this process. Nitrogen fixation by cyanobacteria, removal of this element *via* coupled nitrification/denitrification or release of phosphorus from sediments by phosphate solubilizing bacteria are just a few examples of such influence (Fisher & Wood, 2004; Arhonditsis & Brett, 2005; Vymazal, 2007; Qian et al., 2010).

Relationships between heterotrophic bacteria and phytoplankton are among the most discussed subjects in contemporary hydrobiology (Eiler &

Bertilsson, 2004; Sapp et al., 2007; Tijdens et al., 2008; Berg et al., 2009; Shen et al., 2011; Šimek et al., 2011; Leão et al., 2012). The reason for such an interest is the prevalence of both groups and the role which they play in ecosystems. In most ecosystems these groups are dominant groups in terms of their biomass and contribution to matter and energy flow (Azam et al., 1983; Whitman et al., 1998; Schmidt & Schaechter, 2011). The phytoplankton is responsible for most of primary production and the heterotrophic bacteria are main decomposers, remineralizers and primary consumers. According to microbial loop theory they utilize most of the dissolved organic matter (DOM) produced by phytoplankton. This matter is partially incorporated into their biomass and transferred to higher trophic levels by zooplankton grazing (Azam et al., 1983; Azam, 1998; Cho & Azam, 1990).

While phytoplankton and heterotrophic bacteria often compete for environmental resources (Drakare, 2002; Sigee, 2005) they constitute a functionally complementary system which plays an important role in energy flow, cycling of elements and biogeochemistry. The fact that discussed groups influence each other is widely recognized but the exact role of each partner in the relationship is not always clear and the question of bacterial impact on phytoplankton blooming remains controversial.

Results of earlier studies (Kent et al., 2007; Tijdens et al., 2008; Berg et al., 2009; Mazur-Marzec et al., 2009; Wilhelm et al., 2011; Šimek et al., 2011; Eiler et al., 2012; Leão et al., 2012) allowed hypothesize that:

- the structures of phytoplankton and heterotrophic bacteria assemblages are shaped by partially different sets of physical and chemical parameters,
- the composition of phytoplankton communities significantly influences the structure of heterotrophic bacterioplankton and *vice versa*.

In order to validate these hypotheses purpose was to establish:

- what are the most important ecological factors shaping the structure of communities of phytoplankton and culturable heterotrophic bacteria,
- what are the relationships between structures of these communities.

2. METHODS

2.1. Study site

The research was carried out on Lake Góreckie – a postglacial, ribbon lake situated in the central part of the Wielkopolski National Park (Western Poland, see Figure 1). Surface area of the reservoir is 101.6 ha, average depth is 8.5 m and maximum depth reaches 16.6 m. The basin is divided into two sub-basins, a deep northern one (10-15 m) and a shallower southern one (5-10 m). About 59% of slopes forming the catchment are covered by pine and oak forests and a small part is under agricultural use (Kolendowicz et al., 2008; Sobczyński et al., 2012). The lake is supplied by ground waters and atmospheric precipitation.

A narrow littoral zone is dominated by reeds, mainly *Phragmites australis* and *Typha angustifolia*. The composition of underwater macrophytes is characteristic for highly eutrophic lakes. While small patches of *Najas marina* and *Potamogeton perfoliatus* can be found, submerged vegetation is weakly developed due to light limitations caused by intense phytoplankton blooming.

Lake water quality has been gradually changing from a meso-eutrophic (in the late 1980s) to a strongly eutrophic (Burchardt et al., 2000; Kolendowicz et al., 2008). Eutrophication was connected with the influx of sewage from the nearby sanatorium (functioning for over forty years) (Lubner, 1988; Sobczyński & Joniak, 2009) but elimination of most of anthropogenic nutrient sources (including the sanatorium) did not stop the process.

2.2. Sample collection

Field works were conducted between June 2009 and April 2010. Samples were collected every month (except January and February 2010 when unstable ice cover made the collection impossible). We studied the vertical profile from deepest part of the lake (15 m, figure 1). Samples for microbiological, phycological and chemical analyzes were collected from depths of 0.5, 2.0, 3.0, 5.0, 7.0, 10.0, 13.0, 14.0, and 15 m using a “Gigant” centrifugal pump (Eijkelkamp). Samples of water from above the sediment were extracted with a Kajak sediment sampler (Kajak et al., 1965). Samples for chemical analyses were poured into plastic containers. Two of each tree were immediately preserved (one with $CHCl_3$ and the other with H_2SO_4). To each sample for the phytoplankton analysis (1.5 l), 2 ml of Lugol's solution was added to preserve algae and cyanobacteria until examination. Samples collected for microbiological (heterotrophic bacteria) analysis (6 ml) were immediately transferred to sterile plastic tubes and mixed with 4 ml of transport medium (LB broth). pH, electric conductivity (EC), water temperature (Temp), concentration of dissolved oxygen (DO) and oxygen saturation (OS) were measured in situ with a YSI6600 multiparameter sonde (YSI Corporation, Yellow Springs, OH, USA). Water transparency was determined using the standard Secchi disk method (with a 30 cm diameter white disc).

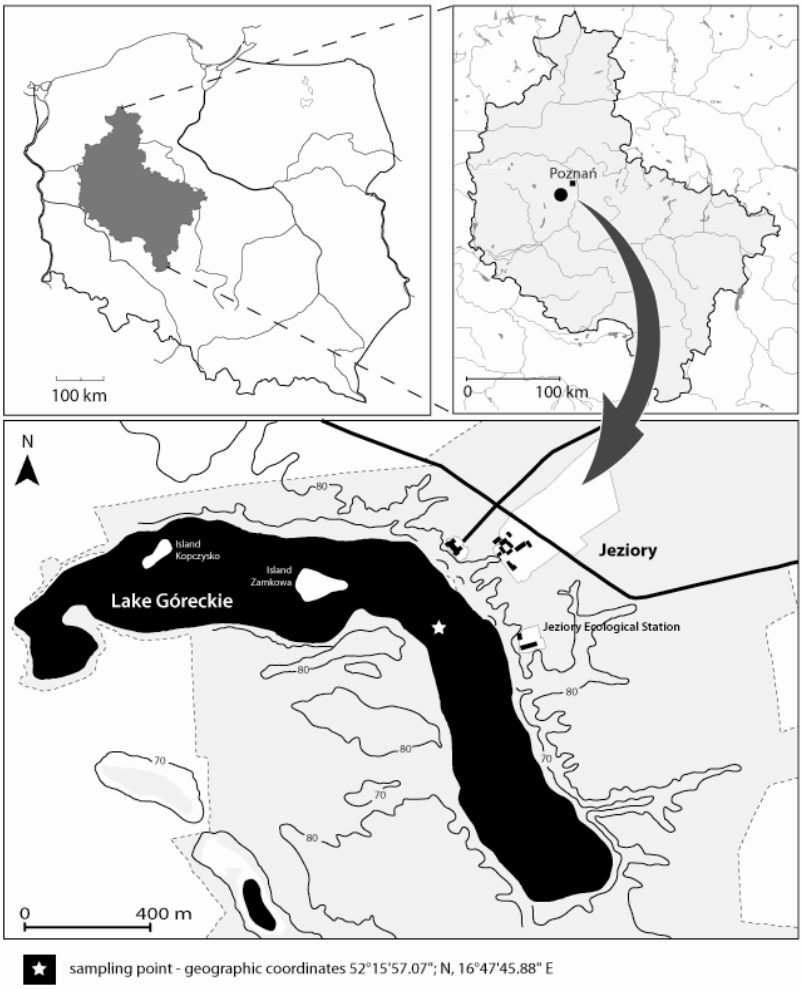

Figure 1. Location of the sampling site (marked with a star).

