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Picophytoplankton predominance in hypersaline lakes (Transylvanian Basin, Romania)

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List of abbreviations:

CyPPP - picocyanobacteria

DCM - deep chlorophyll *a* maximum

DGGE - denaturing gradient gel electrophoresis

DO - dissolved oxygen

EuPPP – picoeukaryotes

HNF - heterotrophic nanoflagellates

PPP - photoautotrophic picoplankton

SC - specific conductance

Abstract

The occurrence and importance of photoautotrophic picoplankton (PPP, cells with a diameter <2 μm) was studied along a trophic and salinity gradient in hypersaline lakes of the Transylvanian Basin (Romania). The studied lakes were found to be rich in PPP, with abundances (maximum: 7.6 x 10^6 cells mL^{-1}) higher than in freshwater and marine environments of similar trophic conditions. The contribution of PPP to the total phytoplankton biovolume did not decrease with increasing trophic state as it was generally found in other aquatic environments. Regardless of the trophic conditions, the contribution of PPP could reach 90-100% in these hypersaline lakes. We hypothesized that the PPP predominance might be the result of the low grazing pressure, since heterotrophic nanoflagellates (the main grazers of PPP) were absent in the studied samples. There were significant differences in community composition among the lakes along the salinity gradient. CyPPP predominated in less saline waters (mainly below 5%), while EuPPP were present along the entire salinity range (up to 18.7%), dominating the phytoplankton between 3 and 13% salinity. Above 13% salinity, the phytoplankton was composed mainly of *Dunaliella* species.

Key words: picoplankton importance, phytoplankton composition, salinity gradient, trophic gradient, inland saline lakes, Transylvania

Introduction

The total volume of continental salt water is approximately equal (~ 45%) to that of freshwater lakes and rivers (Hammer 1986; Last 2002). Although lakes are considered to be salt lakes above 0.3 % salt content, this determination covers various habitats with a wide range of salinity (e.g. in extreme saline lakes the salt content can be as high as 30 or 40 %; Williams 1998; Boehrer and Schultze 2008), different ion composition (soda lakes with Na^+ and HCO_3^- ion dominance, salt lakes with Na^+ and Cl^- ion dominance, etc.) and lake morphometry (from shallow ponds to deep, meromictic lakes). According to Hammer (1986), hypersaline lakes, which are common in the arid and semi-arid regions of the world, have a salt content above 5% (50 g L^{-1}) and, are formed in endorheic basins. They also occur, however, outside these regions e.g. as a result of human activities such as solar salt production or mining (Boehrer and Schultze 2008).

Hypersaline lakes of the Transylvanian Basin (Romania) are artificial water bodies with Na^+ and Cl^- ion dominance and surface areas between 380 and 12.100 m^2, which formed in the last century on a middle Miocene salt stratum (Alexe 2010). This phenomenon was a result of the collapse and inundation of abandoned salt mines (Bulgareanu 1996; Alexe 2010) established at the edges of the Basin, where the salt stratum (with an average thickness of 250-300 m) reaches the surface (Irimuş 1998; Alexe 2010). Most of these lakes experience significant anthropogenic impact, as they are popular bathing resorts. Among them, Lake Ursu (Sovata) is the best studied, where the rare phenomenon of heliothermy can lead to a summer water temperature of as much as 60 ºC at 2 m depth (Kalecsinszky 1901; Alexe and Serban 2008; Máthé et al. 2014). Publications on these lakes focused mainly on the physical and chemical properties of the water, the characteristics of the mud, and on the bacterioplankton and nanophytoplankton communities (Muntean et al. 1996; Irimuş 1998; Ionescu et al. 1998; Alexe 2010; Alinei et al. 2006; Nagy and Péterfi 2008; Borsodi et al. 2013). Based on the results of Ionescu et al. (1998), the nanophytoplankton of these hypersaline lakes was mainly composed of cyanobacteria and green algae.

Photoautotrophic picoplankton (PPP, < 2 μm) is ubiquitous in both marine and freshwater environments (Stockner 1991; Callieri 2008). The contribution of PPP to the total phytoplankton biomass and primary production could be very significant: in open oceans up to 90 % (Li et al. 1983; Agawin et al. 2000; Vaulot et al. 2008 and references therein), in continental waters up to 75-80 % (Craig 1984; Weisse 1993; Callieri 2008 and references therein). The occurrence and

dynamics of PPP are influenced by several environmental factors, such as light intensity, water temperature, salinity, nutrient supply, grazing and viral infection (Stockner 1991; Callieri 2008). Although it is very hard to find an appropriate explanation for picophytoplankton success in aquatic systems (Callieri 2008), a widely accepted trend is the increase of picophytoplankton abundance and the decrease of their contribution (to the total phytoplankton) with increasing trophic state (Stockner 1991; Bell and Kalff 2001; Callieri 2008).

PPP research in salt waters is mainly focused on marine environments in spite of the fact that saline lakes are common throughout the world (Hammer 1986; Last 2002). As a result, there are only a few publications about PPP occurrence in hypersaline lakes (Roesler et al. 2002; Estrada et al. 2004; Elloumi et al. 2009; Fanjing et al. 2009; Schapira et al. 2010; Krienitz et al. 2012), which nonetheless clearly indicate the importance of these minute algae in these water bodies. For example, the phytoplankton of a soda lake with 18.8 % salt content in Inner Mongolia, China (Dagenoer Soda Lake) was exclusively dominated by a picoeukaryote alga (*Picocystis salinarum*) despite the hypertrophic conditions in winter 2003 (Fanjing et al. 2009). In a hypersaline soda lake of the East African Rift Valley (Lake Nukuru) the same species composed 53-68 % of the total phytoplankton biomass in winter 2010 (Krienitz et al. 2012). According to our best knowledge, there are no published results about PPP abundance in European hypersaline lakes. On the other hand, the diversity of PPP communities in hypersaline lakes of the Transylvanian Basin has already been studied by molecular methods (Keresztes et al. 2012). As a result, PPP were represented by a simple community consisting of two major genotypes: one from the picoeukaryote *Picochlorum oklahomense* and the other related to marine picocyanobacteria (*Synechococcus* sp.). Our aim was therefore to study the occurrence and importance of PPP in these water bodies along a trophic and salinity gradient.

