"BABES-BOLYAI" UNIVERSITY

Department of Molecular Biology and Biotechnology,

Faculty of Biology and Geology

Summary of the PhD thesis

STUDIES ON PICOPHYTOPLANKTON BIODIVERSITY IN SOME ROMANIAN SALT LAKES

PhD Coordinator
Prof. Dr. Dragoş Nicolae

PhD Student
Keresztes Zsolt Gyula

Cluj-Napoca

2012

KEYWORDS: Picophytoplankton, hypersaline lakes, DGGE, *Synechococcus, Picochlorum*

THEORETICAL BACKGROUND

Picophytoplankton (PPP) includes picocyanobacteria and eukaryotic cells within the size range of 0,2-2 µm disposing photosynthetic pigments, they are distributed worldwide and ubiquitous in all types of lakes and oceans having different trophic state (Stockner, 1991). Eukaryotic picophytoplankton cells are generally larger than picocyanobacterial cells, due to their more complex internal cell structures (Callieri, 2007), (**Fig. 1**)

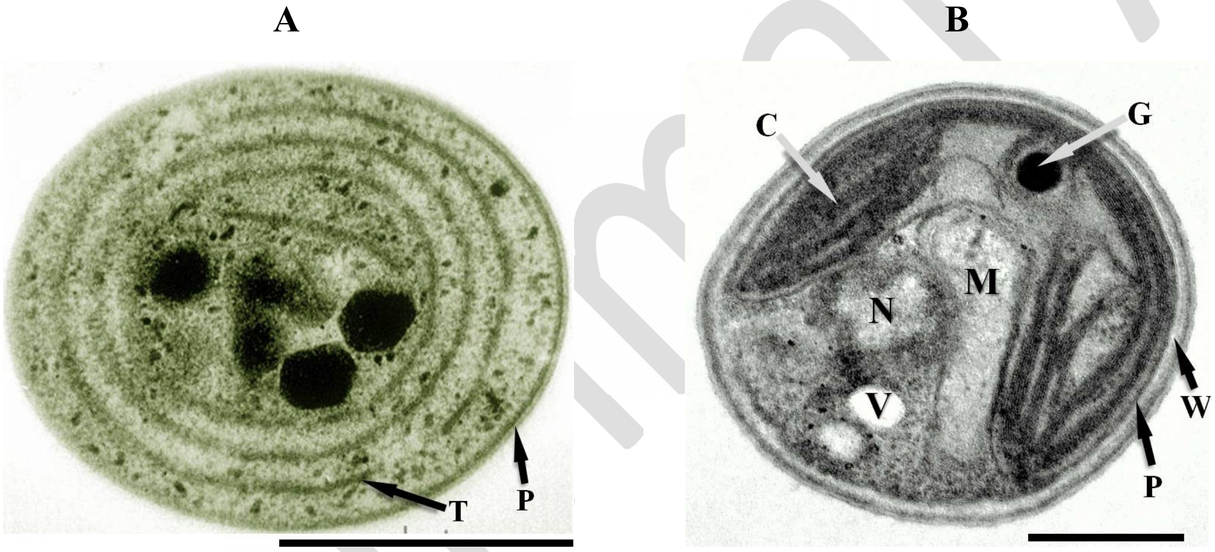

Figure 1. Transmission electron micrographs of *Synechococcus sp.* (A), (Terry Beveridge, 2012) and *Chloroparva pannonica* (B), (Somogyi et al., 2011), C = chloroplast; W = cell wall; T= Thylacoid membranes M = mitochondrion; N = nucleus; G = plastoglobule; P= plasma membrane; V = vacuole, scale bar = 0,5 µm.

The widespread occurrence of pico-sized algae was discovered in the early 1980s, thanks to the new techniques of epifluorescence microscopy and flow cytometry (Callieri and Stockner, 2002). Using these techniques it was possible to quantify PPP routinely. Three cell types were found: yellow autofluorescing phycoerythrin (PE) rich picocyanobacteria and red autofluorescing phycocyanine (PC) rich picocyanobacteria and picoeukaryotes. The picophytoplankton term is generally used for solitary living unicells, however in freshwater environments microcolonies (up to 50 cells) of non blooming species are included in the picophytoplankton (Stockner et al., 2000; Callieri, 2007). The colonial forms of the PPP are more typical for lakes, in seas and oceans the presence of colonial PPP is not characteristic (MacIsaac & Stockner, 1993). The cell size of the eukaryotic PPP usually is greater than the cells size of the picocyanobacteria, but generally the abundance of the picoeukaryotes is smaller with an order of magnitude (Callieri, 2007). In oceans and seas the abundance of the PPP generally is 1.5-3.5 times smaller than in lakes with similar trophic conditions (Bell & Kalff, 2001).

The underwater light climate is a major influencing factor in the occurrence and distribution of picocyanobacteria. In water bodies the underwater light quality rapidly changes with depth because of the spectral selectivity of water itself and of the dissolved and particulated material suspended in it (Kirk, 1992). The underwater light spectrum is one of the key element which determines the photosynthetical pigment dominance of picocyanobacteria. In oligo- and mesotrophic lakes and in the pelagial zone of the oceans, the blue light absorbing phycoerythrin-rich picocyanobacteria are dominant, while in eutrophic conditions (shallow lakes, fish ponds, littoral zones) the phycocyanin-rich picocyanobacteria are the dominant prokaryotic PPP (Vörös et al., 1998; Stomp et al., 2007). Beside the light spectrum the light intensity also determines the phytoplankton distribution in aquatic environment.

The picophytoplankton has a great advantage against the micro- and nanoplankton due to their small size. The intracellular photosyntethic pigment concentration is decreasing with increased cell size (Bricaud et al., 1988) to prevent the autoshading effect of the chloroplasts. From this reason smaller cells absorb more light in respect to their volume than bigger cells and have competitive advantages in light limited environments (Agustí, 1991). The simple structure and small cell size have a decisive role in the ecological success of the PPP in nutrient poor, oligotrophic environments.

It was found that the relative importance (percent of the PPP biomass in the total phytoplankton biomass) of the PPP declines with the increasing trophic of status of the aquatic environment both marine waters and lakes (**Fig. 4**, Bell & Kalff, 2001).

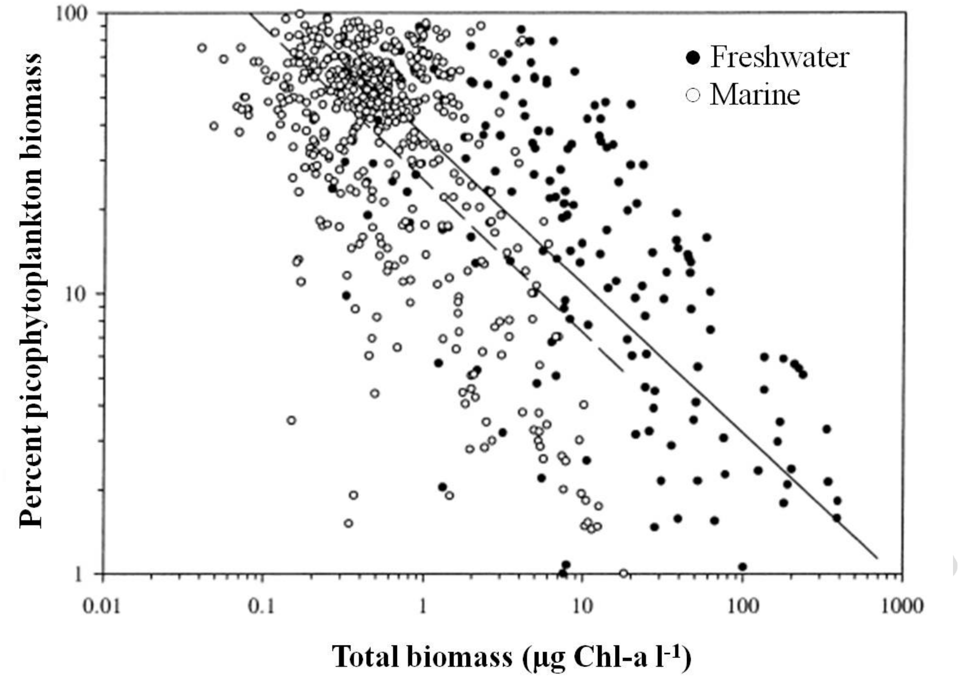

Figure 4. Relationship between the percent contribution of picophytoplankton biomass to total phytoplankton biomass and total phytoplankton biomass (µg Chl-a L^{-1}). The solid regression line is for freshwater data, and the dashed line is for marine data (Bell & Kalff, 2001).

Methodological problems of the picophytoplankton investigation

In many cases, morphological features of picophytoplankton are insufficiently distinct to provide any basis for taxonomic discrimination (Callieri, 2007). Ultrastructural studies, ecological analysis and, in particular, molecular methods have substantially improved the criteria for classification of the PPP. The combination of molecular and ecophysiological approaches offers the most favorable approach to understanding picophytoplankton diversity (Callieri, 2007).

Saline lakes in the Transylvanian-Basin

In the Transylvanian Basin a thick salt stratum was formed during the Badenian period (middle Miocene), at this time the Transylvanian Basin partly separated from the Paratethys sea and the precipitation of high purity salt (NaCl) has begun. Nowadays it is a single, concave shaped salt layer in the Transylvanian Basin in different depth and variable thickness. The thickness of the salt horizon ranges between a few meters to a couple of hundred meters, with an average of 250-300 m. The salt stratum is situated at the surface or near the surface in the edges of the basin, while on the middle of the basin is approximately at 2-4 km depth (Irimuş, 1998, Alexe, 2010). In the Transylvanian Basin saline lakes emerged in high number after the collapse and flooding of abandoned salt mine pits (**Tab. 4**). These lakes are situated within settlements and are used as very popular spas during summer. The inflow water of this lake is mainly rainwater. Relatively many articles were published about these salt lakes, dealing with their appearance, physiochemical properties of the water, microbial and enzymological characterization of the mud and algological investigation (Irimuş, 1998, Alexe, 2010, Muntean et al, 1996, Alinei et al, 2006, Nagy & Péterfi, 2008), most of them in Romanian.

OBJECTIVES

Although, the phytoplankton research has a great tradition in Romania both in freshwater and saline environments, the picophytoplankton communities were not studied, therefore the occurrence and diversity of the picophytoplankton in the hypersaline lakes of our country also remained unknown. From this reasons our objectives in this study were as follows:

- To determine the major physical and chemical properties of the saline lakes in the Transylvanian Basin and to elucidate the stratification conditions of these meromictic lakes in summer and winter.
- To determine the trophic state of some hypersaline lakes in the Transylvanian basin.
- To describe the occurrence, abundance, dynamics and importance of the picophytoplankton in these hypersaline lakes.
- To evaluate the unknown genetic diversity of phototrophic plankton assemblages in distinct salt lakes with special emphasis on the prokaryotic and eukaryotic picophytoplankton.

MATERIAL AND METHODS

Lakes differing in surface area, depth, salt concentration and ecological condition were choosen as study sites. These lakes are situated in the Transylvanian Basin (Romania, Central Europe), within 150 km distance from Cluj-Napoca (**Fig. 15**).

Figure 15. Geographical location of the sampling sites: Ocna Dej: Lake Cabdic (1), Sic: Lake Băilor (2) and Lake Săpat (3), Cojocna: Lake Băilor Cojocna (4) and Lake Durgău Cojocna (5), Turda: Lake Tarzan (7), Lake Ocnei (8) and Lake Rotund (9), Ocna Sibiului: Lake Ocniţa-Avram Iancu (6) and Lake Fără Fund (10). Squares represent some main cities and full circles mark sampling sites (with the numerical codes of lakes in parentheses).

