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Research Article

THE SMALLEST PHOTOTROPHIC ORGANISMS IN A LOWLAND RIVER: COMPARISON WITH OTHER COMPONENTS OF THE PHYTOSESTON*

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Abstract

The phytoseston of the Warta, a polluted eutrophic river in western Poland, was studied from spring to autumn 2003. The focus of the study was autotrophic picoplankton (APP). APP abundance fluctuated from 1.3×10^6 to 1.05×10^8cells L^{-1} and was characterized by a maximum in late July. APP biomass was low, ranging from 0.5 to 19.7 µg C L^{-1}. Both cyanobacteria and eukaryotic organisms were found within the picoplankton. The picocyanobacterial community was often dominated by colonial forms, mostly *Aphanocapsa* spp. and *Cyanogranis ferruginea* (Wawrik) Hindák. Eukaryotic picoplankton included chlorophytes of the genera *Chlorella, Choricystis*, and *Pseudodictyosphaerium jurisii* (Hindák) Hindák. Among the phytoplankton larger than 2 µm, the most abundant species were diatoms and chlorophytes, except in late August, when filamentous cyanobacteria dominated. The contribution of APP to the total phytoplankton biomass was usually below 1%, and the mean (0.6%) was close to the lower limit of values found in lakes.

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INTRODUCTION

Since the popularization of epifluorescent microscopy in the 1980s, the smallest phototrophic organisms, termed autotrophic picoplankton (APP) or picophytoplankton, have been studied frequently in temperate lakes (Stockner and Antia 1986, Hawley and Whitton 1991a, Weisse 1993, Stockner *et al.* 2000). In contrast, very little information is available about APP in rivers, including those in Poland (Marshall and Affronti 1992, Gołdyn *et al*. 1999). Primarily due to methodological difficulties, these small-sized organisms have long been either ignored or treated as bacterioplankton or detritus.

The objective of this study was to analyze the qualitative and quantitative structure of phytoplankton, with a particular focus on APP in a typical lowland river in Poland – the Warta River. Its phytoplankton has been studied for about 80 years, but the picoplankton fraction has been ignored to date.

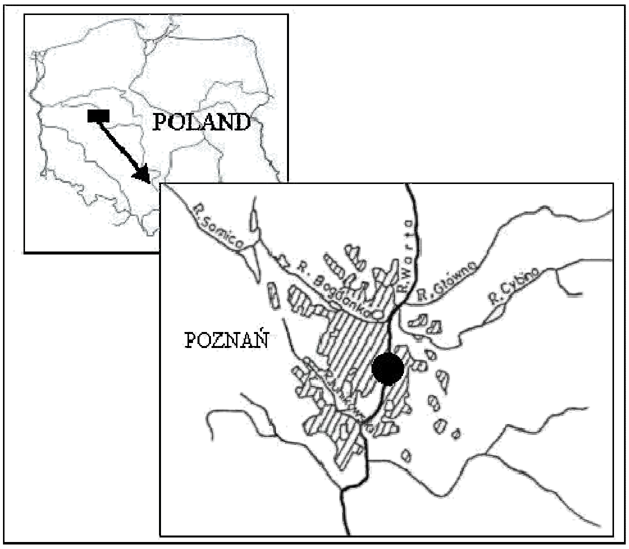

Fig. 1. Location of the sampling station

MATERIAL AND METHODS

Water samples were collected with a 5-L water sampler from the current, 0.2 m below the water surface, ten times between May and October 2003, at one sampling site in the middle section of the Warta River (Wielkopolska Lake District, Poznań Water-Gap) (Fig. 1).

The samples for analysis of picoplankton were preserved with buffered glutaraldehyde to a final concentration of 1%, and with Lugol's solution for the remaining size fractions of phytoplankton. In the laboratory, 1–5 mL subsamples were filtered through Nuclepore black filters with a diameter of 25 mm and a pore size of 0.2 μm at low vacuum pressure. Picoplanktonic cells were counted on the basis of the autofluorescence of their photosynthetic pigments at a magnification of 1500x under an epifluorescence microscope (BX-60 Olympus, purchased by the Foundation for Polish Science within the SUBIN Program). A mercury lamp and standard filter sets were applied to generate blue and green excitation light (MacIsaac and Stockner 1993). Samples for the study of phytoplanktonic organisms larger than 2 μm were analyzed with an inverted microscope (MOD-2, PZO) after sedimentation in settling chambers 9 mL in volume (magnifications: 40, 150, and 600×). APP abundance was expressed as cell numbers per 1 L of water. Cell volume was calculated on the basis of the shape, dimensions, and number. Their biomass was expressed as wet weight, assuming that the volume of 10^6 μm^3 was equivalent to 1 μg. The conversion factors used for carbon estimation conformed to those of Sondergaard (1991): 220 fg C μm^{-3} for picoeukaryotes, 250 fg C μm^{-3} for picocyanobacteria and carbon : chlorophyll ratio 22.5.

