Distribution of the diatom *Cocconeis scutellum* in the karstic estuary (Zrmanja, eastern Adriatic Sea)

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The small karstic river Zrmanja (69 km) discharges into the eastern–central Adriatic Sea, forming shallow, highly stratified, oligotrophic estuary. The abundance of periphytic pennate diatoms (attached to artificial substrates) and their presence in the plankton community were analysed in the upper estuary, in July 2000. Attached diatoms were most abundant in the halocline, while *Cocconeis scutellum* Ehrenberg competed for space with other algae that accumulated in the halocline. Suspended pennate diatoms were composed of freshwater species transported from the river and epiphytic diatoms detached from estuarine aquatic plants. The abundance of detached estuarine *Cocconeis scutellum* cells decreased downstream in the estuary, due to the absence of the host vegetation. This diatom rapidly sank and accumulated along the halocline, died in the strong gradient of salinity and continued to sink to the bottom. Such a fate of diatoms may be linked with the problem of silica cycling in the estuary that should be quantified during the forthcoming research.

Key words: *Cocconeis scutellum*, periphytic diatoms, phytoplankton, halocline, stratified estuary, Adriatic Sea, Croatia.

Introduction

Microphytobenthos contributes significantly to total production in estuaries (de Jong & de Jonge, 1995; de Jonge & van Beusekom, 1995). Benthic-pelagic exchange of microalgae can have far-reaching implications to the flux of organic matter (Lucas et al., 2000).

The diatom *Cocconeis* is cosmopolitan widespread genus living as epiphyte in fresh to marine waters (Krammer & Lange-Bertalot, 1991; Riaux-Gobin, 1991; Romero & Navarro, 1999; DeStefano et al., 2000; Sar et al., 2003). It lives in periphyton, individually attached to other algae and aquatic plants. Cells are sometimes found in plankton, usually as a result of having been physically separated from their substrate (Patrick & Reimer, 1966). *Cocconeis scutellum* Ehrenberg is a heterovalvate diatom with one valve bearing a raphe-sternum and the other one only a sternum (Ehrenberg, 1838). Valves are girdle shallow, so that cells almost always lie in valve view (Round et al., 1996). The raphe valve striae are usually punctate and on both valves the striae tend to be-

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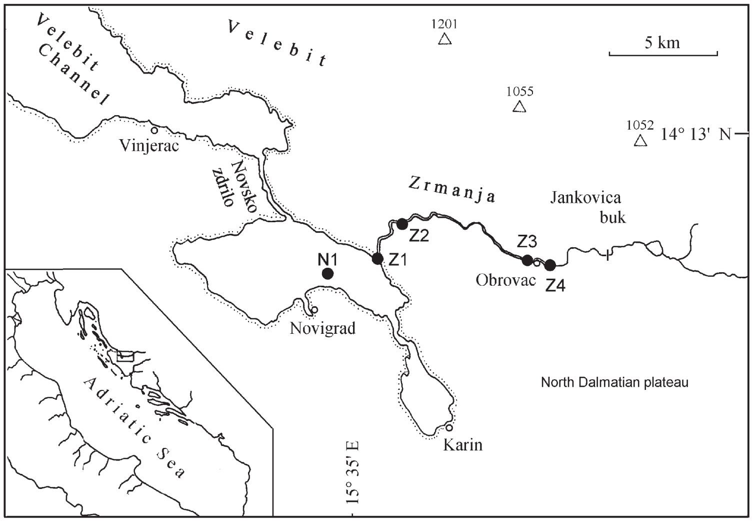

Fig. 1. Position of stations in the Zrmanja Estuary.

come more or less distinctly curved-radiate toward the extremities. The valves are 13–40 μm long and 8–23 μm wide (Peragallo, 1984).

In this paper we present a fragment of the research of periphytic diatoms attached to artificial substrates in the karstic Zrmanja Estuary. Colonisation and vertical distribution of the attached diatoms and particularly *Cocconeis scutellum* were analysed during summer reduced riverine inflow. We tried to compare abundance of the attached *Cocconeis* cells and those suspended in plankton, and correlate with hydrological and hydrographical conditions.

