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VARIABILITY OF PICOPHYTOPLANKTON COMMUNITIES DURING THE COLD-WATER PERIOD IN A SMALL LOWLAND RIVER*

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ABSTRACT. Picophytoplankton community structure, abundance and biomass were studied in the Cybina River above and below the lowland shallow Antoninek Reservoir during the cold-water period 2004/2005. Eukaryotic cells numerically predominated in the picophytoplankton community throughout the study period. Their contribution to total picophytoplankton varied between 73% and 88% in abundance and 58% to 89% in biomass. Numbers of picophytoplankton cells were usually lower at the outlet than at the inlet of the reservoir. The mean difference was 2.1×10^4 cells·ml^{-1}, i.e. 14% of the value at the inlet, and the number of picoeukaryotes decreased on average by 16%, while picocyanobacteria by 6%. However, the differences in both eukaryotic and prokaryotic picophytoplankton between the inlet and outlet were not statistically significant.

Key words: picophytoplankton, picocyanobacteria, eukaryotic picoplankton, lowland river, shallow reservoir

Introduction

Planktonic photoautotrophs can be divided into several size fractions. The smallest, picoplankton fraction (0.2-2.0 μm) is the least studied. Data on this fraction have been collected mainly since the late 1970s. It proved to be a widely distributed component of plankton in both marine and freshwater ecosystems (**Stockner** and **Antia** 1986, **Stockner** 1991, **Hawley** and **Whitton** 1991, **Weisse** 1993). Like the larger fractions, picophytoplankton is subject to seasonal variation. Usually two peaks of abundance are observed: in spring and in late summer or autumn (**Happey-Wood** 1991, **Sime-Ngando** 1995, **Stockner** et **al.** 2000). However, most studies focus on the warm part of the year

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and on water bodies of a low or medium fertility. In such conditions, usually pico-cyanobacteria dominate, so more attention is paid to them than to eukaryotic organisms. Picoeukaryotes, distinguished by means of fluorescence microscopy, are regarded as less numerous, preferring colder and more fertile waters (**Fahnenstiel** et **al.** 1991, **Pick** and **Agbeti** 1991). This group is poorly studied because of not only their low abundance but also the difficulty to observe them under a microscope, due to the fast disappearing of fluorescence.

The objects of this study were: (1) a qualitative and quantitative analysis of picophytoplankton in the lowland Cybina River (rich in nutrients) in a period when water temperature did not exceed 5°C; and (2) an assessment of changes in the picophytoplankton community under the influence of the shallow Antoninek Reservoir.

Study site and methods

Field research was conducted in the lower course of the Cybina River, within the borders of the city of Poznań (mid-west Poland), at the inlet and outlet of the Antoninek Reservoir. This is the first reservoir of the cascade of four reservoirs, with an area of 7.2 ha, volume of 3×10^4 m^3, mean depth of 0.4 m, and theoretical mean water residence time of 0.5 day. The cascade was created to improve the water quality of the Cybina River, which about 1 km above the Antoninek Reservoir leaves the hypertrophic Swarzędzkie Lake, providing water rich in nutrients and plankton (**Gołdyn** and **Grabia** 1998, **Kowalczewska-Madura** 2005).

Samples were taken biweekly from the current of the Cybina River at the inlet and outlet of the reservoir, from November 2004 to March 2005. They were preserved immediately with 25% buffered glutaraldehyde to a final concentration of 1%. In the laboratory the samples were kept in darkness at 5ºC until counting. They were analysed using epifluorescence microscopy and protocols described earlier (**Szeląg-Wasielewska** 2004 a). In addition, a scanning electron microscope was used for identification of small chlorophytes. Abundance was expressed as numbers of cells per 1 ml. The biovolume of each species was calculated on the basis of cell shape, size, and number. Biomass was expressed as wet weight, estimated assuming that the biovolume of $10^9 \mu m^3$ is equivalent to 1 mg. To assess the statistical significance of the changes in the picophytoplankton community between the inlet and the outlet of the reservoir, the Wilcoxon matched pairs test was applied, using the Statistica 5 software.