2.3. Laboratory analysis

Chemical analyses were performed according to standard methods for hydrochemical analyses (APHA, 1998). Concentrations of the ammonia (NH_4^+), nitrate (NO_3^-), nitrite (NO_2^-), total nitrogen (TN), phosphate (PO_4^{3-}) and total phosphorus (TP) were measured. Chlorophyll *a* (Chl-a) concentration was determined in ethanol extracts from material deposited on the GF/F filters according to the ISO 10260 standard method. Descriptive statistics of the recorded variables are summarized in table 1.

Table 1. Descriptive statistics of the physicochemical properties of water in the analyzed profile, between June 2009 and April 2010 (n=84).

Parameter (unit)	**Symbol**	**Min.**	**Max.**	**Mean**	**Std. Dev.**
Depth (m)	Deep	0	15	7.1	4.9
Temperature (°C)	Temp	4.9	23.1	11.1	5.8
pH	pH	6.45	9.04	7.81	0.59
Oxygen saturation (%)	OS	n.d.	99	32.9	25.8
Dissolved oxygen (mg×l^{-1})	DO	n.d.	9.0	3.3	2.6
Electric conductivity (µS×cm^{-1})	EC	252	602	478.8	53.2
Nitrite (mg×l^{-1})	NO_2	0.001	0.007	0.002	0.002
Nitrate (mg×l^{-1})	NO_3	0.001	0.950	0.187	0.319
Ammonium (mg×l^{-1})	NH_4	0.412	6.537	1.406	1.171
Total phosphorus (mg×l^{-1})	TP	0.013	0.716	0.169	0.126
Phosphates (mg×l^{-1})	PO_4	0.001	0.713	0.140	0.149
Chlorophyll a (µg×l^{-1})	Chl-a	0.01	38.62	14.42	9.61

2.4. Phytoplankton analysis

Natural units (e.g. single cells, colonies, and trichomes) of particular phytoplankton species were counted under a light microscope (magnification 200× and 400×) in five different grid quadrants chosen at random across the counting chamber. Thread-like forms were distinguished by the presence of 100 µm trichom. The biomass of algae was determined using the volumetric method (Edler, 1979; Rott, 1981) and the species richness was described as the number of algal taxa in each sample. The identification of species was carried out based on current taxonomical criteria (Bucka & Wilk-Woźniak 2002; Burchardt 2014 and included other references there).

2.5. Bacteria isolation and identification

200 µl of each sample was spread onto a Petri dish containing culture medium (Water Plate Count Agar). Bacteria were cultured overnight at 37°C and (in the case of samples collected from the surface and above the sediment) at 20°C. Colonies of culturable bacteria were counted and pure cultures obtained.

Cells from a single colony were lysed with detergent (0.5% IGEPAL CA - 630 in 10 mM Tris-HCl buffer pH 8.5) and high temperature (95°C, 10 min). The mixture was cooled in ice (5 min) and the cell debris was removed by centrifugation. Clear supernatant (containing DNA) was collected, diluted (5×) and used as a template for polymerase chain reaction. Each reaction mixture contained 2.0 µl of the lysate, 0.6 U of recombinant Taq DNA Polymerase (EP0404, Fermentas), 1×Buffer for Taq Polymerase (with $(NH_4)_2SO_4$), 30 nM of MgCl, 40 pM of each deoxyribonucleotide triphosphate and 20 nM of each primer in a final volume of 20 µl. For primers and cycling conditions see table S1 (Online Resources) (Weisburg et al., 1991; Edwards et al., 1989). The PCR product was purified with a QIAquick PCR Purification Kit (Qiagen) and sequenced with a 3130x Genetic Analyzer (Applied Biosystems) in the Faculty of Biology, Adam Mickiewicz University in Poznań (using the same set of primers as in PCR). The obtained sequences were compared with those available in the GenBank database using a megaBLAST tool (NCBI) (Zhang et al., 2000; Sayers et al., 2009).

2.6. Numerical analyzes

Ordination methods were used to find the relationships between chemistry of the water, phytoplankton and the bacteria. Detrended correspondence analysis (DCA) was performed in order to determine the most important ecological gradients for each group of organisms. Species-environment relationships of phytoplankton and bacteria were explored by redundancy analysis (RDA). Because of the skewed distributions of most variables, square root transformation was performed on data to normalize their distribution. To reduce the number of variables a forward selection procedure using the Monte Carlo test with 999 permutations was performed (variables were discarded until a significance threshold of $p<0.05$ was reached). Variables with significance levels below $p<0.05$ were passively projected into diagrams. The ordination was performed using the CANOCO software package (ter Braak, 1998).

3. RESULTS

3.1. Diversity and density

198 different phytoplankton taxa were found in the studied profile. They belonged to classes: *Chlorophyceae* (47), *Bacillariophyceae* (38), *Cyanophyceae* (31), *Trebouxiophyceae* (18), *Conjugatophyceae* (16), *Dinophyceae* (14), *Euglenophyceae* (12), *Cryptophyceae* (9), *Xanthophyceae* (5), *Chrysophyceae* (4), *Klebsormidiophyceae* (3) or *Nephrophyceae* (1). The species density of phytoplankton is clearly connected with depth and sampling time. The smallest number (25) of taxa was observed in December 2010, and the greatest (50) in July 2009. The observed number of natural units per milliliter varied between 1,726 and 42,454 (10,699 on average). The highest abundance was recorded in the surface layer of the water column (27,234 units×ml^{-1}). From early spring to autumn algal communities were dominated by *Cyanobacteria*. *Planktothrix agardhii* was detected in all samples collected during summer. Also, *Aphanizomenon flos-aquae*, *Limnotrix redekei* and *Pseudanabaena limnetica* were commonly observed. For further information see figure 2.

241 bacteria isolates were collected during the study. The sequences of the 16S rRNA region were obtained for 114 of them. Among the identified bacteria we found members of the genera: *Acinetobacter*, *Aeromonas*, *Enterobacter*, *Hafnia*, *Pantoea*, *Pseudomonas*, *Shewanella*, *Bacillus*, *Paenibacillus*, *Carnobacterium*, *Exigobacterium*, *Flavobacterium* and *Deinicoccus*. The most often detected species were *Bacillus pumilus*, *Pseudomonas fluorescens* or members of *Aeromonas hydrophila* and *Enterobacter cloacae* complexes. A

full list of phytoplankton and bacterial taxa is presented in table S2 (Electronic Supplementary Material).