Investigated area

The Zrmanja River is 69 km long karstic river that discharges into the eastern Adriatic Sea (Fig. 1) forming highly stratified estuary (Viličić et al., 2000). The estuary and the adjacent coastal sea are situated between the coastal Velebit Mountain ridge and North Dalmatian plateau. The karstic system of the region provides number of connections between swallow holes in the karstic hinterland and underwater springs along the estuary. The annual maxima of freshwater discharge appear during rainy and snow melting periods (October–December and March–May). The tides are rather weak, M2 amplitudes are below 10 cm, and K1 amplitudes are close to 13 cm (Kasumović, 1960). The average river outflow equaled 38 m^3s^{-1} (calculated for the period 1953–1990), and ranged between 456 m^3s^{-1} (December 1959) and 0.09 m^3s^{-1} (June 1986).

The research was performed in the upper reach of the estuary, on stations Z1, Z2, Z3 and Z4 (Fig. 1). The upper reach is 14 km long and quite shallow (average depth about 5 m), with abundant submerged macrophytes along the banks. The area is scarcely inhabited. Only slight anthropogenic influence could be detected near the settlement of Obrovac (about 1000 inhabitants) in the upper estuary (Viličić et al., 2001).

Material and methods

Periphyton samples were collected at two sampling stations (Z3 and Z4), in the easilly accesible locations of the canyon formed upper estuary. It was collected from artificial substrates (plexiglas plates) which were fixed on a vertical rope at 0, 0.5, 1, 1.5 and 2 m below the surface. The position of plates provided living conditions from brackish to marine environment. At both stations sampling was done after 15 and 30 days of colonization. To determine colonization rate samples were collected at Station Z4, every third day from artificial substrates fixed on iron frame in the 0–0.5 m layer. To remove periphyton, samples were scrapped of the plate surface by the scalpel and adapted toothbrush, with repeated rinsing with water. Samples were preserved in 4 percent (final concentration) formaldehyde solution.

Determination of periphytic diatoms was performed after the cleaning treatment, using mixture of concentrated nitric and sulphuric acids, and KNO_3 (Round et al., 1996). Diatoms were counted at magnification 400× according to the Utermöhl method (Utermöhl, 1958) under the Zeiss Axiovert inverted microscope equipped with phase contrast. Taxonomic analysis was additionally performed at magnification 1000× with phase contrast. Cells were identified using standard manuals (Zabelina et al., 1951; Hindák et al., 1978; Peragallo, 1984; Hustedt, 1985; Patrick & Reimer, 1966, 1975; Witkowski et al., 2000). The nomenclature was adjusted according to the checklist of diatoms by Hartley (1986).

Samples of planktonic diatoms were taken by Nansen sampler and preserved in a 2 % (final concentrations) neutralized formaldehyde solution. The cells from the 50 mL subsamples were counted using the inverted microscope, after a sedimention time of 24 hours. Cells were counted at a magnification of 400× (1 transect) and 200× (transects along the rest of the counting chamber base plate), respectively.

Fine vertical distribution of salinity and temperature was determined using a conductivity, temperature and depth profiler (SEA Bird Electronics Inc., USA). Nutrient and oxygen concentrations were determined using standard methods (STRICKLAND & PARSONS, 1972; IVANČIĆ & DEGOBBIS, 1984).

Results

During 1999/2000, the river inflow ranged from 0.90 $m^3 s^{-1}$ to 230 $m^3 s^{-1}$ with two maxima detected in December–January (175-230 $m^3 s^{-1}$) and in April (190 $m^3 s^{-1}$). The summer inflow ranged from 0.94 $m^3 s^{-1}$ to 3.84 $m^3 s^{-1}$. A slight increase of the inflow appeared on June 24 and during 12–20 July (Fig. 2). High stratification was documented by the sharp halocline in the 1–1.5 m layer, providing the boundary between brackish and marine layers (Fig. 3). Thermocline was close to the halocline. The surface salinity varied between 8 and 14 PSU, and temperature between 19–24 °C. Below the halocline salinity increased up to 34, and temperature ranged between 21 and 23 °C.