The major physicochemical characteristics of the water such as temperature, conductivity, pH, dissolved oxygen concentration, Secchi-disk transparency, and ice-thickness were determined in the field. The chlorophyll a concentration, the abundance and the biomass of the phytoplankton were determined in laboratory. The abundance and composition of picophytoplankton was determined with epifluorescence microscopy, in the case of nanoplankton the composition and abundance were determined with inverted (plankton) microscope. Molecular biology techniques (DGGE, cloning, sequencing) were applied for the identification of phytoplankton taxa.

RESULTS

Ion composition of the lakes

The major ion composition of the mixed surface water from Lake 1 and Lake 4 collected in the summer of 2010 is shown in **Table 8.** In the investigated lakes, among cations, Na^+ is clearly dominant, among anions, Cl^- is the major ion. The amount of total dissolved solids (TDS) was greater in Lake 4 representing 284.9 g L^{-1}, whereof 277.75 g L^{-1} is given by NaCl (97.5% from total TDS). In Lake 1 the ion composition was similar to Lake 4, the TDS was 208.51 g L^{-1} with a 206.03 g L^{-1} share of NaCl (98.8 % from total TDS).

Table 8. Major ion composition of Lake 1 and Lake 4 of the mixed surface water collected in the summer of 2010.

Major ions (g L^{-1})	Lake 1	Lake 4
K^+	0.03	0.845
Na^+	102.62	138.49
Ca^{2+}	1.388	2.0
Mg^{2+}	0.217	1.1
CO_3^{2-}	0.004	0.003
HCO_3^-	0.316	0.372
Cl^-	103.41	139.26
SO_4^{2-}	0.526	2.847

Stratification

The thermal and chemical stratification is a very characteristic phenomenon for hypersaline lakes which can define the biodiversity of these formally meromictic lakes.

During winter the investigated lakes were highly stratified, this phenomenon in most cases was not observable in summer. The stratification of the upper layers (3-4 m depth) was hindered in summer by the extremely intensive bathing. For example, in the summer of 2010, during the sampling in the Lake 4 (Lake Bailor, Cojocna) the abundance of bathers was 1164 people/ha. In Lake 5, which is ~10 0m farther from Lake 4, this value was “only” 675 people/ha.

The bathers were present in similar density in all of the lakes during summer, except for Lake 10, where, in the summer of 2011, the trespassing to the lake was denied for bathers.

In most of the lakes a reverse double stratification was observable during winter, but this was not detectable in summer. The depth profiles of the lakes were characteristic to winter and summer and the pattern of the profiles were similar between the lakes.

Water stratification in Lake 7 (Turda, Lake Tarzan)

During summer (2010), in the case of Lake 7, the upper layer, which had a thickness of 3 m, there was a constant conductivity and temperature, 32.3 mS cm^{-1} (~22.9 g L^{-1} NaCl) and 28.8 °C, respectively. Below 3m, the conductivity increased while the temperature decreased proportionally with the increasing depth. At 9m depth the SC reached 98.5 mS cm^{-1} (~73.3 g L^{-1} NaCl) and the temperature decreased to 17.9 °C. The Chl-a concentration ranged between 3.5-4.35 9 µg Chl-a L^{-1} in the upper 1.5m thick layer. The dissolved oxygen concentration was not measured (**Fig. 23)**

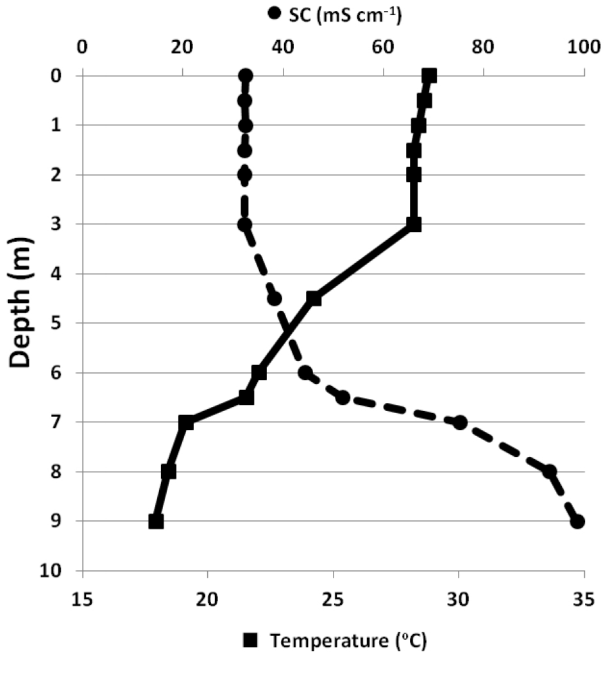

Figure 23. Depth profile of Lake 7 in summer of 2010. SC– specific conductivity of the water,

In the case of Lake 7 in the winter of 2011 the SC in the surface water was 35.6 mS cm^{-1} (~24.4 g L^{-1} NaCl) and this value did not change significantly in the 1.5m thick upper water layer. At 6 m depth the conductivity increased slightly, reaching 58.2 mS cm^{-1} (~37.8 g L^{-1} NaCl). The halocline was present between 6 and 8 m depth, at 10 m the SC rose up to 180 mS cm^{-1} (~190 g L^{-1} NaCl). The temperature on the surface was -0.6 °C, which did not show significant changes in the upper water layer, similar to the SC. Below 1.5 m, the temperature increased proportionally with the depth, reaching 15 °C at 8 m depth (**Fig. 24/A**). The Chl-a concentration showed a maximum value at 3 m depth representing 8.7 µg Chl-a L^{-1}. The dissolved oxygen concentration in the upper layers ranged between 14.6-15.1 mg L^{-1}. Below 1.5 m the oxygen concentration started to decline and the water became anoxic from 4 m depth (**Fig. 24/B**).

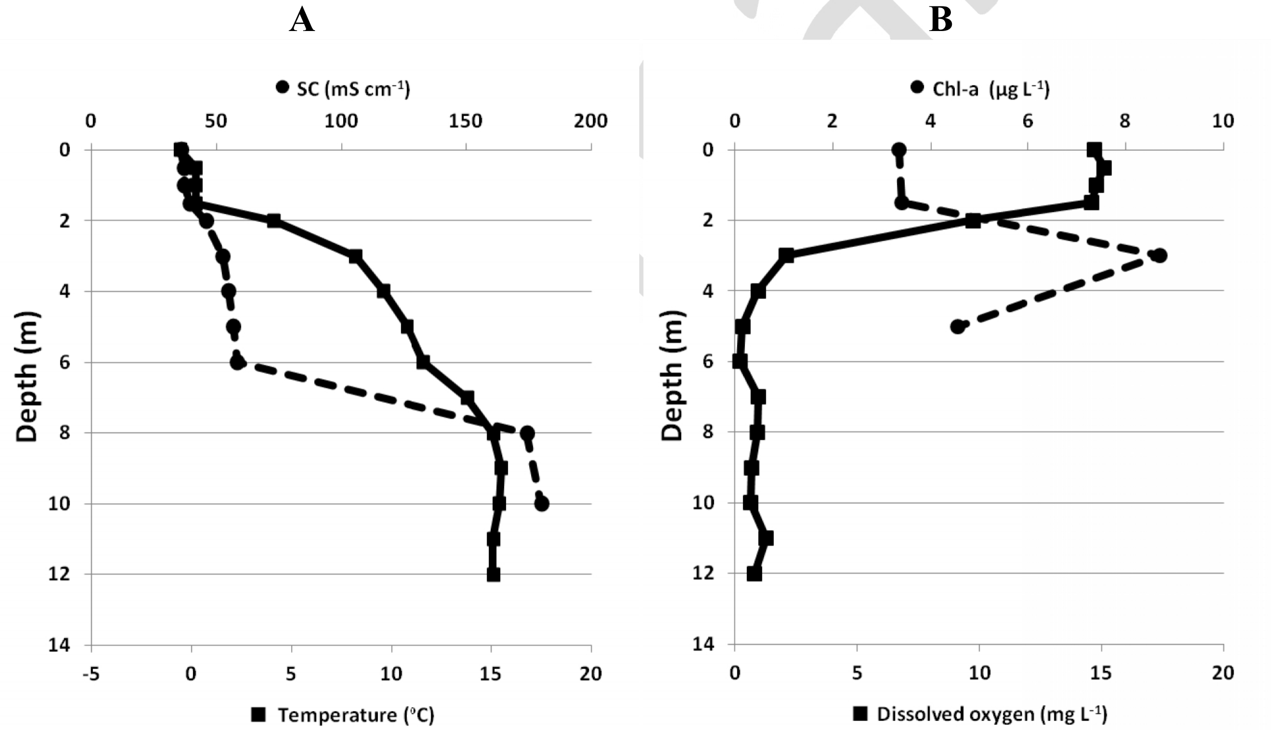

Figure 24. Depth profiles of Lake 7, in winter of 2011. SC– specific conductivity of the water, Chl-a – concentration of chlorophyll-a.

Water stratification in Lake 10 (Ocna Sibiului, Lake Fără-Fund)

In the case of Lake 10, during winter the SC was 101 mS cm^{-1} (~76 g L^{-1} NaCl) on the surface, within 0,5 and 2 m depth the SC was approximately 185 mS cm^{-1} (~200 g L^{-1} NaCl), representing the first halocline. The second halocline was present between 2-3 m depth; below this the water was saturated (~359 g L^{-1} NaCl) with salt. The changes in the temperature profile were parallel with the salinity profile. On the surface the water temperature was -0.4°C, at 0.5 m depth the temperature increased to 6.5 °C, from 0.5 m to 2 m the temperature did not change significantly. Starting from 2 m, the temperature increased proportionally with depth, until it reached 13.5°C at 4 m (**Fig. 27/A)**. High Chl-a values were detected in Lake 10, with a maximum at 1.5 m depth, were the Chl-a concentration was 116 µg L^{-1} (**Fig. 27/B)**. The oxygen concentration was not measured in this case. The pH ranged between 9-9.4 in the surface water and was 6.7 from 3 m below.

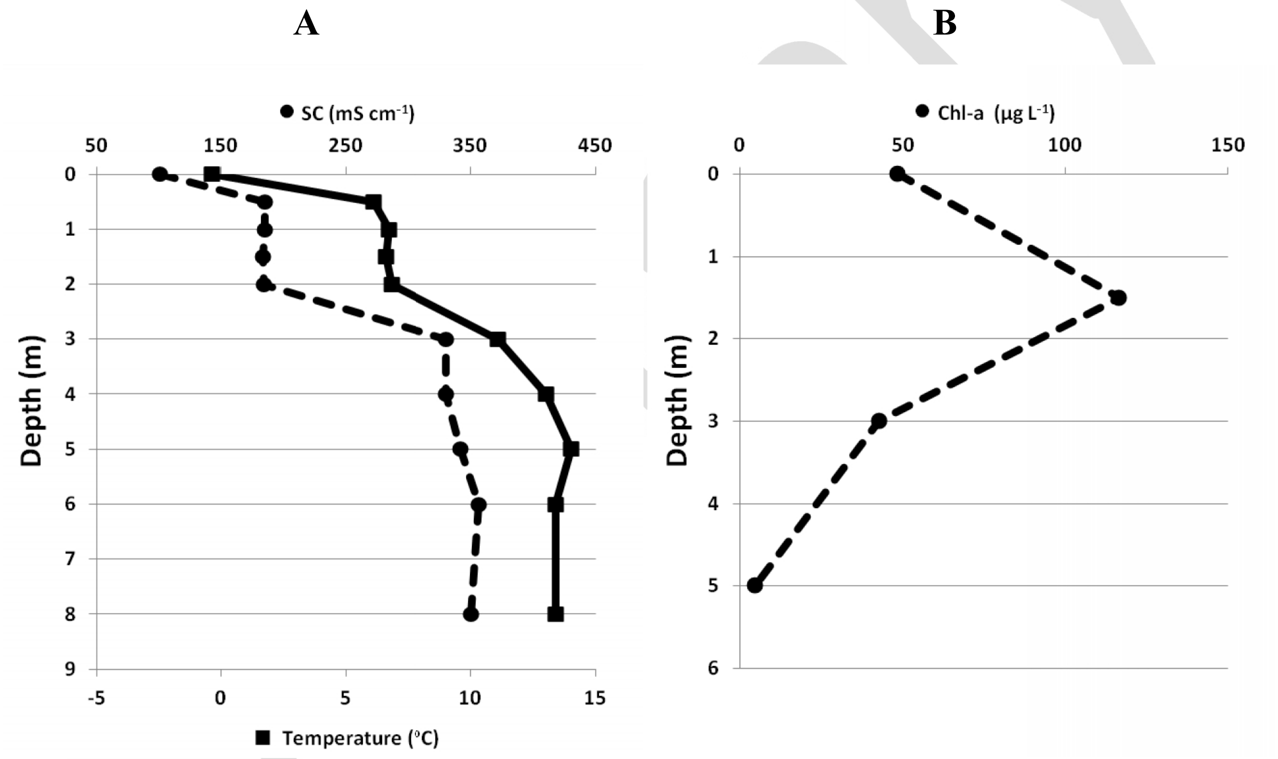

Figure 27. Depth profiles of Lake 10 in winter of 2011. SC– specific conductivity of the water, Chl-a – concentration of chlorophyll-a.