Chlorophyll *a* concentrations were determined spectrophotometrically from 0.5 L samples filtered through Whatman GF/F glass fiber filters. Pigments were extracted with 90% acetone during 24 hours in the dark at 4ºC. The calculations were carried out using Lorenzen's formula (1967). Suspended solids (seston) were weighed after condensation on GF/F filters and dried at 105ºC. Statistical analyses were done with STATISTICA 5.5 software.

STUDY AREA

The Warta River, the major right-side tributary of the Order River, drains 54.5×10^3 km^2 of the Wielkopolska region (midwestern Poland). Its length is 808 km and the mean discharge at its mouth is 210 m^3s^{-1}. It can be divided into several hydromorphologically and ecologically different segments but the present study was restricted to the Poznań segment of the river. The river channel has been regulated within the borders of the city of Poznań. Above and below the city, the valley is covered chiefly by woodland. In the suburbs, wedges composed of grassland and woodland separate built-up areas from the river (Kondracki 1998).

In the upper course, the Warta River flows through the man-made reservoir Jeziorsko (created in 1986), with an area of 42 km^2 and volume of 203 million m^3. This reservoir is eutrophic and rich in planktonic organisms (Szyper and

Bilińska 2003). The Warta River is exposed to human activities such as agriculture and urban and industrial activity, so phosphorus and nitrogen concentrations are high indicating the river's eutrophic state. The river waters within the city of Poznań are very fertile (mean total phosphorus – 0.176±0.058 mg P L^{-1}, mean total nitrogen – 3.39±1.49 mg N L^{-1}) and carry large amounts of phytoplankton (mean chlorophyll *a* – 20.6 µg L^{-1}). Among the cations, calcium and sodium prevail over magnesium and potassium and among the anions - bicarbonates (Bożek *et al.* 2001).

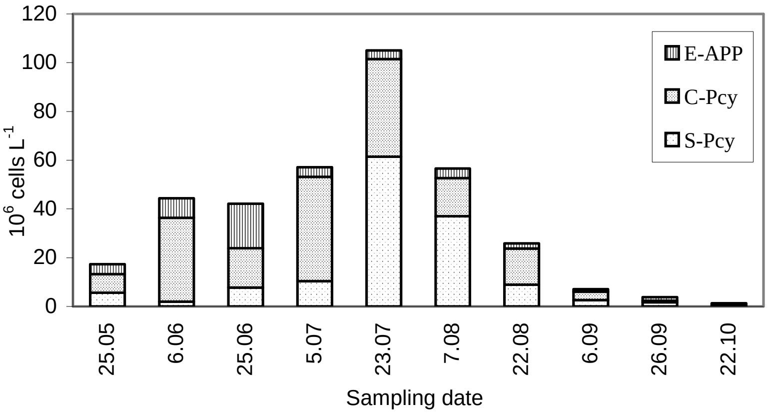

Fig. 2. Abundance of autotrophic picoplankton (APP) groups: single-celled picocyanobacteria (S-Pcy), colonial picocyanobacteria C-Pcy), and picoeukaryotes (E-APP) in the Warta River in 2003.

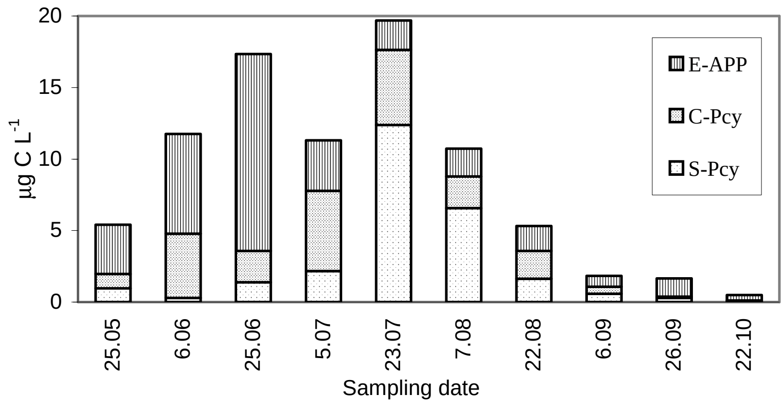

Fig. 3. Biomass of autotrophic picoplankton (APP) groups: single-celled picocyanobacteria (S-Pcy), colonial picocyanobacteria C-Pcy), and eukaryotes (E-APP) in the Warta River in 2003.

