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Vertical distribution of periphytic diatoms in the karstic Zrmanja River (Croatia)

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The small karstic Zrmanja River (69 km long) discharges into the central-eastern Adriatic Sea. High oxygen saturation and water transparency, as well as low nutrient concentrations, indicated the oligotrophic character of the river. We present the taxonomic composition and vertical distribution of diatoms attached on an artificial substrate (Plexiglass) in the lower reach of the Zrmanja River, in July 2000. Thirty attached diatoms were determined on Plexiglass throughout the 2.5 m deep water column, and *Achnanthes* sp. and *Fragilaria* sp.were found to be the most frequent (> 89%) and abundant (> 10^5 cells cm^{-2}) diatoms. The highest abundance of attached diatoms (1.2 x 10^6 and 7.5 x 10^5 cells cm^{-2}) was found at the depth of 0.5 m. The maximum number of diatom taxa was also found below the surface.

Key words: periphyton, diatoms, artificial substrate, Plexiglass, vertical distribution, taxonomic composition, karstic river, Zrmanja, Croatia

Introduction

Periphytic algae have an important role in the production of shallow, aquatic habitats (DODDS et al. 1999). Periphytic communities cover different types of submerged substrates (COVER and HARREL 1978, BARBIERO 2000), and are composed of autotrophic, mixotrophic and heterotrophic organisms. Algae attached on the bottom have the ability to sequester nutrients from pore-water of sediment (SAND-JENSEN and BORUM 1991). Integrating the physical and chemical characteristics of the aquatic environment over time, periphyton may be considered a tool for monitoring water quality (KELLY et al. 1998, LANGE-BERTALOT 1979, SLÁDEČEK 1986). The diatoms dominate in assemblages of benthic microalgae (SNOEIJS 1999, ÁCS and KISS 1991), and their dominance in freshwater periphyton was found especially during the summer (ROEMER et al. 1984). Methodological problems appearing during periphyton investigation have been solved with the use of artificial substrates, a more favorable tool for experimental growth than natural substrates (WAHL and MARK, 1999, ÁCS and KISS 1993 a, b).

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No investigations of the diatoms in the karstic Zrmanja River have been performed to date. To improve knowledge of the taxonomic composition and ecology of the diatoms in this river we present the results of their vertical distribution on artificial substrates throughout the water column. Summer minimum riverine water discharge and higher temperature conditions provide favorable and stabile conditions for the development of periphytic diatoms (HILLEBRAND and SOMMER 1997, ÁCS and KISS 1991). This was the reason for carrying out the research during the minimum river discharge period, in July.

The area investigated

The small karstic Zrmanja River (69 km long) is located in the NW Dalmatian plateau of the Croatian Dinaric karst region (Fig. 1). The river discharges into the central-eastern Adriatic Sea, forming a highly stratified estuary (VILIČIĆ et al. 1999). The surrounding karst is characterized by numerous underground connections between surface waters in the hinterland and the coastal sea. The exact hydrological catchment area and boundaries are not known, although numerous investigations and groundwater tracing have been carried out (BONACCI 1999).

The climate is influenced by the Mediterranean Sea and the eastern European continental area. The mean annual rainfall varies from 1050 to 1216 mm in the area of the river flow, and from 1493 to 3419 mm in the south Velebit mountain area (PERICA and OREŠIĆ 1995). Average air temperature in Novigrad varies from 6.0 °C in January to 24.3 °C in July. The

