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PICOPLANKTON IN SODA LAKES OF THE CARPATHIAN BASIN

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Abstract: The present study documents the findings that picophytoplankton is one of the fundamental contributors to primary biomass production not only in aquatic environments with very low content of inorganic nutrients, but also in lakes with higher trophicity. The autotrophic picophytoplankton (APP) comprises bacterial-sized (0.2–2μm) picocyanobacteria and eukaryotic phototrophs (mainly green algae). These organisms can provide up to 90% of the primary production of shallow soda lakes. Shallow soda pans are very characteristic of the Pannonic Ecoregion (Austria, Hungary and Serbia) and they represent a considerable part of the lakes present in this region.

Between 11 May and 20 June 2009, the authors determined the total biomass of the phytoplankton (based on chlorophyll-a) in 65 soda pans (with an average depth of 17 cm) of the Pannonic Ecoregion. They also estimated the biomass and abundance of the picophytoplankton, using epifluorescence microscopy. The physico-chemical characteristics of the water, relevant for the phytoplankton, were also investigated. The chlorophyll-a (Chl-a) concentration ranged between 1 $\mu g\ l^{-1}$ and 1400 $\mu g\ l^{-1}$ with an average of 115 $\mu g\ l^{-1}$. The abundance varied between 1000 cells ml^{-1} and 88 million cells ml^{-1}. Picoplankters were represented by 1.5 μm-sized green algae and 1.0 μm-sized phycocyanin-rich bacteria. Phycoerythrin-rich picocyanobacteria have not been detected. Based on the present findings, in these water bodies, the contribution of picoplankton to total phytoplankton did not show a decreasing tendency with increasing trophic state, as it was recorded earlier by other authors in different types of aquatic environments. The present results reveal that in soda lakes the picophytoplankton has a crucial contribution to the total primary production not only under conditions of nutrient deficiency, but also in lakes with higher trophicity.

The total phosphorus (TP) concentration in the soda lakes studied varied between 70 $\mu g\ l^{-1}$ and 59 000 $\mu g l^{-1}$. Based on TP, four of the lakes were considered eutrophic and 61 hypertrophic. Based on phytoplankton biomass, half of the lakes were classified as oligotrophic, respectively mesotrophic, while the rest were considered eutrophic, respectively hypertrophic.

Keywords: Pannonic Ecoregion, picoalgae, picocyanobacteria, phytoplankton, soda pans, trophicity

Introduction

The picophytoplankton comprises bacterial-sized (size range: 0.2–2 μm) picocyanobacteria and eukaryotic phototrophs (mainly green algae) [8]. These organisms are distributed worldwide and their occurrence is common in all types of lakes and trophic states. The picophytoplankton is present in all types of aquatic ecosystems, both in freshwater and saltwater. It represents an important component of total phytoplankton biomass and ensures a great part of primary production in many aquatic ecosystems [13]. The widespread occurrence of pico-sized algae was discovered in the early 1980s, thanks to the new techniques of epifluorescence microscopy and flow cytometry [3]. The photoautotroph picoplankton (APP) can be distinguished from the heterotrophic planktonic organisms by the autofluorescence of its components, due to the photosynthetic pigments. With these techniques it becames possible to routinely quantify APP. Three cell types were found: yellow autofluorescing phycoerythrin (PE)-rich picocyanobacteria, red autofluorescing phycocyanin (PC)-rich picocyanobacteria, and picoeukaryotes (EuAPP) without phycobilin pigments. Under blue-violet excitation light, picoeukaryotes appear red, due to chlorophyll-*a* fluorescence, whereas PE-rich cyanobacteria fluoresce yellow and PC-rich cells fluoresce red. With green excitation light, all types of APP fluoresce red, but the intensity of fluorescence of picocyanobacteria increases significantly, while that of picoeukaryotes decreases.

