



Planktonic diatoms and their environment in the lower Neretva River estuary (Eastern Adriatic Sea, NE Mediterranean)

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With 7 figures and 3 tables

Abstract: The seasonal distribution of planktonic diatoms was investigated at one station in the lower Neretva River estuary (Middle Adriatic) at monthly intervals from April 1999 to April 2000. According to the TRIX trophic index, the lower estuary is mesotrophic.

Ninety-six diatom taxa within 51 genera were identified. Of these, 32 occurred in at least 10% of all samples and 12 rare taxa occurred in $\leq 1\%$ of samples. Nine, mostly rare, taxa were only found in brackish layer, and 27 taxa were exclusively presented in marine layer. ANOSIM analysis revealed that no significant difference occurred between these two layers in terms of diatom taxa abundances. The continuous presence of some benthic taxa, underlines the importance of wind-induced sediment resuspension, currents and tides.

Unidentified phytoflagellates (55.68–99.60%) and diatoms (0.20–42.10%) represented the major proportion of total phytoplankton abundance. The diatom community of marine origin varied over five orders of magnitude ($30\text{--}1.2 \times 10^6$ cells L^{-1}) and showed three peaks throughout the year: April 1999 (1.2×10^6 cells L^{-1}), August 1999 (7.9×10^6 cells L^{-1}), and October 1999 (1.3×10^6 cells L^{-1}), while the lowest abundance was observed in November 1999. The most abundant diatoms (exceeding 10^5 cells L^{-1}) were *Nitzschia longissima*, *Leptocylindrus minimus*, *Skeletonema marinoi*, and *Thalassiosira* sp. *N. longissima* (max. 7.9×10^6 cells L^{-1}) dominated the highest peak (August 1999) and represented the 98.08% and 29.26%, respectively, of total diatom and phytoplankton abundance.

Canonical Correspondence Analysis (CCA) showed that temperature, salinity and TIN were the main factors influencing diatom community structure in the estuary. No relationship was noted between the monthly distribution of diatom abundances, water column transparency, and microzooplankton abundance. A main feature of the diatom assemblages was the dominance of more than one species during the blooms. This, likely, is owed to the frequent and rapid environmental changes typical of this dynamic estuarine ecosystem.

Key words: Diatoms, phytoplankton communities, microzooplankton, environmental variables, CCA, estuary, Croatia, eastern Adriatic Sea.

Introduction

Studies on phytoplankton communities in estuaries have shown that diatoms are the most important taxonomic group, either in terms of abundance or of diversity or both (Trigueros & Orive 2001, Gameiro et al. 2007). These communities are composed of dynamic multi-species assemblages characterized by high diversity and rapid successional shifts in species composition in response to environmental changes (Mendes et al. 2009). As primary producers, diatoms play a key role in aquatic food webs. Moreover, it has been shown that, compared to other non diatom phytoplankton species, they are more edible for organisms at higher trophic levels, such as large fish and shellfish (Lopes et al. 2007). Identifying the ecological variables that regulate the seasonal succession of the diatom communities is essential to understand the ecosystem functioning (Quigg & Roerborn 2008).

Estuaries are transition zones between freshwater and marine systems and so are naturally characterized by chemical, physical, and biological gradients. Estuarine circulation, by which marine water enters at the bottom and freshwater flows out at the surface (Day et al. 1989), is responsible for the transport of nutrients (Mackass & Harrison 1997), organic matter (Cadée & Hageman 1974, Wolff 1977), phytoplankton (Tyler & Seliger 1978, Malone et al. 1980), and zooplankton (Soetaert & Herman 1994) from coastal water into estuaries. Human activities have a variety of impacts on estuaries, including modification of the direction and intensity of flow patterns (Humborg et al. 1997, Turner & Rabalais 1994) and exploitation of estuarine living and mineral resources (Wolff 2000).

In the eastern Adriatic, several highly stratified estuaries occur (Viličić et al. 1989, 1999). Such estuaries develop where high volume river discharge combines with low tides (Dyer 1991). This type of estuary is well known around the Mediterranean (cf. Sierra et al. 2002).