The number of colony forming units (cfu) of culturable bacteria varied with sampling date and depth. Generally, larger counts were obtained during summer months. Titer reached a maximum in August 2009 (average count 426 cfu×ml^{-1}) and minimum in December 2009 (average 11 cfu×ml^{-1}). The highest counts were obtained for samples of water collected above the sediment (average 642 cfu×ml^{-1}), but in samples collected between the 1st and the 4th meter they were also relatively high (average 359 cfu×ml^{-1}). The observed abundance of culturable bacteria was much lower at a depth of 5 meters (average 58 cfu×ml^{-1}) and the dropped to 26 in the zone between the 6th and 15th meter (probably because of the predominance of strictly anaerobic microflora).

3.2. Phytoplankton structure and environmental gradients

Monte Carlo tests revealed that temperature, concentration of ammonium and total phosphorus are only independent environmental gradients significantly related to the composition of phytoplankton assemblages ($p < 0.05$, see Table 2).

The contents of ammonium and phosphorus were also correlated with the first of the DCA axes (along with the concentration of PO_4^{3-}, $p<0.001$) and the second axis was associated with temperature, NO_2^- and NO_3^- (see Table 2). Comparison of Monte Carlo test results with correlation coefficients suggests that temperature and ammonium concentration were the two most important independent factors explaining the distribution of phytoplankton species.

Table 2. Results of the forward selection of environmental parameters (Monte Carlo permutation test in RDA, $p < 0.05$ are statistically significant and given in bold).

Parameter	Phytoplankton			Bacteria		
	λ	F	P	λ	F	P
Chl-a	0.02	1.74	0.131	0.26	11.99	0.001
NO_3	0.02	1.28	0.245	0.06	2.96	0.002
NO_2	0.02	1.81	0.102	0.07	3.39	0.016
EC	0.00	0.52	0.771	0.06	3.37	0.007
Depth	0.01	1.35	0.222	0.02	1.38	0.173
pH	0.01	0.43	0.824	0.02	1.13	0.306
OS	0.02	2.44	0.037	0.01	0.75	0.598
DO	0.01	1.49	0.187	0.02	0.64	0.717
Temp	0.04	3.77	0.004	0.01	0.52	0.812
TP	0.10	8.80	0.001	0	0.32	0.965
NH_4	0.08	8.14	0.001	0.01	0.4	0.881
PO_4	0.00	0.48	0.821	0.01	0.49	0.859

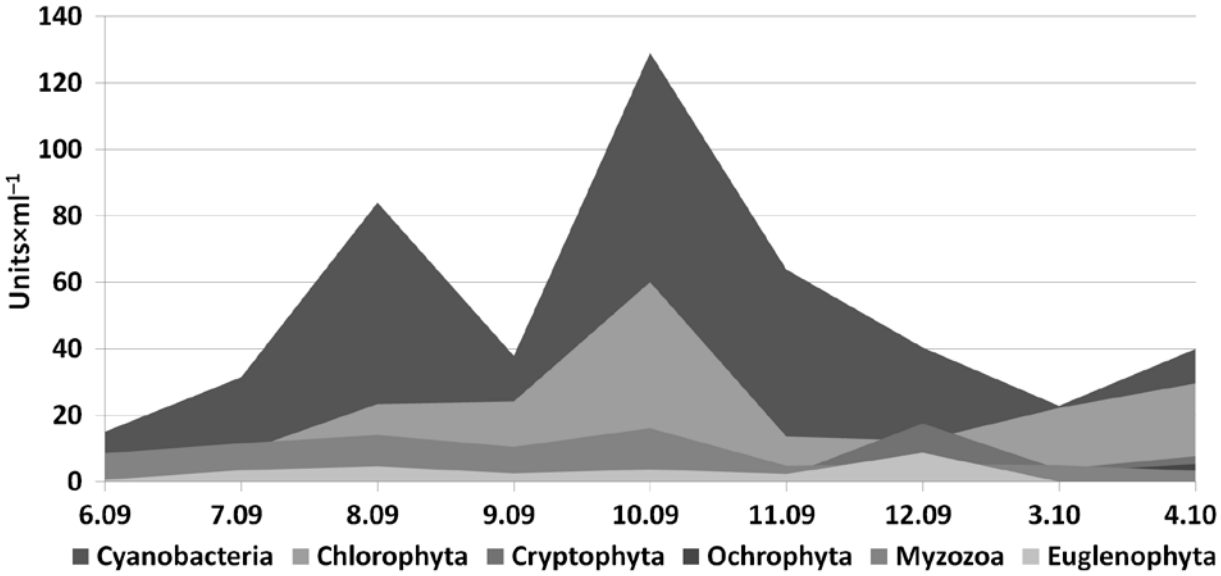

Figure 2. Abundance and taxonomic structure of the phytoplankton community in Lake Góreckie during the study.

RDA biplots for phytoplankton and environmental factors (Fig. 3) showed that the occurrence of *Planktothrix agardhii* (the most abundant species) was connected with high temperatures and substantial oxygenation, but a low level of the nutrients. Two other cyanobacteria: *Chroococcus minutissimus* and *Merismopedia glauca* or the diatom *Fragilaria ulna* could be associated with warm waters, rich in phosphorus and ammonium while chlorophytes *Monoraphidium contortum* and *Koliella spirotaenia* or cryptomonad *Chroomonas acuta* occupied habitats with a high NO_2^- and NO_3^- content.

3.3. Heterotrophic bacteria and environmental factors

According to the results of the Monte Carlo tests (Table 2), nitrate and chlorophyll *a* concentrations are the only independent environmental gradients significantly related to the species structure of heterotrophic bacteria. One pronounced gradient determines the majority of this structure. The first DCA axis representing the gradient correlates with concentrations of NO_2^- and NO_3^- ($p < 0.005$) while the second axis only with conductivity (see Table 3).

RDA analysis revealed the environmental requirements of individual taxa and allowed their distribution into different ecological groups (see Fig. 4). The presence of several *Pseudomonas* species was strongly connected with high concentrations of NO_2^-, oxygen and a more alkaline pH. The occurrence of *Bacillus pumilus*, bacteria from *B. cereus* group, *Enterobacter amnigenus* or *E. aerogenes* seems to be connected with a cool, ammonium and phosphate-rich environment that can be found in greater depths, while *Bacillus megaterium*, *B. simplex*, *Paenibacillus amylolyticus*, *Enterobacter cancerogenus*, *Deinococcus aquaticus* and all detected members of the genus *Exiguobacterium* constitute a cluster of microorganisms associated with high concentrations of chlorophyll a (and thus with phytoplankton), high temperature and more oxygenated water (Fig. 3).

3.4. Relationships between bacteria and phytoplankton

No significant correlation was found between DCA axes of phytoplankton and heterotrophic bacteria (Table 3). Nevertheless, analysis of species-environment relationships using the Monte Carlo test revealed that the concentration of chlorophyll *a* is one of the most important factors relating to the composition of bacterial communities (Table 2, as mentioned above). Also, the presence of several cyanobacteria species (e.g. *Planktothrix agardhii*, *Limnothrix redekei* or *Pseudanabaena limnetica*) was strongly correlated with this parameter, suggesting that they may be a primary source of the pigment (Fig. 3).

Table 3 Pearson's correlation coefficients between DCA ordination scores (first three axes) for phytoplankton (DCA phy1. DCA phy2. DCA phy3), bacteria (DCA bac1. DCA bac2. DCA bac3) and environmental factors (n = 84).