During the summer season (2011) in Lake 10, the SC was ~190 mS cm^{-1} (209.5 g NaCl L^{-1}) and the temperature was ~25.5°C, at a depth of 2m. The halocline was situated between 2-3 m. Below this, the water became nearly saturated with salt. In the zone of the halocline a significant increase of temperature was detectable; here the temperature rose from 25.5 °C to 29 °C at 2.25 m **(Fig. 28/A)**. Below this point the temperature decreased proportionally with the depth to 14.5

°C at 6 m depth. In Lake 10, at 9 m depth, the difference in temperature between winter and summer measurements was ~1 °C. In the zone of the halocline a Chl-a and an oxygen peak was detected. The oxygen peak appeared at 2 m depth, being 10.6 mg L^{-1}, approximately five times greater than in the surface water. The Chl-a peak was located 0.5 m deeper than the oxygen peak. At 2.5 m depth the Chl-a concentration was 19.1 µg Chl-a L^{-1}. Below the Chl-a peak, at 3 m the water suddenly became anoxic **(Fig. 28/B)**.

The pH decreased gradually with depth from 7.2 to 5.4.

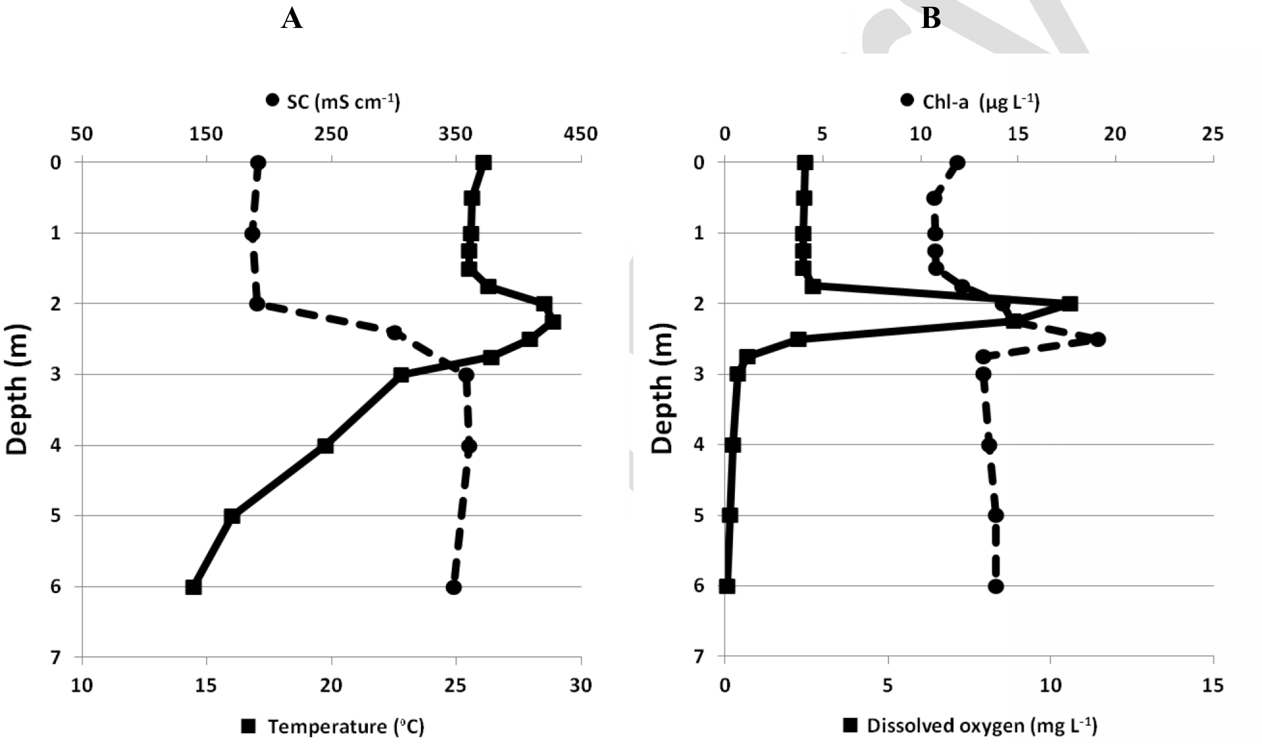

Figure 28. Depth profiles of Lake 10 in summer of 2011. SC– specific conductivity of the water, Chl-a – concentration of chlorophyll-a.

Trophic state of the investigated water bodies

The maximum Chl-a and total phosphorus (TP) concentration of the lakes during the investigation period are presented on **Figure 29**. The maximum Chl-a concentration ranged between 6.15 (Lake 5) and 1713 µg L^{-1} (Lake 3). According to OECD (Organisation for Economic Co-operation and Development) trophic categories, based on Chl-a concentrations, one (Lake 5) of our lakes is mesotrophic, two (Lake 7, 8) of them are eutrophic and seven lakes

(Lake 1, 2, 3, 4, 6, 9, 10) are hypertrophic. The concentration of TP ranged between 37 (Lake 3) and 531 µg L^{-1} (Lake 5). Based on the TP value, Lake 3 and Lake 7 is considered eutrophic, the rest of our water bodies are categorized as hypertrophic lakes according to OECD trophic categories.

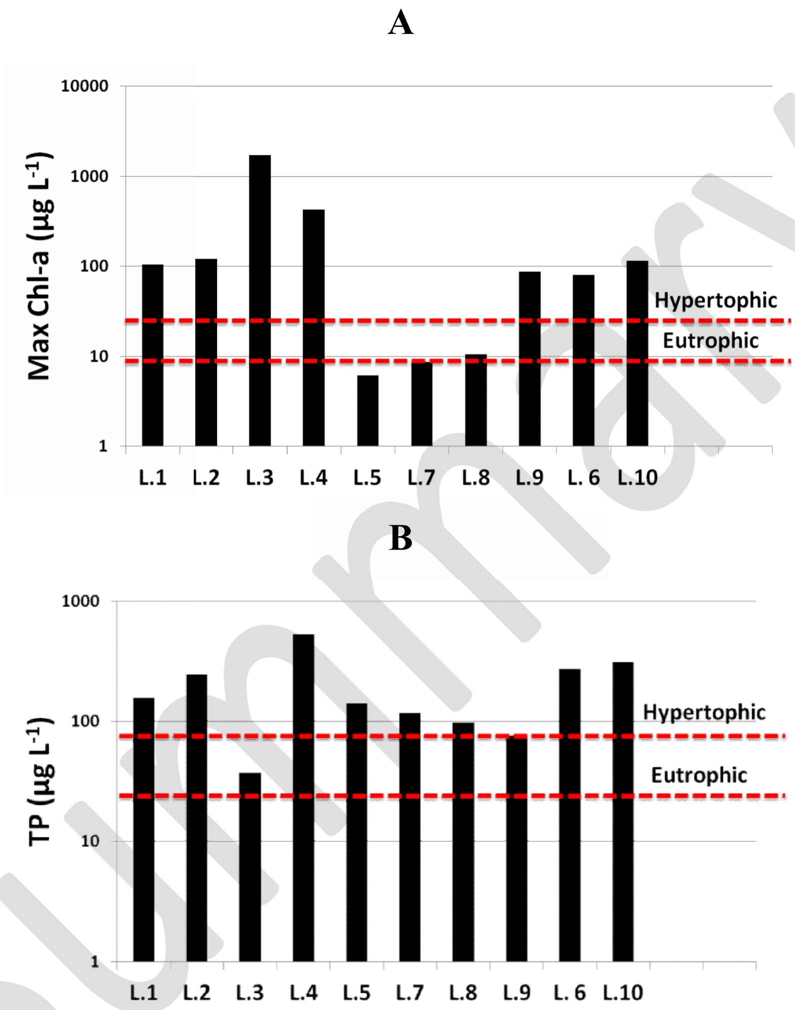

Figure 29. Maximum Chl-a concentrations (A) and the total phosphorous concentrations (B) in the investigated lakes.

Phytoplankton abundance and composition of the saline lakes

Summarized results regarding to algological investigation are presented in **Table 9**. Within the picocyanobacteria (PCy) fraction mostly phycocyanin rich cells were present in the water (**Tab. 9**), phycoerythrin-rich picocyanobacteria was only observable in negligible number (data not shown) in Lake 7 during summer. The number of nanoplankton species was very low.

Beside the PPP, *Dunaliella, Rhodomonas/Cryptomonas, Chaetoceras* and *Dinophyta* species and unidentified pico-size eukaryotic flagellates were present in the investigated saline lakes.

Phytoplankton in Lake 1 (Ocna Dej, Lake Cabdic)

The highest picophytoplankton (PPP) abundance (~7.3 million cells ml^{-1}), represented by picocyanobacteria (PCy, **Fig. 30**) was detected in Lake 1 in the summer of 2010 from the mixed surface layer, when the water temperature was 28.2 ºC and the Chl-a content was 104 µg L^{-1}. Picoeukaryotic (PEu) cells were not detectable. The PCy biomass was 3797 µg L^{-1} while nanoplankton (NP) biomass made up to 570 µg L^{-1}. The contribution of PCy to total phytoplankton biomass (TPB) was 86.9 %. In the following winter, the number of PPP, represented by PEu cells was smaller by an order of magnitude (161,837 cells mL^{-1}), but their biomass (1,864 µg L^{-1}) was half as the PC in the previous summer due to the larger cell volume of the PEu cells. The PEu was present in high biomass (1,864 µg L^{-1}) in contrast with NP (90 µg L^{-1}). The PEu predominated the TPB by 95.4 %. In the summer of 2011, the PPP was composed exclusively by PEu. During this time the biomass of NP was 2,313 µg L^{-1} composed by *Rhodomonas/Cryptomonas* and diatoma, while the PE was present in scant (217 µg L^{-1}).