Despite recent works addressed the phytoplankton ecology of some eastern Adriatic estuaries (Burić et al. 2005, 2007, Cetinić et al. 2006), there are a number of estuaries that have not yet been investigated. Among these is the Neretva River estuary, a small, low tidal (25 cm average daily amplitude, Hydrographic Institute of Republic of Croatia 2000) estuary located on Croatia's southern coast. The Neretva estuary is of the salt-wedge type: the inflow of river water is much larger than that of seawater (Matić 2005) and the vertical mixing is restricted to the thin transition boundary between the freshwater at the surface and the saltwater below.

In this study, we investigated the distribution of the estuarine planktonic diatoms in relation to some key environmental variables. We also compared the diatom community of the Neretva with those of other estuaries of temperate areas.

Material and methods

Study area

The Neretva River offers a typical example of a major river flowing to the sea from nearby mountains (Blondel & Aronson 1999). It is 218 km long with its source deep in the hinterland of Bosnia and Herzegovina and discharges into the middle Adriatic Sea. The catchment area is estimated to be 10,100 km². The Neretva estuary and its surrounding area have been intensively exploited for a variety of economic purposes, including transportation, fisheries, agriculture, and, more recently, tourism and recreation.

Regulation of the Neretva flow began in earnest in the late 1960 s. These intentional changes of the estuary hydrology (Juračić 1998, Jasprica et al. 2003) have affected its water quality (Štambuk-Giljanović 2003). For example, prior to construction of the hydropower plant in the upper Neretva River in Bosnia and Herzegovina, large quantities of suspended material had been

deposited in the lower part of the river. According to estimates from the beginning of the 20th century, this amounted to about 500,000 m³ annually (Juračić 1998). Since then, this has been substantially diminished.

The 20-km long estuary extends from the Croatian town of Metković to the river's mouth near the port of Ploče. The rate of freshwater discharge and the water level depend on the quantity of rainfall gathered in the catchment basin (Jasprica et al. 2003, 2005). The river's mean annual flow (near Metković) is 345 m³ s⁻¹ (Juračić 1998). During the summer dry period from June to September, however, the average flow is only 4–5 m³ s⁻¹.

The present study of the Neretva estuary began in April 1999, in the framework of an interdisciplinary research project investigating the oceanography at a station Lake Vlaška situated near the village of Rogotin in the lower estuary (Fig. 1). The Crna ("Black") Rijeka River comes from the northeast and the Parila Channel is a natural connection with the Adriatic Sea on the south. This area thus has the features of a highly stratified estuary with a very pronounced separation between brackish and marine layers.

Detailed data on the hydrology, climate, geology, and vegetation of the area have been presented by Jasprica et al. (2005).

Physical-chemical parameters and plankton

Lake Vlaška station (43° 02' 47.22" N, 17° 28' 18.46" E) located in the Neretva estuary (Fig. 1) was visited monthly between April 1999 and April 2000. Water samples for the analysis of nutrients, chlorophyll *a* (Chl *a*), phytoplankton and microzooplankton community were taken by 5-L Niskin bottles from the surface to the bottom (10.5 m) at one-meter depth intervals. We collected 133 samples for each parameter.