Picoplankton ecology is well studied in both marine and inland waters [3]. Several studies have shown that the relative importance of picophytoplankton (PP) declines with increasing trophic status. In ultra-oligotrophic freshwaters, where the community biomass represents less than 0.54 µg Chl-*a* l^{-1}, PP typically contributes more than 50% to the total biomass. Its contribution declines in hypertrophic waters to an average of 3% at a concentration of 100 µg Chl-*a* l^{-1} [10]. This tendency is also valid in marine waters; however, the relative contribution of PP to total community biomass is smaller in marine systems [2, 7]. Shallow soda pans are particularly characteristic of the Pannonic Ecoregion (e.g. Austria, Hungary and Serbia) and, due to their special features, they represent outstanding natural values. These are intermittent, shallow alkaline lakes (max. depth = 1 m) that frequently dry out entirely by the end of summer [16]. Their salinity varies between hypo- (3-20 g l^{-1}) and meso-saline (> 20 g l^{-1}) ranges, as a function of the season and water level. These water bodies are characterized by the dominance of Na^+, HCO_3^-, CO_3^{2-} and Cl^- ions, with pH values between 9 and 10 [11]. In most cases, large amounts of suspended inorganic (clay) particles cause very high turbidity and impart a light grey colour to the water. Algological investigation of Hungarian soda lakes/ponds was intense in the last century, and yielded an exhaustive, long list of species, but did not give satisfactory quantitative information about the planktonic and benthic algal assemblages [11]. According to the latest research on some turbid soda pans [15], in Southern Hungary the phytoplankton of lakes was predominantly formed by prokaryotic and eukaryotic picoplankton. In May and June 2009 the picoplankton populations were investigated in a large series of soda pans. The aim of this study was to assess the occurrence and distribution of phototrophic picoplankton in these particular water bodies.

Materials and Methods

Samples were taken from 65 soda pans in Hungary (35), Austria (26) and Serbia (4). The relevant physico-chemical characteristics of the water were also measured (water depth, water temperature, pH, electrical conductivity). Phytoplankton samples were filtered through a GF/5 glass-fibre filter and total chlorophyll was extracted using the hot methanol technique [17]. After extraction, Chl-*a* content was determined spectro-photometrically (absorbance at 750 nm, 666 nm and 653 nm), using the following equation:

Chl-*a* (µg l^{-1}) = [17.12 $*$ (OD666 – OD750) – 8.68 $*$ (OD653 – OD750)] / (Cw/Cm) $*$ 100],

where OD750, OD666 and OD653 represent the optical density of methanolic extracts at wavelengths of 750 nm, 666 nm and 650 nm, Cw is the quantity of filtered water in ml, and Cm is the quantity of methanol (in ml) used for extraction.

Phytoplankton abundance was determined in freshly collected samples using epifluorescence microscopy. Samples were diluted with particle-free lake water and filtered through black polycarbonate membranes (0.2 µm pore size). These were mounted on slides with 50% glycerol, covered with a coverslip and examined by an epifluorescence Nikon Optiphot 2 microscope at X1000 magnification. Each visual field was examined under blue-violet and green excitation light [9]. Picophytoplankton biomass (fresh weight) was calculated based on picoplankton abundance and cell volumes (considering that 10^9 μm^3 = 1 mg).

Results and Discussion

The majority of the Pannonic Ecoregion lakes investigated showed hypertrophic character, the Chl-*a* concentration ranging between 1.0 and 1450 µg l^{-1} with an average of 115 μg l^{-1}. Their water depth varied between 2 cm and 100 cm, with an average depth of 17 cm. The pH ranged between 8.8 and 10.4, and most showed hyposaline character (conductivity average 8500 μS cm^{-1}). The total picoplankton abundance varied between 1000 cells ml^{-1} and 88 million cells ml^{-1}. Two picoplankton types were distinguishable in the water bodies investigated: I. – red-fluorescing eukaryotes, 1.5 μm sized (1-2 μm) spherical or ovoid green algae, and II. – red-fluorescing (phycocyanin-rich), 1.0 μm sized (0.8-1.2 μm) spherical or ovoid picocyanobacteria. Phycoerythrin-rich (yellow-fluorescing) picocyanobacteria could not be detected.

Picoeukaryotes were the most abundant organisms in our studied water samples. In 35 water bodies the contribution of eukaryotes exceeded 50% of total picoplankton abundance (Fig. 1). However, the highest abundance of picocyanobacteria (74 million cells ml^{-1}) was significantly higher than that of picoeukaryotes (15 million cells ml^{-1}), demonstrating that the density of picocyanobacteria populations is much higher than that of picoeukaryotes.

