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Phytoplankton abundance and pigment biomarkers in the oligotrophic, eastern Adriatic estuary

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Abstract Phytoplankton distribution and environmental characteristics were determined in a shallow, highly stratified and oligotrophic estuary (Zrmanja, eastern Adriatic). Samples were collected in two contrasting seasons; winter (February 2000), when river discharge was high, and in summer (July 2003), a period of drought. Phytoplankton distribution was closely related to salinity gradients, nutrient levels, and water residence time. Microscopic analysis revealed that phytoplankton was composed mainly of marine diatoms, dinoflagellates, cryptophytes, green flagellates, and coccolithophorids. The dominant biomarker pigments were fucoxanthin, alloxanthin and 19′-hexanoyloxyfucoxanthin, while lower, but indicative contributions of peridinin and chlorophyll *b* were also noted. Maximum abundance and biomass were found in the middle estuary in winter and in the upper estuary in summer. The estuary is mostly P-limited. Development of chain-forming marine diatoms was evident in winter. Due to the reduced nutrient input in summer, the biomass accumulated in the upper estuary (1,000 ng chlorophyll *a* l^{-1}) was composed mostly of nanoplanktonic unicellular diatoms, nanoplanktonic marine dinoflagellates, cryptophytes, and chlorophytes. The concentrations of about 200 ng l^{-1} hex-fuco, suggested that the contribution of prymnesiophytes to total biomass was compa-

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rable to that of diatoms and dinoflagellates. In the middle estuary and coastal sea, PO_4 and TIN were 3.5 times lower, resulting in a fivefold decrease in biomass (<100 ng chlorophyll *a* l^{-1}). The oligotrophic Zrmanja and other karstic rivers discharging in the eastern Adriatic Sea, provide insufficient source of nutrients and low productivity of the eastern Adriatic Sea.

Keywords Estuary · Phytoplankton · Biomarkers · Salinity · Nutrients · Trophic state · Adriatic Sea

Introduction

Most phytoplankton studies have been limited to highly productive macrotidal and partially stratified estuaries (i.e. Cloern et al. 1983; Malone et al. 1988; Gallegos 1992; Lucas et al. 1999; Pinckney et al. 1998; Sin et al. 2000; Trigueros and Orive 2000; Seoane et al. 2005); few yet have dealt with microtidal, highly stratified estuaries, such as those found along the Mediterranean Sea (Viličić et al. 1989; Jalliffier-Merlon et al. 1991; Terzić et al. 1995; Moreira-Turcq et al. 2001; Naudin et al. 2001; Puigserver et al. 2002).

The estuaries on the eastern Adriatic coast are small highly stratified salt-wedge estuaries, and the two largest of those of the Zrmanja and Krka Rivers.

This paper analyzes the distribution of phytoplankton in the Zrmanja Estuary during two seasons characterized by distinct hydrographic conditions: in winter, immediately after a prolonged period of increased river discharge; and in summer, during the period of drought. The approach, based on a combination of microscopy and chemotaxonomic characterization of phytoplankton as reflected by biomarker pigments, is applied to quantify unrecognizable and fragile cells which may constitute the bulk of the phytoplankton abundance (Seoane et al. 2005), for the first time in the eastern Adriatic estuary.

Materials and methods

Investigated area

The Zrmanja River is a small karstic river that discharges into the eastern Adriatic Sea. From its source in the Dinaric Alps to the Jankovica Buk waterfall, the river spans 69 km. The estuary, lying below the falls, and the adjacent coastal sea are oriented westerly between the Velebit Mountain in the north, and North Dalmatian plateau in the south and east. The 14 km long, canyon-like upper estuary (Fig. 1) has steep and narrow banks that are strongly eroded. The estuarine bed thus is relatively shallow, mostly about 5 m deep (Table 1). The wider and indented middle estuary is about 5 km long and 30 m deep. The lower estuary – 3.5 km long and 40 m deep – is a narrow strait (Novsko ždrilo) connecting the middle estuary and the Velebit Channel.

As a consequence of rather weak tides (M2 amplitudes below 10 cm, and K1 amplitudes near 13 cm, according to Kasumović, 1960) and relatively high river outflow, the estuary is highly stratified throughout the year (Viličić et al. 1999).

Mean annual rainfall of 966 mm over the drainage area (650 km^2) resulted in an annual discharge of about 6×10^8 m^3 s^{-1} during 1999–2000. Average outflow (1953–1990) was 38 m^3 s^{-1}; this ranged from a high of 456 m^3 s^{-1} (December 1959) to a low of 0.09 m^3 s^{-1} (June 1986).

Water circulation in the Zrmanja catchment basin is complex (Bonacci 1999). In a typical karstic region, numerous springs – both permanent and temporary – along the river are connected with sink holes ("ponors") in the hinterland. Underwater springs ("vruljas") discharge into the estuary during periods of rain (October–December) and snow melting (March–May).

Water column sampling

Water samples were collected at seven stations: one in the Velebit Channel coastal sea (V2) and six along the middle (N1) and upper (Z1–Z4, and Z4A) Zrmanja Estuary (Fig. 1). The sampling campaigns were performed in winter (February 26, 2000) during high river discharge and in summer (July 18, 2003) during the dry period. Samples for phytoplankton abundance, biomarker pigments, and nutrients were collected with 5-l Niskin bottles at one-meter intervals in the upper estuary and at 0, 2, 4, and 10 m in the middle estuary and coastal sea.

Samples for phytoplankton abundance and composition were preserved in 2% (final concentration) neutralized formalin. Samples for pigment analysis

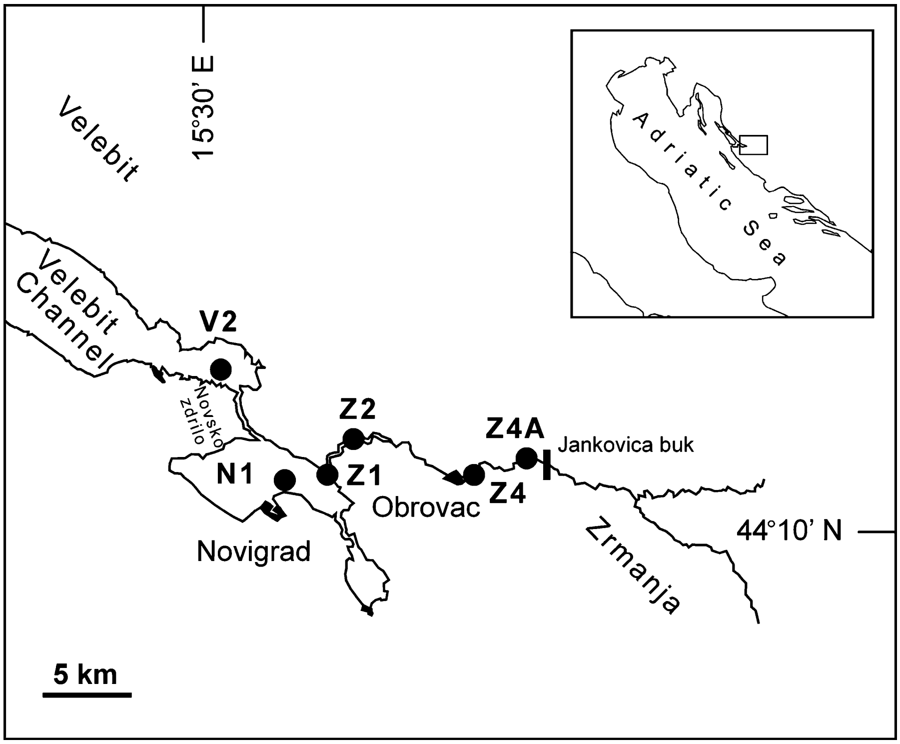


Fig. 1 Position of stations along the Zrmanja Estuary; upper estuary (*Z1–Z4A*), middle estuary (*N1*) and the coastal sea (*V2*)

Table 1 General morphometrical, hydrographical and biological characteristics in the Zrmanja Estuary (1998–2000)

Parameter	Min	Max	Average	STD	Total	Unit
Length					22.5	km
Width	0.05	3				km
Depth	5	40				m
Area					22.05	km^2
Volume					0.37	km^3
Tidal amplitude			20			cm
Depth of the halocline (at Station Z4)	1.2	5	2.7			m
Depth of the halocline (at Station Z2)	0.5	5	2.2			m
Drainage area					650	km^2
Annual rainfall (Station Knin)	835	1133	966			mm
Monthly rainfall (Station Knin)	0	221	80.60			mm
Monthly river discharge (in 2000)	0.95	162	19			m^3
Annual river discharge (in 2000)					5.9×10^8	m^3
Temperature	6.70	26.55	16.34			°C
Salinity	0.00	38.00	18.05	13.35		PSU
Secchi disc visibility	3	11	5.60	1.66		m
Microphytoplankton abundance	0.8	2128	163	361		10^3 cells l^{-1}
Phytoplankton biomass (conc. chlorophyll a)	40	1130	280	240		ng l^{-1}
PO_4	0.00	0.25	0.07	0.06		μmol l^{-1}
NO_3	0.05	22.36	6.78	5.32		μmol l^{-1}
TIN	0.00	23.10	7.60	5.35		μmol l^{-1}
TIN/PO_4	1	4002	149	299		
SiO_4	0.10	43.90	11.30	9.77		μmol l^{-1}
Oxygen saturation	64	115	95	35.70		%

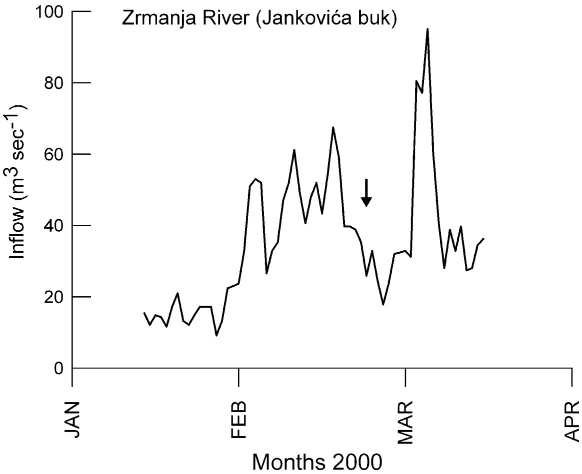


Fig. 2 The inflow of Zrmanja River water into the estuary during January, February and March 2000. Measurements in front of Jankovića Buk Station. *Arrow* indicates day of sampling in the estuary

(0.5–1 l) were filtered onto 47 mm GF/F filters (0.7 μm mean pore size), and frozen immediately in liquid nitrogen.