Materials and methods

Sampling and laboratory measurements

Water samples were collected with Meyer bottles and an electrical layer sampler in July 2010 and in February and August 2011 from eight meromictic, hypersaline lakes in the Transylvanian Basin (Fig 1). The surface area of the lakes was relatively small, ranging between 600 and 3600 m^2, but their maximum depth was between 12 and 69 m (Table 1). In summer, only surface sampling was possible as a result of intensive bathing (700-1160 people/ha), excluding Lake 5 in

2010 and 2011 as well as Lake 2 and Lake 8 in 2011. In winter the investigation was supplemented with depth profile sampling. Temperature, specific conductance (SC), pH and dissolved oxygen (DO) were measured in the field using a HI9033 multimeter (Hanna Instruments, Woonsocket, RI, USA). DO was not measured at one sampling station (Lake 8) in winter 2011 due to technical difficulties. Salt concentration was estimated from SC using the empirical equation presented in Keresztes et al. (2012). Freshly collected water samples were transferred to the laboratory within two hours in dark conditions (thermo boxes) for further analyses. Chlorophyll *a* concentration was determined spectrophotometrically (Shimadzu 160A UV-VIS spectrophotometer) after hot methanol extraction using the absorption coefficients determined by Wellburn (1994).

Epifluorescence microscopy

PPP was studied by epifluorescence microscopy according to MacIsaac and Stockner (1993) in frozen samples within one week after sampling. Briefly, the samples were concentrated on 0.4 µm pore-size black cellulose-acetate filters (Macherey-Nagel), which were embedded into 50 % glycerol. The slides were examined with a Nikon Optiphot 2 epifluorescence microscope at 1000 x magnification. At least 20 fields (400 cells) were photographed with a Spot RT colour camera and picoalgae were counted on these pictures to avoid fluorescence fading. First the cells were located under blue-violet excitation (BV-2A), where picoeukaryotes (EuPPP) show deep red fluorescence due to chlorophyll *a*. Phycoerythrin-rich picocyanobacteria fluoresce bright yellow-orange under this excitation, while phycocyanin-rich picocyanobacteria show only weak red autofluorescence. Switching to green excitation (G-2A) for the same field, picoeukaryotic cells do not show (or just a very weak) autofluorescence. The main property that makes picocyanobacteria (CyPPP) distinct from picoeukaryotes under an epifluorescence microscope is the presence of phycobiliproteins, which show greatly enhanced (red) autofluorescence when using the green waveband (MacIsaac and Stockner 1993).

Heterotrophic nanoflagellates (HNF) were studied according to Sherr et al. (1993). Formalin-fixed samples (2% final concentration) were stained with proflavine (6.47 mg l-1 final concentration) for 5 minutes, filtered onto black, 0.8 µm pore size polycarbonate filters (Millipore) at low vacuum pressure and examined at 1000 x magnification using blue excitation (B-2A) with a Nikon Optiphot 2 epifluorescence microscope.

Phytoplankton biovolume calculation

The abundance and composition of nano- and microplankton were determined in Lugol-fixed samples with an inverted microscope using routine enumeration protocol (Utermöhl 1958). The total biovolume of the phytoplankton was calculated on the basis of cell volume and abundance values assuming a specific gravity of 1.0. Cell size measurements of nano- and microplankton were performed on each species at least on 10 individuals. Picoplankton cell volumes were calculated by measuring the dimensions of 50 cells using an Olympus BX51 differential interference contrast microscope.

Statistical analysis

Relationships between environmental parameters and biological variables were studied using Spearman's rank correlation with OriginPro 8.6 software. Relationship was considered to be significant at $p < 0.05$.

Results

Physical and chemical characteristics of the lakes

The upper layer of the studied lakes had a salt content above 5% (50 g L^{-1}), with the exception of Lake 5, where lower salinity values (2.3-3.4%) were found (Table 1). Strong stratification was observed in the case of deep profile sampling: beneath the mixolimnion, salinity sharply increased, resulting in the halocline between 1.5 and 4 m, with further increases in a number of less distinct steps deeper in the water column. The salinity of the monimolimnion varied between 15.6 and 31% (Table 1). In Lake 5, anthropogenic deep water extraction leads to a decrease in salinity (7.2-21% in the monimolimnion) and a deeper halocline (7-8 m). In winter, Lake 5 had an ice cover of approximately 25 cm, while on the other lakes there was only very thin ice (1-2 cm) and/or a lens of fresher water at the surface due to precipitation. As a result, the salinity of this surface layer was between 2.1 and 7.6% (Table 1).

The temperature profiles of the lakes also show strong stratification. In summer, temperature was found to be nearly constant in the mixolimnion (27-31 °C in 2010, 25-29 °C in 2011), while beneath the halocline it decreased to 15-17 °C. Thermal stratification above the

halocline occurred only in Lake 5, decreasing to 18°C at 6 m depth and showing only a slight decrease below the halocline. Heliothermy was observed in the most saline lake (Lake 8)in summer 2011: temperature increased from 25 °C to 29 °C down to a depth of 2.4 m, then started to decrease with increasing depth. Thermal stratification in winter was evident, with water temperature ranging from -1.7 to 2 °C in the surface water layer (within the lens of fresher water), while the mixolimnion had a constant temperature (3-7 °C) excluding Lake 5, where a continuous increase (from 3 to 13 °C) was observable. A pronounced increase was found in the halocline, and the temperature of the monimolimnion was between 14 and 17 °C in all of the studied lakes.