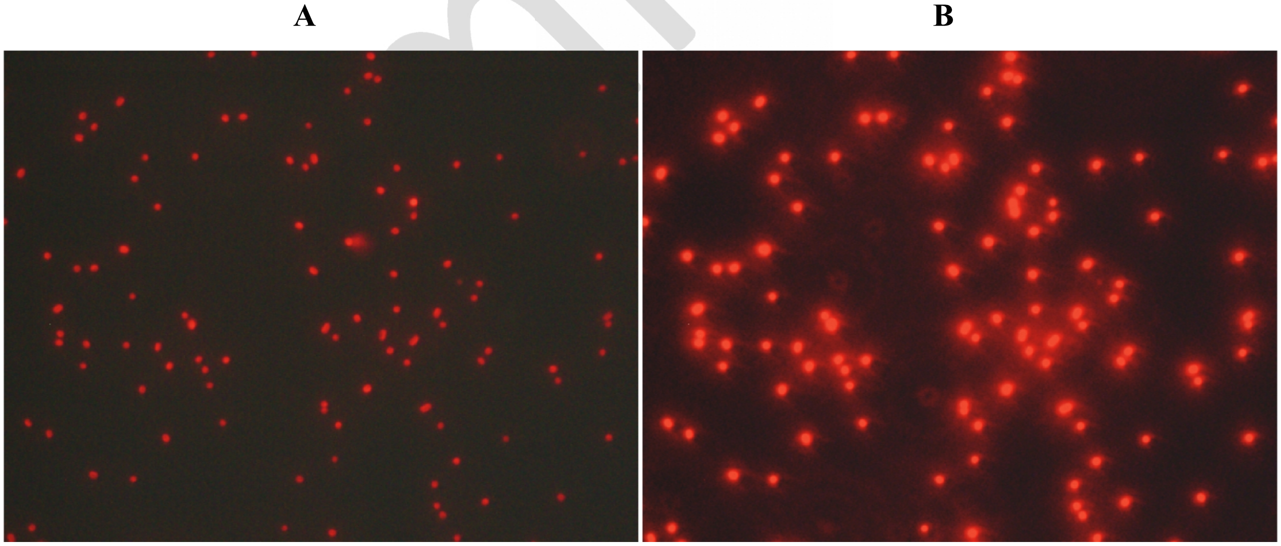

Figure 30. Epifluorescence micrographs (magnification= 1000x) of picoyanobacteria dominated the plankton of Lake Cabdic, in summer of 2010, (A= Blue-Violet excitation, B= Green excitation).

Phytoplankton in Lake 4 (Cojocna, Lake Băilor)

In the summer of 2010 the water temperature was high, 30.5 ºC, and the phytoplankton was composed almost exclusively by PEu cells (**Fig. 31**), consisting 99.7% from TPB at high Chl-a concentration (247 µg L^{-1}). In the following summer the water temperature was 28 ºC and the PEu cells also dominated the phytoplankton (7 million cells mL^{-1}, 99,9 %) in the lake with the highest recorded Chl-a concentration (431 µg L^{-1}). During the winter of 2010, the PPP was present with 0.44 million PE cells mL^{-1} at -0.5 ºC water temperature. *Dunaliella* and *Dinophyta* species were present in variable numbers in the lakes during the investigated period. Small (<4 µm) unidentified flagellates were also present.

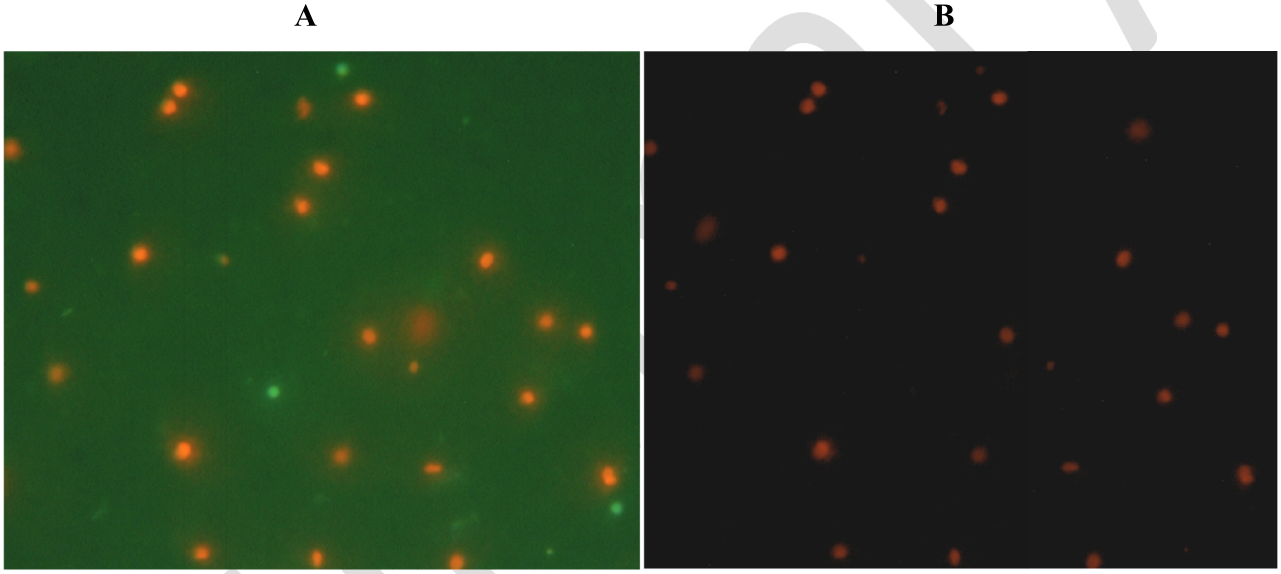

Figure 31. Epifluorescence micrographs (magnification= 1000x) of picoeukaryote dominated plankton of Lake Băilor, in summer of 2010, (A= Blue-Violet excitation, B= Green excitation).

Molecular diversity of the phytoplankton

A total of 27 samples from ten hypersaline lakes (**Tab. 10**) were investigated with DGGE analysis using the 16S rRNA and 18S rRNA genes. Overall, both PCR-DGGE protocols were highly specific for the phototrophic community, cyanobacteria and eukaryotic algae.

Reamplification and sequencing of representative 16S rRNA bands resulted in 15 sequences of unambiguous quality related to cyanobacteria and eukaryotic plastids (**Fig. 32; Fig. 33**). According to the 16S rRNA analysis, sequence C17 from summer surface water of Lake 1 was grouped with marine *Synechococcus* isolates (100 % pairwise sequence similarity with *Synechococcus sp*. RS9918 from clade VIII sensu Fuller et al. 2003; **Fig. 33**, **Tab. 11**).

Another 16S rRNA gene sequence group retrieved that was related to pico-sized algae contained sequence C2 originating from Lake 6 in summer and sequence C8 from Lake 4 in winter (**Fig. 33, Tab. 11**). These sequences were closely related (99.4% pairwise similarity) to *Picochlorum* sp. (Chlorophyta) plastids.

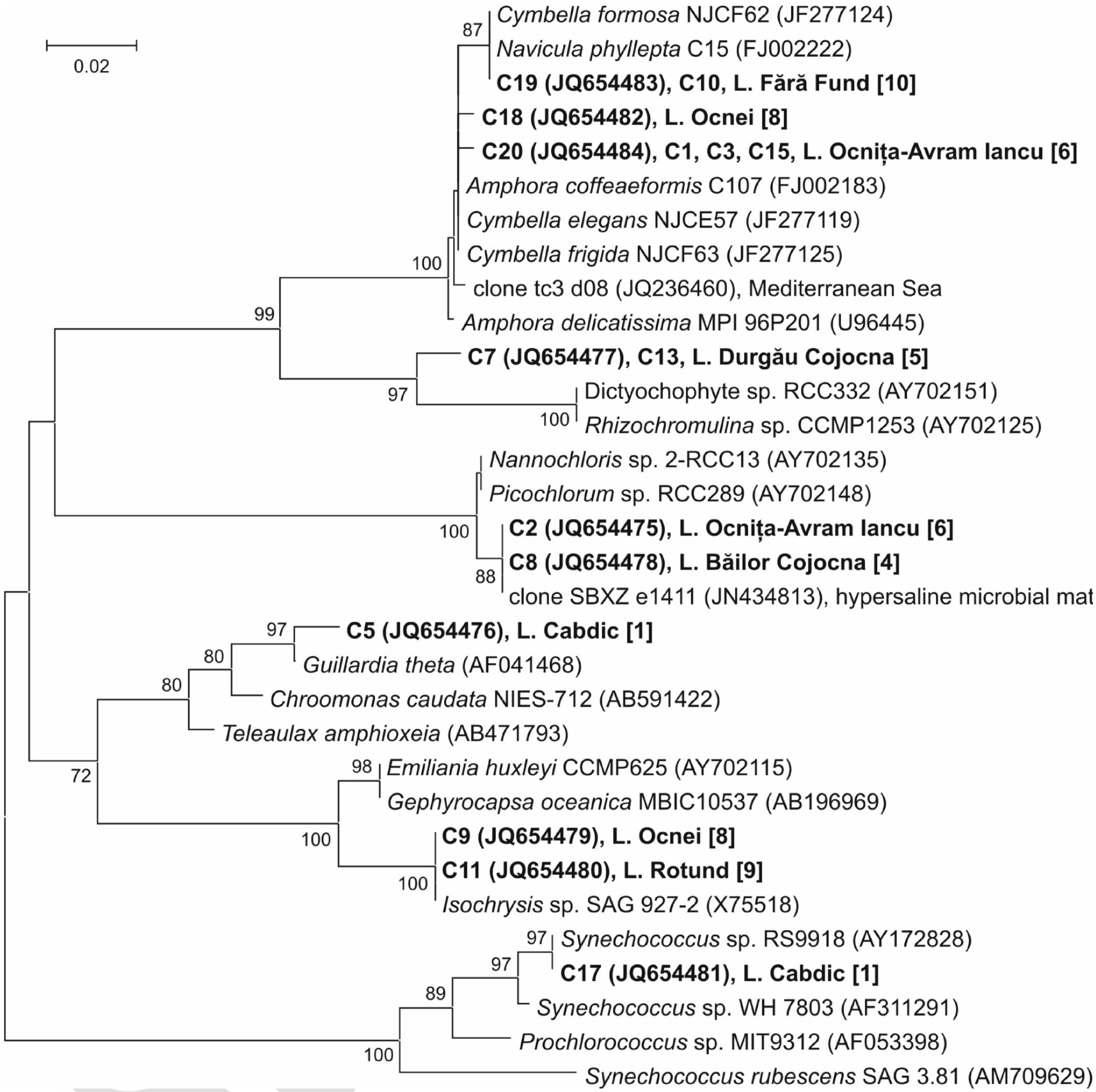

Figure 33. Neighbour joining phylogenetic tree of sequences recovered from 16S rRNA gene-based DGGE. Evolutionary distances were calculated using the Maximum Composite Likelihood method. The tree is based on 297 unambiguously aligned nucleotide positions. Bootstrap values lower than 70 were removed from the branches. Sequences determined in this study appear in bold letters. Lake codes are given in square brackets.

The presence to *Picochlorum* (Chlorophyta) genus was also verified by means of the 18S rRNA analysis: sequences E19 and E20 (from Lake 1 in winter) as well as sequence E12 (from

Lake 6 in summer) showed 100% nucleotide similarity to *Picochlorum oklahomense* UTEX 2795 (**Fig. 34** and **Fig. 35, Tab. 11**).