Phytoplankton, hydrography and nutrients

Phytoplankton abundance was determined using an inverted microscope (Utermöhl 1958; Hasle 1978a, b; Venrick 1978). Sub-samples of 50 ml were analyzed microscopically within one month, after 24 h of sedimentation. One transect along the counting chamber bottom was scanned at 400× and two at 200× with a Zeiss Axiovert 200 phase-contrast inverted microscope. Larger nanoplankton (>5 μm) and abundant microphytoplankton (>20 μm) were counted and identified at 400×. At 100× a total bottom count was completed for taxa greater than 30 μm. The minimum concentration that can be detected by this method is 20 cells l^{-1}. Recognizable nanoplankton was composed mostly of coccolithophorids, dinoflagellates, chlorophytes, and cryptophytes. The entire phytoplankton community was identified to species or genus after image analysis and processing using the Karl Zeiss AxioVision 3.1 System and AxioCam Camera. Classic and recent taxonomic references used in this work have been described elsewhere (Viličić et al. 2002).

The five most prominent biomarkers such as fucoxanthin, peridinin, 19′-hexanoyloxyfucoxanthin, alloxanthin, and chlorophyll *b*, were chosen to illuminate temporal and spatial variability of diatoms, dinoflagellates, coccolithophorids, cryptophytes, and green algae, respectively (Barlow et al. 1993, 1997; Ahel et al. 1996; Ahel and Terzić 1998). Filters containing phytoplankton were extracted in 4 ml of cold 90% acetone using sonication, and then centrifuged to clarify the extract. Pigments were separated by reversed-phase high-performance liquid chromatography (HPLC; Barlow et al. 1993). Briefly, extracts were mixed (1:1 *v*/*v*) with 1 M ammonium acetate and injected into a HPLC system incorporating a C_{18} 3 μm Pecosphere column (3.3×0.45 cm, Perkin Elmer). A binary linear gradient was used to separate the pigments. Solvent A consisted of 80:20

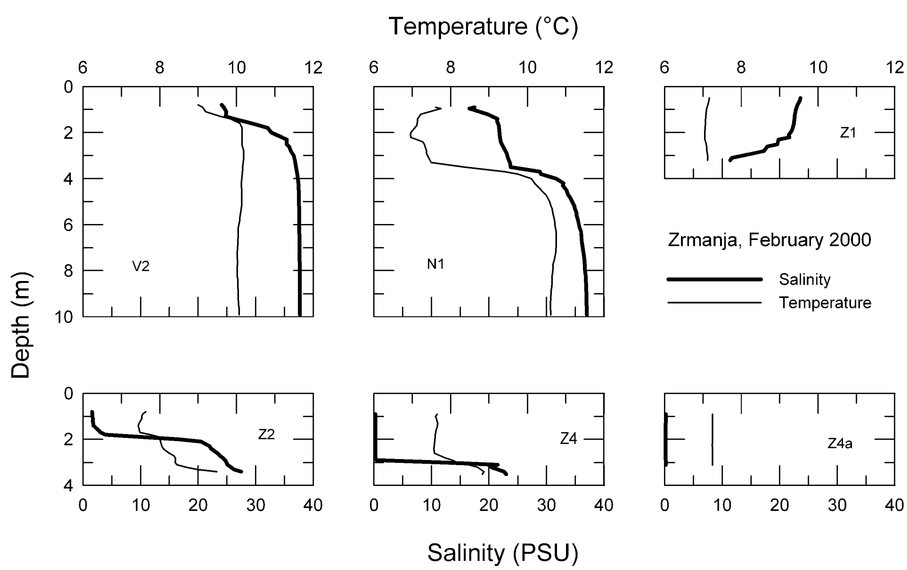


Fig. 3 Vertical distribution of temperature and salinity in the coastal sea (*V2*) and along the Zrmanja Estuary (*N1–Z4A*), on February 26, 2000

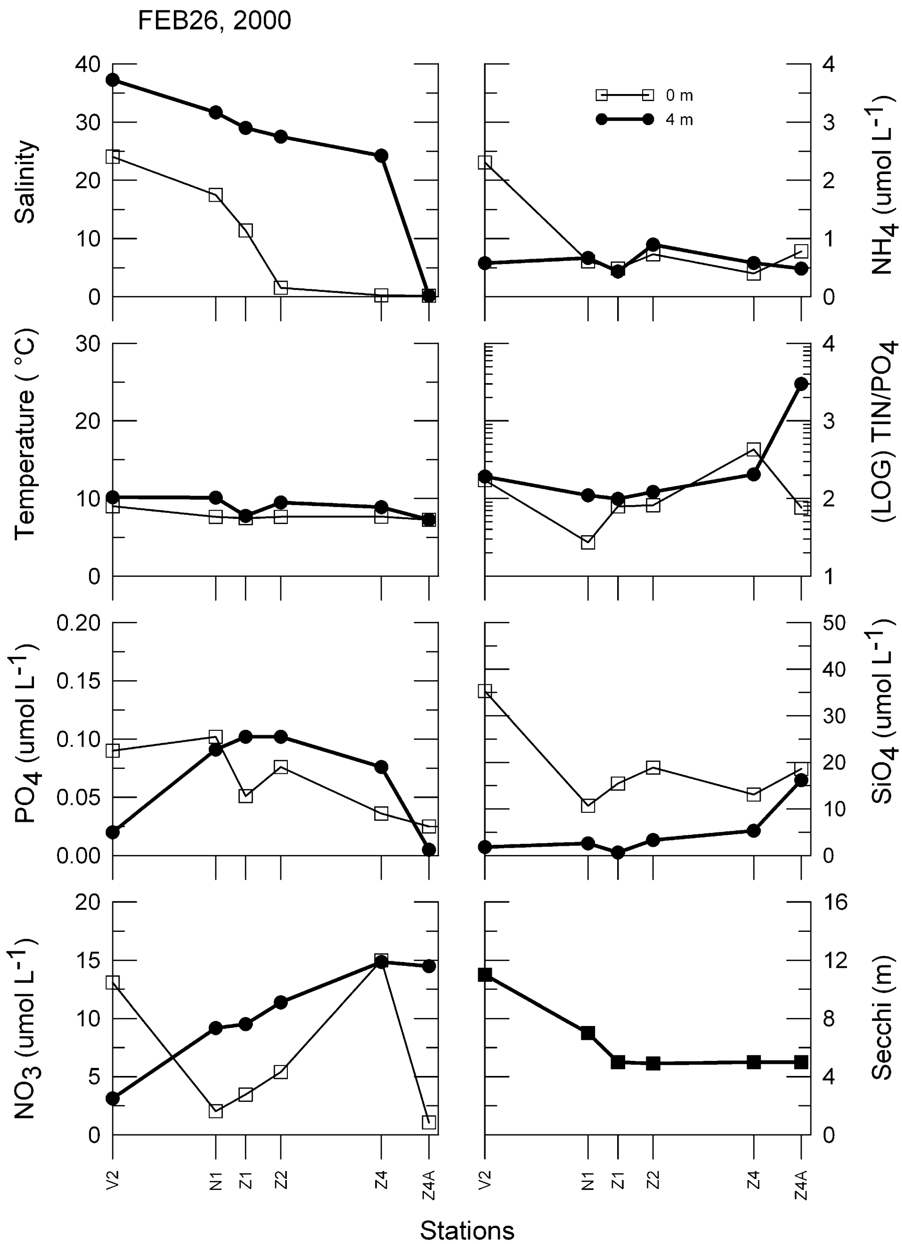


Fig. 4 Distribution of salinity, temperature, orthophosphates, nitrates, ammonia, Redfield ratio, orthosilicates and transparency, along the Zrmanja Estuary, on February 26, 2000

(*v*/*v*) methanol:1 M ammonium acetate and solvent B contained 60:40 (*v*/*v*) methanol:acetone. Chlorophyll and carotenoids were detected by absorbance at 440 nm (Spectra Physics, Model UV 2000). Qualitative and quantitative analyses of individual pigments were performed by external standard calibration using authentic pigment standards (VKI, Denmark).

Fine-scale vertical distribution of salinity and temperature was determined with a CTD profiler (SEA Bird Electronics Inc., USA). A white Secchi disc (30-cm diameter) was used to estimate transparency.