Results

The numbers of picophytoplankton measured in samples from the inlet and outlet, are shown in Figure 1. At the inlet they varied from 4.4×10^4 to 2.9×10^5 cells·ml^{-1} (mean 1.5×10^5 cells·ml^{-1}), while at the outlet from 3.5×10^4 to 2.7×10^5 cells·ml^{-1} (mean 1.3×10^5 cells·ml^{-1}). It was low at the beginning of the study period, in November, but later on it increased and reached a maximum in March. Picophytoplankton abundance and biomass were not correlated significantly with water temperature. Within

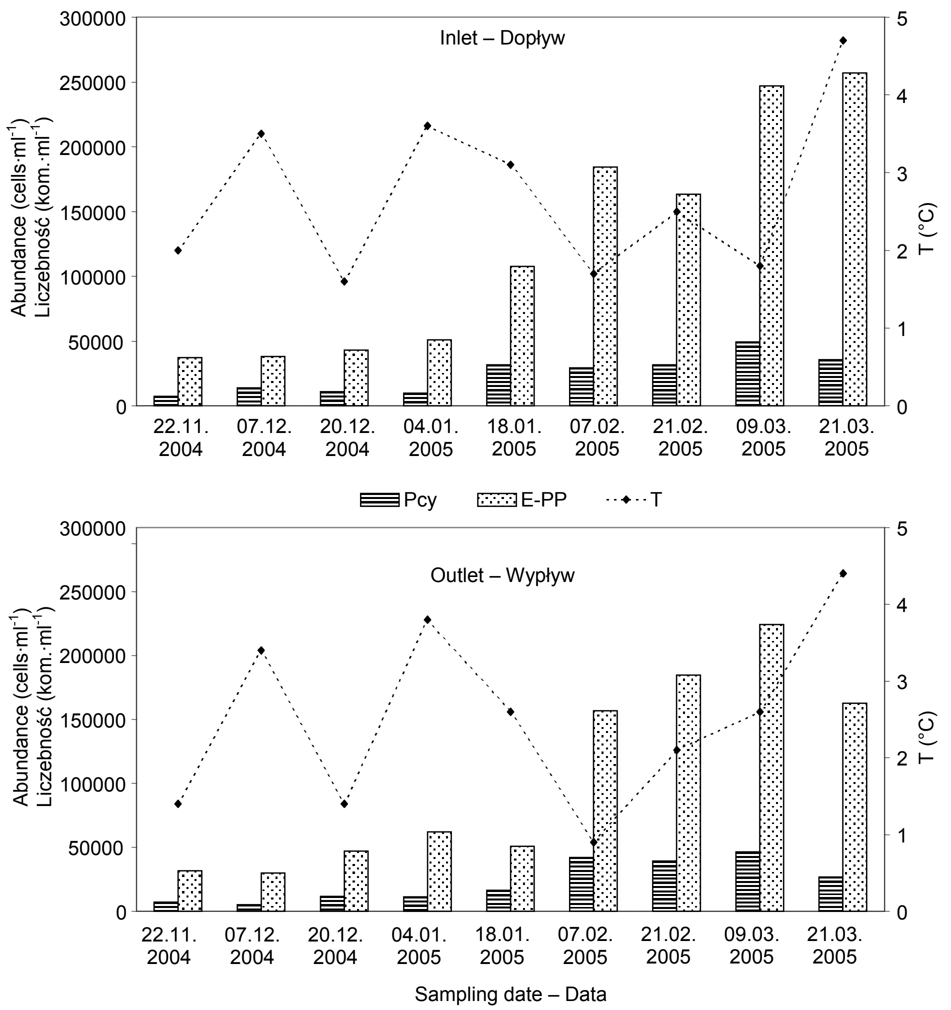

Fig. 1. Abundance of picocyanobacteria (Pcy) and eukaryotic picoplankton (E-PP) in the Cybina River at the inlet and outlet of the Antoninek Reservoir. Dotted lines indicate water temperature (T)