Concentration of orthophosphates was low at the surface (<0.05 μmol L^{-1}), and slightly increased in and below the halocline (0.075 μmol L^{-1}). Concentrations of inorganic nitrogen (TIN) and silicates were higher above the halocline (7.5 μmol TIN L^{-1}) , >10 μmol SiO_4 L^{-1}), than below the halocline (<2.5 μmol TIN L^{-1}, <5 μmol SiO_4 L^{-1}). The oxygen saturation was high (80–87%) throughout the water column (Fig. 3). The secchi

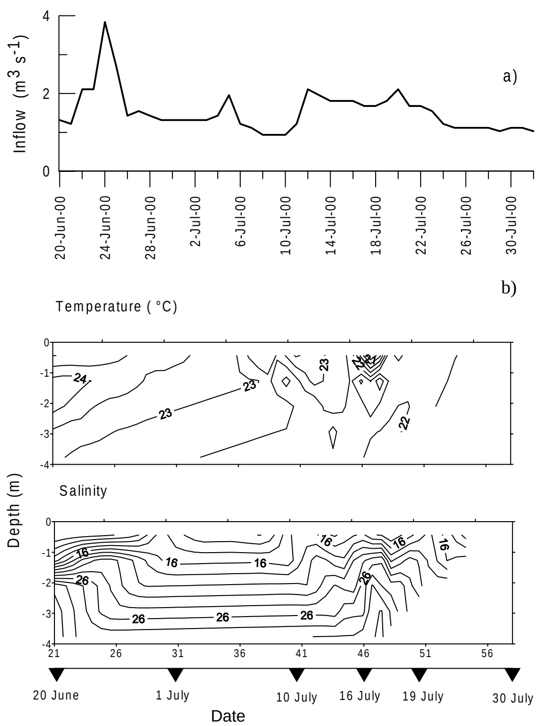

Fig. 2. Inflow of Zrmanja River into the estuary (a) and termohaline conditions at Station Z4 (b) in the period of 20 June – 28 July, 2000.

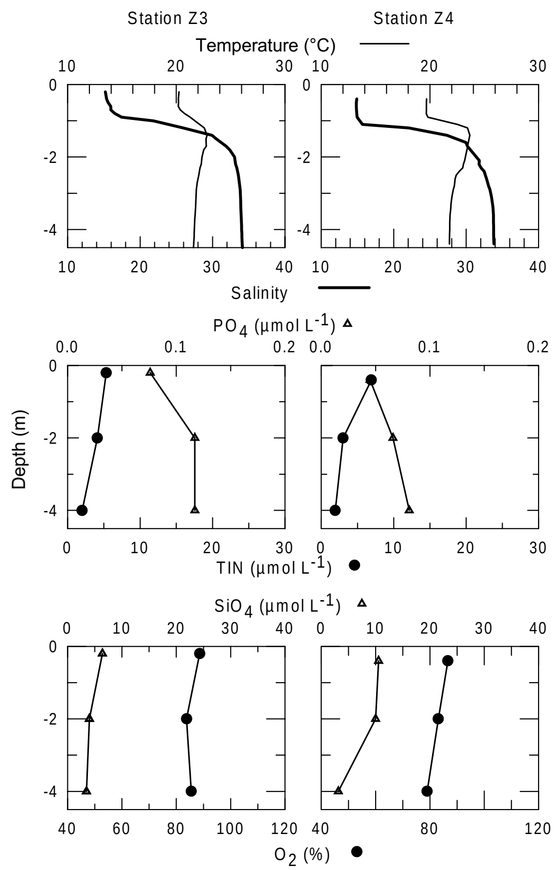

Fig. 3. Vertical distribution of temperature, salinity, orthophosphates (PO_4), total inorganic nitrogen (TIN), silicates (SiO_4) and oxygen saturation, in the upper Zrmanja Estuary, on July 17, 2000.