RESULTS

The abundance of APP varied from 1.3×10^6 to 1.05×10^8cells L^{-1} (mean 3.6×10^7cells L^{-1}) and biomass from 0.5 to 19.7 μg C L^{-1} (mean 8.6 μg C L^{-1}), with maximum values in late July (Fig. 2, 3). The ratio of total maximum to minimum abundance was 81 and the ratio of maximum to minimum biomass, only 39. Total APP abundance was positively and significantly correlated with water temperature (r=0.69).

Throughout the study period, prokaryotic APP, *i.e.*, cyanobacteria, were more numerous than eukaryotic APP (Fig. 2). The mean abundance of picocyanobacteria was 3.1×10^7cells L^{-1} and its contribution to the total abundance of the APP community varied from 57 to 97% (mean 80%). Picocyanobacteria included single-celled species of the genus *Synechococcus* and colony-forming species of the genera *Aphanocapsa* and *Cyanogranis*. Within the last genus, *C. ferruginea* (Wawrik) Hindák was identified. This species formed small colonies with spherical, densely packed cells and often with ferric precipitates near the cells. Colony-forming picocyanobacterial cells often dominated during the study period. Their contribution to the total abundance of picocyanobacteria varied between 29 and 95% (mean 57%). The biomass of picocyanobacteria was the highest in July and early August, and the lowest in October, when the water temperature was only 6.8ºC (Fig. 3, 4).

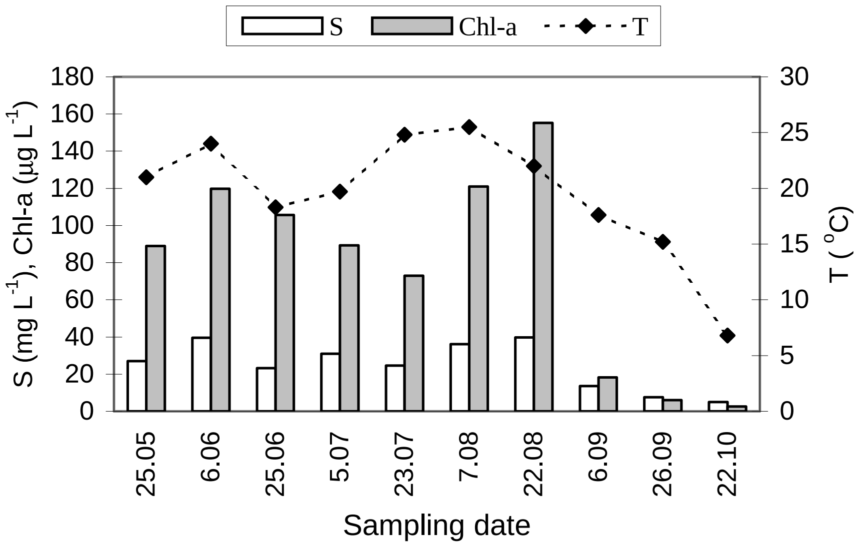

Fig. 4. Seston (S, mg L^{-1}), chlorophyll *a* (Chl-*a*, µg L^{-1} L), and water temperature (T, ºC) in the Warta River in 2003.

The abundance of picoeukaryotes fluctuated between 4.2×10^5 and 1.8×10^7cells L^{-1} (mean 4.7×10^6cells L^{-1}). Picoeukaryotes usually accounted for a small proportion of the total APP abundance (3-43%, mean 15%) and were dominated by *Pseudodictyosphaerium jurisii*. However, the cells of this species often exceeded 2 μm in diameter, and were thus classified as nanoplankton. Picoeukaryotes also included cells of *Chlorella* and *Choricystis* spp. The biomass of picoeukaryotes varied from 0.4 to 13.8 μg C L^{-1}. During half of the sampling sessions it was higher than the biomass of picocyanobacteria. The contribution of eukaryotes to the APP biomass community ranged from 12 to 80% and was 52% on average.