Ryc. 1. Liczebność pikocyjanobakterii (Pcy) i eukariotycznego pikoplanktonu (E-PP) w dopływie i wypływie rzeki Cybiny ze zbiornika Antoninek. Linie przerywane wskazują temperaturę wody (T)

the picophytoplankton community, throughout the study period at both sampling stations, picoeukaryotes were more numerous than picocyanobacteria. The latter (probably *Cyanobium* sp.), usually reached 10^3-10^4 cells·ml^{-1} and never exceeded 5×10^4 cells·ml^{-1}. By contrast, the abundance of picoeukaryotes (mainly *Choricystis* sp.) only in November and December was lower than this value, whereas later on, until the end of the study period in late March, it varied between 1×10^5 and 2×10^5 cells·ml^{-1}.

Generally, the maximum contribution of picocyanobacteria to total picophytoplankton abundance was below 30%, and on average reached about 18%. It must be emphasized that at the inlet of the reservoir, the range of variation was wider (12-27%) than at the outlet (14-24%). The contribution of eukaryotes to total picophytoplankton abundance was never lower than 70% and on average it amounted to 82% (Table 1).

Table 1

Abundance of picocyanobacteria (Pcy) and eukaryotic picoplankton (E-PP) and their contribution to the total abundance of picophytoplankton in the Cybina River at the inlet and outlet of the Antoninek Reservoir from November 2004 to March 2005
Liczebność pikocyjanobakterii (Pcy) i eukariotycznego pikoplanktonu (E-PP) oraz ich udział w liczebności ogólnej pikofitoplanktonu w dopływie i wypływie rzeki Cybiny ze zbiornika Antoninek od listopada 2004 do marca 2005

Group Grupa	Abundance (cells·ml^{-1}) Liczebność (kom.·ml^{-1})		Contribution (%) Udział (%)	
	range zakres	mean średnia	range zakres	mean średnia
Inlet – Dopływ				
Pcy	7 300-49 439	24 391	12.2-26.9	17.9
E-PP	37 076-256 967	125 315	73.1-87.8	82.1
Outlet – Wypływ				
Pcy	5 149-46 359	22 870	14.1-24.3	18.1
E-PP	29 900-224 250	105 557	75.7-85.9	81.9

Picophytoplankton abundance was usually (on six out of nine sampling dates) higher at the inlet of the Antoninek Reservoir than at its outlet. The mean difference was 2.12 × 10^4 cells·ml^{-1}, i.e. 14% of the mean value at the inlet. The decreasing trend was observed in both picocyanobacteria and picoeukaryotes, but in the case of picocyanobacteria the mean difference was only 1.5 × 10^3 cells·ml^{-1}. A larger difference was observed in picoeukaryotes, whose abundance decreased from 1.25 × 10^5 cells·ml^{-1} at the inlet to 1.06 × 10^5 cells·ml^{-1} at the outlet, i.e. by 1.97 × 10^4 cells·ml^{-1}, which accounts for 16% of the mean value at the inlet. In both groups the differences were not statistically significant (Table 2).

Picophytoplankton biomass varied from 0.060 mg·l^{-1} to 0.340 mg·l^{-1} (mean 0.181 mg·l^{-1}) at the inlet and from 0.049 mg·l^{-1} to 0.469 mg·l^{-1} (0.177 mg·l^{-1}) at the outlet. Its variation pattern was similar to that of picophytoplankton abundance and the biomass was also higher in the second part of the study period. Again, picoeukaryotes dominated, because their biomass exceeded 0.37 mg·l^{-1} at the end of the study period (Table 3, Fig. 2). Their mean biomass was 5.2 times higher at the inlet and 4 times higher at the outlet than the biomass of picocyanobacteria, which never exceeded 0.1 mg·l^{-1}. Total picophytoplankton biomass was on average 6% higher at the inlet of the reservoir than at its outlet, but the decreasing trend was observed only in picoeukaryotes, as the biomass of picocyanobacteria was 21% lower at the inlet than at the outlet. Anyway, the differences were not statistically significant in either of the groups (Table 2).