The halocline exhibited also sharp changes in oxygen and pH: the mixolimnion was saturated with oxygen and had a pH between 7.5 and 9.1, while the monimolimnion was mostly anoxic with a pH from 5.9 to 7.7 (Table 1). A deep DO maximum along with a pH increase was found in the monimolimnion of Lake 2 at 2.5 m (DO: 14 mg L^{-1}, 120%; pH 8.2) and Lake 6 at 4 m (DO: 17.8 mg L^{-1}, 195%; pH 7.2) in winter 2011. In Lake 8, the heliothermy of summer 2011 was coupled with oxygen supersaturation: the concentration of DO increased to more than 20 mg L^{-1} (>200%) with a pH value of 7.7 at 2.4 m depth.

Distribution of chlorophyll *a*

In summer 2010, chlorophyll *a* concentration ranged between 4 and 247 µg L^{-1} at the surface layers. In the case of deep profile sampling in Lake 5, chlorophyll *a* distributed homogenously (3.5-4.4 µg L^{-1}) through the mixolimnion. In winter 2011, chlorophyll *a* concentrations were lower (3-44 µg L^{-1}) at the surface layers than in summer 2010, except in Lake 8 (10 µg L^{-1} in summer 2010 and 48 µg L^{-1} in winter 2011). Together with the DO peak, a deep chlorophyll *a* maximum (DCM) was found in Lake 2 at 2.5 m and in Lake 6 at 4 m with values of 127 and 9.5 µg L^{-1}, respectively. However, a DCM was also observable in Lake 4 and Lake 5 (9 µg L^{-1} at 3 m depth on both sites) without a significant increase in dissolved oxygen concentration and in the case of Lake 8 (117 µg L^{-1} at 1.5 m water depth, DO was not measured). In summer 2011, chlorophyll *a* ranged between 0 and 430 µg L^{-1} at the surface layers. In Lake 2, Lake 5 and Lake 8, chlorophyll *a* concentration increased with increasing water depth up to 21 µg L^{-1} at 3 m, 12

$\mu g\ L^{-1}$ at 5 m and 4.5 $\mu g\ L^{-1}$ at 2.5 m, respectively. In the heliothermal layer of Lake 8, where DO reached its maximum (2.4 m), chlorophyll *a* concentration was only 1.7 $\mu g\ L^{-1}$.

Photoautotrophic picoplankton and heterotrophic nanoflagellates

The PPP community was composed of phycocyanin-rich picocyanobacteria and picoeukaryotic algae with highly variable abundance values between 0 and 7.6×10^6 cells mL^{-1} (Fig.2). In summer 2010 the PPP community was dominated by picocyanobacteria (4.3×10^4 - 7.3×10^6 cells mL^{-1}) in the majority of the surface samples, however in Lake 3 and Lake 8 only picoeukaryotes (1.3×10^6 and 7×10^3 cells mL^{-1}, respectively) were found. In the case of deep profile sampling in Lake 5, picocyanobacteria distributed homogenously through the mixolimnion.

In winter 2011, picoeukaryotic algae dominated in the surface layers with abundances between 2.6×10^3 and 4.8×10^5 cells mL^{-1} except in Lake 5, where picocyanobacteria were found with lower abundances (1.6×10^3 and 1.8×10^4 cells mL^{-1}). In the deeper layers, an abundance peak of picoeukaryotes was observed in Lake 2 at DCM depth (2.5 m) with an abundance value of 1.3×10^6 cells mL^{-1} and within the anoxic monimolimnion of Lake 1 (4.4×10^5 cells mL^{-1}) at 5 m and in Lake 8 (1.1×10^5 cells mL^{-1}) at 3 m. Similarly to the latter, picocyanobacteria were observed in the anoxic monimolimnion of Lake 5 and Lake 7 (4.4×10^5 and 2.1×10^4 cells mL^{-1}) at 3 m depth.

In summer 2011, the PPP community was dominated by picoeukaryotes with abundances between 2.6×10^4 cells mL^{-1} and 7.1×10^6 cells mL^{-1} in most of the surface samples. Picocyanobacteria dominated only in Lake 5 and Lake 6 (6.1×10^4 and 1.5×10^6 cells mL^{-1}, respectively). In the case of deep profile sampling, the abundance of picoalgae was homogenous through the mixolimnion in Lake 2 (EuPPP) and Lake 5 (CyPPP). Picoalgae were absent from the mixolimnion of Lake 8, but in the anoxic monimolimnion the abundance of picocyanobacteria reached 3.2×10^5 cells mL^{-1} at 4 m depth.

Heterotrophic nanoflagellates were not observed in any of the samples.

Phytoplankton community structure and importance of PPP along the salinity gradient

Significant differences were found within the PPP community along the salinity gradient: CyPPP were mainly found in less saline waters (up to a salinity of 11%, but mainly below the lower limit of the hypersaline category), while EuPPP does not seem to be affected by salinity, as they were present in consistently high numbers along the entire salinity range (up to 18.7% salinity). The highest abundance values of EuPPP, however, were observed between 5.2% and 11.5% (Fig. 3.).

Regarding the composition of the total phytoplankton, the PPP community (CyPPP and/or EuPPP) was dominant (more than 50% of the total phytoplankton biovolume) in the majority of the studied samples (Fig. 4). Dinophytes were found in small numbers only below 5% salinity. CyPPP, cryptophytes, small chrysophytes or EuPPP were the dominant group between 2 and 6% salinity, while diatoms were observed only with low contribution (Fig. 4). Above 6% salt content, mainly EuPPP predominated the phytoplankton up to 13% salinity, while *Dunaliella* sp. up to 31% (Fig. 4.). In winter 2011, the phytoplankton community was exclusively composed of the latter group at the DCM in Lake 6 (at 4 m depth) and in Lake 8 (at 1.5 m depth).

Importance of PPP along the trophic gradient

PPP abundance increased with increasing trophic state and the obtained data were mostly in good agreement with PPP abundance values found in freshwater lakes and oceans (expressed here as empirical regression models described by Bell and Kalff (2001); Fig. 5.). In some cases, however, these values were found to be one order of magnitude higher than in lakes or oceans with similar trophic state (Fig. 5.).