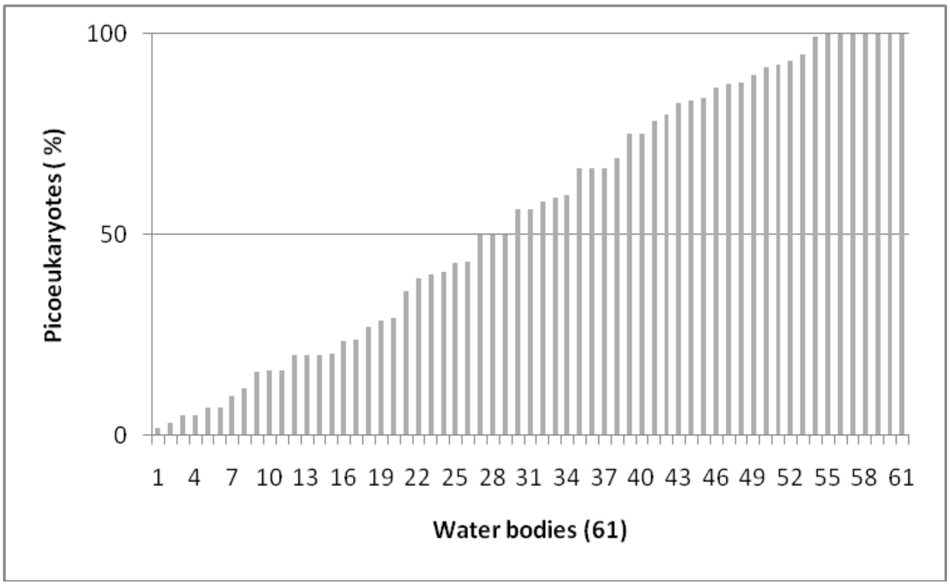

Fig. 1: Contribution of picoeukaryotes to the total picoplankton abundance in the investigated water bodies

In the majority of the world's lakes picocyanobacteria abundance usually exceeds the abundance of eukaryotes by 1–2 orders of magnitude [4]. The present results do not accord to this general pattern, because in the alkaline, hyposaline turbid waters of the Pannonic Ecoregion in late spring, the abundance of picoeukaryotes was as high as that of picocyanobacteria. To explain this finding, further investigations are required, such as seasonal sampling and the investigation of the abundance of picoeukaryotes and picocyanobacteria, and finally the comparison of results. It is possible that the abundance of the two types of organism is changing according to temperature, light conditions and nutrient content. Somogyi *et al*. (2009) observed a very high abundance and dominance of picoeukaryotes in some Hungarian soda pans in winter and spring periods.

The present authors found a significant positive relationship between total phytoplankton biomass (reflected by the Chl-*a* concentration) and picoplankton abundance (Fig. 2). A similar relationship occurred between the total phytoplankton biomass and picophytoplankton biomass (Fig. 3). These results suggest that autotrophic picoplankton constitutes a major part of the total phytoplankton community in the waters studied.

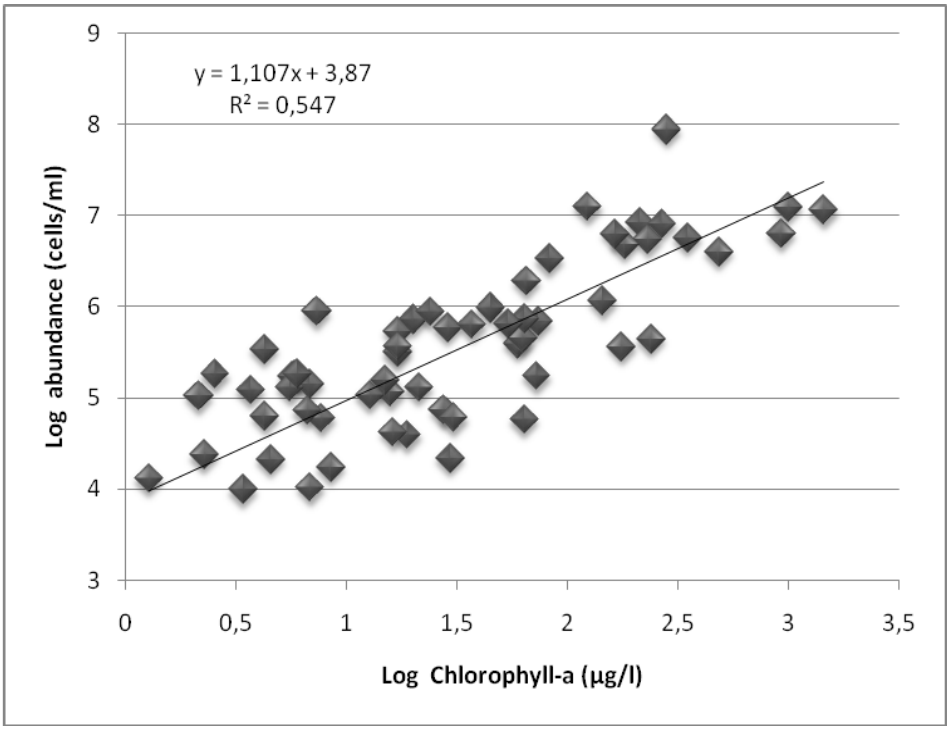

Fig. 2: Relationship between total phytoplankton biomass (based on chlorophyll-*a* content) and picoplankton abundance in the investigated water bodies