Table 2

Differences in cells number and biomass of picocyanobacteria (Pcy), eukaryotic picoplankton (E-PP) and total picophytoplankton (Total) community in the Cybina River between the inlet and outlet of the Antoninek Reservoir from November 2004 to March 2005
Różnice liczebności i biomasy pikocyjanobakterii (Pcy), eukariotycznego pikoplanktonu (E-PP) i całkowitego pikofitoplanktonu (Total) między dopływem i wypływem rzeki Cybiny ze zbiornika Antoninek od listopada 2004 do marca 2005

Parameter Parametr	Group Grupa	Difference Różnice	Percentage of changes Procent zmian	Significance level Poziom istotności
Abundance (10^3cells·ml^{-1}) Liczebność (10^3kom.·ml^{-1})	Total	–21 279	–14.2	0.173
	Pcy	–1 521	–6.2	0.594
	E-PP	–19 758	–15.8	0.139
Biomass (mg·l^{-1}) Biomasa (mg·l^{-1})	Total	–0.003	–5.9	0.594
	Pcy	0.007	20.5	0.441
	E-PP	–0.010	–4.7	0.594

Table 3

Biomass of picocyanobacteria (Pcy) and eukaryotic picoplankton (E-PP) and their contribution to the total biomass of picophytoplankton in the Cybina River at the inlet and outlet of the Antoninek Reservoir from November 2004 to March 2005
Biomasa pikocyjanobakterii (Pcy) i eukariotycznego pikoplanktonu (E-PP) oraz ich udział w biomasie ogólnej pikofitoplanktonu w dopływie i wypływie rzeki Cybiny ze zbiornika Antoninek od listopada 2004 do marca 2005

Group Grupa	Biomass (mg·l^{-1}) Biomasa (mg·l^{-1})		Contribution (%) Udział (%)	
	range zakres	mean średnia	range zakres	mean średnia
Inlet – Dopływ				
Pcy	0.009-0.048	0.029	11.0-41.8	18.8
E-PP	0.048-0.295	0.162	58.2-89.0	81.2
Outlet – Wypływ				
Pcy	0.007-0.097	0.036	13.5-32.7	20.0
E-PP	0.041-0.372	0.142	67.3-86.5	80.0