In the studied hypersaline lakes, the share of PPP from the total phytoplankton biovolume did not decrease with increasing trophic state as it was described for freshwater and marine environments (Fig. 6.). Regardless of the phytoplankton biovolume, the contribution of PPP could reach 90-100% in these aquatic environments (Fig. 6.).

Phytoplankton and environmental variables

A positive correlation was found between temperature and the relative biovolume of CyPPP, while negative correlation between temperature and the biovolume of *Dunaliella* sp. (Table 2). The biovolume and contribution of CyPPP and cryptophytes (correlated negatively with salinity, while. *Dunaliella* sp. showed a positive correlation (Table 2). Significant correlations were not found between salinity and other algal groups. With regard to trophic state, there was a clear positive correlation between chlorophyll *a* concentration and PPP/EuPPP biovolume (Table 2).

Discussion

The rich PPP community (maximum abundance of 7.6 x 10^6 cells mL^{-1}), which was found in the studied lakes, showed no clear depth distribution pattern. Deep-water CyPPP or EuPPP populations were found, however, in many lakes within the anoxic monimolimnion, which might be the result of sinking as was described in Mono Lake (Budinoff and Hollibaugh 2005). Seasonal succession of the PPP community - the dominance of CyPPP in summer and EuPPP in winter -, which has often been described in temperate freshwater and soda lakes as well as in Mediterranean lagoons (Callieri 2008; Vörös et al. 2009; Somogyi et al. 2009; Bec et al. 2011), was not observed in the studied hypersaline lakes. In spite of the fact that CyPPP is mainly found in summer and there was a positive relationship between temperature and CyPPP contribution to total phytoplankton biovolume, EuPPP can dominate the PPP community either in winter or in summer. Similarly to that, Fanjing et al. (2009) described the exclusive dominance of *Picocystis salinarum* in the hypersaline Dagenoer Soda Lake through the whole year.

Salinity seems to influence the PPP communities better than temperature: CyPPP was found mainly below 5% salt content and their biovolume/contribution decreased with increasing salinity. EuPPP, however, was observed with high abundance and contribution values between 5 and 11.5 % salinity. Above that, EuPPP was found only with lower abundances up to 18.7% salt content. In the most saline lake (Lake 8.), where the salinity of the mixolimnion ranged between 19 and 21%, PPP was barely found. Similar results were obtained in a coastal lagoon system, where the environmental variable that best explained the picophytoplankton abundance pattern along the lagoon was salinity (Schapira et al. 2010). According to Schapira et al. (2010), CyPPP was mainly abundant below 3% salinity, while at salinities ranging from 4.5% to 14.0% the PPP was dominated by EuPPP. However, at salinity values greater than 14.0%, the community shifted into a *Prochlorococcus*-like population (Schapira et al. 2010). In a solar saltern system

(Tunesia), the PPP was exclusively composed of EuPPP with maximum abundances between 7.9 and 19% salinity (Elloumi et al. 2009). In the crystallizer pond (43 % salinity), however, picoeukaryotes were not found (Elloumi et al. 2009). These results are in good correlation with our findings. However, available nutrients (N and P forms and ratios) may have significant influence on PPP community composition and dynamics as described by Crosbie et al. (2003).

Within the picosize range, mainly eukaryotic algae were isolated from hypersaline environments. Among them, *Picochlorum* and *Picocystis* are the most thoroughly studied (Henley et al. 2002; Roesler et al. 2002; Fanjing et al. 2009). In the studied hypersaline lakes, a previous DGGE analysis detected *Picochlorum* sequences (Keresztes et al. 2012). Henley et al. (2002) studied the salinity tolerance of *Picochlorum oklahomense*, which originated from the Salt Plains National Wildlife Refuge (Oklahoma, USA). As a result, *Picochlorum* was able to grow from 0 to 10% salinity, however it exhibited decreasing growth rate with increasing salinity (Henley et al. 2002). A *Picocystis* isolate from an Inner Mongolian soda lake exhibited a broader salinity tolerance, as it could grow over a salinity range of 2.9-17.5% (Fanjing et al. 2009). Another *Picocystis* strain, which was isolated from Mono Lake, was able to grow from 0 to 26% salinity, with a peak at 4% (Roesler et al. 2002). In comparison, a picocyanobacterium strain isolated from the same lake had lower salinity tolerance (growing from 0% to 10% salinity with a maximum specific growth rate at 3% and a minimum at 8%) than *Picocystis* (Budinoff and Hollibaugh 2007). The higher salinity tolerance of EuPPP could explain their success in waters of higher salinity, as was observed in lagoon and solar saltern systems (Elloumi et al. 2009; Schapira et al. 2010), in agreement with the present study.

Microscopic observations on the composition of nano- and microplankton corresponded well with the results of the DGGE analysis, which showed the presence of mainly flagellated chlorophytes (*Dunaliella* spp., *Chlamydomonas* spp.), besides cryptophytes, haptophytes and diatoms (Keresztes et al. 2012). A significant change appeared in the community structure as the salinity increased. Below 5% salinity, CyPPP predominated in the majority of the samples. EuPPP was dominant between 3 and 13% salinity, but above that, the communities were exclusively dominated by *Dunaliella*. In the most saline lake (Lake 8.) the phytoplankton was dominated by *Dunaliella* sp. at all sampling dates. The broad halotolerance of *Dunaliella* sp. was described in many studies. In a solar saltern system (Tunesia), chlorophytes (*Dunaliella* sp.) and cyanobacteria (*Aphanothece* sp.) dominated the community above 19% salinity (Elloumi et al.