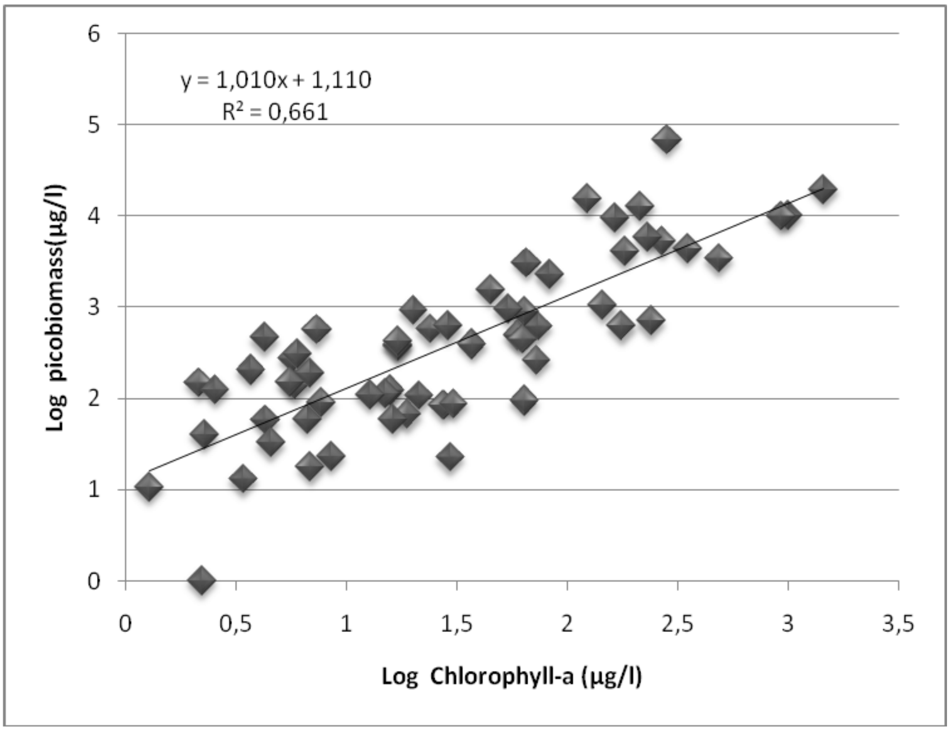

Fig. 3: Relationship between total phytoplankton biomass (based on chlorophyll-*a*) and picoplankton abundance in the investigated soda lakes

Gaulke *et al*. (2010) investigated the contribution of PP of the total phytoplankton productivity in a river-dominated estuary, and demonstrated that the PP Chl-*a* concentration accounted for 30–44% of the total Chl-*a* on an annual basis, this parameter showing a negative correlation with total Chl-*a.* The primary productivity of PP showed a negative correlation with the total productivity. When these values from all sampling stations with distinctive trophic status and all seasons were pooled, the PP productivity was positively correlated with the total productivity.

Bell and Kalff (2001) established in a large series of lakes (mainly deep lakes) a significant positive relationship between total phytoplankton biomass (Chl-*a*) and picoplankton abundance. The present results correspond with this general trend, but there is a very important difference between our results and the results based on deeper lakes and/or marine waters. The difference between the investigated soda pans and other examined lakes and seas is the different slope of the above-mentioned relationships (Table 1).