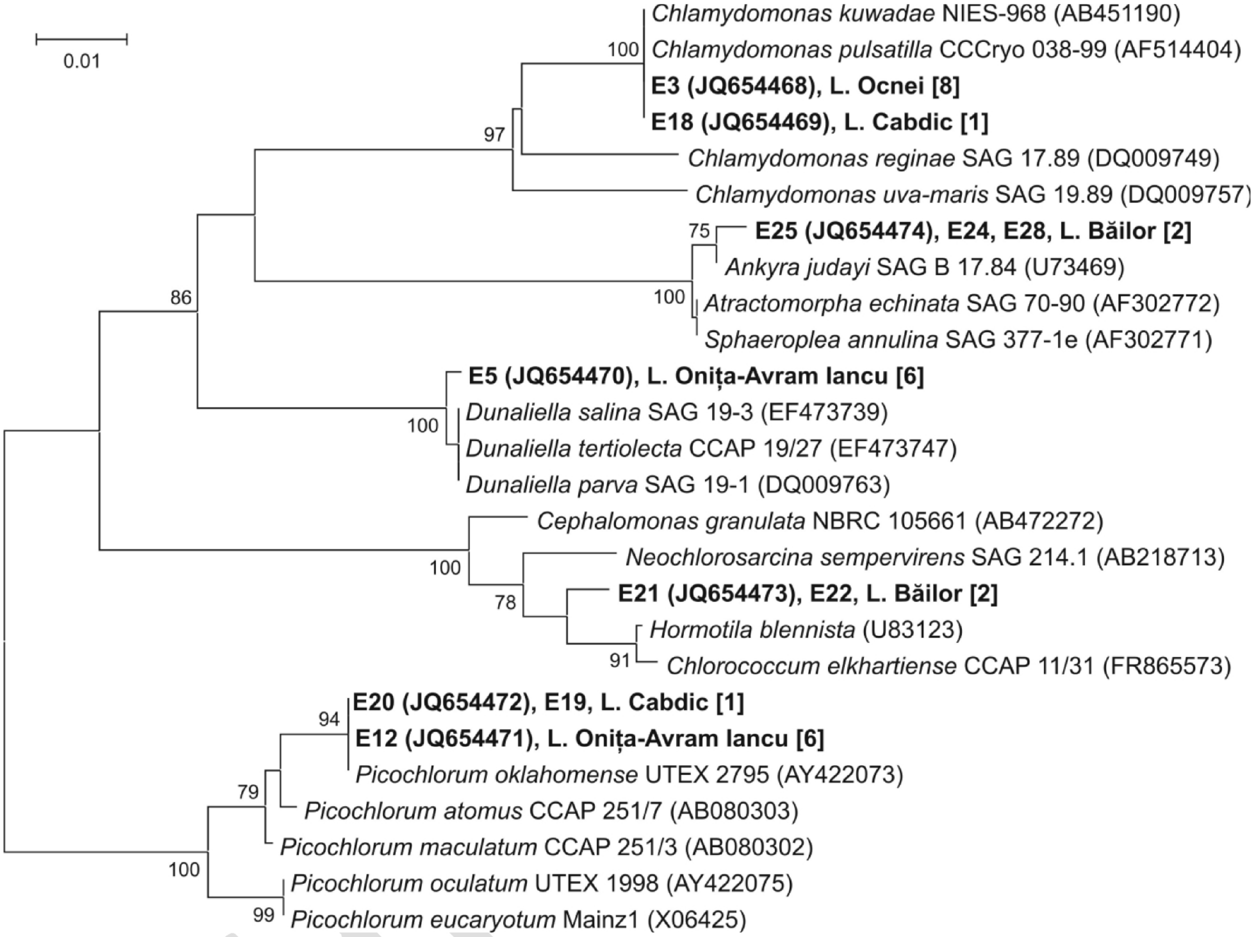

Fig. 35. Neighbour joining phylogenetic tree of sequences recovered from 18S rRNA gene-based DGGE. The tree is based on 331 unambiguously aligned nucleotide positions. Bootstrap values lower than 70 were removed from the branches. Sequences determined in this study appear in bold letters. Lake codes are given in square brackets.

Sequence C7 and C13 from the winter sample of Lake 5 showed moderate similarity (95.6 %) to *Rhizochromulina* sp. CCMP 1253 (Heterokontophyta) and to an undescribed pico-sized flagellate, dictyochophyte sp. RCC332 (Heterokontophyta) (**Fig. 33, Tab. 11**). Plastid 16S rRNA gene sequences of these strains were completely identical within the investigated region, even though they differ significantly in their morphology and their 18S rRNA sequences (online available, unpublished data from Roscoff Culture Collection; Vaulot et al. 2004). Therefore, sequence C7 and C13 may represent an unknown dictyochophyte genotype (a potential picoflagellate) but the identification of these microorganisms was not possible based on their 16S rRNA gene.

Other genotypes detected in this study were closely or distantly related to larger eukaryotic algae. Plastid 16S rRNA gene sequences were affiliated with members of the genera *Guillardia* (Cryptophyta), *Isochrysis* (Haptophyta), *Amphora* and *Navicula* (Heterokontophyta; Table 3). *Guillardia theta* was found with 99.1 % pairwise similarity from Lake 1 and *Isochrysis* sp. were identified with 100% pairwise similarity from Lake 8 and 9 in winter (**Fig. 32 and Fig. 33, Tab. 11**). Based on the investigated region of the plastid 16S rRNA gene, the retrieved diatom sequences were indistinguishably closely related, to a clade formed by members of the genera *Amphora*, *Cymbella* and *Navicula*.

Table 11. Phylogenetic affiliation of sequences obtained from DGGE bands and origin of most related isolates. Picoplanktonic taxa were marked with asterisk.

Sequence (Lake code)	Closest relatives (Accession number)	Similarity (%)	Division	Habitat	Reference
C17 (1)	*Synechococcus sp.* RS9918 (AY172828)*	100	Cyanobacteria	Marine	Fuller et al. 2003
C5 (1)	*Guillardia theta* (AF041468)	99.1	Cryptophyta	Marine	Douglas and Durnford 1989
C9 (8), C11 (9)	*Isochrysis* sp. SAG 927-2 (X75518)	100	Haptophyta	Marine	Huss et al., unpublished
C18 (8), C1, C3, C15, C20 (6)	*Amphora coffeaeformis* C107 (FJ002183)	99.7	Heterokontophyta	Marine/ Brackish	Rampen et al. 2009
C10, C19 (10)	*Navicula phyllepta* C15 (FJ002222)	100	Heterokontophyta	Marine	Rampen et al. 2009
C7, C13 (5)	*Rhizochromulina sp.* CCMP1253 (AY702125)	95.6	Heterokontophyta	Marine	Fuller et al. 2006
	Dictyochophyte sp. RCC332 (AY702151)*	95.6	Heterokontophyta	Marine	Fuller et al. 2006
E12 (6), E19, E20 (1)	*Picochlorum oklahomense* UTEX 2795 (AY422073)*	99.7	Chlorophyta	Hypersaline	Henley et al. 2004
C2 (6), C8 (4)	*Picochlorum sp.* RCC289 (AY702148)*	99.4	Chlorophyta	Marine	Fuller et al. 2006
	Nannochloris sp. 2-RCC13 (AY702135)*	99.4	Chlorophyta	Marine	Fuller et al. 2006
E3 (8), E18 (1)	*Chlamydomonas pulsatilla* CCCryo 038-99 (AF514404)	100	Chlorophyta	Marine/ Snow	Leya et al., unpublished
	Chlamydomonas kuwadae NIES-968 (AB451190)	100	Chlorophyta	Freshwater	Nakada and Nozaki 2009
E5 (6)	*Dunaliella salina* SAG 19-3 (EF473739)	99.7	Chlorophyta	Hypersaline	Di Giuseppe and Dini, unpublished
	Dunaliella tertiolecta CCAP 19/27 (EF473747)	99.7	Chlorophyta	unknown	Di Giuseppe and Dini, unpublished
	Dunaliella salina SAG 19-1 (DQ009763)	99.7	Chlorophyta	Saline lake	Buchheim et al., unpublished
E24, E25, E28 (2)	*Ankyra judayi* SAG B17.81 (U73469)	99.7	Chlorophyta	Freshwater	Buchheim et al. 2001
E21, E22 (2)	*Hormotila blennista* (U83123)	98.8	Chlorophyta	Freshwater	Booton et al. 1998

Based on the Chlorophyta-specific 18S rRNA gene analysis, we found sequences related to the genera *Chlamydomonas, Dunaliella, Ankyra* and *Hormotila/Chlorococcum* (**Fig. 35, Tab. 11**). Some of these taxa also harbor species that inhabit marine or saline aquatic habitats Regarding the 18S rRNA gene-based DGGE (**Fig. 35)**, the band representing the genus *Chlamydomonas* (E3) was observable in most of the samples investigated, while *Ankyra*- and *Hormotila*-related genotypes were restricted to a few samples (bands E21, E22, E24, E25 and E28).

DISCUSSION

Physiochemical properties of the lakes

Investigating the ion composition of our lakes we found the expected results that Na^+ and Cl^- were dominant. Our results fit well with the findings of Poplăcean (2007) who reported similar ion composition and content in the saline lakes from Ocna Sibiului. In our investigated lakes the salt concentration exceeded the salinity of the world oceans (e.g. Atlantic ocean, Puyate & Rim-Rukeh, 2008) and can be categorized as hypersaline water bodies based on many salt lake classification systems (Covardin et al, 1979, Hammer, 1986).

Analyzing the temperature profiles of Lake 10 from the summer of 2011, we found a slight but definite heliotermy. The appearance of heliotermy coincides with the disappearance of human disturbance. If the bathers- and their mixing- effect ceases from the lake, the essential conditions - like thin diluted surface water layer- for heliotermy can be developed. We conclude that the intensive bathing during summer significantly influence the formation of natural stratification in these hypersaline environment. In Lake Ursu the heliotermy is maintained by the continuous inflow of freshwater and the well regulated bathing periods. We note the fact that in the absence of continuous freshwater input, developing and maintaining of the heliothermic conditions is more difficult in our investigated lakes than in lake Ursu, if there is such a intention on the part of lake management.

Phytoplankton in the hypersaline lakes of the Transylvanian-Basin

Trophic state

In the majority of our investigated lakes the total phosphorous concentrations indicate hypertrophic conditions, which is undoubtable result of the high human disturbance. The highest TP concentration was measured in Lake 4 where in the time of sampling the abundance of bathers were the highest per unit lake surface (1164 persons/ha). According to our results the recreational uses of these lakes significantly modify the physical structure of the water body. Furthermore the high density of humans significantly increase the nutrient concentration resulting in heavy phytoplankton blooms, where the Chl-a concentration can be extremely high, for example in Lake 3 the Chl-a concentration was 1713 μg L^{-1} in the summer of 2010. The observed high phytoplankton biomass values (Chl-a concentration) are very rare in natural aquatic habitats they are more characteristics for highly productive fish ponds. In marine environments the bloom of toxic dinoflagellates (red tide) is a worldwide phenomenon as a result of the eutrophication. These algae seriously deteriorate the water quality and they are harmful for the marine aquaculture and the humans. Fortunately in these saline lakes we have never detected the occurrence of these toxic dinoflagellates.

Picophytoplankton

During the study period, picocyanobacteria and picoeukaryotes were both present in the investigated lakes at least one time in except of Lake 3 which was sampled only in summer of 2010. Despite the extremely high salt concentration in the investigated lakes, high Chl-a concentrations and PPP in high abundance and biomass was detected. The PPP communities were present in these lakes with high abundance, comparable to saline lakes found in literature (**Tab. 12**)

Table 12. The maximal PPP abundance of some well studied saline lakes compared with our results

Maximum PPP abundance (10^6 cells mL^{-1})	Origin	Reference
0.03 (picoeukaryotes)	Tibetian hypersaline lake	Wu et al., 2009
0.09 (picoeukaryotes)	Saline mining lakes	Zippel &Schimmele, 1998
0.48 (picocyanobacteria)	Deep hyposaline lake	Okada et al., 2007
1.4 (picocyanobacteria and picoeukaryotes)	Coastal lagoon	Schapira et al., 2010
1.6 (picocyanobacteria and picoeukaryotes)	Warm monomictic tropical saline lake	Macek et al., 2009
7.06 (picoeukaryotes)	Transylvanian hypersaline lakes	This study
7.30 (picocyanobacteria)	Transylvanian hypersaline lakes	This study
8.0 (picocyanobacteria)	Saline Antarctic lake	Powel et al., 2005

We found that the PPP dominated the phytoplankton in the lakes at least in one case during the investigation, with a relative large contribution ranging between 63-100%, in except of the Lake 3 were no PPP cells were detected and in Lake 6 were the maximum contribution of the PPP to the total phytoplankton biomass was only 18%. We compared our data on the relative importance of the PPP biomass with 91 literature data (22 freshwater and 69 marine studies) synthesized by Bell & Kalff (2001). Our results show that the PPP from the Transylvanian Basin does not follow the generally accepted trend expressed in many publications (Szelag-Wasielewska, 1997, Bell & Kalff, 2001), that the relative abundance of the PPP decreases with the increasing trophic level of aquatic habitats (marine, freshwater), (**Fig. 36**).