Nutrient concentrations were measured by standard methods (Strickland and Parsons 1972; Ivančić and Degobbis, 1984). The total inorganic nitrogen/ortophosphate ratio $\left(\mathrm{TIN}/\mathrm{PO}_4^-\right)$ was calculated according to Redfield et al. (1963). The limiting nutrients for phytoplankton growth at any salinity were determined using the graphic method according

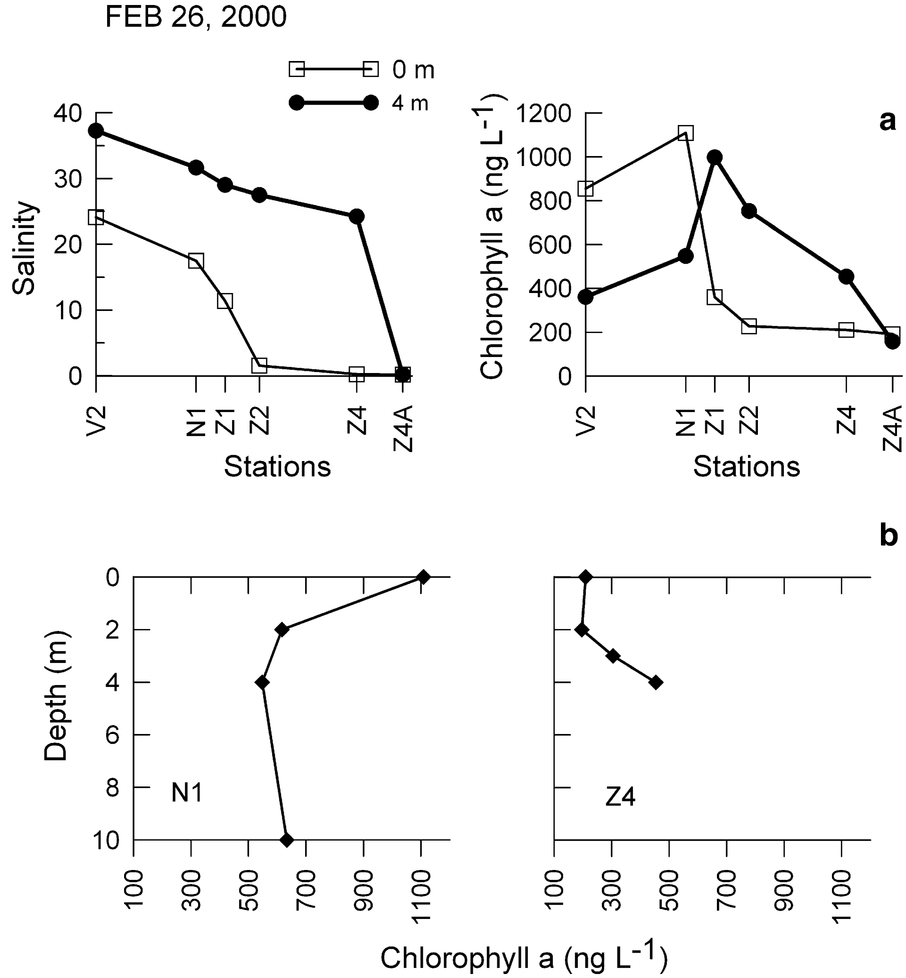


Fig. 5 Longitudinal (**a**) and vertical (**b**) distribution of salinity and chlorophyll *a*, on February 26, 2000

to Neill (2005). Graphs are prepared for TIN vs salinity and for o-phosphate vs salinity with the vertical axes set at a ratio of N:P=16:1 (i.e. the scales for N and P in the graphs are set so that they are proportional to the average rate at which these nutrients are absorbed by phytoplankton during growth). When these graphs are superimposed, the lowermost trendline indicates the limiting nutrient for phytoplankton growth at any salinity.

Results

February 2000

River discharge for February 2000 ranged from 17.8 to 67.5 m^3 s^{-1} (Fig. 2), with the maximum on February 18. The average discharge for the year 2000 was 19.4 m^3 s^{-1}. Discharge on the day of sampling (February 26) was 24.4 m^3 s^{-1}. This was, however, preceded by a 3-week period of higher discharge that displaced the mouth of the estuary 2 km seaward from the Jankovića Buk waterfall (Fig. 3). The downstream section – stations Z4, Z2, and Z1 – was characterized by a sharp halocline with a vertical salinity gradient of 10.88 m^{-1} between 2 and 4 m. There were also considerable longitudinal salinity gradients in the brackish layer: salinity of the brackish layer above the halocline in the upper estuary (Z1, Z2, and Z4) ranged between 3 and 20; in the middle estuary (N1), from 17 to 25. The brackish layer at the coastal station (V2) was about 1 m deep, with salinity between 20 and 30. Salinity in the estuarine layer bellow the halocline ranged from 25 (Z4) to 37.94 (V2).

Temperatures in the upper estuary were 7–8°C throughout the water column, while in the middle

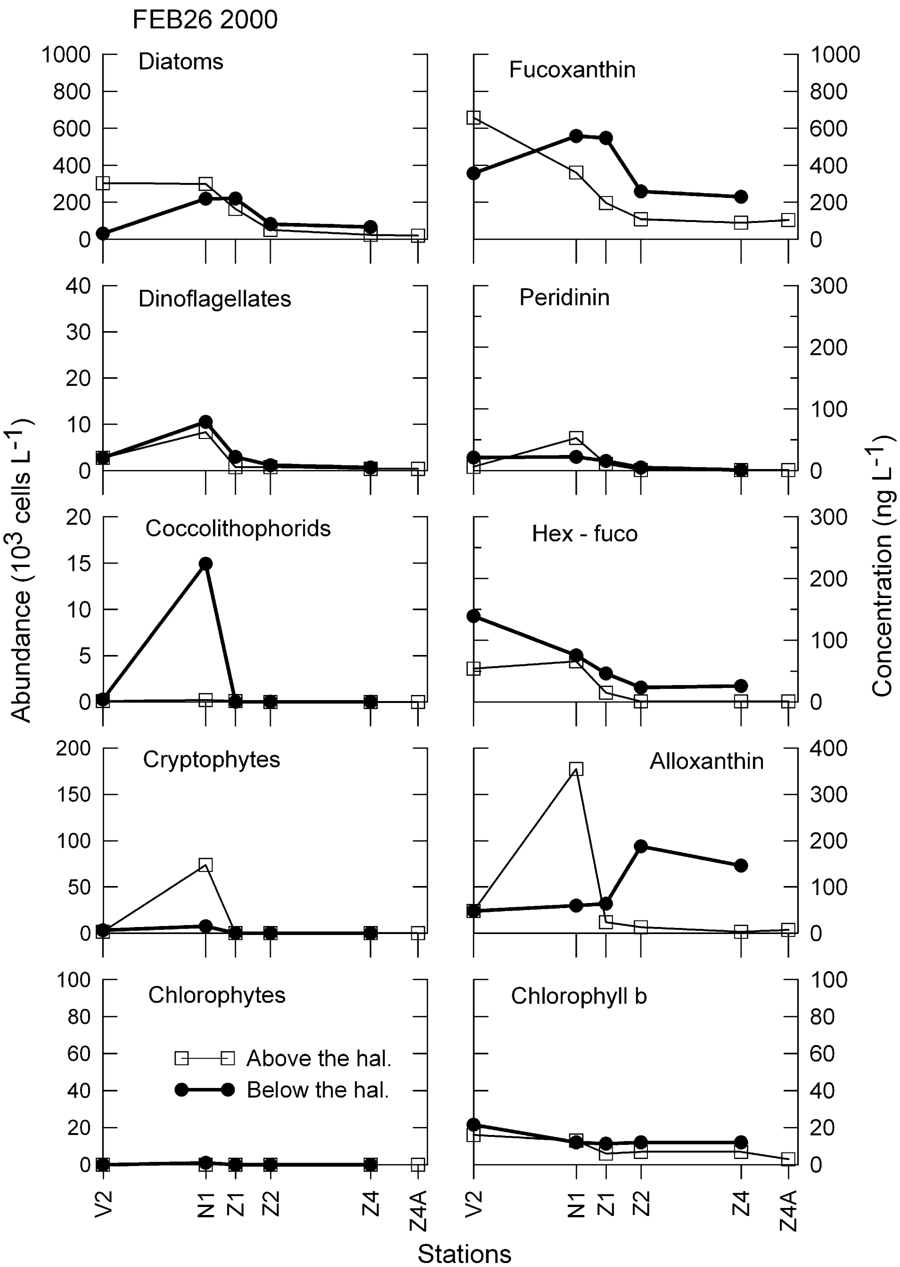


Fig. 6 Distribution of phytoplankton abundance and biomarker pigments along the Zrmanja Estuary on February 26, 2000

estuary similar temperatures (from 6 to 8°C) were found only above the thermocline (Fig. 3). The thermocline of the marine layer was slightly above 10.5°C, which is relatively low for this area. This is likely the result of intense cooling during January 2000, when ice cover formed for a short time in the middle estuary.

Nutrient concentrations in the estuary were relatively low due to the oligotrophic nature of the riverine inputs (Fig. 4). Orthophosphate concentration was generally lower than 0.1 μmol l^{-1}, while total inorganic nitrogen and silicate were always lower than 14 and 20 μmol l^{-1}, respectively. Phosphate and nitrate were higher below the halocline. Phosphate was slightly higher at downstream stations below the halocline, probably as a result of more advanced microbial regeneration.