Axis or parameter	Phytoplankton			Bacteria		
	DCA phy1 Eigenvalue: 0.52	DCA phy2 Eigenvalue: 0.18	DCA phy3 Eigenvalue: 0.06	DCA bac1 Eigenvalue: 0.24	DCA bac2 Eigenvalue: 0.15	DCA bac3 Eigenvalue: 0.10
DCA phy1	-	n.s.	n.s.	n.s.	n.s.	n.s.
DCA phy2	n.s.	-	n.s.	n.s.	n.s.	n.s.
DCA phy3	n.s.	n.s.	-	n.s.		n.s.
DCA bac1	n.s.	n.s.	n.s.	-	n.s.	n.s.
DCA bac2	n.s.	n.s.	n.s.	n.s.	-	n.s.
DCA bac3	n.s.	n.s.	n.s.	n.s.	n.s.	-
Depth	-0.59**	n.s.	0.39*	n.s.	n.s.	n.s.
pH	0.44*	n.s.	-0.55**	n.s.	n.s.	n.s.
EC	-0.48*	n.s.	0.50**	n.s.	0.40*	-0.39*
DO	n.s.	n.s.	n.s.	n.s.	n.s.	0.45*
OS	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Temp	n.s.	-0.43*	n.s.	n.s.	n.s.	0.48*
PO_4	-0.79***	n.s.	n.s.	n.s.	n.s.	n.s.
Chl-a	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
NH_4	-0.81***	n.s.	0.56**	n.s.	n.s.	n.s.
NO_2	n.s.	0.60**	-0.47*	0.72***	n.s.	n.s.
NO_3	n.s.	0.65***	0.49*	0.56**	n.s.	n.s.
TP	-0.67***	n.s.	n.s.	n.s.	n.s.	-0.39*

Significance levels: *** $p < 0.001$; ** $p < 0.01$; * $p < 0.05$; n.s. – not significant.

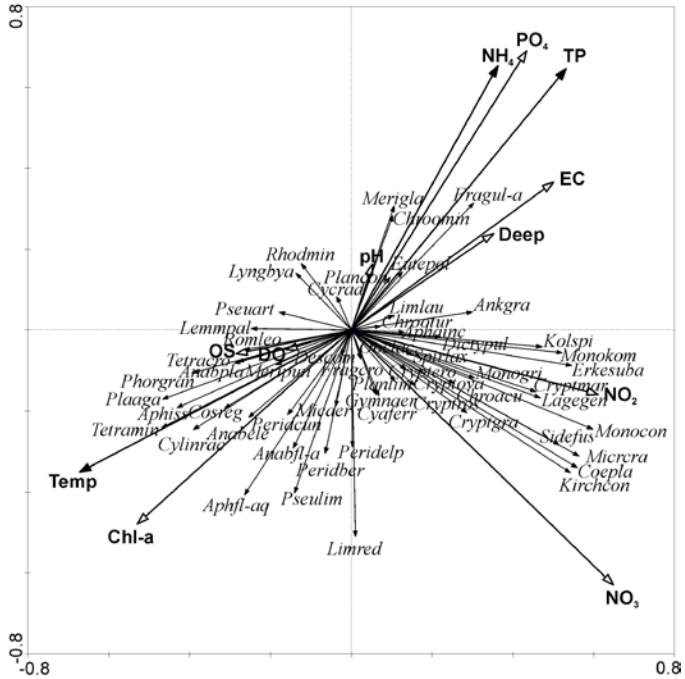

Figure 3. Biplots of redundancy analysis (RDA) of phytoplankton species (only species that reached 5% frequency threshold were shown). The variables that remained significant after forward selection (Temp, TP, NH_4^+) are shown with solid arrowheads, while the variables that projected passively, with hollow arrowheads. Species list: Chroacu – *Chroomonas acuta*, Cryptero – *Cryptomonas erosa*, Cryptgra – *Cryptomonas gracilis*, Cryptmar – *Cryptomonas marssonii*, Cryptova – *Cryptomonas ovata*, Cryptros – *Cryptomonas rostrata*, Rhodmin – *Rhodomonas minuta*, Erkesuba – *Erkenia subaequiciliata*, Gymnaer – *Gymnodinium aeruginosum*, Peridber – *Peridiniopsis berolinense*, Peridcun – *Peridinium cinctum*, Peridelp – *Peridiniopsis elpatiewskyi*, Anabele – *Anabaenopsis elenkinii*, Anabfl-aq – *Anabaena flos-aquae*, Anabpla – *Anabaena planctonica*, Aphfl-aq – *Aphanizomenon flos-aquae*, Aphiss – *Aphanizomenon issatschenkoi*, Aphainc – *Aphanocapsa incerta*, Chroominu – *Chroococcus minimus*, Chrootur – *Chroococcus turgidus*, Cyaferr – *Cyanogranis ferruginea*, Cylinraci – *Cylindrospermopsis raciborski*, Lemmpal – *Lemmermaniella pallida*, Limlau – *Limnothrix lauterbornii*, Limred – *Limnothrix redekei*, Merigla – *Merismopedia glauca*, Meripun – *Merismopedia punctata*, Micaer – *Microcystis aeruginosa*, Phorgran – *Phormidium granulatum*, Plancon – *Planktolyngbya contorta*, Planlim – *Planktolyngbya limnetica*, Plaaga – *Planktothrix agardhii*, Lyngbya – *Lyngbya* sp., Pseulim – *Pseudanabaena limnetica*, Pseuart – *Pseudanabaena articulata*, Romleo – *Romeria leopoliensis*, Spirlax – *Spirulina laxissima*, Cycrad – *Cyclotella radiosa*, Fragcro – *Fragilaria crotonensis*, Fragul-ang – *Fragilaria ulna* var. *angustissima*, Ankgra – *Ankistrodesmus gracilis*, Coepla – *Coenocystis planctonica*, Cosreg – *Cosmarium regnellii*, Dictypul – *Dictyosphaerium pulchellum*, Eutepol – *Eutetramorus polycoccus*, Kirchcon – *Kirchneriella contorta*, Kolspi – *Koliella spirotaenia*, Lagegen – *Lagerheimia genevensis*, Micrcra – *Micractinium crassisetum*, Monocon – *Monoraphidium contortum*, Monogri – *Monoraphidium griffithii*, Monokom – *Monoraphidium komarkovae*, Ooclac – *Oocystis lacustris*, Descom – *Desmodesmus communis*, Sidefus – *Siderocystopsis fusca*, Tetracro – *Tetradesmus crocini*, Tetramin – *Tetraedron minimum.*

4. DISCUSSION

4.1. Bacteria isolation and identification techniques

Culturable bacteria usually represent only a fraction of microbial diversity (in many cases less than 1%) and culture-dependent methods usually fail to detect important freshwater taxa including many aquatic *Actinobacteria*, *Alphaproteobacteria*, *Betaproteobactertia* and *Verrucomicrobia* (Muyzer et al., 1993; Amann et al., 1995; Pernthaler & Amann, 2005; Newton et al., 2011).