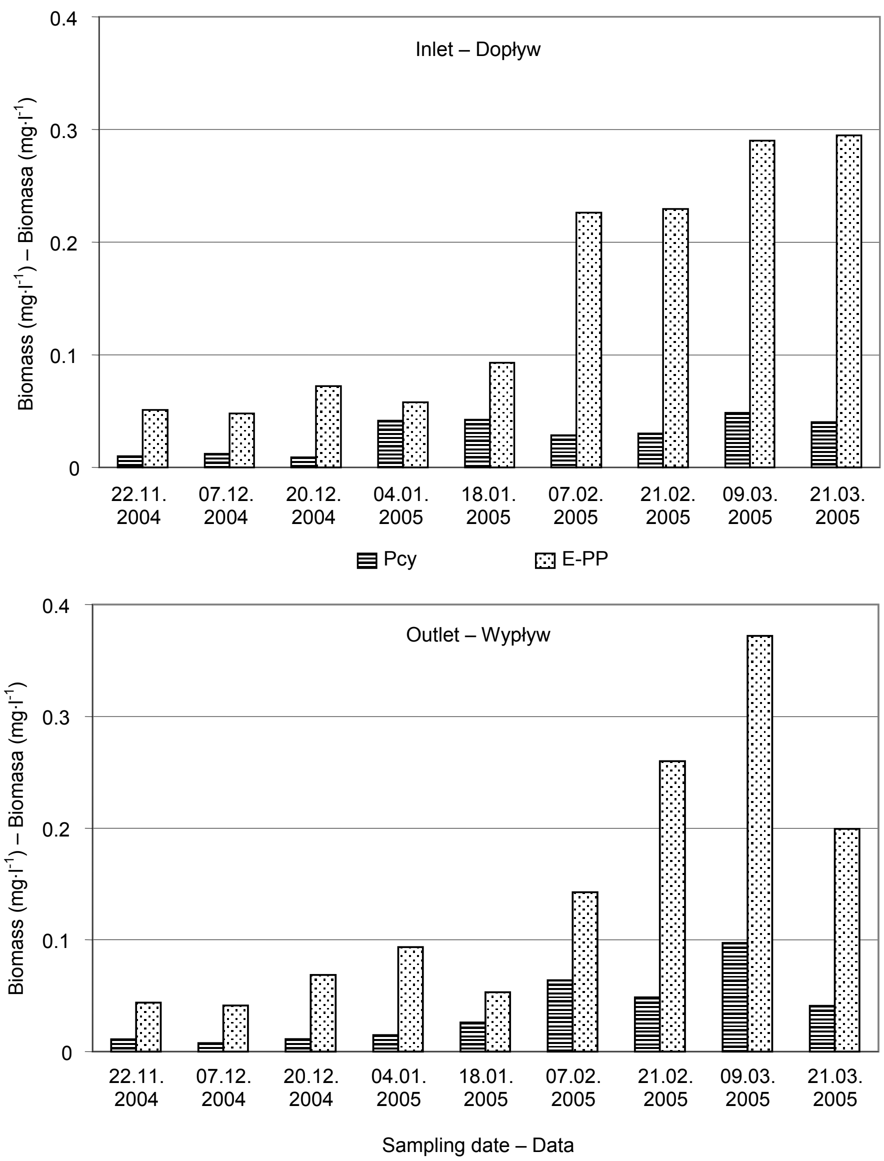

Fig. 2. Biomass of picocyanobacteria (Pcy) and eukaryotic picoplankton (E-PP) in the Cybina River at the inlet and outlet of the Antoninek Reservoir
Ryc. 2. Biomasa pikocyjanobakterii (Pcy) i eukariotycznego pikoplanktonu (E-PP) w dopływie i wypływie rzeki Cybiny ze zbiornika Antoninek

Discussion

Results of this study show that prokaryotic and eukaryotic picophytoplankton, distinguished by means of epifluorescence microscopy (thanks to the natural fluorescence of chlorophyll and phycobilins), were present throughout the study period. In contrast to many previous studies of freshwater ecosystems, picoeukaryotes dominated over picocyanobacteria. The abundance of picoeukaryotic algae reached a maximum of 2.6×10^5 cells·ml^{-1} in March, when water temperature was only about 5ºC. Also in other water bodies the highest numbers of picoeukaryotes are recorded when water temperature is low, i.e. in early spring, autumn or winter, whereas in summer only in deeper zones of stratified lakes. High densities of picoeukaryotes, in the order of 10^5, are rarely reported. It is noteworthy that such high values are reported from various lake types and from various regions, e.g. the small and shallow dystrophic Lake Skrzynka in western Poland (**Szeląg-Wasielewska** 2004 b), the large oligotrophic Lake Baikal in Russia (**Nagata** et **al.** 1994, **Belykh** et **al.** 2000), the eutrophic and dimictic Lake Aydat in France (**Sime-Ngando** 1995), and the highly eutrophicated Lake Feldberger Haussee in north-eastern Germany (**Hepperle** and **Krienitz** 2001). An extremely high abundance of eukaryotic picophytoplankton, which can be regarded as a water bloom caused by solitary picochlorophytes, was observed by **Hepperle** and **Krienitz** (2001) in early April in a hypertrophic pond located in the village Prosigk in Germany. These organisms reached there a density of about 6.6×10^6 cells·ml^{-1} and biomass of about 27.9 mg·l^{-1}. However, there is generally much less information on picoeukaryotes than on picocyanobacteria. This results mainly from methodological difficulties during their epifluorescence analysis, as emphasized e.g. by **Fahnenstiel** et **al.** (1991).