In the studied hypersaline lakes the relative importance of PPP does not show changes with the increasing trophic level. The PPP can dominate the phytoplankton community (up to 100%) even at a very high trophic condition (99% at 431 µg Chl-a L^{-1}, **Tab. 9.**).

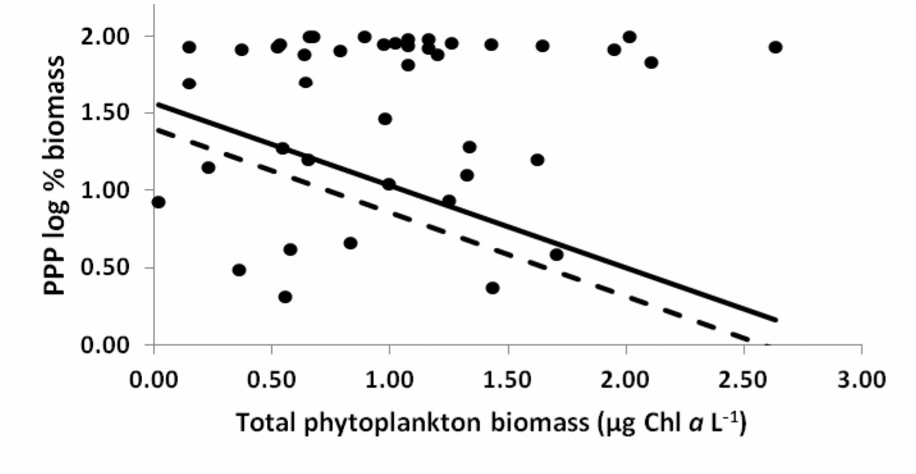

Figure 36. Relationship between the contribution (log%) of picophytoplankton (PPP) to total phytoplankton biomass (µg Chl *a* L^{-1}) based on literature data (lines) and in the Transylvanian hypersaline lakes (dots). Solid line represents PPP contribution from freshwater-, dashed line for PPP contribution from marine environment (Bell and Kalff, 2001)

Phytoplankton along a salinity gradient

The vast majority of algal studies in hypersaline systems showed the prevalence of *Dunaliella* spp. and cyanobacteria (Kirkwood & Henley, 2006). The 23 species of the genus *Dunaliella* are wall-less flagellate eukaryotic algae found in saline environments and exhibiting optimal growth at different salt concentrations with varying abilities to turn orange–red under particular environmental conditions (Massyuk, 1973, García et al, 2007). It was found that *Dunaliella* species are able to grow over an extremely wide range of salinities and at extreme salinities of up to NaCl saturation (□5 M NaCl) was due to their production of glycerol. Our findings are in accordance with these statement as *Dunaliella* species occurred in all of the investigated water bodies, covering a wide range of salinity form 32 mS cm^{-1} to 354 mS cm^{-1} (23-311 g NaCl L^{-1}), (**Fig. 39**).

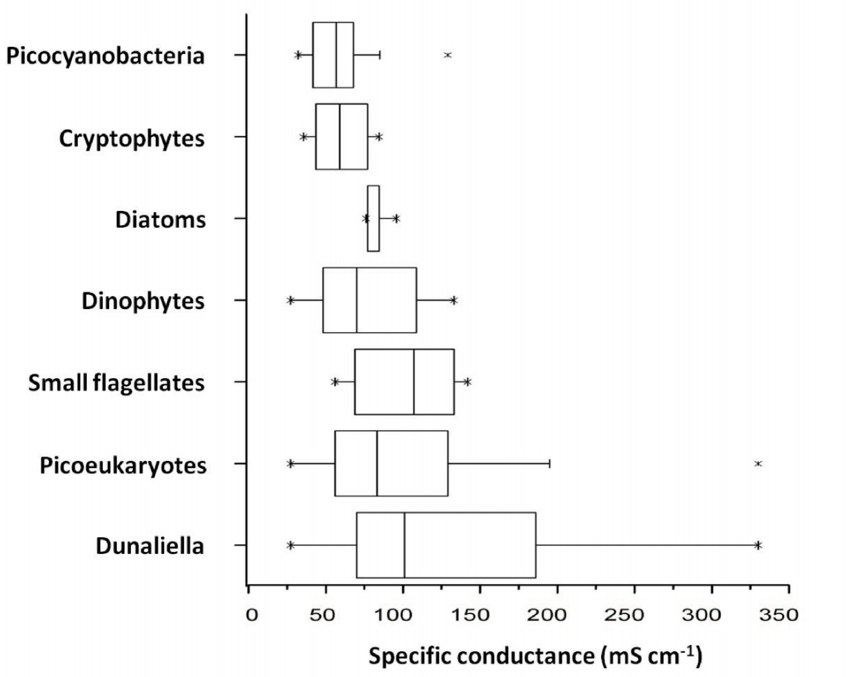

Figure 39. Box plot of the occurrences of different phytoplankton taxa along the salinity gradient in the Transylvanian hypersaline lakes.

The second most halotolerant microorganisms were the picoeukaryotes (**Fig. 39**). They occurred between 25 mS cm^{-1} to 200 mS cm^{-1}(19-230 g NaCl L^{-1}) The less halotolerant microorganisms were picocyanobacteria and cryptophytes, they were detectable up to 80 mS cm^{-1} (55 g NaCl L^{-1}), (**Fig. 39**). Schapira et al. (2010) investigated the distribution of PPP along a salinity gradient from brackish to hypersaline (salinity:18-155 g L^{-1}) water, in coastal lagoons. They observed that the abundance of PPP was the highest (1,3 million cells ml^{-1}) between a salinity of 80-110 g L^{-1}. This salinity interval corresponds well with the salinity of our lakes, but the upper limit of the salt tolerance of the picophytoplankton in our lakes were much higher (230 g NaCl L^{-1} instead of 150 g NaCl L^{-1}) than in the coastal lagoons in Australia. In spite of these differences it seems that the salinity optimum of the picophytoplankton did not differ significantly between the Transylvanian hypersaline lakes and Australian coastal lagoons. Our results suggest high difference between the halotolerance of picocyanobacteria and picoeukaryotic algae, but further research is needed to get further evidences.

Phylogenetic diversity of the phytoplankton

According to the 16S rRNA gene analysis we found that the prokaryotic fraction of the picophytoplankton was composed by marine *Synechococcus* species from clade VIII sensu Fuller et al. (2003). Members of the picocyanobacterial genus *Synechococcus* are commonly distributed worldwide, many strains have been isolated and studied (sometimes under incorrect names) and their whole taxonomy awaits comprehensive revision (Komárek, 2010). Picocyanobacterial sequences retrieved from continental habitats are separated from the obligate marine picophytoplankton clade (comprising *Prochlorococcus* and marine *Synechococcus)* (Crosbie et al. 2003). Previous reports on saline or hypersaline environments, such as the Great Salt Lake (UT, USA), the hypersaline Mono Lake (CA, USA), East Tibetan saline lakes (China) or hyposaline soda pans (Hungary), have indicated that the members of their picocyanobacterial communities were distantly related to the above-mentioned marine clade (Budinoff and Hollibaugh 2007; Xing et al. 2009; Wu et al. 2010; Felföldi et al. 2011a). *Synechococcus* phylotypes of the marine picophytoplankton clade are well known inhabitants of seas and oceans (e.g. Fuller et al. 2003; Zwirglmaier et al. 2008), although the saline environment and the high dispersive potential of these microorganisms could explain their presence in the investigated continental lakes.

Picochlorum oklahomense UTEX 279 found in Lake 6 was isolated from an ephemeral saline pool (Henley et al. 2004). Members of the picoeukaryotic genus *Picochlorum* were found in various marine and saline environments (Henley et al. 2004), and were also detected in the heliothermal Lake Ursu that is located in Sovata, Romania. The presence of this genus in the hypersaline lakes of the Transylvanian Basin corresponds well with the broad halotolerance of *P. oklahomense* (Henley et al. 2002).

In the present study, we detected marine cryptophytes and haptophytes, as well as members of the marine picocyanobacteria for the first time in hypersaline lakes. The presence of some taxa detected in the Transylvanian lakes by our analysis (e.g. *Picochlorum* and *Dunaliella*) agrees well with former studies on hypersaline environments; but additionally, we have retrieved that marine taxa (cryptophytes, haptophytes and picocyanobacteria) are also characteristic inhabitants of these aquatic ecosystems.

CONCLUSIONS

The Transylvanian hypersaline lakes are under high human impact, they serve as popular spas in summer. The intensive bathing destroys their thermal stratification. The thermal and chemical stratification can develop after the bathing season. In one lake, where the bathing was restricted, heliotermy was observable in summer. In winter a well expressed inverse stratification was detectable in these meromictic lakes.

In all of our investigated lakes the total phosphorus concentrations indicate hypertrophic conditions which are undoubtable result of the high human disturbance. According to the high total phosphorus content these lakes are rich in planktonic algae. The nanophytoplankton was composed by the common halotolerant algal species. *Dunaliella* sp. dominated the nanoplankon in most of these lakes.

Picocyanobacteria and picoeukaryotic algae occurred in all of the lakes. At least in one case they became the dominant phytoplankters, even in hypertrophic conditions. The seasonal dynamics of the picophytoplankton in these lakes is different from the most of the lakes of the temperate zone, where the picocyanobacteria dominates the picophytoplankton fraction during summer and the picoeukaryotes are dominant in winter. In the Transylvanian hypersaline lakes summer blooms of picoeukaryotes are common phenomenon. According to our result these lakes has unique character as they not follow the general trend as the relative contribution of picophytoplankton to the total phytoplankton biomass did not decrease with the increasing trophic state. The observed highest abundance values are amongst the published maximum picophytoplankton abundances from saline (NaCl dominated) environments.

In summary, to the molecular investigation of the Transylvanian hypersaline lakes the PCR-DGGE method was highly specific to cyanobacteria and green algae, and no heterotrophs were retrieved in our analysis (that could be a significant bias in similar analyses). We found eleven algal taxa in the Transylvanian saline lakes and these taxa were related mainly to marine or hypersaline species. PPP was represented by the marine picophytoplankton clade of *Synechococcus* (Cyanobacteria) and the marine/hypersaline genus *Picochlorum* (Chlorophyta). This is the first record for both groups in Central Europe. Additionally, a putative dictyochophyte picoflagellate may also present. Within larger algae, the occurrence of marine cryptophytes and haptophytes was also verified. The presence of the identified marine and hypersaline species could be explained by wind, precipitation and waterfowl transfer. However, this latter could have smaller importance because of the small surface and urban environment of the studied lakes.

ACKNOWLEDGEMENT

I am thankful to Prof. Dr. Nicolae Dragoş from Babeş-Bolyai Univerisity for giving me the opportunity to be his Ph.D. student and to coordinating my work during this three year study.

I want to thank my supervisor Prof. Dr. Lajos Vörös from the Balaton Limnological Institute (HAS, CER) for his support and lots of good ideas throughout my PhD studies.

I also thank to Prof. Dr. Biró Péter (Director, Balaton Limnological Institute, HAS, CER) and Prof. Dr. Károly Márialigeti (Head of Department of Microbiology, Eötvös Loránd University) for giving me the opportunity to work in their institutes.