On the other hand, results of comparative studies using culture-dependent as well as independent methods show that culturable bacteria are as responsive to environmental gradients and changes as the rest of community (Kisand & Wikner, 2003; Camu et al., 2008; Rodrigues et al., 2013). This suggest that simple and inexpensive culture-dependent approach may be sufficient for the purposes of some ecological studies that aim in understanding of relationships and changes rather than the diversity itself.

To sum up, obtaining a complete picture of a lake microflora would require broader approach, but chosen methodology allows to draw meaningful conclusions concerning relationships between environmental variables, phytoplankton and certain groups of bacteria.

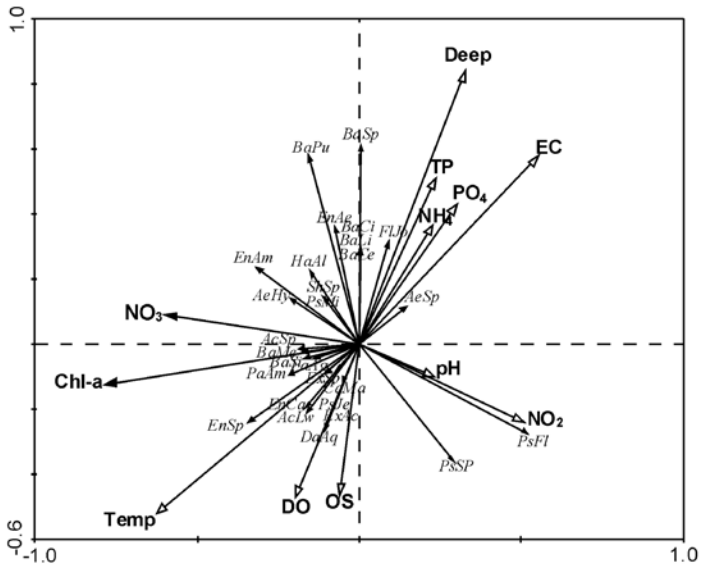

Figure 4. Biplots of redundancy analysis (RDA) of bacteria species. The variables that remained significant after forward selection (NO_3^-, Chl-a) are shown with solid arrowheads, while the variables that projected passively, with hollow arrowheads. Species list: AcLw – *Acinetobacter lwoffii*, AcSp – *Acinetobacter* sp., AhGr – *Aeromonas hydrophila* group, BaLi – *Bacillus licheniformis*, BaMe – *Bacillus megaterium*, BaPu – *Bacillus pumilus*, BaSi – *Bacillus simplex*, BaSp – *Bacillus* sp., BcGr – *Bacillus cereus* group, CaMa – *Carnobacterium maltaromaticum*, CiKo – *Citrobacter koseri*, DaAq – *Deinococcus aquaticus*, EcCc – *Enterobacter cloacae* complex, EnAe – *Enterobacter aerogenes*, EnCa – *Enterobacter cancerogenus*, EnSp – *Enterobacter* sp., EsCo – *Escherichia coli*, ExAc – *Exiguobacterium acetylicum*, ExSi – *Exiguobacterium sibiricum*, ExSp – *Exiguobacterium* sp., FlJo – *Flavobacterium johnsoniae*, HaAl – *Hafnia alvei*, MoMo – *Morganella morganii*, PaAg – *Pantoea agglomerans*, PaAm – *Paenibacillus amylolyticus*, PsAe – *Pseudomonas aeruginosa*, PsFl – *Pseudomonas fluorescens*, PsJe – *Pseudomonas jessenii*, PsMa – *Pseudomonas mandelii*, PsMi – *Pseudomonas migulae*, PsSP – *Pseudomonas* sp., ShBa – *Shewanella baltica*, ShSp – *Shewanella* sp., SpKi – *Sphingobacterium kitahiroshimense.*

4.2. Phytoplankton along the ecological gradients

Overall distribution of phytoplankton species was shaped mostly by temperature, total phosphorus and the ammonium concentration. The later relation could be explained by the fact that, while some cyanobacteria (e.g. *Aphanizomenon flos-aquae*) utilize dinitrogen, many phytoplankton species preferably consume the ammonium instead (South & Whittick, 1996; Whitton & Potts, 2000; Ferber et al., 2004).

Almost constant blooms of cyanobacteria become one of the hallmarks of the studied reservoir. At the time of the summer stratification *Planktothrix agardhii* blooms in well-oxygenated surface zones of the lake. During spring and autumn it is replaced by *Aphanizomenon flos-aquae* and *Pseudanabaena limnetica* and these species (usually found in colder, rich in nutrients deep zones of the lake) dominate in the whole vertical profile (Pełechata et al., 2009). This scenario is reflected in the results of redundancy analysis. They indicate that the occurrence of *Planktothrix agardhii* is connected with high temperatures, while presence of *Aphanizomenon flos-aquae* and *Pseudanabaena limnetica* seems to be dependent on high nitrate concentrations.

4.3. Culturable heterotrophic bacteria along the ecological gradients

Observed composition of bacterial communities suggest that we couldn't avoid the bias typical for all culture-dependent studies. As expected, neither *Actinobacteria*, *Alphaproteobacteria*, *Betaproteobactertia* nor *Verrucomicrobia* were detected – these bacteria are abundant in freshwater habitats but relatively difficult to culture (Newton et al., 2011). The cause of apparent underrepresentation of phylum *Bacteroidetes* (as compared to studies using culture independent approaches) may be similar. Relative abundance of *Gammaproteobacteria* may be connected with copiotrophic lifestyle that favors their disproportionate isolation (Zavarzin et al., 1991; Newton et al., 2011). On the other hand, large number of detected members of phylum *Firmicutes* may not be incidental. We cannot rule out that they are overrepresented (due to their nutrient preferences or ability of some members to form endospores) but there are indications that these bacteria are undersampled in many 16S rDNA libraries. Resilient gram-positive cell wall seems to hinder DNA extraction and hence they may be omitted in studies

adhering solely to culture independent methodology (Frostegård et al., 1999; Pernthaler & Amann, 2005).

Structure of culturable fraction of the bacterial community was predominantly dependent on the gradient set by concentrations of NO_2^- and NO_3^-. Importance of these ions (and other forms of inorganic nitrogen) was previously highlighted by studies assessing bacteria community composition in eutrophic and meso-eutrophic lakes (Shade al., 2007; Rösel et al., 2012). Relationship between bacterioplankton composition and the content of oxidized nitrogen forms may be connected with the ability of some bacteria (including the members of the genera *Bacillus* and *Pseudomonas* that represented 25.7% and 14.8% of our identifications) to utilize NO_2^- and NO_3^- for respiratory purposes. This seems to be the case, especially considered the anaerobic conditions commonly found in deeper zones of eutrophic lakes.

4.4. Relationships between phytoplankton and bacteria

During the study, phytoplankton and culturable heterotrophic bacteria responded to environmental gradients in a roughly independent manner. While distribution of both groups was connected with nitrogen content, they seem to rely on different forms of this element (NH_4^+ and NO_3^- for phytoplankton; NO_2^- and NO_3^- for bacteria). This suggests that production of the studied communities may be determined by an interplay between nitrogen fixation by cyanobacteria and coupled nitrification/denitrification. It remains unclear what may shape the equilibrium between these processes. In case of the studied lake it might be the above mentioned factors that control microbial communities in bottom-up fashion. On the other hand, balance may also be regulated by top-down acting factors like zooplankton grazing or viral lysis – phage epidemic that affected one of *Bacillus* strains was even observed in spring and summer of 2010 (Barylski et al., 2014).