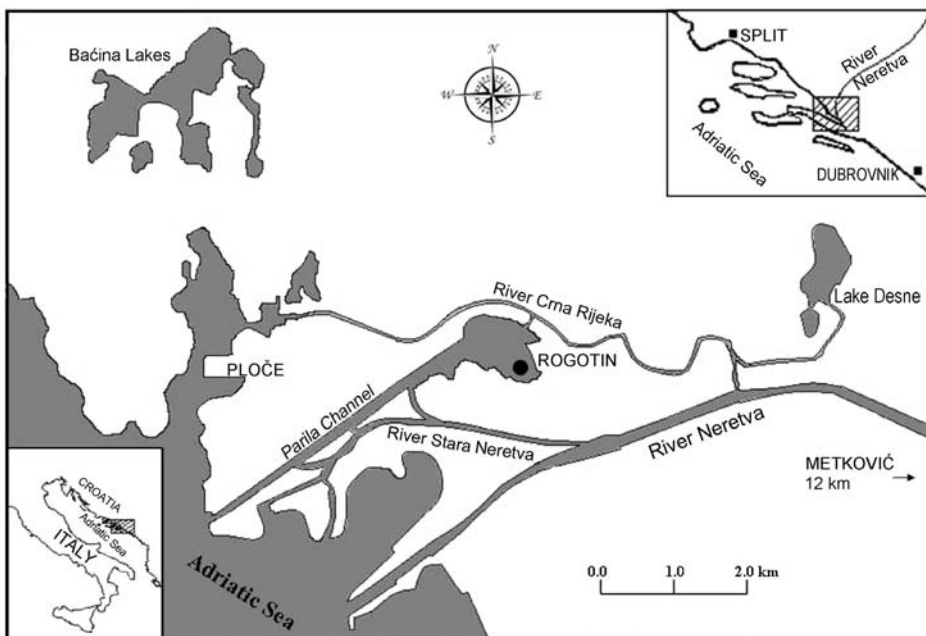


Fig. 1. Station location in the Neretva River estuary.

Nutrient concentrations were measured using standard methods (Strickland & Parsons 1972). Salinity was determined by argentometric titration (Grasshoff et al. 1983). Dissolved oxygen was determined by the Winkler method while oxygen saturation (O_2/O_2') was calculated from solubility of oxygen in seawater as a function of temperature and salinity (Weiss 1970, UNESCO 1973). Temperature was measured using inverted thermometers. A Secchi disc (30-cm diameter) was used to estimate transparency and the euphotic zone depth was calculated as 2.5 times the Secchi depth (Strickland 1958).

The TRIX index, commonly employed in coastal marine areas in the Mediterranean (Vollenweider et al. 1998), was used to evaluate trophic status:

$$TRIX = (\log [Chl\ a \times DO\% \times DIN \times TP] + k) / m$$

Each factor in the argument of the logarithm is a component of trophic state: *Chl a* = chlorophyll *a* concentration [$\mu\text{g L}^{-1}$], *DO%* = dissolved oxygen [absolute % deviation from saturation], *DIN* = ($\text{NO}_3 + \text{NO}_2 + \text{NH}_4$) = dissolved inorganic nitrogen [$\mu\text{g L}^{-1}$], and *TP* = total phosphorus [$\mu\text{g L}^{-1}$]. The parameters $k = 1.5$ and $m = 1.2$ adjust the TRIX scale from 0–10.

Chl a was determined from 500-mL sub-samples filtered through Whatman GF/F glass-fiber filters and stored at $-20\text{ }^\circ\text{C}$ for a period less than a month. Filtered samples were homogenized and extracted in 90 % acetone for 24 hours at room temperature (Holm-Hansen et al. 1965). *Chl a* was determined fluorometrically using a Turner TD-700 Laboratory Fluorometer (Sunnyvale, CA) calibrated with pure *Chl a* (Sigma).

Phytoplankton samples were preserved in 2 % neutralized formalin and observed with an inverted microscope (Olympus IX-71) according to the Utermöhl method (Utermöhl 1958) using phase contrast and bright field illumination. Sub-samples (25–50 mL) were settled for 24–48 hours in the counting chambers Wild Hydro-Bios (Kiel-Holtenau, Germany). Counting of diatoms and other phytoplankton cells was performed on 1–2 transects along the counting chamber bottom at a magnifications of 400 x (1 transect) and 200 x (2 transects). In addition, the entire chamber was subsequently scanned at magnification of 100 x to obtain a more correct evaluation of rarer taxa. The minimum abundance that can be detected by this method is 20 cells L^{-1} . Phytoflagellates were counted in 30 randomly selected fields-of-view at a magnification of 400 x. Cells smaller than 2 μm generally escaped detection. Results are expressed as number of cells per liter (abundance). When filamentous taxa (cyanophytes) were encountered, we counted single cells of filament.