The river was the major source of silicate and nitrate. Increased nitrate in the surface layer of station

Table 2 Vertical distribution of phytoplankton abundance (cells l^{-1}) in the Zrmanja Estuary (at stations Z2 and Z4), on February 26, 2000

Station	Depth (m)	Salinity (PSU)	Syn ul	Syn ac	Dia elo	Bac del	Ch cur	Ch div	Lep med	Cyc	Pseudo
Z2	0	1.55	800	0	0	0	0	0	0	0	0
	1	1.62	1,600	800	16,800	1,200	0	0	0	0	0
	2	16.65	800	2,400	8,000	14,400	520	200	40	9,600	2,000
	3	24.92	1,000	0	0	62,010	1,200	200	160	12,800	2,400
	4	27.48	0	0	0	23,200	160	320	80	16,000	1,600
Z4	0	0.24	1,000	600	0	0	0	0	0	0	0
	2	0.24	2,400	800	0	0	0	0	0	0	0
	3	12.34	200	1,600	0	1,600	320	200	0	1,200	600
	4	24.24	400	0	0	20,400	360	2,800	40	3,600	2,4000

Syn ul *Synedra ulna*; Syn ac *Synedra acus*; Dia elo *Diatoma elongatum*; Bac del Bacteriastrum *delicatulum*; Ch cur *Chaetoceros curvisetus*; Ch div *Chaetoceros diversus*; Lep med *Leptocylindrus mediterraneus*; Cyc, *Cyclotella coctawhatcheeana*; Pseudo *Pseudo-nitzschia* spp. 0 denotes abundance <40 cells l^{-1}.

Z4 indicates the slight anthropogenic influence of the small settlement of Obrovac.

Secchi disc transparency varied between 5 and 7 m (Fig. 4), indicating good transparency throughout the water column.

Chlorophyll *a* concentration exhibited a pronounced vertical and longitudinal variability (Fig. 5). The average concentration was higher in the middle (N1: 1180±310 ng chlorophyll *a* l^{-1}) than in the upper (Z4: 330±145 ng chlorophyll *a* l^{-1}) estuary

Table 3 Distribution of phytoplankton taxa, according to the average water column abundance (cells l^{-1}) along the Zrmanja Estuary (stations V2, N1, Z1, Z2, Z4), on February 26, 2000

Station	V2	N1	Z1	Z2	Z4
km	0	7.6	9.8	12.4	19.8
Salinity (water column average)	35.89	30.16	22.76	26.20	18.29
Temperature (water column average)	10.12	9.22	7.33	8.94	8.53
Taxa					
1					
Chaetoceros curvisetus Cleve	17070	7200	4330	680	340
Guinardia striata (Stolterfoth) Hasle	12000	1870	270	100	100
2					
Bacteriastrum delicatulum Cleve	530	63780	83620	42600	11000
Cerataulina pelagica (Cleve) Hendey	670	1470	550	120	20
Chaetoceros compressus Laud.	0	20200	24270	0	0
Chaetoceros diversus Cleve	0	3200	5470	260	1500
Chetoceros socialis Laud.	10	47700	4800	10	10
Cyclotella coctawhatcheeana Prasad	10	21870	66330	14400	2400
Diatoma elongatum (Lyngb.) Agardh.	0	10	4000	10	0
Leptocylindrus mediterraneus (Perag.) Hasle	10	800	2800	120	20
Green flagellates	10	1067	10	10	10
Gymnodinium spp.	670	1730	130	0	200
Dinoflagellates (cells 10–20 µm)	1600	4270	1870	800	10
Pseudo–nitzschia spp.	530	25600	7400	2000	12300
Scrippsiella sp.	10	1330	130	0	0
3					
Synedra acus Kütz.	0	0	0	0	800
Synedra ulna (Nitzsch) Ehrenb.	0	130	70	500	300

Three groups of taxa are differentiated in the coastal sea (Group 1), middle estuary (Group 2), and upper estuary (Group 3). 0 denotes abundance <40 cells l^{-1}

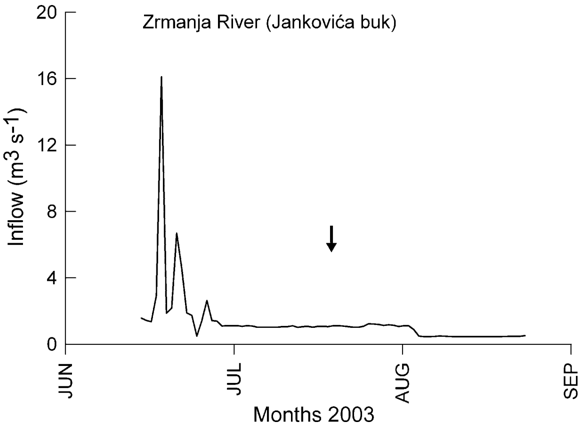


Fig. 7 The inflow of Zrmanja River water into the estuary during June, July and August 2003. Measurements in front of Jankovića Buk Station. Arrow indicates day of sampling in the estuary

(Fig. 5a). Its vertical distribution (Fig. 5b) in the upper estuary (Z4) was characterized by a higher concentration below (450 ng chlorophyll *a* L^{-1}) than above the halocline (240 ng chlorophyll *a* l^{-1}). In contrast, the chl *a* maximum in the middle estuary (N1) was in the surface layer (1,160 ng chlorophyll *a* l^{-1}). The concentration in the underlying layers (2–10 m) was significantly lower and rather uniform (about 600 ng chlorophyll *a* l^{-1}).

Diatoms dominated the microphytoplankton. The middle estuary had high abundance of diatoms, as well as of nanoplanktonic dinoflagellates, prymnesiophytes, coccolithophorids, cryptophytes, and chlorophytes (Fig. 6). Cryptophytes made up a considerable percentage of small autotrophic and mixotrophic nanoplankton in the middle and upper estuary (the maximum abundance was detected in the middle estuary). Some smaller cells could be hardly recognizable, and biomarker alloxanthine helped to detect their real distribution. Alloxanthin concentration increased gradually towards the head of the estuary, while biomass of green algae increased slightly toward the sea but nevertheless remained very low (1–20 ng chlorophyll *b* l^{-1}) throughout the estuary. In the upper part of the estuary cryptophytes (alloxanthin) contributed significantly to total phytoplankton biomass only below the halocline, while in the middle estuary (N1) their maximum was in the surface layer.

Two freshwater diatoms, *Synedra ulna* and *S. acus*, were transported to the estuary with discharge from the nearby hydroelectric plant that runs on freshwater. Both were found above the halocline (Table 2). Freshwater input was not sufficient to support abundant populations in the brackish layer of the upper estuary (Z2 and Z4). A third diatom, *Diatoma elongatum,* developed within the estuary itself and was found along the halocline.

Diatoms were composed of 15 taxa with a maximum abundance >200 cells l^{-1} on February 26, 2000 (Table 3). The most abundant were marine diatoms: *Bacteriastrum* sp., *Chaetoceros curvisetus*, *Ch. compressus*, *Ch. socialis*, *Cyclotella choctawhatcheeana*, *Guinardia flaccida*, and *Pseudo–nitzschia* sp. Among dinoflagellates, naked and thecate nanoplanktonic species (10–20 μm) were abundant. Other abundant phytoplankton included one silicoflagellate, as well as six dinoflagelattes and green nanoflagellates.

With respect to longitudinal taxonomic composition, phytoplankton assemblages were distributed heterogeneously. Three different sections; upper estuary, middle/lower estuary, and coastal marine section were characterized by different phytoplankton assemblages (Table 3). The first contained marine taxa occurring primarily in the coastal sea (Velebit Channel). The second was composed of taxa mainly distributed in the middle estuary and the lower reach of the upper estuary. The third group was composed of scarce freshwater diatoms that were distributed mainly in the brackish layer of the upper estuary.

July 2003

The Zrmanja River discharge in July 2003 ranged from 1.03 to 1.39 m^3 s^{-1} (Fig. 7), values typical for the dry summer of the Adriatic coast. The discharge on the day of sampling (July 17, 1.12 m^3 s^{-1}) was similar to those during the previous 15 days and represented only about 5% of the annual daily average. These very low discharges resulted in maximum intrusion of marine water, up to the Jankovica buk waterfalls where the halocline was clearly detected (Z4a; Fig. 8). A sharp halocline was evident in the upper estuary (from Z4a to Z4) with a vertical salinity gradient of 17.5 (14.50 to 32.0) in the 0.5–1.8 m layer. Salinity below the halocline ranged between 33.83 and 33.30 at 2.5 m. There were moderate longitudinal surface salinity gradients, from 23.31 to 36.30, from Z4A to V2.