Information available up to date support the hypothesis that the phytoplankton assemblages strongly affect heterotrophic bacteria and *vice versa* (Eiler & Bertilsson, 2004; Berg et al., 2009; Mazur-Marzec et al., 2009; Šimek et al., 2011; Leão et al., 2012). One mechanism of such influence is connected with the release of extracellular products (EPP) by the autotrophic organisms (Baines & Pace, 1991; Sundh, 1992). Since solitary filamentous species such as *Planktothrix agardhii*, *Limnothrix redekei* and *Pseudanabaena limnetica* are adapted to small light intensity, compounds released from their cells may be a significant source of carbon for bacteria in dark waters of the studied lake.

Studies concerning cyanobacteria-associated heterotrophic bacteria often emphasize importance of *Bacteroidetes*, *Betaproteobactertia* (especially from genus *Limnohabitans*) and *Alphaproteobacteria* or (less frequently) *Actinobacteria* and *Gammaproteobacteria* (Eiler & Bertilsson, 2004; Berg et al., 2009; Šimek et al., 2011; Eiler et al., 2012; Bagatini et al., 2014). During this study we identified a group of culturable microorganisms connected with high concentrations of chlorophyll *a* and thus with presence of the phytoplankton. The group of chlorophyll-related bacteria comprises members of the genera *Bacillus*, *Exiguobacterium* and *Deinococcus*. Related strains had previously been associated with cyanobacteria. They were reported either as promoting their growth (Berg et al., 2009; Ya-Ping et al., 2010) or, to the contrary, inhibiting it and killing their cells (Wright & Thompson, 1985; Nakamura et al., 2003; Berg et al., 2009).

5. CONCLUSIONS

Paradoxical as it may seem, in the nitrogen-rich environment of a eutrophic lake nitrogen may still be a limiting factor for the development of some phytoplankton and bacterial taxa. This phenomenon can be explained as a result of the dynamic equilibrium between the energy consuming process of nitrogen fixation and the removal of this element *via* coupled nitrification/denitrification. Our work highlights the importance of microbial communities as driving factors of the nitrogen cycling. It is the next step towards an understanding of the functioning of the microbial food web in eutrophic lakes, an understanding crucial in the context of the protection and management of these ecosystems.

Acknowledgements

We thank the Administration of Wielkopolski National Park for support and cooperation during this study and Tomasz Sobkowiak for help in improving the manuscript. This work was supported by a grant from the Polish Ministry of Science and Higher Education (No. N N303 391136).

REFERENCES

Amann, R.I., Ludwig W. & Schleifer, K.H., 1995. *Phylogenetic identification and in situ detection of individual microbial cells without cultivation.* Microbiol. Rev. 59, 1, 143-169.

APHA (American Public Health Association), 1998. *Standard methods for the examination of water and wastewater*. Washington, DC.

Arhonditsis, G.B. & Brett, M.T., 2005. *Eutrophication model for Lake Washington (USA): Part I. Model description and sensitivity analysis.* Ecol. Modell., 187, 140-178.

Azam, F., 1998. *Microbial control of oceanic carbon flux: The Plot Thickens.* Science, 280, 5364, 694-696.

Azam, F., Fenchel, T., Field, J.G., Gray, J.S., Meyer-Reil, L.A. & Thingstad, F., 1983. *The ecological role of water-column microbes in the sea.* Mar. Ecol. Prog. Ser., 10, 257-263.

Bagatini, I.L., Eiler, A., Bertilsson, S., Klaveness, D., Piton Tessarolli, L. & Henriques Vieira, A.A., 2014. *Host-specificity and dynamics in bacterial communities associated with bloom-forming freshwater phytoplankton.* PLoS ONE, 9, 1, doi: 10.1371/journal.pone.0085950.

Baines, S.B. & Pace, M.L., 1991. *The production of dissolved organic matter by phytoplankton and its importance to bacteria: patterns across marine and freshwater systems.* Limnol. Oceanogr., 36, 1078-1090.

Barylski, J., Nowicki, G. & Goździcka-Józefiak, A., 2014. *The discovery of phiAGATE, a novel phage infecting Bacillus pumilus, leads to new insights into the phylogeny of the subfamily Spounavirinae.* PLoS One, doi: 10.1371/journal.pone.0086632.

Berg, K.A., Lyra, C., Sivonen, K., Paulin, L., Suomalainen, S., Tuomi, P. & Rapala, J., 2009. *High diversity of cultivable heterotrophic bacteria in association with cyanobacterial water blooms.* ISME J., 3, 3, 314-325.

Bucka, H. & Wilk-Woźniak, E. 2002. *Monografia. Gatunki kosmopolityczne i ubikwistyczne wśród glonów pro- i eukariotycznych występujących w zbiornikach wodnych Polski południowej. (A monograph of cosmopolitan and ubiquitous species among pro- and eukaryotic alga from waterbodies in southern Poland).* Zakład Biologii Wód im. K. Starmach PAN Press, Kraków, 233 pp.

Burchardt, L. 2014. *Key to identification of phytoplankton species in lakes and rivers. Guide for laboratory classes and field research.* W. Szafer Institute of Botany Press, Cracow, 175 pp.

Burchardt, L., Cerbin, S., Kuczyńska-Kippen, N., Messyasz, B., Nagengast, B. & Pelechaty, M. 2000. *Wpływ zlewni rolniczej na stan biologiczny dwóch jezior Wielkopolskiego Parku Narodowego (Impact of an agricultural drainage area on the biological state of two lakes of the Wielkopolski National Park),* pp. 227-234. In: Radwan, S. & Lorkiewicz, Z. (eds), *Problemy ochrony i użytkowania obszarów wiejskich o dużych walorach przyrodniczych (Problems of the protection and use of rural areas of high environmental value).* UMCS Press, Lublin.

Camu, N., González, A., De Winter, T., Van Schoor, A., De Bruyne, K. & Vandamme, P., 2008. *Influence of turning and environmental contamination on the dynamics of populations of lactic acid and acetic acid bacteria involved in spontaneous cocoa bean heap fermentation in Ghana.* Appl. Environ. Microbiol., 74, 1, 86-98.

Caron, A.D., 1994. *Inorganic nutrients, bacteria, and the microbial loop.* Microb. Ecol., 28, 295-298.

Cho, B.C. & Azam, F., 1990. *Biogeochemical significance of bacterial biomass in the ocean's euphotic zone.* Mar. Ecol. Prog. Ser., 63, 253-259.

Conley, D.J., Paerl, H.W., Howarth, R.W., Boesch, D.F., Seitzinger, S.P., Havens, K.E., Lancelot, C. & Likens, G.E., 2009. Ecology. *Controlling eutrophication: nitrogen and phosphorus.* Science, 323, 5917, 1014-1015.

Drakare, S., 2002. *Competition between picoplanktonic cyanobacteria and heterotrophic bacteria along crossed gradients of glucose and phosphate.* Microb. Ecol., 44, 4, 327-335.