Table 1: Summary of the relationships between abundance (abund, cells ml^{-1}) and biomass (μg l^{-1}) of autotrophic picoplankton (APP) and total phytoplankton biomass (TChl *a*; μg l^{-1}) for turbid soda pans, deep lakes and marine waters

Water type	Model	r^2	n	p	Reference
soda pans	$\log_{10}$(APPabund) = 3,87 + **1.107** $\log_{10}$ (TChl *a*)	0.548	65	< 0.0001	this study
soda pans	$\log_{10}$(APPbiomass) =1.109 + **1.010** $\log_{10}$ (TChl *a*)	0.662	56	< 0.0001	this study
deep lakes	$\log_{10}$(APPabund) = 4.16 + **0.74** $\log_{10}$ (TChl *a*)	0.25	137	< 0.0001	*Bell & Kalff model
seas	$\log_{10}$(APPabund) = 3.98 + **0.33** $\log_{10}$ (TChl *a*)	0.03	122	0.051	*Bell & Kalff model

When they analyzed a large-scale database on the productivity of PP, Agawin *et al.* (2000) ascertained that the PP was present with a higher biomass than the nano- and micro-phytoplankton fraction in less productive waters. In more productive waters, the biomass of the larger algae was higher than the PP biomass.

In marine and freshwater ecosystems the contribution of picoplankton to the total phytoplankton biomass decreases with the increasing trophic state (the slope is significantly lower than 1.0) [2, 5], while in the soda pans it was equal and even higher than 1.0 (Table 1). Consequently, in these water bodies the contribution of picoplankton to the total phytoplankton biomass did not show any decreasing tendency with increasing trophic state.

Conclusions

In contrast with previous findings, the present research provides new data supporting the fact that in soda lakes the contribution of picoplankton to the primary biomass production of the overall phycoplankton does not decrease with a higher degree of trophicity. By contrast, picoplankton remains a crucial contributor to primary production even when there is no nutrient deficiency for the autotrophic organisms. Furthermore, it has been demonstrated that in this particular type of lake, picocyanobacteria are not much more abundant than green picoalgae, and the eukaryotic picoplankton may be even more abundant than the prokaryotic.

The results clearly demonstrate the unique character of the shallow turbid soda pans, but the understanding of the observed particular behaviour of picoplankton requires further field analysis and experimental investigations. The picophytoplankton community of Pannonic Ecoregion sodic waters is constituted by both picoeukaryotes and picocyanobacteria, showing roughly similar abundance, at least in the late spring period.

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STUDIUL PICOPLANCTONULUI AUTOTROF DIN LACURI SODICE ALE BAZINULUI CARPATIC

(Rezumat)

Picoplanctonul autotrof (APP) are o răspândire globală în mediile acvatice, fiind alcătuit din cianobacterii şi alge cu dimensiuni cuprinse între 0,2-2 µm. APP are un rol deosebit de important în habitatele acvatice, unde poate asigura pâna la 90% din producţia primară. Distincţia între APP şi microorganismele acvatice heterotrofe, precum şi examinarea diversităţii APP este posibilă datorită autofluorescenţei pigmenţilor fotosintetici. Pe baza autofluorescenţei putem distinge trei grupe majore de organisme care compun picoplanctonul: cianobacterii bogate în ficoeritrină, cianobacterii în care predomină ficocianina şi alge fără ficobiliproteine. Apele saline puţin adânci, prezente în număr semnificativ, sunt foarte caracteristice Bazinului Carpatic.

Între 11 mai şi 20 iunie 2009, s-a determinat biomasa totală a fitoplanctonului (pe baza concentraţiei clorofilei *a*), biomasa şi abundenţa picofitoplanctonului (prin microscopie de epifluorescenţă) şi s-au măsurat caracteristicile fizico-chimice relevante pentru fitoplancton în 65 de lacuri sodice din Bazinul Carpatic.

Concentraţia clorofilei *a* a variat între 1 µg l^{-1} şi 1400 µg l^{-1}, cu o medie de 115 µg l^{-1}. Abundenţa fitoplanctonului oscila între o mie şi 88 milioane de celule per mililitru. Picofitoplanctonul a fost reprezentat de alge verzi de 1,5 µm şi de cianobacterii cu o dimensiune medie de 1,0 µm, bogate în ficocianină. Picocianobecterii bogate în ficoeritrină nu au fost detectate în apele examinate.

Pe baza rezultatelor noastre, în lacurile sodice examinate, contribuţia picofitoplanctonului la alcătuirea biomasei totale fitoplanctonice nu a prezentat tendinţă de scădere, proporţional cu creşterea troficităţii, aşa cum a fost observat de diverţi autori, referitor la alte tipuri de habitate acvatice. Rezultatele de faţă arată că în lacurile sodice picofitoplanctonul are o contribuţie crucială la producţia primară totală nu numai în condiţiile unui deficit de nutrienţi, dar şi în lacuri cu troficitate ridicată.

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