I would like to thank my colleagues Boglárka Somogyi, Tamás Felföldi, Balázs Németh, Gyöngyi Székely, Csaba Bartha, Károly Pálffy, László Bartha, Dorina Moldovan, Adriana Bica, Cristian Coman, Drugă Bogdan, Elena Rákosy for their help and technical contribution to this study.

I am grateful to László Fodorpataki, Horia Banciu and Vasile Muntean for the oversee of my research.

Special thanks for Katalin V.-Balogh for teaching me goodness.

I am very thankful to Erika Nagy for her continuous support and understanding during these years.

Thanks to my family: Köszönöm a családomnak, különösképpen Édesanyámnak és Édesapámnak, hogy áldozatos munkával lehetővé tették számomra a továbbtanulást.

This work was supported by the Human Resources Development Program (POSDRU 88/1.5/S/60185, Doctoral studies: through science towards society); CNCSIS/UEFISCDI TE306/70; the Hungarian Scientific Research Fund (OTKA K 73369); Collegium Talentum (Edutus, Tatabánya, Hungary); and Domus Hungarica Scientiarum et Artium.

REFERENCES

Agustí S (1991) Allometric scaling of light absorption and scattering by phytoplankton cells. *Can. J. Fish. Aquat. Sci.*48: 763-767.

Alexe M (2010) A study on the salt lakes of the Transylvanian Basin, Cluj University Press, Cluj-Napoca, Romania. (In Romanian)

Alinei R, Ionica A, Cheratoiu C, Cicu A, Gheorghita D, Guzu D, Falamas R, Bucurenciu C, Arimie B, Morinescu M, Oprean L (2006) Isolation of halophylic microorganisms in the saline lakes from Ocna Sibiului and analysis of red pigments production. Acta Univ Cibiensis E Food Technol X/2:21-33

Bell T, Kalff J (2001) The contribution of picophytoplankton in marine and freshwater systems of different trophicstatus and depth. *Limnol. Oceanogr.* 46: 1243-1248.

Bricaud A, Bédhomme A-L, Morel A. 1988. Optical properties of diverse phytoplanktonic species: experimental results and theoretical interpretation. *J. Plankton Res.* 10: 851-873.

Budinoff CR, Hollibaugh JT (2007) Ecophysiology of a Mono Lake picocyanobacterium. Limnol Oceanogr 52:2484-2495

Callieri C (2008) Picophytoplankton in freshwater ecosystems: the importance of small-sized phototrophs. Freshwat Rev 1:1-28

Callieri C, Stockner JG (2002) Freshwater autotrophic picoplankton: a review. *J. Limnol.* 61: 1-14.

Cowardin LM, Carter V, Golet FC, Laroe ET (1979) Classification of wetlands and deepwater habitats of the United States. U.S. Fish and wildlife servive rep. FWS/OBS-79/31. US Govt Printing Office, Washington D.C.: 103pp.

Crosbie ND, Pöckl M, Weisse T (2003) Dispersal and phylogenetic diversity of nonmarine picocyanobacteria, inferred from 16S rRNA gene and cpcBA-intergenic spacer sequence analyses. Appl Environ Microbiol 69:5716-5721.

Felföldi T, Somogyi B, Márialigeti K, Vörös L (2011) Notes on the biogeography of non-marine planktonic picocyanobacteria: re-evaluating novelty. J Plankton Res 33:1622-1626.

Fuller NJ, Marie D, Partensky F, Vaulot D, Post AF, Scanlan DJ (2003) Clade-specific 16S ribosomal DNA oligonucleotides reveal the predominance of a single marine Synechococcus clade throughout a stratified water column in the Red Sea. Appl Environ Microbiol 69:2430-2443

Fuller NJ, West NJ, Marie D, Yallop M, Rivlin T, Post AF, Scanlan DJ (2005) Dynamics of community structure andphosphate status of picocyanobacterial populations in the Gulf of Aqaba, Red Sea. *Limnol. Oceanogr.* 50: 363-375.

Fuller NJ, Campbell C, Allen DJ, Pitt FD, Zwirlgmaier K, Le Gall F, Vaulot D, Scanlan DJ (2006) Analysis of photosynthetic picoeukaryote diversity at open ocean sites in the Arabian Sea using a PCR biased towards marine algal plastids. Aquat Microb Ecol Vol.43:79-93

García F, Freile-Pelegrín Y, Robledo D (2007) Physiological characterization of *Dunaliella* sp. (Chlorophyta, Volvocales) from Yucatan, Mexico . Bioresource Technology, 98/7: 1359–1365

Hammer UT (1986) Saline lake ecosystems of the world. Dr W. Junk Publishers, Dordrecht, The Netherlands

Henley WJ, Major KM, Hironaka JL (2002) Response to salinity and heat stress in two halotolerant chlorophyte algae. J Phycol 38:757-766

Henley WJ, Hironaka JL, Guillou L, Buchheim MA, Buchheim JA, Fawley MW, Fawley KP (2004) Phylogenetic analysis of the 'Nannochloris-like' algae and diagnoses of *Picochlorum oklahomensis* gen. et sp. nov (Trebouxiophyceae, Chlorophyta). Phycologia 43:641-652

Kirkwood AE, Henley WJ (2006) Algal community dynamics and halotolerance in a terrestrial hypersaline environment. Phycol. Soc. Am. DOI: 10.1111/j.1529-8817.2006.00227.x

Komárek J (2010) Recent changes (2008) in cyanobacteria taxonomy based on a combination of molecular background with phenotype and ecological consequences (genus and species concept). Hydrobiologia 639:245-259

Macek M, Alcocer J, Lugo Vázques A, Martínez-Pérez ME, Peralte Soriano L, Vilaclara Fatjó G (2009) Long term picoplankton dynamics in a warm-monomictic, tropical high altitude lake. J. Limnol, 68/2:183-192

MacIsaac EA, Stockner JG (1993) Enumeration of phototrophic picoplankton by autofluorescence microscopy. In: Kemp PF, Sherr BF, Sherr EB, Cole JJ (eds) The handbook of methods in aquatic microbial ecology. CRC Press, Boca Raton FL, pp 187-197

Massyuk, 1973. Morphology, taxonomy, ecology and geographic distribution of the genus Dunaliella Teod. and prospects for its potential utilisation. Kiev: Naukova Dumka. Massyuk. pp. 312.

Muntean V, Crişan R, Paşca D, Kiss S, Drăgan-Bularda M (1996) Enzymological classification of salt lakes in Romania. Int J Salt Lake Res 5:35-44

Nagy L, Péterfi LS (2008) Preliminary data on the diatom communities from “Lacul Sulfuros” (“Lake No. 6”) near Turda (Cluj County), Romania. Contrib Bot XLIII:105-111

Irimuş IA, (1998) Relieful pe domuri şi cute diapire în Depresiunea Transilvaniei. Editura Presa Universitară Clujeană, Cluj-Napoca, 311 pag.

Terry Beveridge 2012: http://www.sciencephoto.com/media/115212/view

Olliver B, Caumettte P, Garcia JL, Mah RA (1994) Anaerobic bacteria from hypersaline environments. Microbiol. Rev. 58, 27–38.

Okada M, Taniuchi Y,·Murakami A, Takaichi S, Ohtake S, Ohki K (2007) Abundance of picophytoplankton in the halocline of a meromictic lake, Lake Suigetsu, Japan. Limnology 8: 271-280

Poplăcean M (2007) *Quality analysis of Ocna Sibiului`s Lakes, phzsical-chemical and bacteriological description of waters*. Acta Universitas Cibiensis, Series E, Food Techn. Vol. XI/2: 27-38.

Powell LM, Bowman JP, Skerratt JH, Franzmann PD, Burton HR (2005) Ecology of a novel *Synechococcus* clade occurring in dense populations in saline Antarctic lakes. Mar Ecol Prog Ser 291: 65–80

Puyate YT, Rim-Rukeh A (2008) Variability with depth of some physico-chemical and biological parameters of Atlantic Ocean water in part of the coastal area of Nigeria. J. Appl. Sci. Environ. Manage. Vol. 12(1) 87 – 91.

Sánchez-Baracaldo P, Handley BA, Hayes PK (2008) Picocyanobacterial community structure of freshwater lakes and the Baltic Sea revealed by phylogenetic analyses and clade-specific quantitative PCR. Microbiology (SGM) 154:3347-3357

Schagerl M. & Oduor SO (2008) Phytoplankton community relationship to environmental variables in three Kenyan Rift Valley saline-alkaline lakes. Mar Freshwater Res 47:189-197

Schapira M, Buscot MJ, Pollet T, Leterme SC ,Seuront L (2010) Distribution of picophytoplankton communities from brackish to hypersaline waters in a South Australian coastal lagoon. Saline Systems 6/2:1-15

Seckbach J (Ed.) (2007) Algae and Cyanobacteria in extreme environments. Springer, Berlin

Shannon S, Chrzanowski T, Grover J (2007) Prey foodquality affects flagellate ingestion rates. *Microb. Ecol*., 53:66-73.

Shi XL, Marie D, Jardillier L, Scanlan DJ, Vaulot D (2009) Groups without cultured representatives dominate eukaryotic picophytoplankton in the oligotrophic South East Pacific Ocean. – PLoS ONE, 4: 1-11.

Stockner JG (1991) Autotrophic picoplankton in freshwater ecosystems: The view from the summit. Int Rev Hydrobiol 76:483-492

Stockner J, Callieri C, Cornberg G (2000) picoplankton and other non-bloom forming cyanobacteria in lakes. In: The Ecology of Cyanobacteria: Their Diversity in Time and Space (eds. Whitton B and Potts M), pp. 195-238. Kluwer Academic Publisher, The Netherlands.

Stomp M, Huisman J, Vörös L, Pick FR, Laamanen M, Haverkamp T, Stal LJ (2007) Colourful coexistence of red and green picocyanobacteria in lakes and seas. *Ecol. Lett.* 10: 290-298.

Szelag-Wasielewska E (1997) Picoplankton and other size groups of phytoplankton in various shallow lakes. Hydrobiologia 342/343: 79–85

Vörös L, Callieri C, V-Balogh K, Bertoni R (1998) Freshwater picocyanobacteria along a trophic gradient and light quality. Hydrobiologia 369/370:117-125

Xing P, Hahn MW, Wu QL (2009) Low taxon richness of bacterioplankton in high-altitude lakes of the Eastern Tibetan Plateau, with a predominance of *Bacteroidetes* and *Synechococcus* spp. Appl Environ Microbiol 75:7017-7025

Williams DW (1998) Guidelines of lake management. Vol 6. Management of inland saline waters. International Lake Environment Committee Foundation, Japan.