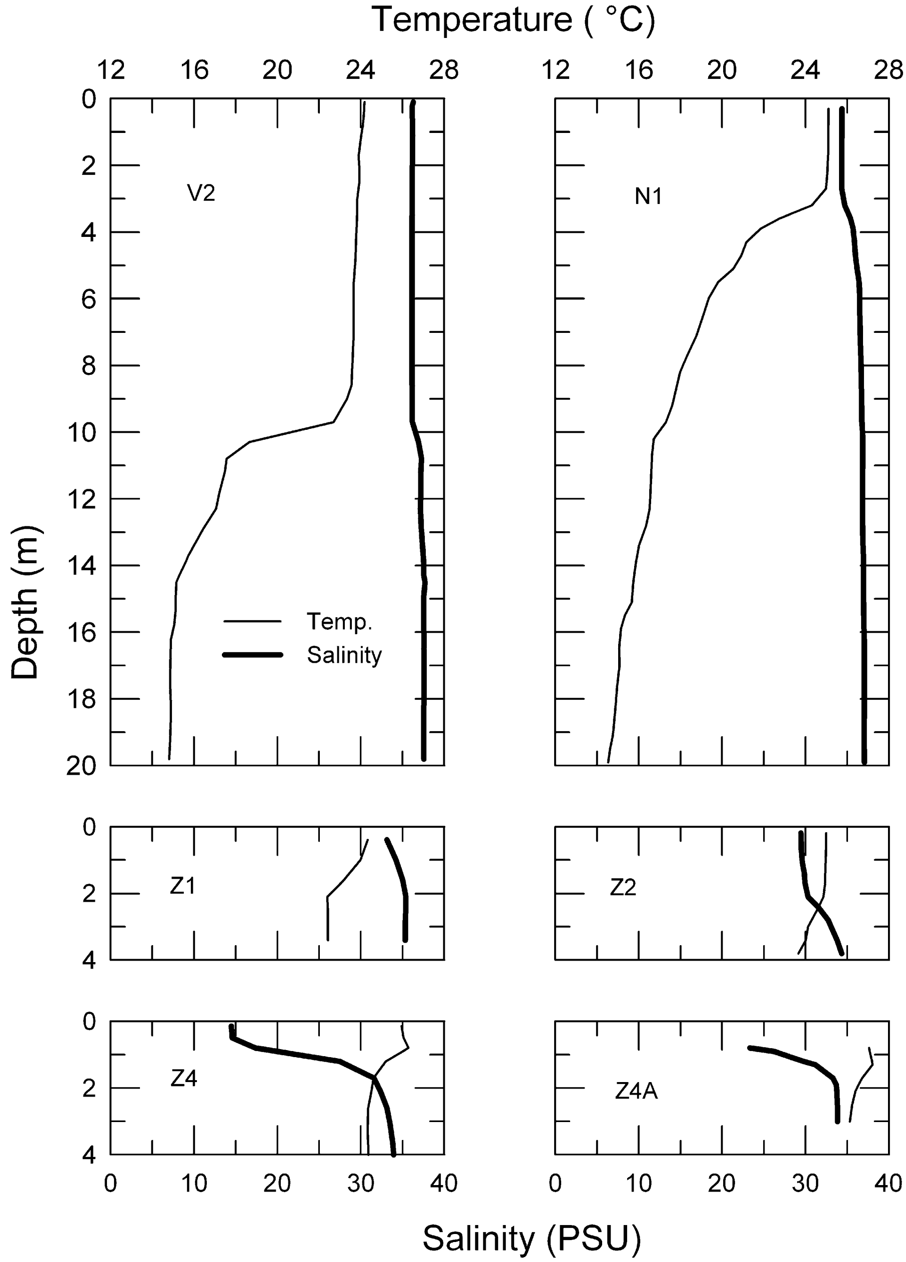


Fig. 8 Vertical distribution of temperature and salinity in the coastal sea (*V2*) and along the Zrmanja Estuary (*N1–Z4A*), on July 18, 2003

Temperatures in the upper estuary were 27.04–24.35°C throughout the water column (Fig. 8). Temperatures above the thermocline (above 0.5 m) ranged from 22 to 27.04°C, with a maximum in the head of the estuary. In the middle estuarine Station N1, the thermocline (and halocline) was at 3 m; at coastal Station V2 it was at 10 m. The subsurface temperature maximum was found in the halocline in the upper estuary due to the selective absorption of solar energy by suspended particles.

Nutrient concentrations in the estuary were relatively low in summer, due to the reduced riverine input (Fig. 9). Ortophosphate was less than 0.11 μmol l^{-1}, while the total inorganic nitrogen and silicate concentrations were less than 3 and 32 μmol l^{-1}, respectively. A slight increase in nitrogen downstream of Z4 indicated a weak influence of anthropogenic activities.

Secchi disc depth varied between 4 and 10 m (Fig. 9), indicating good transparency in the estuarine water column.

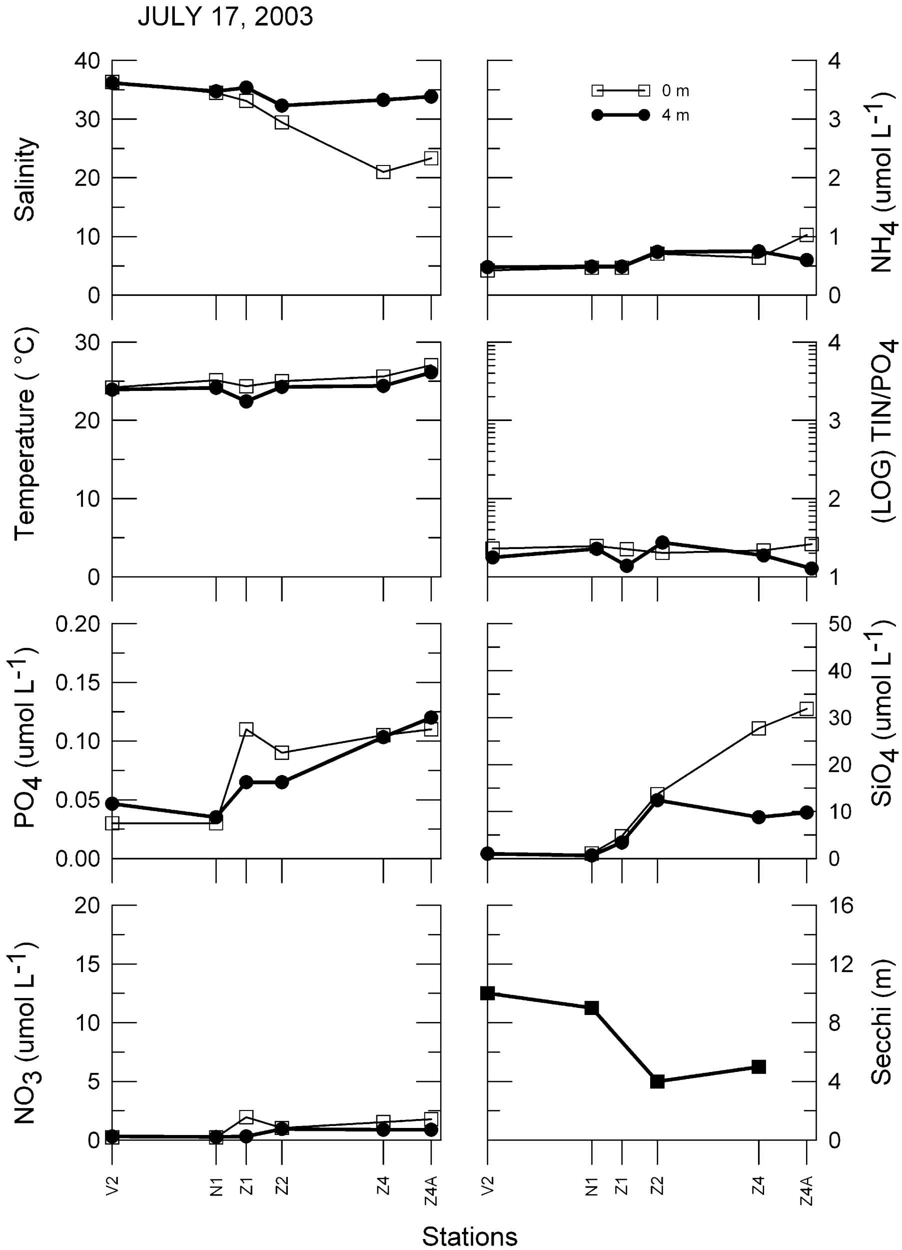
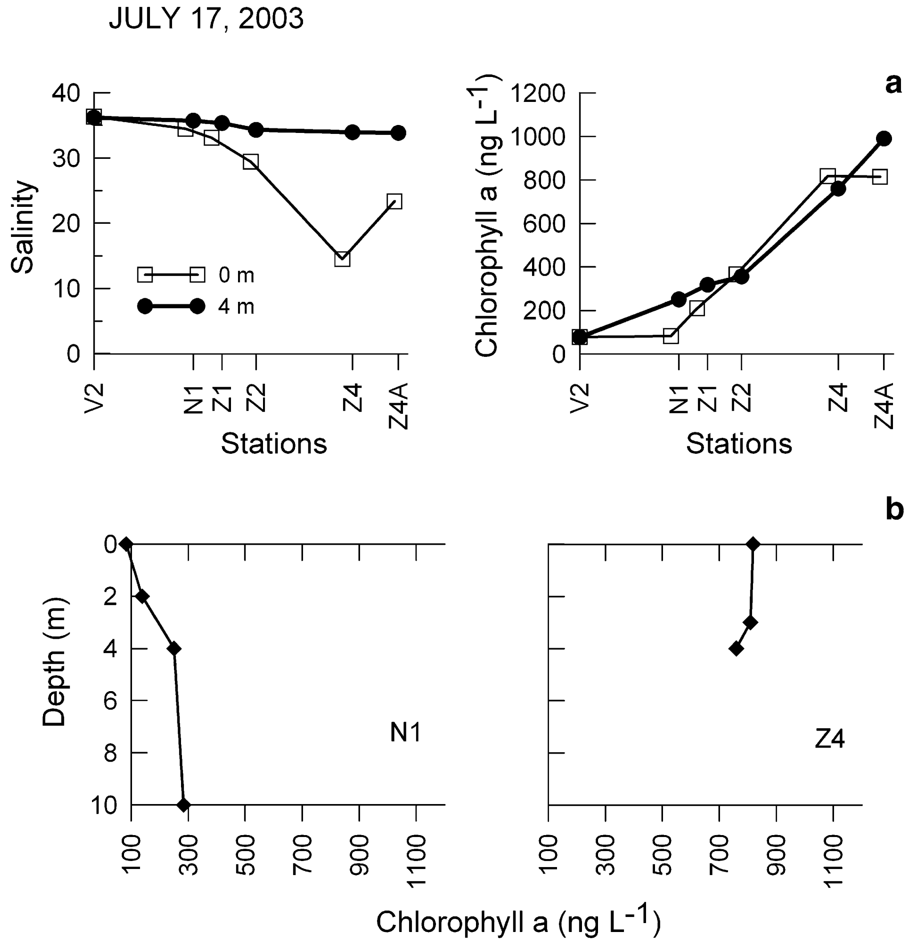


Fig. 9 Distribution of salinity, temperature, orthophosphates, nitrates, ammonia, Redfield ratio, orthosilicates and transparency, along the Zrmanja Estuary, on July 18, 2003

Concentrations of PO_4 and TIN were 3.5 times lower in the middle estuary and the coastal sea (Fig. 9), resulting in a fivefold decrease in biomass (<200 ng chlorophyll *a* l^{-1}; Fig. 10). Chlorophyll *a* was 1000 ng l^{-1} in the head of the estuary and gradually decreased downstream to 100 ng l^{-1} in the coastal sea. In the middle estuary (N1) biomass was three times higher in the marine layer (290 ng chlorophyll *a* l^{-1}) than in the surface (100 ng chlorophyll *a* l^{-1}).