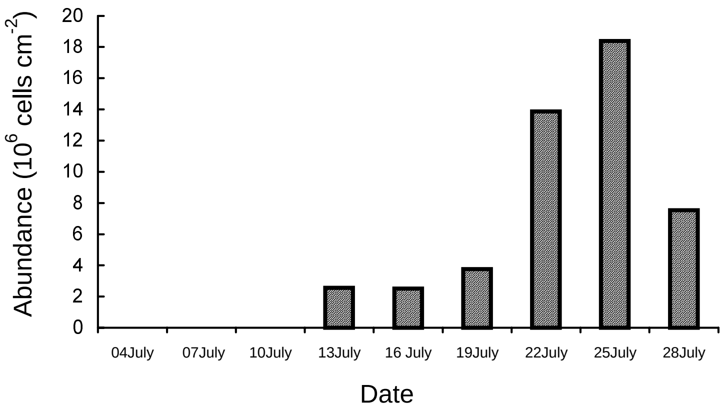

Fig. 4. Successive development of attached diatoms at Station Z4 (0–0.5 m layer) in July 2000.

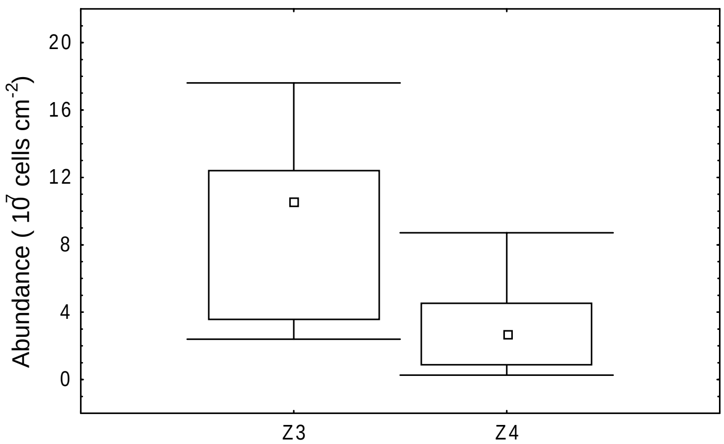

Fig. 5. Abundance of attached diatoms at Stations Z3 and Z4 (0, 0.5, 1, 1.5 and 2 m depth) in the upper Zrmanja Estuary taken on July 16 and 28, 2000. Range, median, as well as lower (25 %) to upper (75 %) quartil values are presented. $n = 21$ samples.

disc transparency varied between 3 and 9 m.

Periphyton was composed of algae from different taxonomic groups: cyanobacteria, rhodophytes, chrysophyceae, xanthophyceae, chlorophytes and diatoms. Diatoms were most abundant and composed of 60 species (5 pennates and 2 centrics). The colonization of attached diatoms evolved to the maximum abundance of 1.83×10^7 cells cm^{-2} after 25 days of colonization (Fig. 4).

Attached diatoms were significantly more abundant ($p < 0.05$) at Station Z3 (avg 6.2×10^7 cells cm^{-2}) than at Station Z4 (avg 3.2×10^7 cells cm^{-2}) (Fig. 5). The most abundant (avg $> 10^6$ cells cm^{-2}) and frequent (100%) taxa were *Achnanthes coffeaformis* (C. A. AGARDH) KÜTZIG, *Melosira moniliformis* O. F. MÜLLER, *Navicula veneta* KÜTZIG and *Nitzschia elongatula* GRUNOV in VAN HEURCK.