2009), similarly to other systems in Spain, where *Dunaliella salina* was found at salinities of 25% and above (Pedrós-Alió et al. 2000; Estrada et al. 2004). Henley et al. (2002) compared the salinity tolerance of the picoeukaryote *P. oklahomense* and *Dunaliella* sp. isolated from the same hypersaline environment. *Picochlorum* preferred lower salt content than *Dunaliella*, which exhibits broad halotolerance, growing faster at 5-10% salinity than at 2% (Henley et al. 2002). According to Jahnke and White (2003), *Dunaliella tertiolecta* was able to grow at up to 17.6% salinity, while other *Dunaliella* species (*D. parva, D. salina* and *D. bardawil*) at up to 29 %. The exclusive *Dunaliella* sp. predominance in the studied hypersaline lakes above 13% salinity is in good agreement with previous findings.

The generally observed trend about the increase of PPP abundance with increasing trophic state (Stockner 1991; Bell & Kalff 2001; Callieri 2008) was clearly observable in the studied hypersaline lakes. However, the maximum PPP abundance values (7.1-7.6 x 10^6 cells mL^{-1}) were higher than in the majority of freshwater and marine environments of similar trophic state (Fig. 5). It is hard to find PPP abundance values from other hypersaline lakes for comparison. On the other hand, Schapira et al. (2010) found PPP abundances in the same order of magnitude (1.3-1.4 x 10^6 cells mL^{-1}) in a coastal lagoon system with a salinity of 8-11% (South Australia), despite the lower biomass of the phytoplankton (chlorophyll *a*: 6 - 14 μl L^{-1}). In some hypersaline soda lakes of the East African Rift Valley, PPP (*Picocystis* sp.*)* was also found in high abundance (3.1 – 3.5 x 10^6 cells mL^{-1}) but the trophic state was not determined (Krienitz et al., 2012). In the case of the hypertrophic Dagenoer Soda Lake (Inner Mongolia, China), Fanjing et al. (2009) also hinted at high PPP abundances (*Picocystis salinarum*), but chlorophyll *a* and picoplankton abundance were not determined. On the basis of these findings, high PPP abundance could be common in hypersaline lakes, but the number of studies describing PPP occurrence in these environments, particularly along with trophic state, is limited.

Predominating in the majority of the samples, the PPP community in the studied hypersaline lakes did not follow the widely observed trend on the decreasing contribution of PPP with increasing trophic state in freshwater and marine ecosystems (Stockner 1991; Bell and Kalff 2001; Callieri 2008). In spite of the fact that the abundance of PPP was studied in lagoon and solar saltern systems along a salinity and/or trophic gradient, the relative importance of PPP within the phytoplankton was not characterized (Elloumi et al. 2009; Schapira et al. 2010; Estrada et al. 2004). The present study is therefore the first observation on the behaviour of PPP

as a function of trophic state in hypersaline lakes. The PPP predominance in hypersaline lakes might be the result of decreased grazing pressure, as heterotrophic nanoflagellates (the main grazers of PPP (Callieri 2008)) were absent in the studied lakes. Similarly to our findings, Wu et al. (2009) described the absence of HNF in hypersaline lakes of the East Tibetan Plateau. According to Pedrós-Alió et al. (2000), who studied the microbial food web along the salinity gradient in solar saltern systems in Spain, the abundance of HNF and ciliates decreased with increasing salinity, disappearing around 25% salt content. However, in other solar salterns, HNF were found to be actively grazing on bacteria even in the most saline (32-37%) ponds (Park et al. 2003; 2006). The study of other potential grazers (such as ciliates and larger zooplankton, such as *Artemia* sp., which grazed heavily on PPP in Lake Mono according to Roesler et al. 2002) along with grazing experiments would be necessary to quantify top-down processes in hypersaline lakes, which might serve as an explanation to the unusual behaviour of PPP in these ecosystems.

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TABLES

Table 1 List of investigated lakes and selected physical, chemical and biological variables. Morphometric data (water surface, average and maximum depth) were adopted from Alexe (2010). Abbreviations: ML – mixolimnion, MM – monimolimnion, ND – no data.

Lake	Coordinates	Surface (m^2)	Depth (m) [a]	pH		Estimated NaCl concentration (g L^{-1}) [b]		Max. chlorophyll *a* (µg L^{-1})	Max. PPP abundance (cells mL^{-1})	Max. PPP contribution (%)
				ML	MM	ML	MM			
Lake 1 (L. Cabdic)	N47°07.712' E23°51.900'	1524	8.9 (38)	7.5-8.9	7.3	(36) 55-60	219- >311	104	7.3×10^6	95
Lake 2 (L. Băilor)	N46º55.913' E23º54.073'	600	2.5 (69)	8.5-8.8	6.9-8.2	(31) 44-52	156- >311	128	7.6×10^6	90
Lake 3 (L. Băilor Cojocna)	N46°44.907' E23°50.441'	2425	3.5 (12)	8.3-8.5	ND	(64) 114-116	ND	431	7.1×10^6	99.9
Lake 4 (L Durgău Cojocna)	N46°44.836' E23°50.442'	2406	9.2 (43)	8.2-8.6	7.0-7.4	110-128	>311	12	1×10^5	82
Lake 5 (L. Tarzan)	N46º34.472' E23º48.549'	3589	4.9 (12)	8.2-9.1	6.7-7.7	23-34	72-211	12	5×10^5	89
Lake 6 (L. Ocnei)	N46°35.158' E23°47.282'	2134	12 (33)	7.6-8.7	6.0-7.2	44-78	>311	11	1.5×10^6	100
Lake 7 (L. Rotund)	N46°35.099' E23°47.210'	624	3.3 (13)	8.1-8.6	5.9-6.9	(21) 44-83	>311	89	9.9×10^5	96
Lake 8 (L. Fără Fund)	N45º52.578' E24º04.064'	1672	6 (32)	7.5-9.1	6.1-7.7	(76) 187-209	>311	117	7.1×10^3	3.8

[a] Maximum values are given in parenthesis. [b] Salinity of freshwater lens on the surface in winter 2011 is given in parenthesis.