Identification of phytoplankton was performed to the lowest possible taxonomical level, according to the following literature: Hustedt (1930), Cupp (1943), Chrétiennot-Dinet (1990), Heimdal (1993), Thronsen (1993), Hasle & Syvertsen (1996), Steidinger & Tangen (1996), Bérard-Therriault et al. (1999), Horner (2002), Viličić (2002), etc. Along with diatoms (Heterokontophyta, Bacillariophyceae), cells were counted for other taxonomic groups: dinoflagellates (Dinophyta, Dinophyceae), silicoflagellates (Heterokontophyta, Dictyochophyceae), coccolithophorids (Prymnesiophyta, Prymnesiophyceae), chrysophytes (Heterokontophyta, Chrysophyceae), chlorophytes (Chlorophyta, including both Charophyceae and Prasinophyceae), euglenophytes (Euglenophyta, Euglenophyceae), and cyanophytes (Cyanophyta, Cyanophyceae). Small (< 15 μm), more or less, spherical mono- or biflagellate specimens, which could not be identified, were included in a group named Unidentified phytoflagellates.

Microzooplankton (small zooplankton taxa) samples were preserved in 2.5 % formalin. Samples were settled in the laboratory for 72 hours (Kršinić 1980), until the original 5 L volume was reduced to 30 mL. The organisms then were counted and identified with an Olympus IX-71 microscope at magnifications of 100 x and 400 x. Microzooplankton included tintinnids (*Tintinnina*), non-loricate ciliates (including all other ciliates > 20 μm), rotifers, copepod nauplii, copepod postnauplii, including copepodites of all copepod species and adult specimens of small copepods of the genera *Oithona*, *Oncaea*, and *Euterpina*. According to the method of Kršinić et

al. (1988) confirmed by ICES Working Group on Zooplankton Ecology (ICES 2007), adults of small copepods (smaller than 200 μm in size) of the genera *Oithona*, *Oncaea* and *Euterpina* were included in microzooplankton size-fraction. In this study, small adult copepods were included within group of postnaupliar copepods.

Statistical analysis

Indicator species analysis based on taxa abundances and frequencies was used to detect importance values for taxa in brackish and marine layers (Dufrêne & Legendre 1997). Significance is determined with 4,999 permutations. This was performed with PC-ORD 5.0 for Windows (McCune & Mefford 2006).

Analysis of similarities (ANOSIM) randomization (Clarke & Warwick 1994) was used to test whether there is a significant difference in diatom community structure between brackish and marine layers. Statistical analyses were performed using PRIMER v5 software (Clarke & Gorley 2001).

Canonical Correspondence Analysis (CCA) was used to relate the abundance of diatom taxa and 124 samples to six environmental variables. CCA extracts synthetic gradients from the biotic and environmental matrices, which are quantitatively represented by arrows in graphical biplots (ter Braak & Verdonschot 1995). The length of the arrow is relative to the importance of the explanatory variable in the ordination, and arrow direction indicates positive and negative correlations. CCA ordination biplot was used to show relation of taxa and environmental variables. A dataset of 45 diatom taxa found in more than 5 % of the total number of samples was selected for this analysis. Neither transformation (e.g., square root or log) of species data nor down-weighting of rare species was performed. The data were centered and standardized before analyses as they were measured on different scales. A Monte Carlo permutation test (reduced model – 499 permutations) was used to test the statistical significance of each variable (expressed with F and P value). Thus eigenvalues calculated measure the importance of each of the ordination axes (0–1). Species–environment correlation measures the strength of the relationship between taxa and the environment for particular axes. Additional CCA diagram was used to show distribution of samples throughout a year. Environmental variable vectors were excluded to ensure better visual appearance of ordination plot. The analysis was carried out using CANOCO for Windows 4.52 software (ter Braak & Šmilauer 2002).

Results

Physical-chemical parameters

Annual ranges, means, modes, and standard deviations of physical, chemical, and biological parameters are reported in Table 1.

Vertical temperature stratification was observed from June to September, and inverse stratification occurred between November 1999 and March 2000 (Fig. 2). Throughout the year, the strongest vertical temperature gradient occurred at 6–7 m ($4.0\text{ }^{\circ}\text{C m}^{-1}$ in September 1999); those of salinity (21.39 in June 1999) and density ($\sigma\text{-t}$) ($15.43\text{ kg m}^{-3}\text{ m}^{-1}$ in February 2000) were above 1 m. The pycnocline, in most cases generated by salinity, created a very distinct separation of brackish ($\sigma\text{-t} \leq 14\text{ kg m}^{-3}$) and marine layers. Position of pycnocline was within the upper meters (0–3 m) of the water column.