Table 1

Major taxa in the phytoplankton and contribution of the APP size fraction to total chlorophyll *a* in the Warta River

Date 2003	The most important taxa larger than 2 μm in relation to their abundance (values in cells 10^6 L^{-1} are given in brackets)	Contribution of APP (%) to total chlorophyll *a*
25.05.	*Stephanodiscus* spp. (42.6), *Cyclotella meneghiniana* (6.3), *Pseudodictyosphaerium jurisii* (5.7)	0.27
6.06.	*Coelastrum microporum* (28.0), *Pseudodictyosphaerium jurisii* (5.7), *Stephanodiscus* spp. (11.2)	0.43
25.06.	*Coelastrum microporum* (33.3), *Pseudodictyosphaerium jurisii* (22.8), *Stephanodiscus* spp. (10.2)	0.73
5.07.	*Coelastrum microporum* (12.8), *Cyclotella meneghiniana* (11.5), *Scenedesmus* spp. (8.9)	0.56
23.07.	*Stephanodiscus* spp. (14.6), *Coelastrum* spp. (8.1), *Scenedesmus* spp. (6.6)	1.21
7.08.	*Stephanodiscus* spp. (87.6), *Pseudodictyosphaerium jurisii* (10.5), *Scenedesmus* spp. (9.5)	0.40
22.08.	*Aphanizomenon flos-aquae* (97.5), *Microcystis aeruginosa* (32.2), *Stephanodiscus* spp. (7.4)	0.15
6.09.	*Scenedesmus* spp. (4.7), *Stephanodiscus* spp. (3.4), *Microcystis aeruginosa* (2.0)	0.45
26.09.	*Pseudodictyosphaerium jurisii* (4.2), *Rhodomonas lacustris* (2.2), *Cyclotella* spp. (1.9)	1.22
22.10.	*Cyclotella* spp. (0.22), *Rhodomonas lacustris* (0.23), *Stephanodiscus* spp. (0.20)	0.88

The dry weight of seston varied from 5 to 40 mg L^{-1} (mean 24.7 mg L^{-1}), while chlorophyll *a* concentration ranged from 2.5 to 155 μg L^{-1} (mean 78 μg L^{-1}) (Fig 4). The highest values of chlorophyll *a* concentration were noted in June and August (>100 μg L^{-1}); the ratio of maximum to minimum value was 36. Until August 1 mg of seston contained 3.0-4.6 μg of chlorophyll *a*, but later its content decreased to about 1 μg, and during the last two sampling sessions it was below 1 μg.

The seasonal contribution of APP biomass to total phytoplankton biomass expressed as chlorophyll *a* concentration, ranged from 0.15% to 1.22%. In most cases it was below 1%, increasing to 1.2% only in late July and late September (Tab. 1). The correlation between APP biomass and chlorophyll *a* concentration was positive but insignificant. The contribution of APP to total phytoplankton was also insignificantly but negatively correlated with chlorophyll *a* concentration.

The study revealed a seasonal variation in species composition of phytoplankton. Cyanobacteria were sometimes dominant, but usually chlorophytes and diatoms were the most abundant. Diatoms were abundant throughout the study period, and centric forms of the genera *Cyclotella* and *Stephanodiscus* were the most numerous among them. Chlorophytes were rich in species, especially the order *Chlorococcales*, including *Scenedesmus* ssp. and *Tetraëdron* ssp. In late August, the phytoplankton was dominated by a mixture of colony-forming cyanobacteria of the genera *Microcystis* and *Aphanizomenon* (Tab. 1). The filamentous colonies of *Aphanizomenon*, forming sickle-shaped clusters (each composed of about a dozen filaments) could even be seen with the naked eye and caused water blooms.

DISCUSSION

Studies of the smallest phototrophic organisms from river waters are rare in Europe in comparison with investigations of the APP of lakes. Rivers with slow-moving currents, like the Warta in its middle part, are comparable with lakes with short water residence times. APP development can occur in such rivers, or in rivers with managed flow regimes. The current authors found that in the Warta, the mean APP abundance was low (3.6×10^7cells L^{-1}) and only once exceeded 10^8 cells per liter. A similar value was recorded in the waters of the Cybina River above a cascade of small reservoirs (Gołdyn *et al*. 1999). By contrast, Marshall and Affronti (1992) noted major APP growth and mean densities of about 10^8 to 10^9cells L^{-1} in the lower James, York, and Rappahannock Rivers. In the riverine zone of the Sardis Reservoir (southeastern

U.S.), the total APP density was also higher at a mean of 4.7×10^{8}cells L^{-1} (Rhew and Ochs 2000). APP density in the Warta River, as in many lakes, declines with seasonal declines in water temperature (Pick and Agbeti 1991, Weisse 1993).