Table 2 Spearman's rank correlation coefficients between biological, physical and chemical variables (*** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$). Coefficients were computed with data from the studied hypersaline lakes at all sampling dates and depths, excluding data from the anoxic monimolimnion (n=47). Abbreviations: CyPPP – picocyanobacteria, EuPPP – picoeukaryotes, PPP – autotrophic picoplankton.

Variable 1	Variable 2	Correlation coefficient
Temperature (°C)	CyPPP contribution to PPP biovolume (%)	0.386*
Temperature (°C)	*Dunaliella* biovolume ($mm^3 L^{-1}$)	-0.420*
Specific conductance ($mS\ cm^{-1}$)	CyPPP biovolume ($mm^3 L^{-1}$)	-0.578***
Specific conductance ($mS\ cm^{-1}$)	CyPPP contribution to PPP biovolume (%)	-0.556***
Specific conductance ($mS\ cm^{-1}$)	CyPPP contribution to phytoplankton biovolume (%)	-0.518***
Specific conductance ($mS\ cm^{-1}$)	*Dunaliella* biovolume ($mm^3 L^{-1}$)	0.357*
Specific conductance ($mS\ cm^{-1}$)	*Dunaliella* contribution to phytoplankton biovolume (%)	0.459**
Specific conductance ($mS\ cm^{-1}$)	cryptophyte biovolume ($mm^3 L^{-1}$)	-0.527**
Specific conductance ($mS\ cm^{-1}$)	cryptophyte contribution to phytoplankton biovolume	-0.588***
Chlorophyll *a* concentration ($\mu g\ L^{-1}$)	EuPPP biovolume ($mm^3 L^{-1}$)	0.449**
Chlorophyll *a* concentration ($\mu g\ L^{-1}$)	PPP biovolume ($mm^3 L^{-1}$)	0.605***

FIGURE LEGENDS

Fig. 1 Geographical location of the sampling sites. Squares represent some major cities and full circles mark sampling sites with the the names of nearby villages and the numerical code of lakes in parentheses

Fig. 2 Relationship between picoplankton abundance (PPP abundance) and temperature in the studied hypersaline lakes. Data from anoxic monimolimnion are not included

Fig. 3 Picoplankton abundance (PPP abundance) along the salinity gradient in the studied hypersaline lakes. Data from anoxic monimolimnion are not included

Fig. 4 Occurrence of different phytoplankton taxa along the salinity gradient in all samples obtained from the hypersaline lakes. Abbreviations: CyPPP – picocyanobacteria, EuPPP – picoeukaryotes. Data from anoxic monimolimnion are not included

Fig. 5 Relationship between picoplankton abundance (PPP abundance) and total chlorophyll *a* concentration in the studied hypersaline lakes. Empirical regression models describing the relationship in freshwater lakes and marine systems are also shown (Bell & Kalff, 2001). Data from anoxic monimolimnion are not included

Fig. 6 Relationship between picoplankton contribution (Percent PPP biovolume) and total chlorophyll *a* concentration in the studied hypersaline lakes. Empirical regression models describing the relationship in freshwater lakes and marine systems are also shown (Bell & Kalff, 2001). Data from anoxic monimolimnion are not included


UKRAINE
N
W
E
S
HUNGARY
MOLDOVA
Dej
Ocna Dej (Lake 1)
Sic (Lake 2)
Cluj-Napoca
Cojocna (Lake 3, 4)
Turda (Lake 5, 6, 7)
Ocna Sibiului (Lake 8)
Sibiu
ROMANIA
SERBIA
Bucharest
Black Sea
BULGARIA

Fig.1.

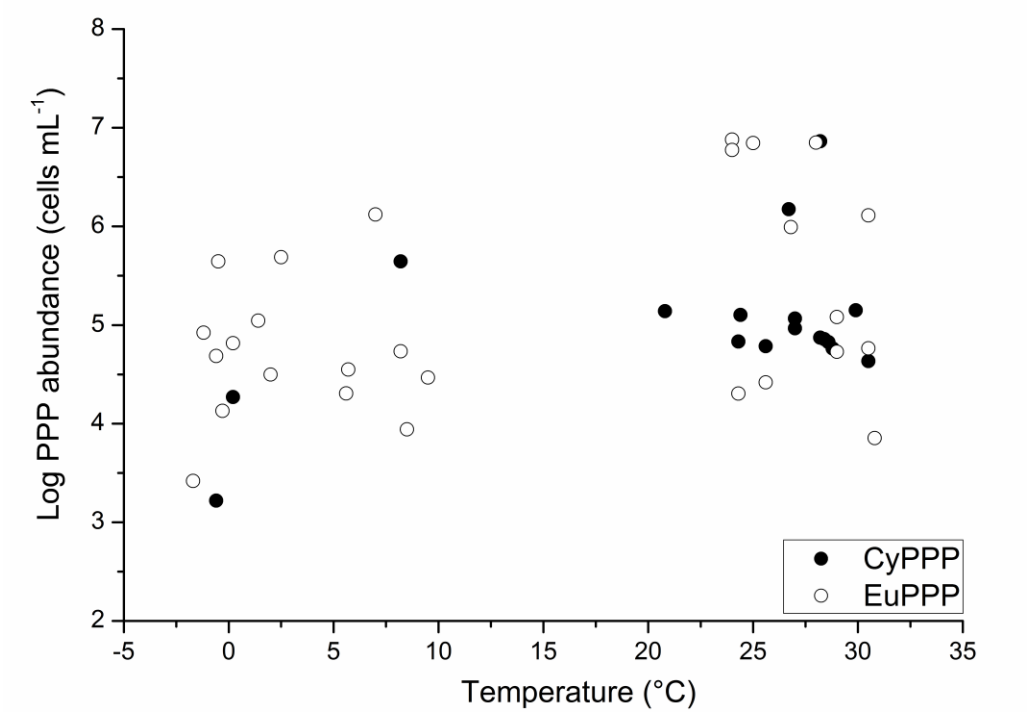
Log PPP abundance (cells mL-1)
Temperature (°C)
CyPPP
EuPPP
8
7
6
5
4
3
2
-5
0
5
10
15
20
25
30
35

Fig.2.