Edler, L., 1979. *Recommendations for marine biological studies in the Baltic Sea: Rep. of UNESCO Working Group 11*, Marine Biologists. National Swedish Environment Protection Board, Stockholm, 38 pp.

Edwards, U., Rogall, T., Blöcker, H., Emde, M. & Böttger, E.C., 1989. *Isolation and direct complete nucleotide determination of entire genes. Characterization of a gene coding for 16S ribosomal RNA.* Nucleic Acids Res., 17, 19, 7843-7853.

Eiler, A., Heinrich, F. & Bertilsson, S., 2012. *Coherent dynamics and association networks among lake bacterioplankton taxa.* ISME J., 6, 2, 330-342.

Eiler, A. & Bertilsson, S., 2004. *Composition of freshwater bacterial communities associated with cyanobacterial blooms in four Swedish lakes.* Environ. Microbiol., 6, 12, 1228-1243.

Ferber, L.R., Levine, S.N., Lini, A. & Livingston, G.P., 2004. *Do cyanobacteria dominate in eutrophic lakes because they fix atmospheric nitrogen?* Freshw. Biol., 49, 6, 690-708.

Fisher, L.H. & Wood, T.M., 2004. *Effect of water-column pH on sediment-phosphorus release rates in Upper Klamath Lake, Oregon, 2001.* U.S. Geological Survey Water-Resources Investigations Report 034271.

Frostegård, A., Courtois, S., Ramisse, V., Clerc, S., Bernillon, D., Le Gall, F., Jeannin, P., Nesme, X. & Simonet, P., 1999. *Quantification of bias related to the extraction of DNA directly from soils.* Appl. Environ. Microbiol., 65, 5409-5420.

Gołdyn, R., Messyasz, B., Domek, P., Windhorst, W., Hugenschmidt, C., Nicoara, M. & Plavan, G., 2013. *The response of Lake Durowskie ecosystem to restoration measures*. Carpathian Journal of Earth and Environmental Sciences, 8, 3, 43-48.

Harris, P.G., 1978. *Photosynthesis, productivity and growth: the physiological ecology of phyoplankton.* Ergebnisse der Limnologie, 10, 1-171.

Kajak, Z., 2001. *Hydrobiologia – limnologia: ekosystemy wód śródlądowych. (Hydrobiology - limnology: ecosystems of inland waters, book in Polish).* Adam Mickiewicz University Scientific Press, Poznań, 259 pp.

Kajak, Z., Kacprzak, K. & Polkowski, R., 1965. *Tube bottom sampler for taking samples of micro and macro benthos, and for sampling of undisturbed structures of mud samples for experimental purposes.* Ekologia Polska B, 159-165.

Kent, A.D., Yannarell, A.C., Rusak, J.A., Triplett, E.W. & McMahon, K.D., 2007. *Synchrony in aquatic microbial community dynamics.* ISME J., 1, 38-47.

Kisand, V. & Wikner, J., 2003. *Combining culture-dependent and – independent methodologies for estimation of richness of estuarine bacterioplankton consuming riverine dissolved organic matter.* Appl. Environ. Microbiol., 69, 6, 3607-3616.

Kolendowicz, L., Hanke, J., Kaczmarek, L. & Lorenc, M., 2008. *Zmiany poziomu wody Jeziora Góreckiego (Wielkopolski Park Narodowy) w latach 2002-2007 na tle wahania poziomu wód wielkopolskiej doliny kopalnej i warunków atmosferycznych (Changes of water level in Góreckie Lake (Wielkopolski National Park) in the years 2002-2007 against the background of water level variation in the Wielkopolska buried valley and atmospheric condition),* pp. 159-160. In: Pociask-Karteczka, J. & Partyka, J. (eds), Wody na obszarach chronionych (Waters of protected areas) Jagiellonian University Press, Kraków.

Leão, P.N., Ramos, V., Vale, M., Machado, J.P. & Vasconcelos, V.M., 2012. *Microbial community changes elicited by exposure to cyanobacterial allelochemicals.* Microb. Ecol., 63, 1, 85-95.

Lubner, H., 1988. *Threats to the water environment of the Wielkopolska National Park and its buffer zone posed by wastewater,* pp. 12. In: Dąmbska, I., Bałazy, S. & Pawuła, R. (eds), Wielkopolski National Park. Problems of protection and development of the natural environment. PWN, Warszawa-Poznań.

Lyche Solheim, A., Austnes, K., Peterlin, M., Kodeš, V., Filippi, R., Semerádová, S., Prchalová, H., Künitzer, A., Spiteri, C., Prins, T., Collins, R.P. & Kristensen, P., 2012. *Ecological and chemical status and pressures in European waters ETC/ICM Technical Report 1/2012.* European Topic Centre on Inland, Coastal and Marine waters.

Mazur-Marzec, H., Toruńska, A., Błońska, M.J., Moskot, M., Pliński, M., Jakóbkiewicz-Banecka, J. & Wegrzyn, G., 2009. *Biodegradation of nodularin and effects of the toxin on bacterial isolates from the Gulf of Gdansk.* Water Res., 43, 11, 2801-2810.

Muyzer, G., de Waal, E.C. & Uitterlinden, A.G., 1993. *Profiling of complex microbial populations by denaturing gradient gel electrophoresis analysis of polymerase chain reaction-amplified genes coding for 16S rRNA.* Appl. Environ. Microbiol., 59, 3, 695-700.

Nakamura, N., Nakano, K., Sugiura, N. & Matsumura, M., 2003. *A novel cyanobacteriolytic bacterium, Bacilluscereus, isolated from a eutrophic lake.* J. Biosci. Bioeng., 95, 2, 179-184.

Newton, R.J., Jones, S.E., Eiler, A., McMahon, K.D. & Bertilsson, S., 2011. *A guide to the natural history of freshwater lake bacteria.* Microbiol. Mol. Biol. Rev., 75, 14-49.

Pełechata, A., Walna, B., Pełechaty, M., Kaczmarek, L., Ossowski, P. & Lorenc, M., 2009. *Sezonowa dynamika zbiorowiska glonów i sinic planktonowych Jeziora Góreckiego na tle cech fizyczno-chemicznych wód powierzchniowych i stopnia rozwoju makrofitow. (Seasonal dynamics of phytoplankton assemblages of lake Góreckie in context of surface water physicochemistry and macrophytes developement),* pp. 27-42. In: Kaczmarek, L., Lorenc, M. & Dondajewska, R. (eds), Wielkopolski Park Narodowy w badaniach przyrodniczych (Wielkopolski National Park in Natural Sciences). Adam Mickiewicz University Scientific Press, Poznań-Jeziory.

Pernthaler, J. & Amann, R., 2005. *Fate of heterotrophic microbes in pelagic habitats: focus on populations.* Microbiol. Mol. Biol. Rev., 69, 440-461.

Pop, A.I., Maihăiescu, R., Maihăiescu, T., Oprea, M.G., Tănăselia, C. & Ozunu, A., 2013. Physico-chemical properties of some glacial lakes in the Romanian Carpathians. Carpathian Journal of Earth and Environmental Sciences, 8, 4, 5-11.