During the study period, the total cells densities of APP ranged within two orders of magnitude. A similar range of density variation was observed in lakes. For instance, Hawley and Whitton (1991b) reported such ranges for most of the lakes studied in the English Lake District. Moreover, as in these lakes, peaks of chlorophyll *a* concentration and APP density were recorded in the Warta in mid to late summer. The ratio of maximum to minimum APP abundance was also within the range reported by these authors, although it was close to the lower limit. In contrast, the ratio of maximum to minimum chlorophyll *a* concentration was many times higher than in the lakes of the English Lake District; this attests to the higher variation in rivers in this respect.

Both cyanobacteria and eukaryotes were found within the picoplankton in the Warta River. Cyanobacteria always dominated in terms of APP abundance but chlorophytes often dominated in terms of APP biomass. This is due to the fact that eukaryotic picoplanktonic cells were slightly larger than picocyanobacteria, which has also been noted by many authors who study lakes (Pick and Agbeti 1991, Fahnenstiel *et al.* 1991, Sondergaard 1991, Hepperle and Krienitz 2001). In the Warta River, picocyanobacterial cells occurred primarily as colonies (on the average 57%), mostly of *Aphanocapsa* spp. and *Cyanogranis ferruginea*. Colonial picocyanobacteria are also common in many lakes, particularly in the summer and autumn when nutrients are limited (Stockner and Shortreed 1991, Stockner *et al.* 2000, Passoni and Callieri 2000, Schallenberg and Burns 2001). However, relatively little is known about the occurrence and distribution of these colonies in rivers. It can be assumed that in rivers the anti-predator mechanism will be less important because of the smaller amount of consumers in these habitats. APP survival and development in rivers are probably more strongly affected by the turbulence and turbidity of river water. In a turbid and turbulent system, it is light access, not nutrients, that is the major factor limiting algal growth. However, it must be emphasized that APP requires less light than larger phytoplankton (Weisse 1993, Sime-Ngando 1995, Gervais *et al.* 1997).

Eukaryotic picoplankton of the Warta River included chlorophytes of the genera *Chlorella*, *Choricystis*, and *Pseudodictyosphaerium jurisii*. These taxa are common in various freshwater habitats: ponds, lakes (of varied trophic levels), and rivers. The last species mentioned (synonymous with *Dactylosphaerium jurisii* Hindák), which was the most numerous of the picochlorophytes in the Warta, has also been recorded, for example, in channels, fish-ponds, the Danube River, flooded gravel pits, and oligotrophic to eutrophic lakes (Hindák 1997, 1984, Padisák *et al*. 1997, Krienitz *et al.* 1999, Hepperle and Krienitz 2001). Its occurrence in the Warta may result from its transport from lakes (by tributaries of the river) or from the reservoir located in the upper section of the river.

Among phytoplankton larger than 2 μm, the most abundant species in the Warta were diatoms, especially centric ones. Diatoms have frequently been reported to dominate in phytoplankton communities in rivers (Holmes and Whitton 1981). Phytoplankton biomass is generally the highest in spring and summer, following which other groups of algae become abundant. In the Warta, filamentous cyanobacteria formed a dense water bloom in August, whereas chlorophytes were numerous during nearly all sampling sessions. Within the last group, *Scenedesmus* and *Coelastrum* spp. were the most numerous, but in several English rivers *Ankistrodesmus* and *Chlamydomonas* were also the most common genera (Happey-Wood 1988).

In the studied river, the contribution of APP to the total phytoplankton biomass was very low and the mean (0.63%) was close to the lower limit of values found in the literature (Stockner 1991). This is consistent with reports indicating that the relative contribution of APP to total phytoplankton biomass is the lowest when the trophic state is high (Hawley and Whitton 1991b). Moreover, the low APP density and biomass suggest that picoplanktonic autotrophic communities do not find suitable conditions for development in heavily polluted aquatic habitats, like the Warta. This may be associated with the sensitivity of APP to eutrophication and contaminant enrichment, especially to heavy metal contamination (Severn *et al.* 1989, Weisse 1991, Weisse and Mindl 2002).

CONCLUSIONS

The results of 10 seasonal sampling sessions in 2003, which were used to analyze the phytoplankton communities of the Warta River, showed that the contributions of the smallest phototrophic organisms (APP) to the total chlorophyll *a* concentration were low. However, since the amount of data on this subject is still insufficient, these investigations will be continued, including the analysis of the transport of APP from other habitats and the possibility of APP development in the river.

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