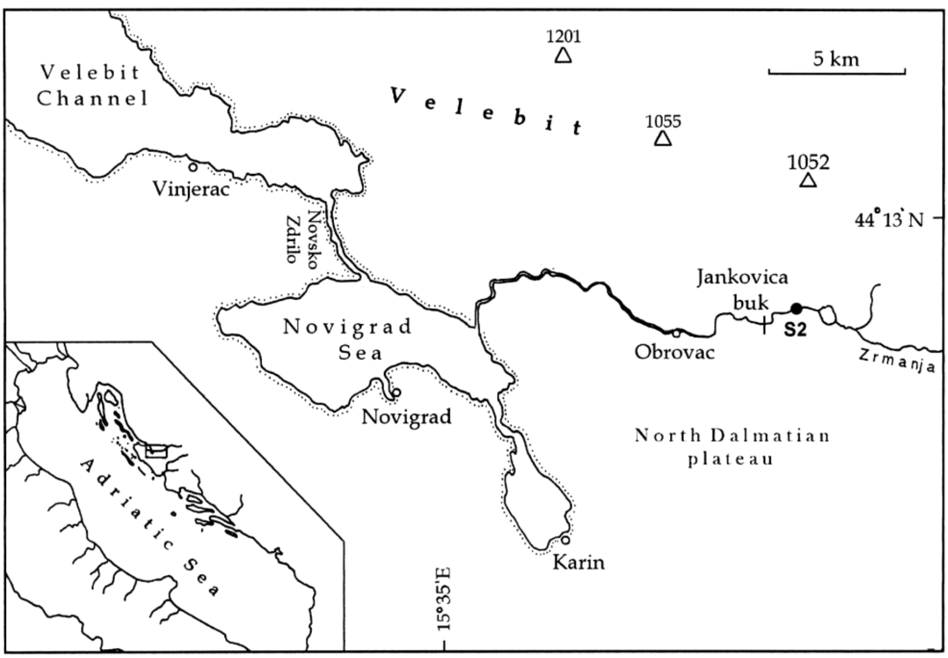

Fig. 1. Investigated area and location of Station S2.

summers are dry and hot with air temperature as high as 35 °C. During winter there is great influence of cold north winds (bora), with temperatures as low as –9.5 °C. The drainage area is sparsely inhabited.

Material and methods

Research was performed in the lower reach of the Zrmanja River at station S2, about 1 km upstream from the Jankovića buk waterfalls (Fig 1). Waterfalls are the end point of the river, while below the waterfalls there is the beginning of the estuary. At S2, the water column was 3 m deep. Periphyton samples were collected from artificial substrates (Plexiglass plates) fixed on rope at depths of 0, 0.5, 1, 1.5 and 2 m, after 15 and 27 days of colonization. The experiment began on July 1, and ended on July 28, 2000. Only one plate was installed at each depth. The rope was fixed to plastic container floating on the surface and anchored in the bottom sandy sediment.

The samples were collected with a scalpel by scraping periphyton from the Plexiglass surfaces. The fine removal of periphyton was performed using an adapted toothbrush, after rinsing surfaces with a tap water (CAPUT and PLENKOVIĆ-MORAJ 2000). Samples were preserved in formaldehyde solution (4% final concentration) and divided in two subsamples. After acid cleaning (ROUND et al. 1996), diatoms were identified under a light microscope (100 x immersion objective) using standard manuals (ZABELINA et al. 1951, HINDÁK et al. 1978, HUSTEDT 1985, PATRICK and REIMER 1966, 1975). The nomenclature was adjusted according to HARTLEY (1986). In unclean samples cells were resuspended and counted using the UTERMÖHL (1958) method. The presence of periphytic diatoms was expressed by the frequency of appearance and abundance. Due to formation of organic aggregates and heterogeneous microdistribution of diatoms, and difficulties in separating dead from living frustules, the counting error could increase up to 30 percent in some samples. Resuspension of diatoms from aggregates could not be achieved by using ultrasonication. Nutrients (NO_3, NH_4, SiO_4, PO_4) were measured in June and July 2000. They determined using the standard method (STRICKLAND and PARSONS 1972, IVANČIĆ and DEGOBIS 1984). N/P nutrient ratio (total inorganic N/PO_4) was calculated according to REDFIELD (REDFIELD et al.1963). Concentration of oxygen was measured according to Winkler's method (STRICKLAND and PARSONS 1972).

Results

During 1999/2000, the maximum river outflow was detected in the December-January period (175–240 m^3 s^{-1}) and in April (190 m^3 s^{-1}), while the minimum inflow was detected in July–August (avg. 1.43 m^3 s^{-1}) (Fig 2). Temperature varied between 19 and 24 °C.

Concentration of inorganic nutrients was low (Tab. 1). Redfield ratios (TIN/PO_4) were considerably higher than 16. Secchi disc visibility extended to the 2.5 m deep bottom. The oxygen saturation in the whole water column ranged between 80 and 90 %.