Wu QL, Chatzinotas A, Wang J, Boenigk J (2009) Genetic diversity of eukaryotic plankton assemblages in Eastern Tibetan lakes differing by their salinity and Altitude. Microb Ecol 58:569-581

Wu QL, Xing P, Liu W-T (2010) East Tibetan lakes harbour novel clusters of picocyanobacteria as inferred from the 16S-23S rRNA internal transcribed spacer sequences. Microb Ecol 59:614-622

Zippel B, Scimmele M (1999) Composition and dynamics of autotrophic picoplankton and spectral light distribution in saline mining lakes of Germany. Aquatic Ecosystem Health and Management 2: 319-329

Zwirglmaier K, Jardillier L, Ostrowski, Mazard S, Garczarek L, Vaulot D, Not F, Massana R, Ulloa O, Scanlan DJ (2008) Global phylogeography of marine *Synechococcus* and *Prochlorococcus* reveals a distrinct partitioning of lineages among oceanic biomes. Environ Microbiol 10:147-161

PUBLICATIONS RELATED TO THE THESIS

1. **Zsolt Gyula Keresztes,** Tamás Felföldi, Boglárka Somogyi, Gyöngyi Székely, Nicolae Dragos, Károly Márialigeti, Csaba Bartha, Lajos Vörös (2012) First record of picophytoplankton diversity in Central European hypersaline lakes. Extremophiles 16:759–769. DOI 10.1007/s00792-012-0472-x **(IF: 2,943)**
2. **Keresztes Zsolt Gyula**, Nagy Erika, Somogyi Boglárka, Németh Balázs, Bartha Csaba, Székely Gyöngyi, Nicoale Dragoş, Vörös Lajos (2011) Trophic conditions of saline lakes in the Transylvanian-Basin. . Hung. Hydrobiol. Soc 91(6):46-48
3. Tamás Felföldi, Boglárka Somogyi, **Zsolt Gyula Keresztes**, Károly Márialigeti, Lajos Vörös (2012) Comments on the reliability of scientific papers using the example of picocyanobacteria inhabiting continental aquatic habitats. Hung. Hydrobiol. Soc 92/6:25-27
4. **Zsolt Gyula Keresztes**, Boglárka Somogyi, Emil Boros, Gyöngyi Székely, Csaba Bartha, Erika Nagy, Nicolae Dragoş, Lajos Vörös (2010) Picoplankton in soda lakes of the Carpathian Basin. Contrib. Bot. XLV: 41-46

Books

1. Fodorpataki László, Papp Judit, Bartha Csaba, **Keresztes Zsolt Gyula:** Növényélettan és ökofiziológia laboratoriumi gyakorlatok. 2010 Kolozsvári egyetemi kiadó, ISBN 978-973-595-109-2
2. V.-Balogh Katalin, **Keresztes Zsolt Gyula**, Németh Balázs, Somogyi Boglárka, Vörös Lajos: Szervesanyagok szerepe a Balaton vízminőségének alakításában. A Balaton kutatásának 2008. évi eredményei, 2009. Szerkesztette: Bíró Péter, Banczerowski Januszné. MTA Budapest, ISSN 1419-1075

Participation on international scientific events:

1. **Zsolt Gyula Keresztes**, Boglárka Somogyi, Tamás Felföldi, Erika Nagy, Csaba Bartha, Nicolae Dragos, Gyöngyi Székely, Lajos Vörös: Picophytoplankton blooms in Transylvanian hypersaline lakes. Water resources and wetlands. 14-16 September 2012, Tulcea, Romania

2. Andreea Baricz, Ştefan Adrian Andrei, Vasile Muntean, Cristian Coman, **Zsolt Gyula Keresztes,** Mircea Alexe and Horia Banciu: Spatial distribution of microbial communities along salinity gradient in six saline and hypersaline lakes from Transylvania (Central Romania). 9th International Congress on Extremophiles, 10-13 September 2012, Sevilla, Spain.

3. **Zsolt Gyula Keresztes**, Tamás Felföldi, Boglárka Somogyi, Csaba Bartha, Nicolae Dragos, Lajos Vörös, Gyöngyi Székely: Unknown picophytoplankton in Transylvanian salt lakes: great biotechnological potential in extreme environment. European Biotechnology Congress, 28.09.2011-01.10.2011, Istanbul, Turkey

4. **Zsolt Gyula Keresztes**, Boglárka Somogyi, Tamás Felföldi, Csaba Bartha, Nicolae Dragoş, Gyöngyi Székely, Lajos Vörös: Trophic state of Transylvanian hypersaline lakes. Interantional Conference on Ecological Problems of Tourist Lakes, 20-23 june,2011, Tihany, Hungary

5. **Zsolt Gyula Keresztes,** Emil Boros, Boglárka Somogyi, Lajos Vörös: Picoplankton in soda lakes of the Carpathian basin. Second International Conference on Phycology, Limnology and Aquatic Sciences, 14-16 febr. 2010, Port Said, Egypt

Participation on national scientific events:

1. Keresztes Zsolt Gyula, Nagy Erika, Somogyi Boglárka, Németh Balázs, Dragoş Nicolae, Bartha Csaba, Székely Gyöngyi, Vörös Lajos : Pikofitoplankton dominancia az Erdélyi-Medence sós tavaiban. XII. Kolozsvári Biologus Napok, Kolozsvár, 2012 03. 30-31
2. **Keresztes Zsolt Gyula**, Nagy Erika, Somogyi Boglárka, Németh Balázs, Felföldi Tamás, Vörös Lajos: Erdély-i sós tavak fizikokémiai jellemzői. A Magyar Hidrológiai Társaság Szikes Vízi Munkacsoportjának éves találkozója. 2011. 06.17., Pusztaszer, Hungary
3. Felföldi Tamás, Somogyi Boglárka, **Keresztes Zsolt Gyula**, Márialigeti Károly és Vörös Lajos: A tudományos közlemények megbizhatóságának problémái a kontinentális vizekben élő legkissebb cianobaktériumok példáján. LIII. Hidrobiológus napok, Tihany, 2011.10.5-7

4. **Keresztes Zsolt Gyula**, Somogyi Boglárka, Németh Balázs, Székely Gyöngyi, Bartha Csaba, Nicolae Dragoş és Vörös Lajos: Néhány erdélyi sóstó rétegzettségi viszonyai 2011 telén. XII. Kolozsvári Biologus Napok, Kolozsvár, 2011.04.8-10
5. **Keresztes Zsolt Gyula**, Nagy Erika, Somogyi Boglárka, Németh Balázs, Batha Csaba, Székely Gyöngyi, Nicoale Dragoş, Vörös Lajos Az erdélyi medence sós tavainak trofitási viszonyai. LII. Hidrobiológus napok, Tihany, 2010.10.6-8.
6. **Keresztes Zsolt Gyula**, V.-Balogh Katalin, Dragoş Nicolae, Vörös Lajos: Diversitatea picoplanctonului autotrof şi contribiţia acestuia la producţia primară a unor lacuri saline din Bazinul carpatic. Sesiunea Ştiinţifică Actualităţi în Biologia vegetală – Biodiversitate şi etnoecosisteme, 24-25 septembrie 2010, Beliş, România.
7. **Keresztes Zsolt Gyula**, Vörös Lajos, Dragoş Nicolae: Epifluorescence microscopy and molecular techniques for investigation the diversity of the picoalgae. Transylvanian Scientific Days, Cluj-Napoca 20-21.11.2009.
8. **Keresztes Zsolt Gyula**, Ágyi Ákos, Vanyovszki József., Somogyi Boglárka, V.-Balogh Katalin, Vörös Lajos, Fodorpataki László:Influence of physical and chemical parameters of the aquatic environment on photosynthesis of microalgae. Transylvanian Scientific Days, Cluj-Napoca 23-24.11.2007

OTHER PUBLICATIONS OF THE AUTHOR

Articles

1. László Fodorpataki, **Zsolt Gyula Keresztes**, Szabolcs Barna (2010) Algal Toxins and their biological effects. Acta Scientiarium Transylvanica-Biologia, 18/1:5-21
2. V.-Balogh, Katalin, Balázs Németh, **Gyula Zsolt Keresztes,** Ákos Ágyi, Boglárka Somogyi, Lajos Vörös (2010) The relationship between organic carbon pool, bacterioplancton, and photolytic degradation of organic substances in shallow lakes. J. Hung. Hydrobiol. Soc 90(6): 154-156
3. Csaba Bartha, László Fodorpataki, Erika Nagy**, Zsolt Gyula Keresztes**, Gyöngyi Székely, Octavian Popescu (2010) Photosyntesis and water relations of leaf cells exposed to salt stress. Annals of RSCB, Vol. XV. Issue, 1/2010: 211-218

4. László Fodorpataki, Csaba Bartha, **Zsolt Gyula Keresztes** (2009) Stress-physiological reactions of the green alga *Scenedesmus opoliensis* to water pollution with herbicides. Anal. Univ. Oradea, Fas. Biol., XVI/1: 51-56
5. **Zsolt Gyula Keresztes**, Boglárka Somogyi, Balázs Németh, László Fodorpataki, Katalin V.-Balogh (2009) The effect of ultraviolet radiation on the biological availability of dissolved organic substances. J. Hung. Hydrobiol. Soc 86(6):126-128
6. **Zsolt Gyula Keresztes**, Katalin V.-Balogh (2009) Photolytical degradation of dissolved organic substances in a shallow freshwater lake. Acta Scientiarium Transylvanica-Biologia. 17/1:35-45
7. **Keresztes Zsolt Gyula**, Fodorpataki László, V.-Balogh Katalin (2008) Photochemical degradation of dissolved organic substances in Lake Balaton, J. Hung. Hydrobiol. Soc 88(6): 81-83

Results presented on international scientific events

1. László Fodorpataki, **Zsolt Gyula Keresztes**, Csaba Bartha, Attila-Laszló Márton, Szabolcs Barna: Suitability of a green microalgal strain for wastewater bioremediation and biomass production. European Biotechnology Congress, 28.09.2011-01.10.2011 Istanbul, Turkey
2. Csaba Bartha, László Fodorpataki, **Zsolt Gyula Keresztes**, Gyöngyi Székely, Octavian Popescu: Influence of Salt Stress on Photosyntesis on different letuce cultivars. Photosintesis Congress, Research for Food Fuel and Future, 22-27 08. 2010, Beijing, People`s Republic of China
3. László Fodorpataki, **Zsolt Gyula Keresztes**, Csaba Bartha, Szabolcs Barna: Methylmethionine (Vitamine U) alleviates negative effects of chemical stressors of photosyntesis of the green alga Scendesmus opoliensis. Photosintesis Congress, Research for Food Fuel and Future, 22-27 08. 2010, Beijing, People`s Republic of China.

Results presented in national scientific events

1. **Keresztes Zsolt Gyula**, Somogyi Boglárka, Németh Balázs, Székely Gyöngyi, Bartha Csaba, Nicolae Dragoş és Vörös Lajos: Néhány erdélyi sóstó rétegzettségi viszonyai 2011 telén. XII. Kolozsvári Biologus Napok, Kolozsvár, 2011.04.8-10
2. **Keresztes Zsolt Gyula**, Somogyi Boglárka, Németh Balázs, Fodorpataki László, V.-Balogh Katalin: Az UV sugárzás hatása a bakterioplankton szaporodására az oldott szervesanyagok biológiai hozzáférhetőségének tükrében. Kolozsvári Biologus Napok, Kolozsvár. 2009.04. 3-4
3. **Zsolt Gyula Keresztes**, Erika Nagy, Csaba Bartha, Szabolcs Barna, László Fodorpataki: Modificări fiziologice şi biochimice în celulele algale, ca bioindicatori ai calitătii a mediului acvatic. A XXVIII-a Sessiune ştiinţifică a societăţii Române de biologie celulară. 9-12 iunie 2010, Constanţa, România.
4. Csaba Bartha, Erika Nagy, **Zsolt Gyula Keresztes**, Gyöngyi Székely, László Fodorpataki, Octavian Popescu: Fotosinteza şi regimul hidric al celulelor foliare la diferite varietăti de Lactuca sativa expuse stressului de salinitate. A XXVIII-a Sessiune ştiinţifică a societăţii Române de biologie celulară. 9-12 iunie 2010, Constanţa, România
5. V.-Balogh Katalin, Németh Balázs, **Keresztes Zsolt Gyula**, Ágyi Ákos, Somogyi Boglárka, Vörös Lajos : *A szerveszén „pool", a bakterioplankton, a szervesanyagok fotolitikus bomlástermékainek in situ vizsgálata sekély tavakban*. LII. Hidrobiológus napok, Tihany, 2009.09.30-10.02

PARTICIPATION IN RESEARCH PROJECTS

Molecular phylogeny of picoalgae from Romanian salt lakes, CNCSIS/UEFISCDI TE306/70