The attached cells of *Cocconeis* were more abundant at station Z4, below the halocline (3 ×

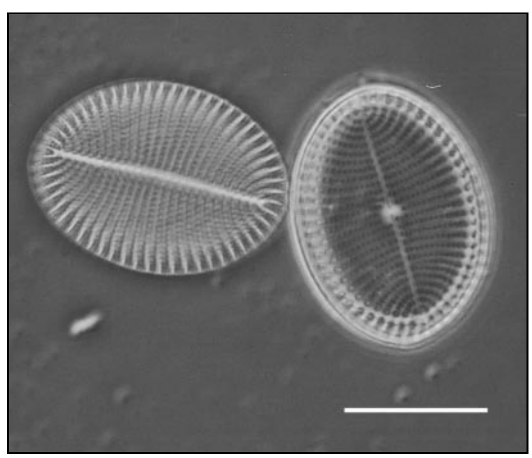

Fig. 6. Heterovalvar diatom *Cocconeis scutellum*. Upper valve with raphe sternum (R-valve) and lower valve lacking raphe but with a corresponding raphless sternum (P-valve) are presented. Scale bar 20 μm.

Table 1. Vertical distribution of the attached diatom *Cocconeis scutellum* and total *Cocconeis* (counted after cleaning treatment) on two sampling days of the 28 days succession, at Stations Z3 and Z4 in the upper Zrmanja Estuary (July, 2001). 0 denotes value <100 cells cm^{-2}. Shaded area denotes halocline.

	Depth (m)/day	Z3		Z4	
		17	28	16	28
Cocconeis scutellum	0–0.5	0	4220	0	2280
	1–1.5	550	0	200	1730
	2–3	660	–	5000	2320
Cocconeis	0–0.5	700	15230	1830	3150
	1–1.5	1100	0	2000	7650
	2–3	5290	–	5200	5350

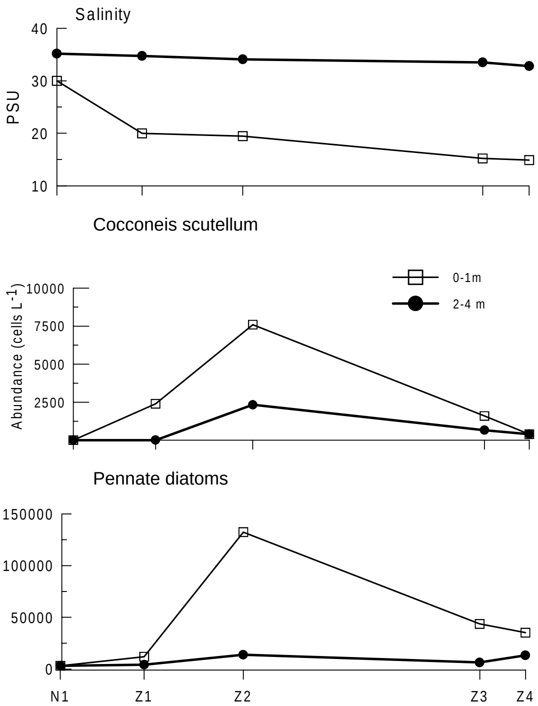

Fig. 7. Horizontal distribution of suspended *Cocconeis scutellum* and other penate diatoms along the upper reach of the Zrmanja Estuary (July, 2000).

10^6 cells cm^{-2}) after 28 days of colonization. *Cocconeis scutellum* (Fig. 6) was more abundant at Station Z4 (avg 1.9×10^3 cells cm^{-2}) than at Z3 (avg 1.1×10^3 cells cm^{-2}). At Station Z4 it provided maximum abundance below the halocline after 16 days, while at Z3 in the surface after 28 days of colonization (Tab. 1).

Suspended pennatae diatoms were most abundant above the halocline with a maximum of 3.7×10^7 cells L^{-1} (Fig. 6).

In the upper estuary the suspended *C. scutellum* was found throughout the water column, but at stations Z3 and Z2 it was most abundant (1600–12800 cells L^{-1}) above the halocline (17–20 PSU). In the seaward direction it was restricted to the surface at Station Z1, and disappeared in the middle estuary, at Station N1 (Fig. 7).