Twenty eight diatoms attached on Plexiglass plates were determined (Tab.2). The dominant group of diatoms, with high frequency (>56%) and high abundance (>avg 2.1 x 10^4 cells cm^{-2}) were *Achnanthes* sp, *Fragilaria* sp. *Cymbella parva* and *C. affinis*. A diatom with high frequency (56%) but low abundance was *Synedra ulna*, while *Synedra* sp. was a

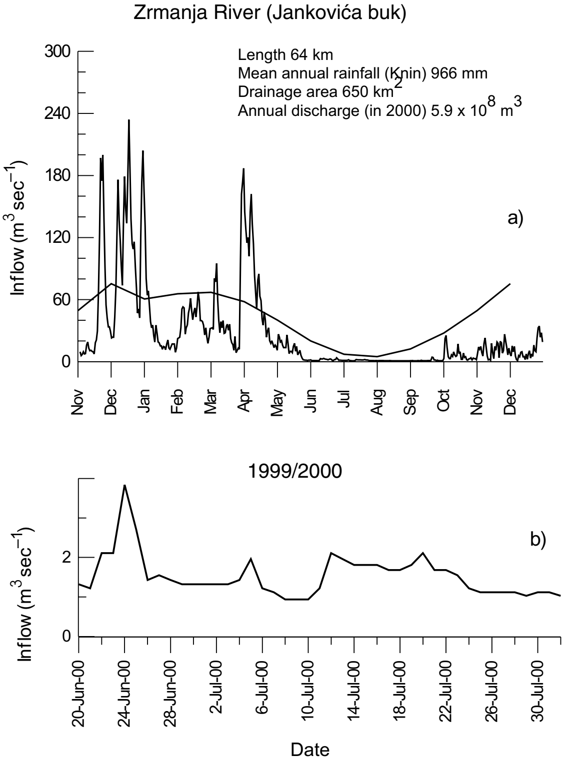

Fig. 2. Inflow of the Zrmanja River water into the estuary. a) 1999/2000 and 10-year average values, b) during June, 2000.

Tab. 1. Concentration of nutrients (μmol L^{-1}) in the Zrmanja River (Station S1) in June–July 2000.

	NO_3	NO_2	NH_4	TIN	PO_4	SiO_4	TIN/PO_4
June, 2000	5.38	0.10	1.04	6.53	0.07	4.96	99
July, 2000	7.62	0.09	0.65	8.37	0.03	5.51	335

taxon with low frequency (11%) and high abundance (avg 7.6 x 10^4 cells cm^{-2}). The minimal abundance (avg <1.6 10^3 cells cm^{-2}) and frequency (11%) were found for the diatoms *S. capitata*, *Navicula radiosa* and *Meridion circulare* (Tab. 2).

After 15 day-colonization, *Fragilaria* sp. (avg 3.1 x 10^5 cells cm^{-2}) and *Achnanthes* sp. (avg 2.9 x 10^5 cells cm^{-2}) were the most abundant taxa. After 27 days of colonization, the diatom *Achnanthes* sp. was the most abundant taxon and *Fragilaria* sp. was codominant (avg 2.1 x 10^5 cells cm^{-2}, avg 7.4 x 10^4 cells cm^{-2}) in the periphytic diatom assemblages.

Regarding the vertical distribution, the highest abundance of diatoms in July was found at the depth of 0.5 m (1.2 x 10^6 on July 16, and 7.5 x 10^5 on July 28) (Fig. 3).

At 0 m depth, *Fragilaria* sp. was the most abundant diatom (max 1.9 x 10^5 cells cm^{-2}, 5.2 x 10^4 cells cm^{-2}). At 0.5 m depth, and *Achnanthes* sp. was the most abundant (max 6.9 x 10^5 cells cm^{-2}, 5 x 10^5 cells cm^{-2}) (Fig 4).

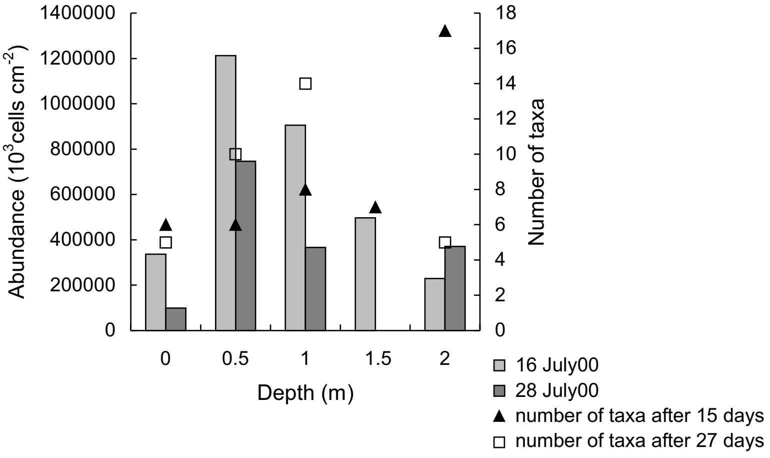

Fig. 3. Abundance (cells cm^{-2}) and diversity of periphytic diatoms throughout the water column in the Zrmanja River, in July 2000. Samples at a depth of 1.5 m were accidentally lost.