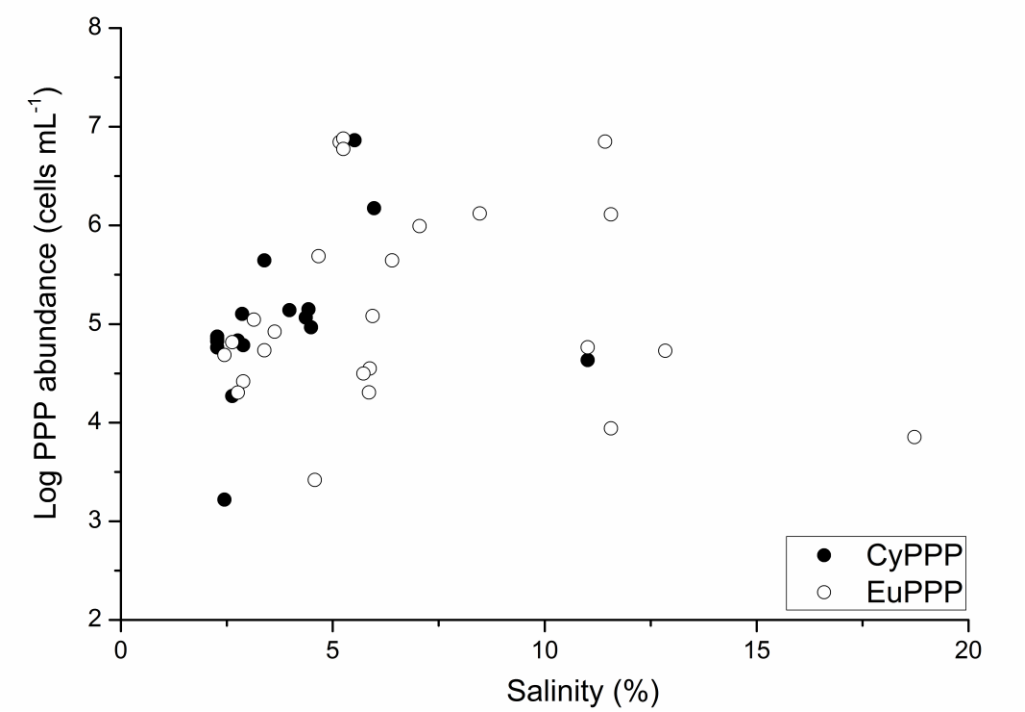
Log PPP abundance (cells mL-1)
Salinity (%)
CyPPP
EuPPP
8
7
6
5
4
3
2
0
5
10
15
20

Fig.3.

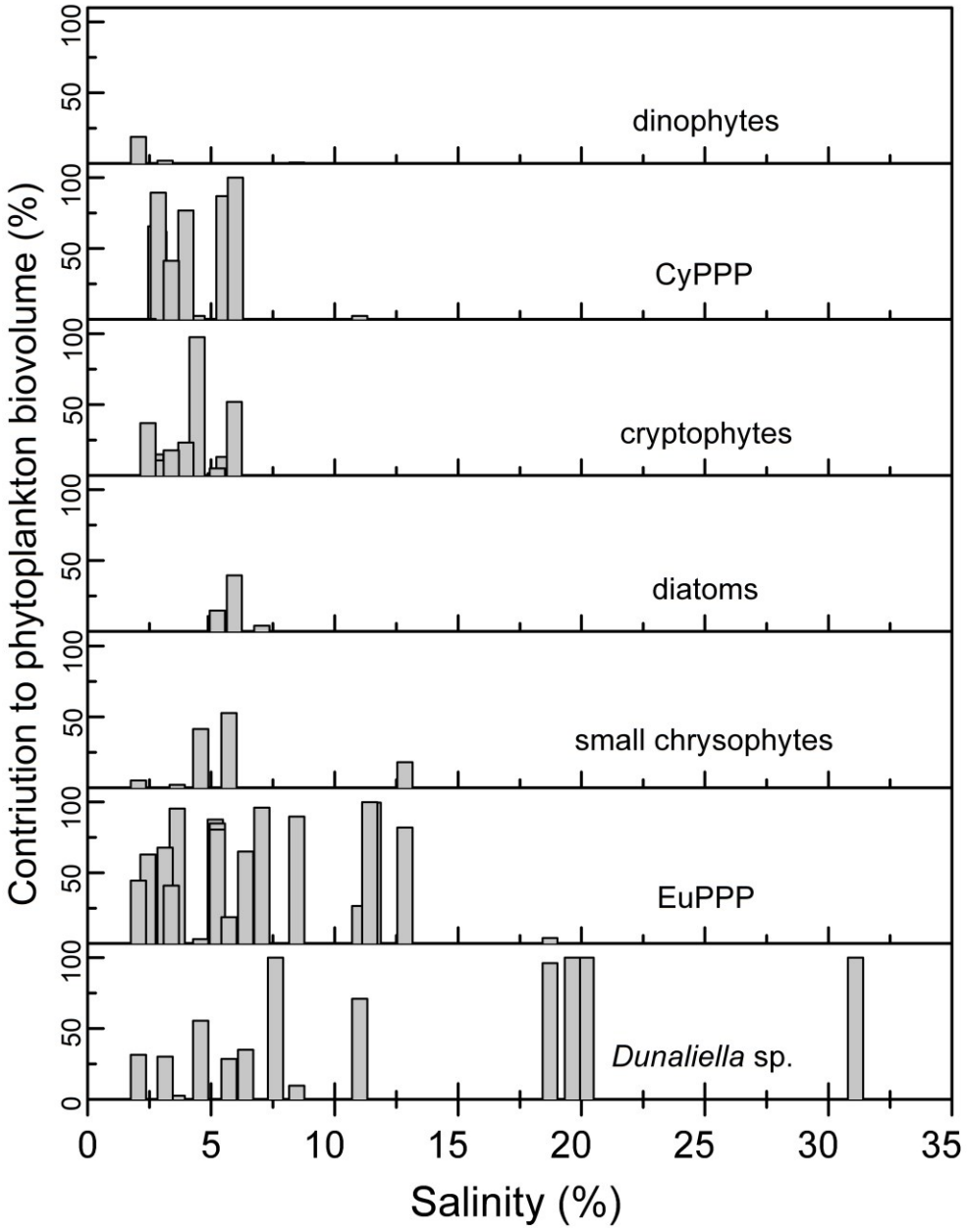
Contriution to phytoplankton biovolume (%)
dinophytes
CyPPP
cryptophytes
diatoms
small chrysophytes
EuPPP
Dunaliella sp.
0
50
100
0
5
10
15
20
25
30
35
Salinity (%)

Fig.4.