Diatoms, dinoflagellates, and cryptophytes dominated the marine phytoplankton (Fig. 11); these, together with green flagellates, dominated the marine layer of the upper estuary. The same distribution was detected for Hex–fuco, the biomarker of prymnesiophytes that hardly could be recognized by microscopy. In the middle estuarine station N1 and nearby Z1, diatoms, coccolithophorids, and cryptophytes were the next most important contributors to abundance and biomass in the marine layer.

Fig. 10 Longitudinal (**a**) and vertical (**b**) distribution of salinity and chlorophyll *a*, on July 18, 2003



In the upper estuary, phytoplankton above the halocline was made up mostly of the nanoplanktonic unicellular diatom *Chaetoceros throndsenii*, the nanoplanktonic marine dinoflagellate *Prorocentrum minimum*, the freshwater crysophyceae *Dynobryon*, as well as cryptophytes and chlorophytes; the latter two were more abundant at Z4 than Z2 (Table 4). Regarding horizontal distribution, three groups of phytoplankton characterized the coastal sea, middle estuary, and upper estuary (Table 5). The marine diatoms *Proboscia alata*, *Rhizosolenia imbricata*, and *Hemiaulus hauckii* were most abundant in the coastal sea and middle estuary. The most abundant of these, *Proboscia alata*, is an indicator of marine source water in the upper estuary. Small diatoms such as *Nitzschia longissima* and *Chaetoceros throndsenii* were distributed in the middle/upper estuary, while small marine dinoflagellates and freshwater species were found mostly in the upper estuary.

Discussion

Orthophosphates are indicated as being the limiting nutrient in the Zrmanja Estuary, and exceptional limitation with nitrogen could be sporadically detected only in summer (Fig. 12).

Phytoplankton seasonality in the estuary is characterized by the distinctive spring maximum (Fig. 13). The weak anthropogenic activities in the surrounding area and low input of nutrients do not provoke phytoplankton blooms in the estuary and the coastal sea (Fig. 14). Chlorophyll *a* concentrations in the Zrmanja Estuary (average less than 0.5 μg l^{-1}) are close to those in the oligotrophic Mediterranean (minimum in Ionian sea equals 0.045, maximum off Marocco 2.2 μg l^{-1}; Claustre et al. 2004). The low level of nutrients, especially in summer, high light transparency, absence of phytoplankton blooms (Fig. 14) suggest that the Zrmanja Estuary could be

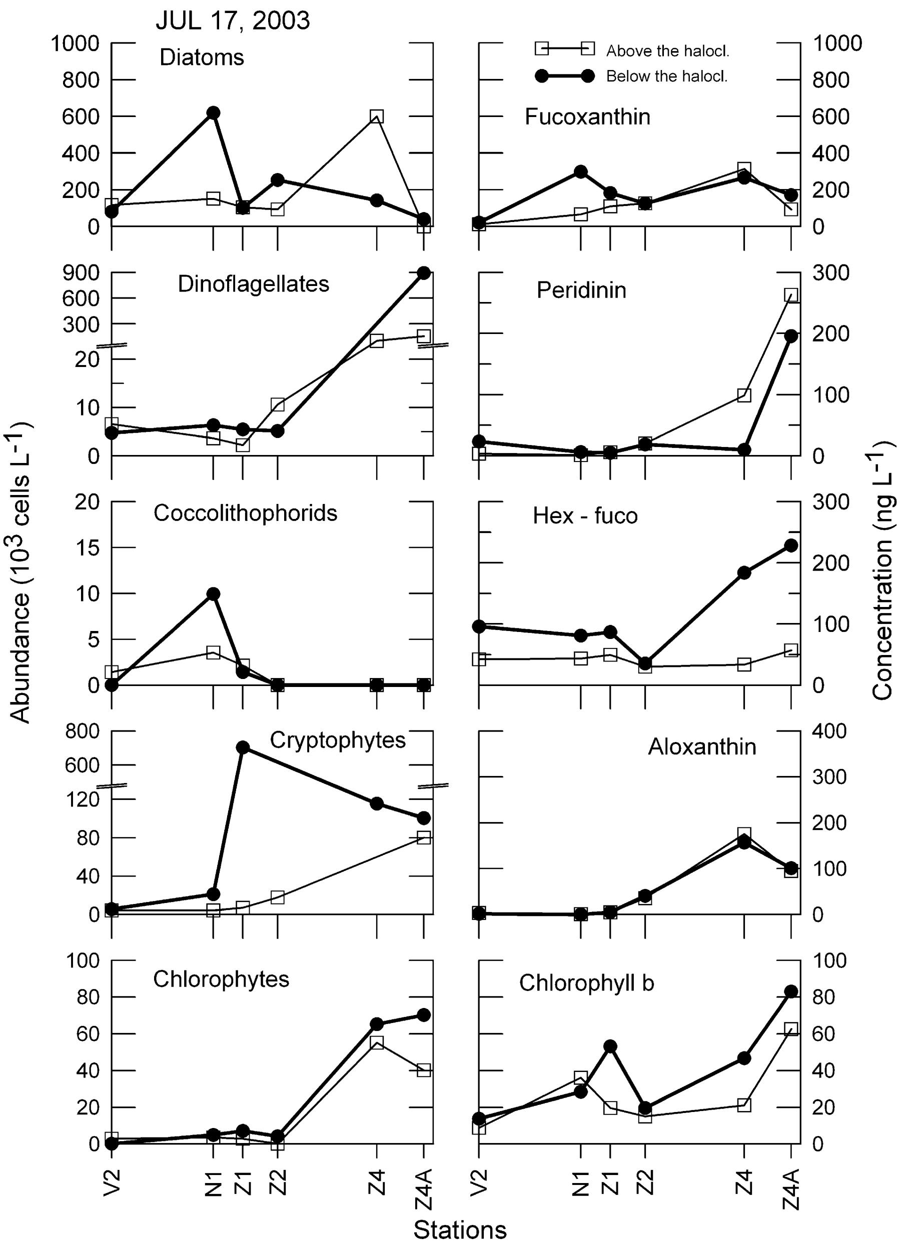


Fig. 11 Distribution of phytoplankton abundance and biomarker pigments along the Zrmanja Estuary on July 18, 2003

characterized as an oligotrophic ecosystem, as well as other eastern Adriatic estuaries (Svensen et al. 2007).

In such conditions, phytoplankton is composed of specific assemblages with two abundant diatoms *Cyclotella choctawhatcheeana* and *Chaetoceros throndsenii* which to the recent state of knowledge, do not abundantly appear elsewhere in the Adriatic and Mediterranean (Burić et al. 2007). Microscopic examination in winter revealed that microphytoplankton in the Zrmanja Estuary was composed predominantly of chain-forming diatoms such as *Bacteriastrum*, *Chaetoceros*, *Cyclotella*, and *Pseudo-nitzschia*, which probably much successfully survive in the period of increased nutrient input. In contrast, small celled phytoplankton (*Chaetoceros throndsenii*, *Nitzschia longissima*, *Prorocentrum minimum*) predominated during the summer oligotrophication. In the more eutrophicated estuaries, the seasonality of phytoplankton groups may be different than in the oligotrophic ones. For example, in the Nervion River estuary, eutrophicated from Bilbao city, maximum abundance/biomass of diatoms and

Table 4 Vertical distribution of phytoplankton abundance (cells L^{-1}) in the Zrmanja Estuary (at stations Z2 and Z4), on July 18, 2003

Stations	Depth (m)	Salinity (PSU)	Ch thrond	N long	Prob alata	P mic	P min	Dynob	Crypto	Green flag	Cil
Z2	0	29.41	160760	0	3790	2270	13640	0	30142	50237	12120
	2	30.29	11350	760	2270	0	3740	0	5680	0	14394
	4	34.32	241140	0	12880	0	1520	0	130617	4260	24320
Z4	0	14.47	854037	380	380	2270	90427	3790	261235	60285	90427
	1	27.48	0	50237	380	1510	90427	19700	80380	50237	90427
	3	33.50	180855	0	380	380	37308	3400	130617	30142	1890
	4	33.92	100475	90	1520	0	15150	1200	100475	100475	5300

Ch thrond – *Chaetoceros throndsenii*, N long –*Nitzschia longissima*, Prob alata – *Proboscia alata*, P mic – *Prorocentrum micans*, P min – *Prorocentrum minimum*, Dynob – *Dynobrion*, crypto – cryptophytes, Green flag – green flagellates, Cil – naked ciliates. 0 denotes abundance <40 cells L^{-1}

haptophytes were found in summer (Seoane et al. 2005; Cebrian and Valiela 1999), not in winter like in the oligotrophic Zrmanja (Burić et al. 2001).