The most abundant picoeukaryotes recorded in the study period in the Cybina River were chlorophytes identified as *Choricystis* sp. on the basis of morphological characters, cell size, and mode of reproduction. This confirms the result of **Hepperle** and **Krienitz** (2001) or **Fawley** and **Fawley** (2003), who found that species from this genus are common components of freshwater picoplankton and are present in lakes of all trophic levels. In the waters of the Cybina River it probably originates from the eutrophic Swarzędzkie Lake, which is located only 1 km upstream from the Antoninek Reservoir. The lake created favourable conditions for picochlorophytes growth during the study period, which was reflected in the high abundance of this fraction, reaching 2.6×10^5 cells·ml^{-1} at the inlet. This value was much higher than that recorded 10 years earlier, also in the cold-water period (November-March), as then total picochlorophytes abundance did not exceed 2×10^4 cells·ml^{-1} (**Gołdyn** 2000, **Gołdyn** and **Szeląg-Wasielewska** 2005).

Results of this study show that the dominance of eukaryotic picoplankton over picocyanobacteria in terms of biomass can be observed not only during the spring isothermal mixing conditions at water temperature below 10ºC, as reported by **Pick** and **Agbeti** (1991) or **Fahnenstiel** et **al.** (1991), but also in late autumn and winter. This indicates that the ecological importance of picoeukaryotes increases in the cold-water period. The present study confirms that eukaryotic picoplankton can be very abundant during the cold-water period, before the spring water bloom, caused by larger phytoplankters (**Weisse** 1993).

In the study period, picophytoplankton biomass at the inlet of the reservoir was not significantly different from the values recorded at its outlet, probably due to the short water residence time. However, our earlier research – conducted at both stations on 12 sampling dates during the whole year – showed that picophytoplankton abundance was higher at the inlet than at the outlet both in the cold-water and warm-water periods. This applied mainly to picocyanobacteria, whose abundance was on average 73% higher at the inlet than at the outlet of the Antoninek Reservoir. The water body was then covered by emergent vegetation (*Phragmites australis*, *Typha* sp.) and submerged waterweeds (*Ceratophyllum* sp.), which caused a reduction of the concentration of all suspended solids, including picophytoplankton (**Gołdyn** 2000). As a result of restoration of the reservoir in 2001-2003, all vascular plants were removed, so the role of primary producers was later played by filamentous algae, which covered the bottom, and by phytoplankton in the pelagic zone (**Gawrońska** 2005).

It can be concluded that the picophytoplankton community composed of prokaryotic and eukaryotic algae, behaves as a pioneer community. On the one hand, they colonize and use the resources of the water body that is not very fertile and is not easily accessible to the more demanding, larger algae. On the other hand, thanks to eukaryotic picoplankton, the ecological importance of picophytoplankton increases in the colder part of the year, when physical conditions are the most unfavourable to the majority of photoautotrophs.

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ZMIENNOŚĆ ZBIOROWISK PIKOFITOPLANKTONU PODCZAS OKRESU CHŁODNEJ WODY W MAŁEJ RZECE NIZINNEJ

S t r e s z c z e n i e

Przedstawiono strukturę, liczebność i biomasę pikofitoplanktonu w wodzie rzeki Cybiny poniżej i powyżej płytkiego, nizinnego zbiornika Antoninek podczas okresu, gdy woda była chłodna, na przełomie lat 2004-2005. W pikofitoplanktonie dominowały wtedy komórki glonów eukariotycznych, których udział w liczebności ogólnej zbiorowiska wynosił od 73 do 88%, natomiast w biomasie od 58 do 89%. Zagęszczenie komórek pikoplanktonowych w wypływie rzeki ze zbiornika było na ogół mniejsze niż w jej dopływie, średnio o $2,1 \times 10^4$ kom. $\cdot$ ml^{-1}, tj. o 14%, przy czym liczebność eukariotycznego pikoplanktonu zmniejszyła się średnio o 16%, natomiast pikocyjanobakterii o 6%. W przypadku obu grup pikofitoplanktonu różnice między dopływem a wypływem były statystycznie nieistotne.

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