Qian, Y., Shi, J., Chen, Y., Lou, L., Cui, X., Cao, R., Li, P. & Tang, J., 2010. *Characterization of phosphate solubilizing bacteria in sediments from a shallow eutrophic lake and a wetland: isolation, molecular identification and phosphorus release ability determination.* Molecules, 15, 11, 8518-8533.

Reynolds, S.C., 1987. *Community organisation in the freshwater plankton,* pp. 297-325. In: Gee, M. & Giller, P.S. (eds), Organization of communities, past and present. Blackwell Scientific Publications, Oxford.

Reynolds, S.C., 1980. *Phytoplankton assemblages and their periodicity in stratifying lake systems.* Holarctic Ecol., 3, 141-159.

Rodrigues, D.F., Jaisi, D.P. & Elimelech, M., 2013. *Toxicity of functionalized single-walled carbon nanotubes on soil microbial communities: implications for nutrient cycling in soil.* Environ. Sci. Technol., 47, 1, 625-633.

Rösel, S., Allgaier, M. & Grossart, H.P., 2012. *Long-term characterization of free-living and particle-associated bacterial communities in Lake Tiefwaren reveals distinct seasonal patterns.* Microb. Ecol., 64, 3, 571-583.

Rott, E., 1981. *Some results from phytoplankton counting intercalibrations*. Schweiz. Z. Hydrol., 43, 1, 34-62.

Sapp, M., Schwaderer, A.S., Wiltshire, K.H., Hoppe, H.G., Gerdts, G. & Wichels, A., 2007. *Species-specific bacterial communities in the phycosphere of microalgae?* Microb. Ecol., 53, 4, 683-699.

Sayers, E.W., Barrett, T., Benson, D.A., Bolton, E., Bryant, S.H. & Canese, K., 2009. *Database resources of the National Center for Biotechnology Information. Nucleic Acids Res.* 41 (Database

issue), D8-D20.

Schmidt, T.M. & Schaechter, M., 2011. *Topics in ecological and environmental microbiology.* Academic Press, San Diego, 102 pp.

Shade, A.L., Kent, A.D., Jones, S.E., Newton, R.J., Triplett, E.W. & McMahon, K.D., 2007. *Interannual dynamics and phenology of bacterial communities in a eutrophic lake.* Limnol. Oceanogr., 52, 487-494.

Shaw, G.R., Moore, D.P. & Garnett, C., 2003. *Eutrophication and algal blooms.* In: Sabljic, A. (ed) *Encyclopedia of Life Support Systems (EOLSS).* Developed under the Auspices of the UNESCO, Eolss Publishers, Oxford, 21 pp.

Shen, H., Niu, Y., Xie, P., Tao, M. & Yang, X., 2011. *Morphological and physiological changes in Microcystis aeruginosa as a result of interactions with heterotrophic bacteria*. Freshw. Biol., 56, 6, 1065-1080.

Sigee, D.C., 2005. *Freshwater microbiology: biodiversity and dynamic interactions of microorganisms in the aquatic environment.* John Wiley, Sons, Chichester, 517 pp.

Šimek, K., Kasalický, V., Zapomělová, E. & Hornák, K., 2011. *Alga-derived substrates select for distinct Betaproteobacterial lineages and contribute to niche separation in Limnohabitans strains.* Appl. Environ. Microbiol., 77, 20, 7307-7315.

Sobczyński, T. & Joniak,T., 2009. *Vertical changeability of physical-chemical features of bottom sediments in three lakes in aspect type of water mixis and intensity of human impact.* Pol. J. Environ. Stud., 18, 6, 1093-1099.

Sobczyński, T., Joniak, T. & Pronim, E., 2012. *Assessment of the multi-directional experiment to restore Lake Góreckie (Western Poland) with particular focus on oxygen and light conditions: first results.* Pol. J. Environ. Stud., 21, 4, 1025-1031.

South, R.G. & Whittick, A., 1996. *Introduction to Phycology.* Oxford, London, Edinburgh: Blackwell Scientific Publications, 341 pp.

Sundh, I., 1992. *Biochemical composition of dissolved organic carbon derived from phytoplankton and used by heterotrophic bacteria*. Appl. Environ. Microbiol. 58, 2938-2947.

ter Braak, C.J., 1998. *CANOCO - an extension of DECORANA to analyze species-environment relationships.* Hydrobiologia, 184, 3, 169-170.

Tijdens, M., Hoogveld, H.L., Kamst-van Agterveld, M.P., Simis, S.G., Baudoux, A.C. & Laanbroek, H.J., 2008. *Population dynamics and diversity of viruses, bacteria and phytoplankton in a shallow eutrophic lake.* Microb. Ecol., 56, 1, 29-42.

Vymazal, J., 2007. *Removal of nutrients in various types of constructed wetlands.* Sci. Total. Environ., 380, 1-3, 48-65.

Weisburg, W.G., Barns, S.M., Pelletier, D.A. & Lane, D.J., 1991. *16S ribosomal DNA amplification for phylogenetic study*. J. Bacteriol., 173, 2, 697-703.

Wetzel, G.R., 2001. *Limnology*. Academic Press, San Diego, 1006 pp.

Whitman, W.B., Coleman, D.C. & Wiebe, W.J., 1998. *Prokaryotes: the unseen majority*. Proc. Natl. Acad. Sci. USA, 95, 12, 6578-6583.

Whitton, B.A. & Potts, M., 2000. *The ecology of cyanobacteria. Their diversity in time and space.* Kluwer Academic Publishers, Dordrecht, 669 pp.

Wilhelm, S.W., Farnsley, S.E., LeCleir, G.R., Layton, A.C., Satchwell, M.F., DeBruyn, J.M., Boyer, G.L., Zhu, G.W. & Paerl, H.W., 2011. *The relationships between nutrients, cyanobacterial toxins and the microbial community in Taihu (Lake Tai), China.* Harmful Algae, 10, 2, 207-215.

Wright, S.J. & Thompson, R.J., 1985. *Bacillus volatiles antagonize cyanobacteria.* FEMS Microbiol. Lett., 30, 3, 263-267.

Yang, X.E., Wu, X., Hao, H.L. & He, Z.L., 2008. *Mechanisms and assessment of water eutrophication.* J. Zhejiang Univ. Sci. B. (Science B), 9, 3, 197-209.

Ya-Ping, S., Hong, Z., Dao-Sen, G. & Rong-Gui, L., 2010. *Isolation and Identification of a Bacterium Strain Promoting the Growth of Cyanobacterium (article in Chinese).* J. Qingdao Univ., 25, 2, 41-46.

Zavarzin, G.A., Stackebrandt, E. & Murray, R.G., 1991. *A correlation of phylogenetic diversity in the Proteobacteria with the influences of ecological forces.* Can. J. Microbiol., 37, 1-6.

Zhang, Z., Schwartz, S., Wagner, L. & Miller, W., 2000. *A greedy algorithm for aligning DNA sequences.* J. Comput. Biol., 7, 1-2, 203-214.

Received at 18. 08. 2014
Revised at: 12. 11. 2014
Accepted for publication at: 17. 11. 2014
Published online at: 20. 11. 2014