The longitudinal distribution of chlorophyll *a* in the brackish and marine layers (Figs. 5 and 10) illustrates clearly the effect of salinity gradients on the distribution of phytoplankton biomass. In February, in the brackish layer of the upper estuary, between Z2 and Z4, chlorophyll *a* remained low despite a fairly healthy nutrient supply. This is due to salinity limitation (salinities <3) for marine species, and short residence time of water masses in the upper estuary. The phytoplankton growth was possible in the middle reach of the estuary, where the concentration of chlorophyll *a* below the halocline (in the salinity from 20–30), was significantly higher despite the fact that nutrient levels were rather similar to those in the upper layer. The impact of salinity gradients is apparent from the vertical profiles of chlorophyll *a* in the upper estuary. There was a conspicuous increase of the biomass below the halocline (salinity >20). In contrast, in the middle estuary (N1) salinities from 15–20 were recorded from the surface to 4 m (Figs. 3 and 4) and, consequently, the maximum chlorophyll *a* (1,100 ng l^{-1}) was detected in the surface layer.

In July 2003, reduced riverine flow led to higher salinity and longer water residence time, both of which could be conducive for phytoplankton development in the upper estuary. Due to the phytoplankton growth in the upper estuary, concentrations of

Table 5 Distribution of phytoplankton taxa, according to the average water column abundance (cells L^{-1}) along the Zrmanja Estuary (stations V2, N1, Z1, Z2, Z4), in July, 2003

	Stations:	V2	N1	Z1	Z2	Z4	Z4A
	Km	0	7.6	9.8	12.4	19.8	23.3
	Salinity (water column average)	36.74	35.69	34.61	32.30	28.34	28.33
	Temperature (water column average)	20.34	20.65	23.04	24.49	24.86	26.58
	Taxa						
1	*Hemiaulus hauckii* Grun.	1140	120	0	0	0	0
	Rhizosolenia imbricata Brightw.	27760	140800	4550	760	0	0
	Proboscia alata (Brightw.) Sund.	672090	311560	97660	6310	670	0
2	*Nitzschia longissima* (Breb.) Ralfs.	380	380	380	760	16900	0
	Chaetoceros throndsenii (Mar. Montr. et Zing.) Mar.	0	0	2840	137750	378460	40190
3	*Prorocentrum micans* Ehrenb.	380	590	380	2270	3000	4930
	Prorocentrum minimum (Pav.) Schiller	380	570	760	6300	58330	461690
	Penatae diatoms	0	0	1330	1520	2150	5300
	Dinobryon spp.	0	0	0	0	7020	44440

Three groups of taxa are differentiated in the coastal sea (Group 1), middle estuary (Group 2), and upper estuary (Group 3). 0 denotes abundance <40 cells l^{-1}

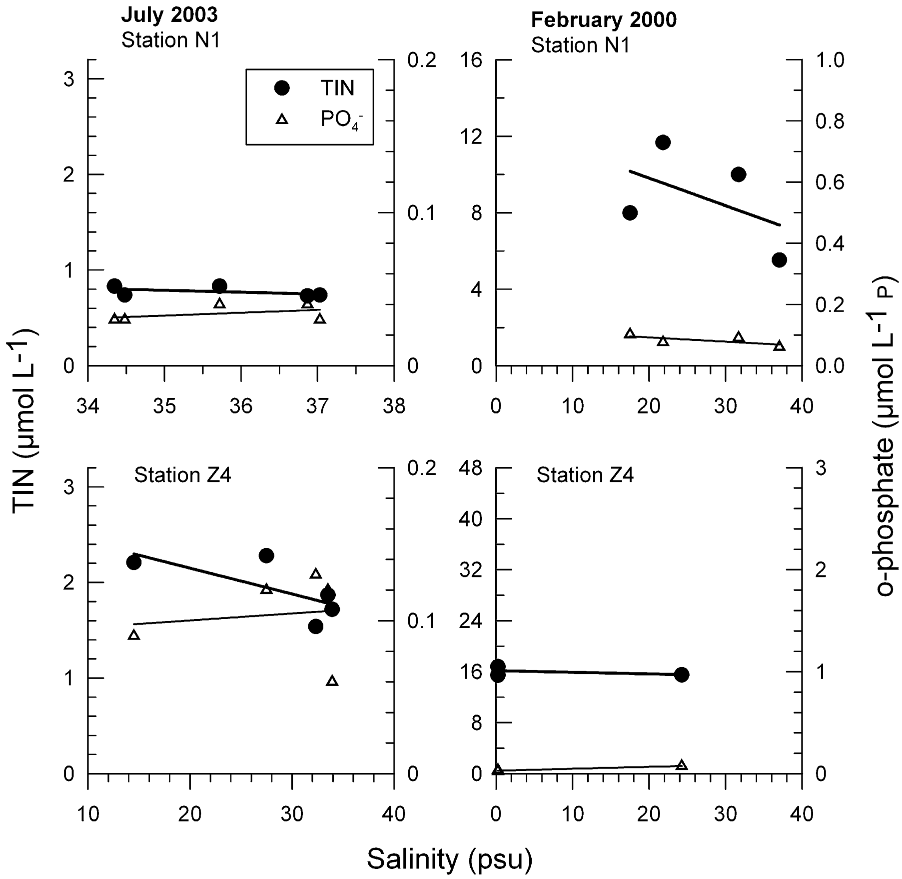


Fig. 12 Demonstration on nutrient limitation at any salinity in the Zrmanja Estuary (from upper estuary to coastal sea). The lower-most trendline denotes the limiting nutrient

PO_4 and nitrogen were 3.5 times lower in the middle estuary, resulting in the five-fold decrease of chlorophyll *a*. Under such nutrient depleted conditions, nanophytoplankton were the most successful primary producers.

The Zrmanja Estuary shows a similarity with the neighbouring Krka Estuary, with respect to the prounounced stratification (Žutić and Legović 1987) and low content of suspended inorganic and organic matter (Moreira-Turcq et al. 1993; Cauwet 1991; Svensen et al. 2007). The halocline provides an interface that accumulates micro- and nanophytoplankton (Viličić et al. 1989; Denant et al. 1991; Ahel et al. 1996), bacteria (Fuks et al. 1991), dissolved organic matter and detritic particles (Žutić and Legović 1987; Cauwet 1991), and pollutants (Mikac et al. 1989) along the brackish water–sea water interface.

In contrast to the neighboring Krka Estuary, where phytoplankton biomass is generally significantly higher above the halocline (Viličić et al. 1989), in the Zrmanja Estuary phytoplankton was concentrated mostly below the halocline. In the Krka Estuary, there is higher input of freshwater phytoplankton and greater nutrient input from wastewaters, emanating from the city of Šibenik (Legović et al. 1994, Cetinić et al. 2006).

The abundance and composition of phytoplankton in the Zrmanja Estuary strongly depend on the hydrodynamic conditions that result from the inflow of freshwater. In winter, phytoplankton is more abundant in the wider, deeper middle estuary. This may result from more stable hydrodynamic conditions (i.e. higher water residence time) compared to those in the narrower upper part. Although current measurements are not available, the average winter flushing period of the brackish layer in the upper estuary (at a discharge of 30 m^3) was estimated at 1–2 days, and this rather short residence time prevents phytoplankton accumulation and growth. This phenomenon has been well described in other estuaries (Alpine and Cloern 1992; Trigueros and Orive 2000). For comparison, under typical winter conditions the residence