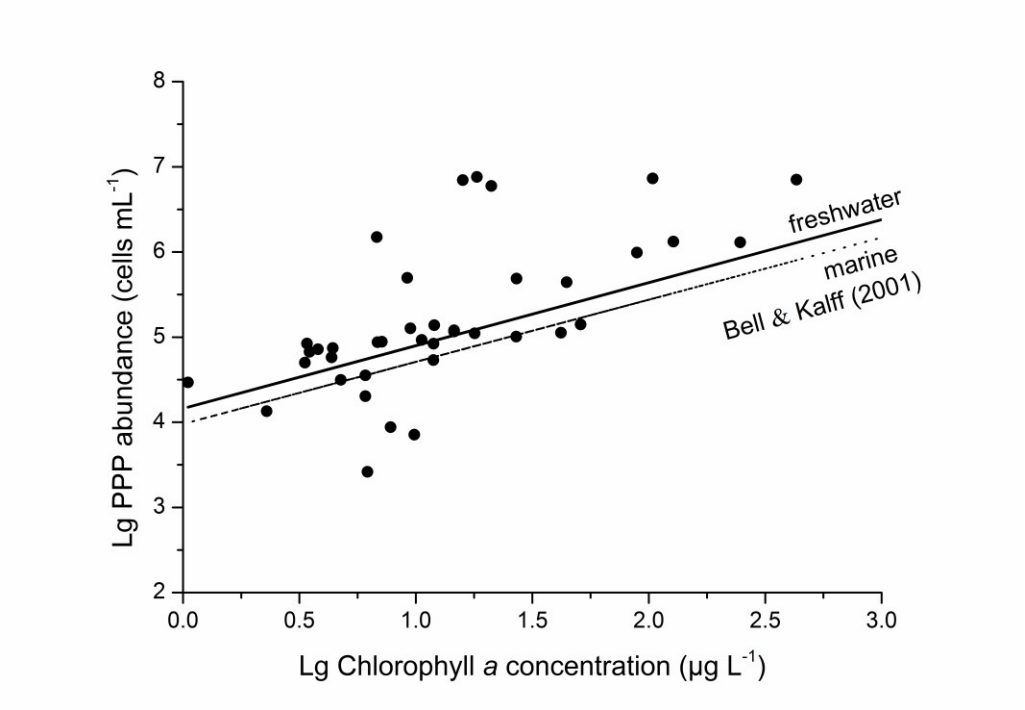
Lg PPP abundance (cells mL^{-1})
Lg Chlorophyll *a* concentration (μg L^{-1})
freshwater
marine
Bell & Kalff (2001)

Fig.5.

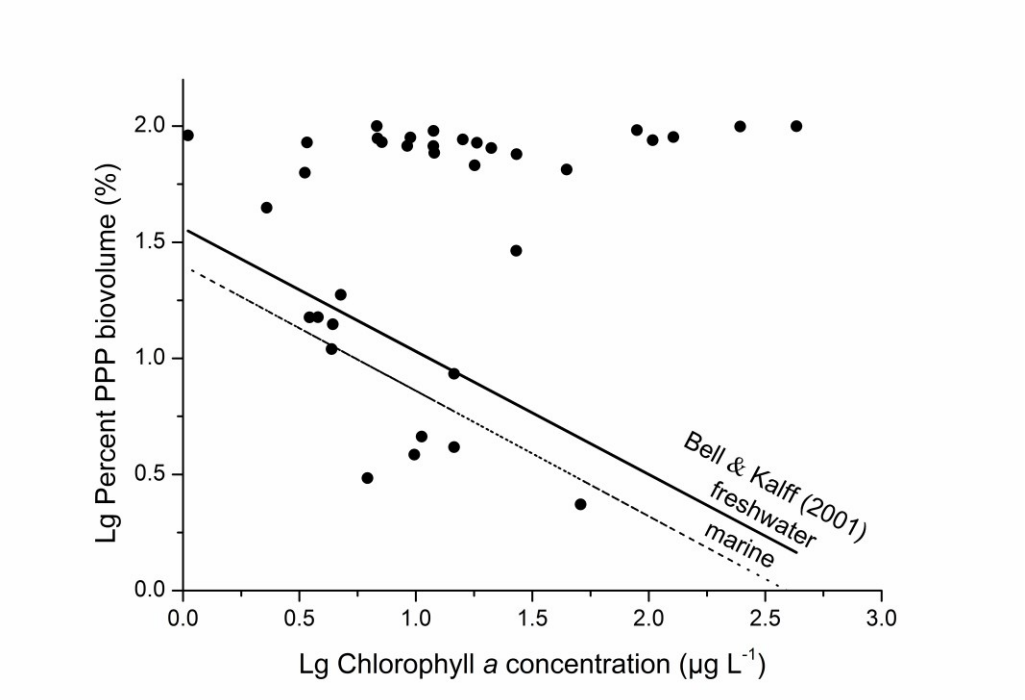
Lg Percent PPP biovolume (%)
Lg Chlorophyll *a* concentration (μg L^{-1})
Bell & Kalff (2001)
freshwater
marine

Fig.6.