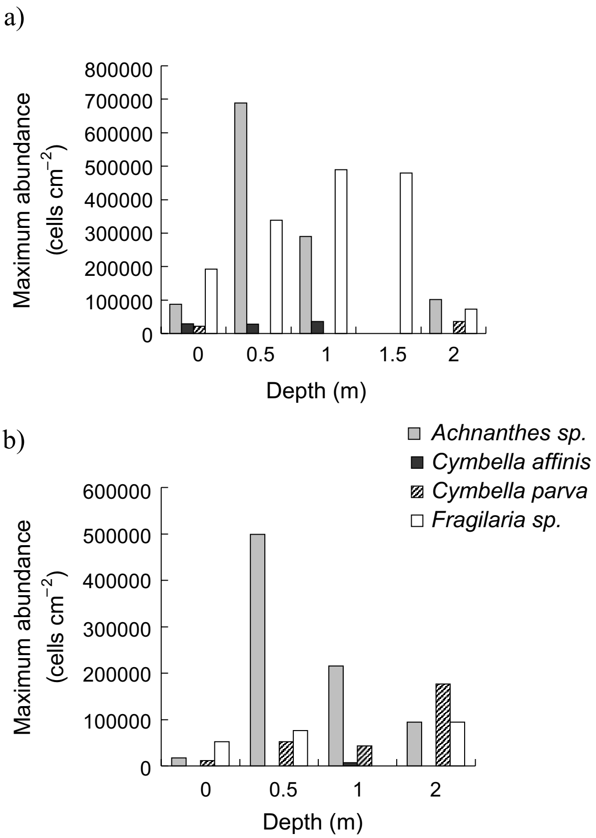

Fig. 4. Depth distribution of maximum abundances of dominant periphytic diatoms (a >10^5 cells) in the Zrmanja River at Station S2. a) 16 July, 2000, b) 28 July, 2000.

At 1 m depth, *Fragilaria* sp. was the most abundant (max 4.9 x 10^5 cells cm^{-2}) on July 16 (Fig 4a), and *Achnanthes* sp. on July 28 (max 2.2 x 10^5 cells cm^{-2}) (Fig 4b).

At 1.5 m depth results were available only on July 16. *Fragilaria* sp. was the most abundant (max 4.8 x 10^5 cells cm^{-2}), while other diatoms were presented in less than 10^4 cells cm^{-2} (Fig 4a).

In the layer below 1.5 m, the dominant species were *Achnanthes* sp. (max 10^5 cells cm^{-2}) on July 16 (Fig 4a), and *Cymbela parva* (max 1.8 x 10^5 cells cm^{-2}) on July 28 (Fig 4b).

The maximum number of diatom taxa was found below the surface (Fig. 3). After 15 days, regarding the vertical distribution, the number of taxa was relatively constant (6–8), except on the plate at the depth of 2 m (17). After 27 days, the number of taxa rose from surface layer to the depth of 1 m. Below that depth, sample was lost.

Tab. 2. Taxonomic composition of periphytic diatoms, frequency of appearance (%) and abundance (cells cm^{-2}) at Station S2, the Zrmanja River, July 2000. n – number of samples, f – frequency, avg – average abundance, max – maximum abundance