The water column was well oxygenated (>0.70) in the 0–4 m layer throughout the year. Hypoxia (<0.40) occurred in the lower (6–10 m) layer from July to December 1999. Anoxic conditions were found at 9–10 m from July to October 1999.

Table 1. Ranges, means (AVG, in bold), modes, and standard deviations (STD) for physical-chemical parameters (A), chlorophyll *a* (Chl *a*), and plankton (B) in the lower Neretva River estuary (April 1999–May 2000, $n = 133$). O_2/O_2' = oxygen saturation, TIN = total inorganic nitrogen, Total PHYTO = total phytoplankton. The values for microzooplankton groups are expressed as the number of individuals per liter.

A

| | Temperature (°C) | Salinity | Density (kg m ⁻³) | Secchi (m) | O_2/O_2' | TIN ($\mu\text{mol L}^{-1}$) | PO_4 ($\mu\text{mol L}^{-1}$) | SiO_4 ($\mu\text{mol L}^{-1}$) |
|------|---------------------|--------------|----------------------------------|---------------|------------|-----------------------------------|---|--|
| MIN. | 7.9 | 0 | 0.02 | 0.5 | 0 | 0.3 | 0.07 | 0.14 |
| MAX. | 24.6 | 38.35 | 28.92 | 4 | 1.4 | 75.66 | 12.28 | 97.29 |
| AVG. | 16.6 | 31.42 | 22.82 | 2.6 | 0.7 | 11.67 | 0.81 | 13.97 |
| STD. | 3.9 | 10.48 | 7.86 | 1.0 | 0.3 | 14.21 | 1.92 | 17.43 |
| MODE | 13.1 | 38.35 | 28.16 | 3.5 | 1.0 | 2.41 | 0.13 | 4.27 |

B

| | Chl <i>a</i> ($\mu\text{g L}^{-1}$) | Total PHYTO (cells L ⁻¹) | Non-loric. ciliates | Tintinnids | Rotifers | Copepod nauplii | Copepod postnaup. |
|------|--|---|------------------------|------------|-----------|--------------------|----------------------|
| MIN. | 0.02 | 153010 | 0 | 0 | 0 | 0 | 0 |
| MAX. | 4.52 | 11804600 | 13536 | 1013 | 496 | 66 | 40 |
| AVG. | 0.55 | 1694950 | 715 | 46 | 51 | 14 | 9 |
| STD. | 0.66 | 1666560 | 1786 | 121 | 98 | 13 | 10 |
| MODE | 0.21 | 1057750 | 0 | 2 | 0 | 2 | 0 |

The lowest transparency (0.5 m) was in December 1999 and the highest (3–4 m) from January to April 2000 (Fig. 3). The euphotic zone was at least 1.25 m deep.

Significantly higher concentrations of total inorganic nitrogen (TIN > 20 $\mu\text{mol L}^{-1}$) were observed in the October–December period, with the maximum in November (Fig. 3). Ammonia accounted for highest proportion (33–85 %) of TIN from April–December; more TIN occurred as NO_3 (46–72 %) from December 1999 to the end of the investigation period. Nitrite generally was almost negligible (1–20 %).

There were two peaks of PO_4 in the estuary, one in August and the other in November 1999. SiO_4 peaked in the same time. The highest Chl *a* concentrations were observed in July–September 1999, with the maximum (4.52 $\mu\text{g L}^{-1}$) in September (Tab. 1, Fig. 3). The trophic index (TRIX) for the whole period was 4.56.

Distribution of diatoms

Altogether, 174 phytoplankton taxa were identified. Of these, 96 were diatoms comprising 51 genera. Thirty-two diatom taxa were identified in more than 10 % of all samples, and 52 taxa occurred in 2–9 % of the samples (Tab. 2). There were 12 rare diatom taxa (≤ 1 % of all samples).

Other phytoplankton groups included the following number of taxa: dinoflagellates, 58; coccolithophorids, 9; chlorophytes, 4; cyanophytes, 3; silicoflagellates, 2; chrysophytes, and euglenophytes, 1 each.