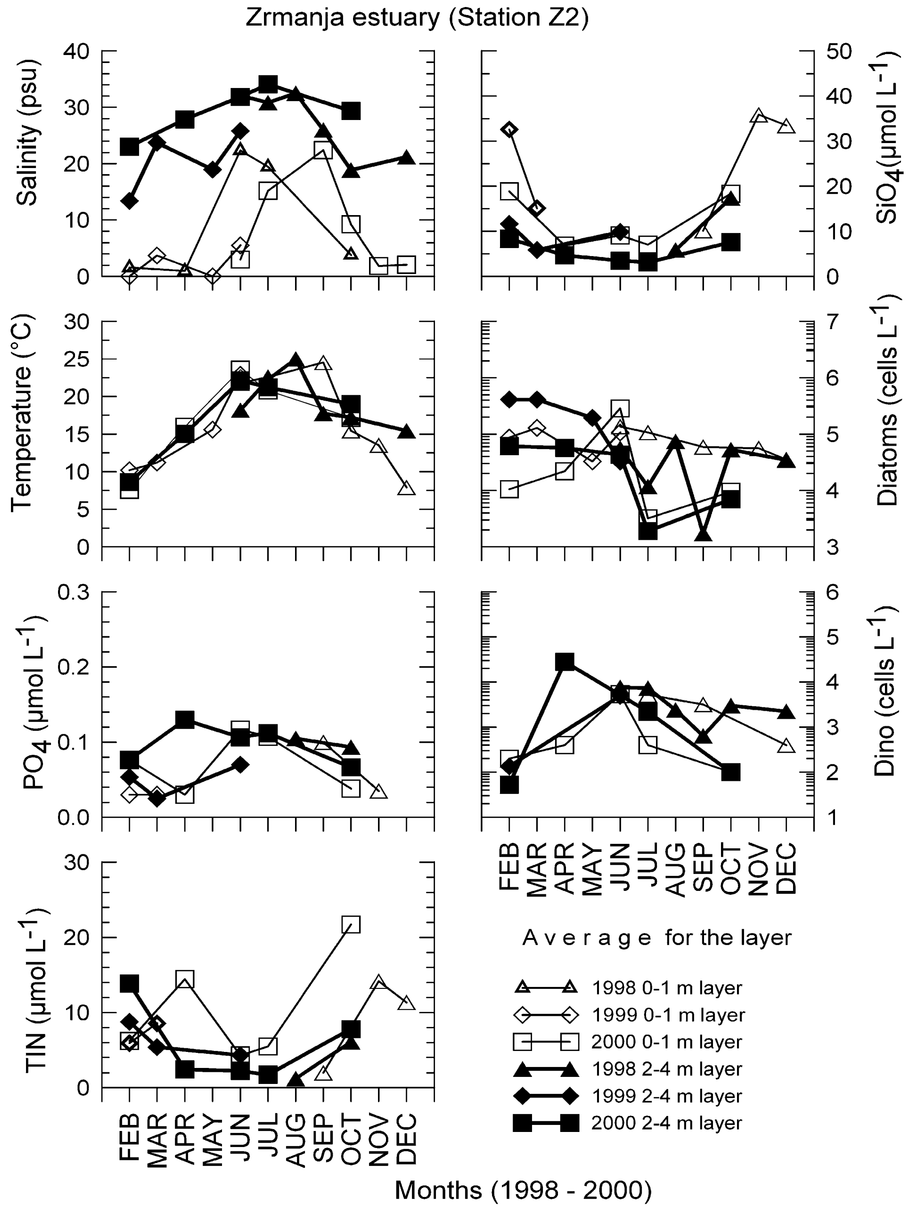


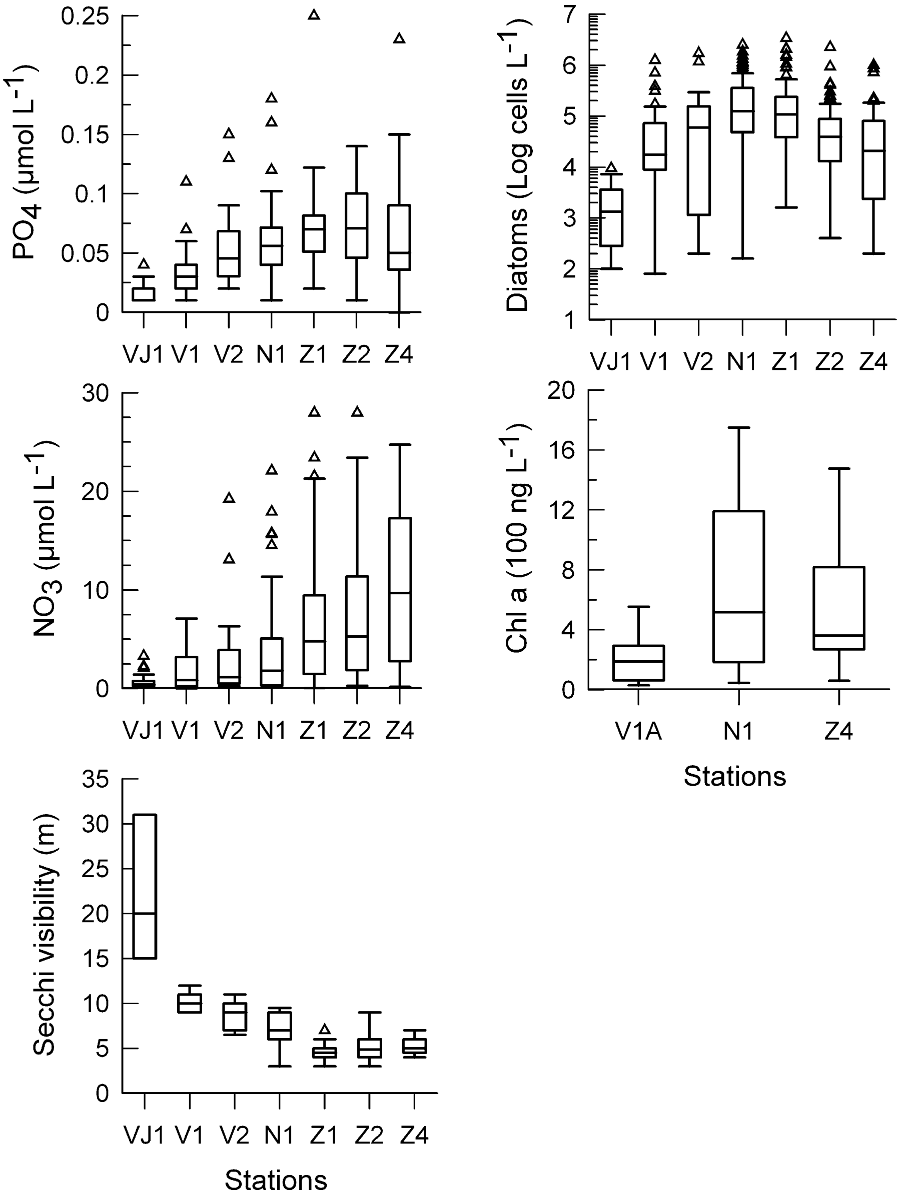
Fig. 13 Seasonal distribution of thermohaline characteristics, nutrients, and microphytoplankton (diatoms and dinoflagellates) abundance in the upper Zrmanja estuary (at station Z2)

time of the brackish and marine layers in the Krka Estuary are, 6–12 and 15–45 days, respectively (Legović 1991).

As to the comparison between microscopy and chemotaxonomic determinations they were generally in a good agreement. The predominant biomarker throughout the estuary was fucoxanthin, which was very well correlated with chlorophyll *a* in all layers and estuarine sections (r^2=0.9002; n=27; p<0.001). As microscopic counts showed that the most numerous phytoplankton were microplanktonic diatoms, it seems likely that fucoxanthin in the Zrmanja Estuary originated mainly from diatoms, consistently as in earlier studies in the eutrophic coastal and estuarine waters (Ahel et al. 1996; Ahel and Terzić 1998; Ansotegui et al. 2001; Seoane et al. 2005).

Among other accessory carotenoids showing an unambiguous chemotaxonomic interpretation are 19′-hexanoyloxyfucoxanthin which can be used as pigment signature for some prymnesiophytes, and peridinin in

Fig. 14 *Box* Whisker plot distribution of nutrients, light transparency (Secchi visibility) and phytoplankton abundance (diatoms and cryptophytes), along the coastal sea – head of the Zrmanja estuary profile, in the period 2000–2004. V1 and VJ1 are 5 and 50 km westerly stations from V2



photosynthetic dinoflagellates (Jeffrey and Wright 1994; Rodriguez et al. 2003).

In the Zrmanja Estuary, some discrepancies arise when comparing distribution of some marker pigments with corresponding taxonomic groups, possibly due to the presence of accesory pigments in different groups (Jeffrey et al. 1997); (1) fucoxanthin in haptophytes and some dinoflagellates (Jeffrey and Wright 1994; Jeffrey et al. 1975; Ansotegui et al. 2001; Seoane et al. 2005); (2) Hex-fuco in many prymnesiophytes, not only in coccolithophores (Carreto et al. 2003). Pigment composition within a strain may be controlled by the combination of light and N/P supply (Leonardos and Geider 2004). Certain disagreements between the nanophytoplankton abundance and specific biomarkers might be due to the uncertainties in the microscopic counts of cells smaller than 5 μm (Breton et al. 2000) or to the specific pigment composition of taxa with endosymbiont plastids (Lewitus et al. 2005). Microscopy is more useful in counting coccolithophores, while less useful in determining small, mostly unrecognizable green algae. This may be the reason why we found better positive correlations between the abundances and biomarker pigments in prymnesiophytes than in green algae.

Cryptophytes containing alloxanthin were found mainly at lower salinies (18–35), with maxima in the surface layer of the middle estuary and bellow the halocline in the upper estuary. Cryptophytes reached higher biomass in the marine layer of the upper estuary, probably owing to their mixotrophic potential and possibility to incorporate more abundant organic particles there. Cryptophyte abundances below the halocline of the middle and upper estuary were below the detection limit and did not match the concentration of alloxanthin. As alloxanthin is considered an exclusive cryptophyte marker, this discrepancy probably is due to the smaller cells that were not detected by microscopy. In those samples in which cryptophytes were found, however, their numbers correlated very well with alloxanthin concentration (r^2=0.961; n=8; p=0.1082). This suggests a relatively uniform content of alloxanthin per cell: 4.9±1.3 pg/cell.

The concentrations of hex-fuco and chlorophyll *b* were much lower than those of fucoxanthin and alloxanthin. These pigments, however, proved very good indicators of the influence of the salinity gradient on phytoplankton biomass. Hex-fuco was much higher at higher salinities, clearly indicating a marine origin of the species carrying this pigment – probably prymnesiophytes (Zapata et al. 2004).

Conclusion

The Zrmanja Estuary is an oligotrophic, highly stratified ecosystem. Prolonged high river discharges could limit phytoplankton growth in the narrow part of the upper estuary due to the intense hydrodynamic flushing and salinity limitations. The maximum phytoplankton biomass develops when residence time of water is sufficiently high. In winter, the maximum biomass was found in the middle estuary; in summer, in the upper estuary. Upper, middle, and coastal parts of the estuary have distinct phytoplankton assemblages. Phytoplankton assemblages were composed of microphytoplanktonic diatoms, as well as nanoplanktonic/picoplanktonic cryptophytes, dinoflagellates, prymnesiophytes, and chlorophytes in the phosphate-limited environment. The general oligotrophy of the Zrmanja and other rivers discharging in the eastern Adriatic Sea influence low productivity of the eastern Adriatic Sea.

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