Taxa n=9	f (%)	max	avg
Achnanthes sp.	100	676267	122611
Amphora sp.	22	35927	18679
Cocconeis sp.	44	7789	5107
Cyclotella sp.	33	18114	7713
Cymbella affinis Kützing	56	36229	20687
Cymbella helvetica Kützing	11	18114	18114
Cymbella parva (W. Smith) Cleve	67	176886	55960
Cymbella sp.	22	28741	16877
Diatoma vulgare Bory	11	1558	1558
Diploneis sp.	22	1558	1137
Epithemia sp.	11	3593	3593
Fragilaria sp.	89	489086	224357
Gomphonema olivaceum (Hornemann) Brebisson	44	28178	15024
Gomphonema sp.	33	14265	7627
Gyrosigma acuminatum (Kützing) Rabenhorst	11	3116	3116
Mastogloia smithii Thwaites	11	3593	3593
Mastogloia sp.I	33	17965	6985
Meridion circulare (Greville) C. A. Agardh	11	1558	1558
Navicula radiosa Kützing	11	1431	1431
Navicula rynchocephala Kützing	33	36229	14948
Nitzschia palea (Kützing) W. Smith	11	1558	1558
Nitzschia sigmoidea (Nitzsch) W. Smith	33	4755	3302
Nitzschia sp.	22	28178	14868
Nitzschia sp. I	33	7185	4599
Rhoicosphaenia curvata (Kützing) Grunow	11	14089	14089
Synedra capitata Ehrenberg	11	716	716
Synedra sp.	11	76080	76080
Synedra ulna (Nitzsch) Ehrenberg	56	34998	17057

Discussion

Apply of artificial substrates for quantification of periphyton has been recommended by WAHL and MARK (1999) who have found them more favorable for experimental growth than natural substrates. The summer season has been found to be a period of rapid succession of periphytic communities in the Mediterranean rivers (CAZAUBON et al. 1995, CERQUEIRA DA SILVA 1992) and also in the River Danube (ÁCS and KISS 1991, ÁCS 1998). Although BARBIERO (2000) found them selective for growing of epilithic diatoms such as *Cymbella microcephala* and *Cymbella affinis*, the dominant diatom community was composed of *Achnanthes-Cymbella-Fragilaria*.

In stabile conditions, the non-attached (planktonic) diatoms *Melosira varians* and *Fragilaria* spp. were also found in periphyton, as loosely attached plokon community (ROUND 1991). On horizontally positioned substrate plates, beside true-epiphytes and seston, SLÁDEČKOVÁ (1962) found plankton, detritus and sediment.

In the later successional stages, species composition changed, and the number of species increased to the depth of 1 m. The reason for the lower diversity below 1 m depth after the 27 days was unknown, although rain accompanied by bora wind provided unstabile hydrological conditions in the period from 19–22 of July. This might have led to a higher removal of diatoms from substrates, because filamentous taxa loosely associated with the substrate are more likely to be eroded in such circumstances (JUGGINS 1992), as has previously been revealed by JOHN and MOORE (1985) in the River Thames, but it could be also result of human vandalism (CATTANEO and AMIREAULT 1992). Species attached by mucilage stalks or pads (i.e. *Achnanthes*, *Cymbella*, *Gomphonema* and *Synedra)* maintained the community.

The supply of nutrients might have affected the species composition of periphyton (HILLEBRAND and SOMMER 2000). The dominant diatom *Achnanthes* sp. has been known as an indicator of nutrient-poor waters (AGATZ et al 1999). The nutrient poor region was colonized by smaller diatoms, such as species of the genus *Achnanthes* (BELEGRATIS and ECONOMOU-AMILLI 2000), which have great surface area in relation to small volume, and a higher nutrient apsorption rate (BAILLIE 1987). The diatom *Cymbella affinis* has been found to be the dominant epiphytic species in hydrochemically stabile and oligotrophic waters (PATRICK and PALAVAGE 1994), and in other karstic water in Croatia, the Plitvice Lakes (epiphyton) (CAPUT and PLENKOVIĆ-MORAJ 2000). High frequency (in 56–100% of samples) and high abundance (7.2×10^3–3.1×10^4 cells cm^{-2}) of this species clearly show the oligotrophic conditions in the Zrmanja River.

In the oligotrophic environment, benthic algae and vascular plants can survive due to their ability to sequester nutrients from the interstitial water of the sediment (SAND-JENSEN and BORUM 1991). Artificial substrates like chemically inert Plexiglass do not supply nutrients to periphytic diatoms, although some nutrients may come during resuspension of sediment. Low concentration of silicates, nitrogen and phosphates in the Zrmanja River probably limited development of periphytic diatoms on artificial substrates, but in summer phosphate-limited conditions, benthic diatoms use cell storage absorbed during the spring period (JÖBGEN et al. 2004).

Relationship between light and nutrients is also important for the growth of diatoms. Due to high transparency in the Zrmanja River, light did not limit the growth of photoautotrophs throughout the water column.

Due to the specific hydrography and oligotrophic character of the karstic Zrmanja River, forthcoming research should provide more information on the ecology of the river, as well as of the estuary.

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