Discussion

Salinity and temperature are important environmental factors influencing distribution of algae (UNDERWOOD, 1994). The high water transparency in the Zrmanja Estuary resulted in the successful colonization of the attached diatoms.Colonization of periphytic algae in the Zrmanja Estuary might be influenced by hydrological and meteorological conditions. Similarly, timing and magnitude of physical forcing events, mainly rainfall, appear critical in determining the susceptibility of the ecosystem to summer and autumn algal blooms (THOMPSON & HOSJA 1996). Rainy weather conditions that started on July 12 (twelfth day of colonization) and increased river water inflow resulted in the decrease of surface salinity. In the same time simultaneous increase of subsurface salinity was detected, probably due to the stronger inflowing current into the estuary below the halocline, appearing during north wind events in the coastal sea. Such conditions resulted in the reduction of diatom abundance in the surface layer during 12 to 19 day of colonization. Due to increased surface current velocity, periphytic diatoms were probably mechanically detached from the substrate. The influence of water discharge on the abundance and diversity of species is widely known. During the phylogenic evolution, periphytic algae have adopted different adhesion strategies to survive in flowing waters. There is selection by velocity of flow of diferent adhesion strategies. The weather stabilized after 22 days of colonization and three days later, the diatoms reached maximum of their abundance.

Higher diatom abundance at Station Z3 than at Z4 is a result of slight anthropogenic input of orthophosphates at Station Z3. On the other hand periphytic genus *Cocconeis* was more abundant at Station Z4 than at Z3.

Due to vertical distribution, all species of *Cocconeis* had minimum abundance in the halocline (especially at Station Z3), probably due to more pronounced competition for space (for attachment) with other algae that were observed to accumulate in the halocline. Explanation could have been the way *Cocconeis* attaches to the substrate to which valves are tightly appressed with the whole surface (CATTANEO, 1978). There are three algal life forms in periphyton, according to the degree of attachment and proximity to the substratum (CATTANEO, 1990). *Cocconeis* is in the group of "tightly attached alga" including forms that grow appressed to the substratum. "Filamentous and long stalked algae" also attach to the substrate but extend outward to form a mat, while "motile planktonic algae" include motile forms and those suspended or trapped in the mat. Algae that form mats are only loosely attached to the substratum and should be more vulnerable to detachment (HOAGLAND, 1983), while euplanktonic algae are the first to be washed out from the coating (ACS & KISS, 1993). Tightly attached algae lack the response to changes in currents due to their size and proximity to the substratum (SILVESTER & SLEIGH, 1985). Changes may be in response to physical force of currents, to direct damage to filaments or removal of individuals, or to indirect effects, such as grazing by herbivores (BERGEY, 1995). *Cocconeis* is a grazer resistant diatom that can be abundant under high grazing pressure (DUDLEY, 1992). It is an early colonist (BLENKINSOPP & LACK, 1994) resistant to currents due to their strong horizontal attachment to the substrate (MONTEANU & MALY, 1981). Higher water discharge and current velocity in the period of 12–19 July, as well as the lack of nutrients, resulted in lower competition and easier recolonization of *Cocconeis* species.

Upper reach of the Zrmanja Estuary is suitable for periphyton growth in the area of *Phragmites*, where flow may be localy reduced. Downstream from Stations Z3 and Z4 there is no *Phragmites* and no further development of periphytic communities. Stronger currents could detache *C. scutellum* cells from the substrate and transport them as suspended cells down the estuary. In such conditions, even benthic macroalgae may temporarily contribute to plankton as considerable biomass in the water column (DE JONGE & VAN BEUSEKOM, 1992). The suspended cells of *C. scutellum* sank to the halocline, died in the layer of strong gradient of salinity and continued to sink to the bottom. The horizontal transport resulted in the 30 % increase of suspended *C. scutellum* abundance at downstream Station Z2. Due to absence of the host macrophytic vegetation, *C. scutellum* was less abundant in suspension, and finally not detectable in the middle reach of the estuary (Station N1).

The fate of *C. scutellum* in the highly stratified environment such as Zrmanja Estuary, could represent the model for other periphytic diatoms which develop in the estuary and detach from the substrate during luxurious growth and higher current velocities. Change in water dynamics affects not only abundance but also diversity of the species.

In the further research it would be interesting to determine growth of periphytic diatoms in different hydrological conditions, and how much they contribute to overall phytoplankton production in the stratified estuary.

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