Three peaks were observed for total phytoplankton abundance (Figs 3 and 4). The first was in May 1999 (2.3×10^6 cells L⁻¹ at 10 m depth), the second in August 1999 (11.8×10^6 cells L⁻¹ at 5 m depth), and the third in February 2000 (1.5×10^6 cells L⁻¹ on the surface). The lowest water column mean ($4.9 \times 10^5 \pm 1.2 \times 10^5$ cells L⁻¹) was in December 1999. The occurring peaks of phytoplankton coincided with the unidentified phytoflagellates peaks during the year.

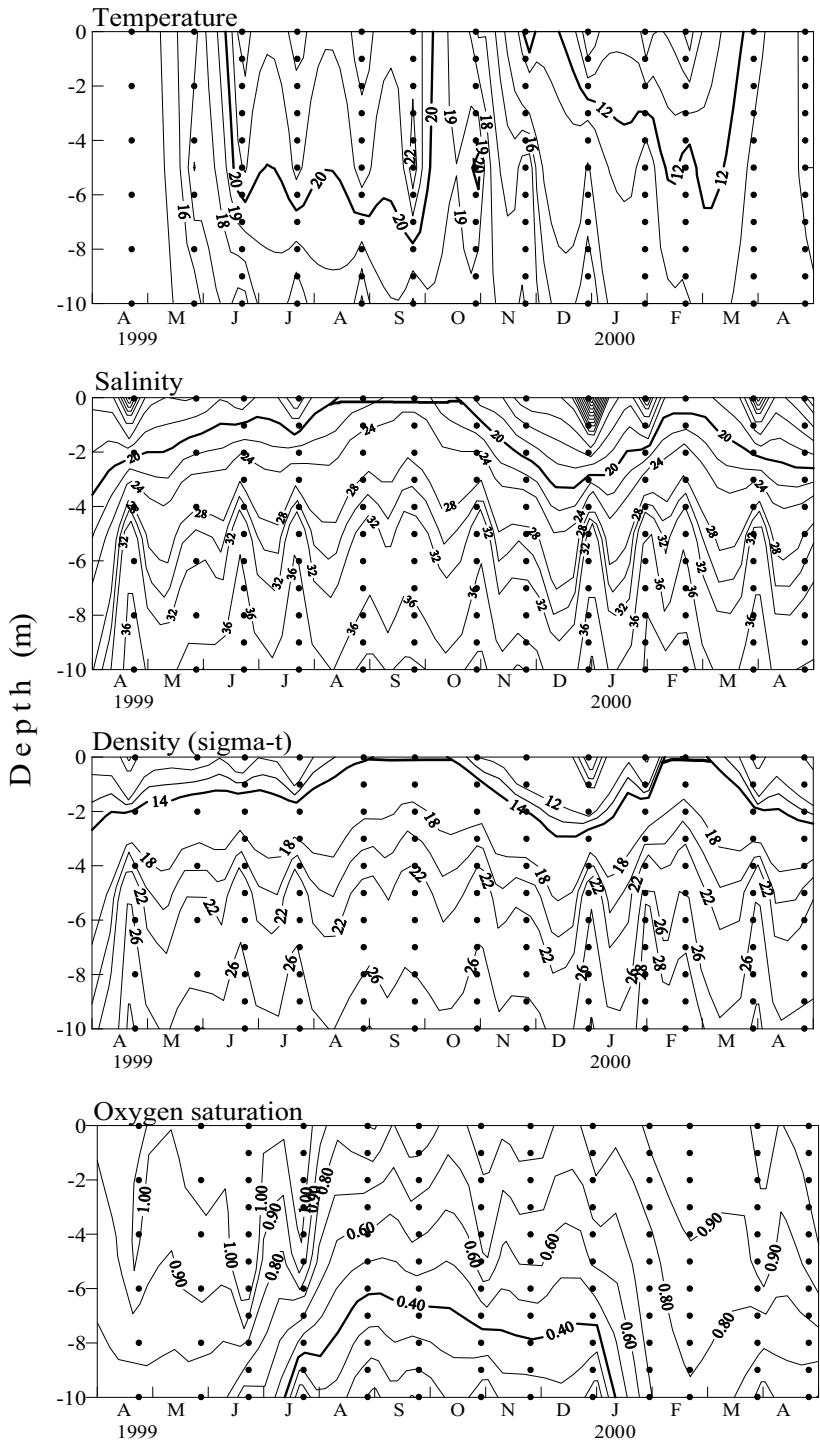
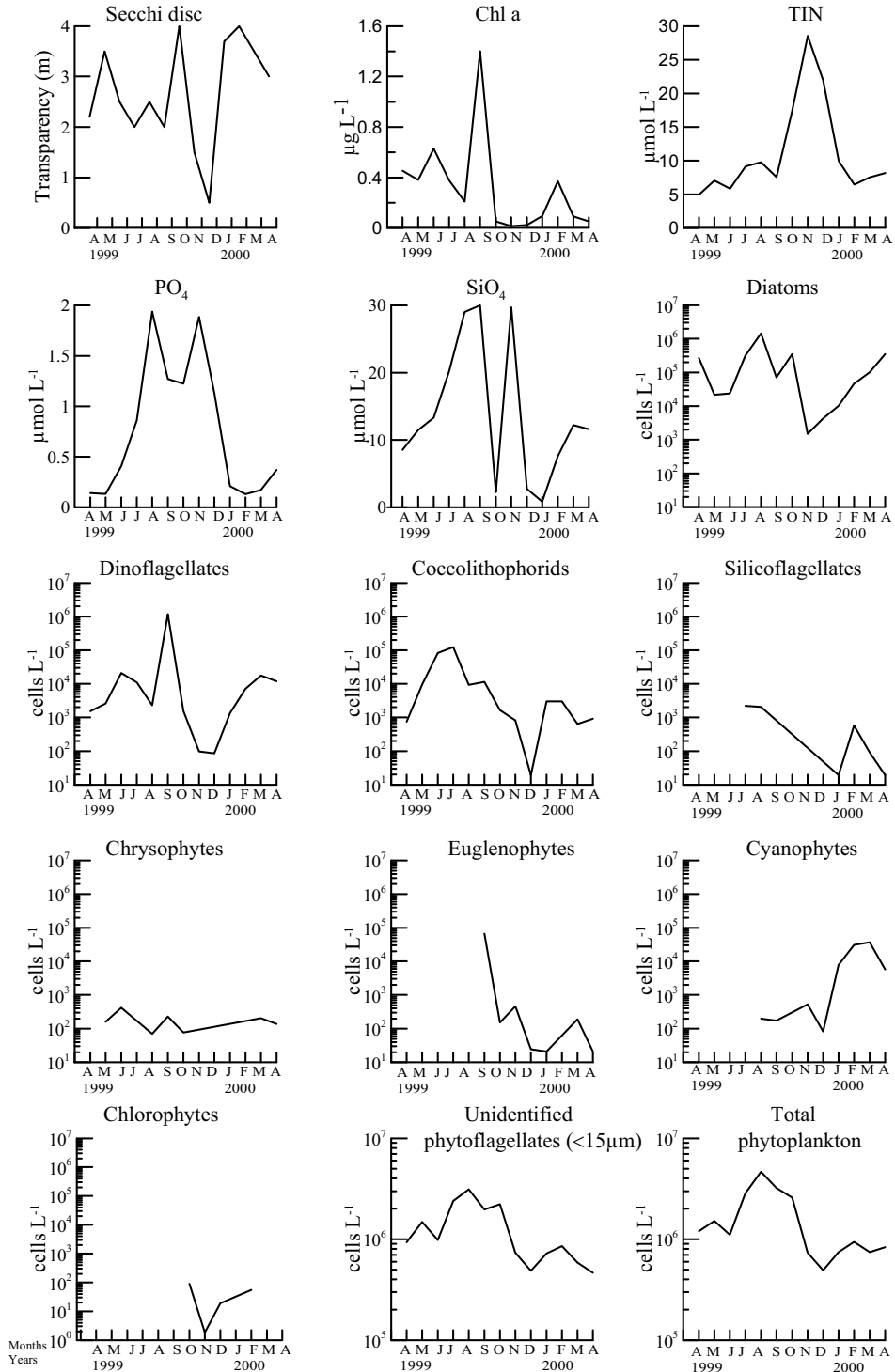


Fig. 2. Distribution of temperature, salinity, density (sigma-t), and oxygen saturation.



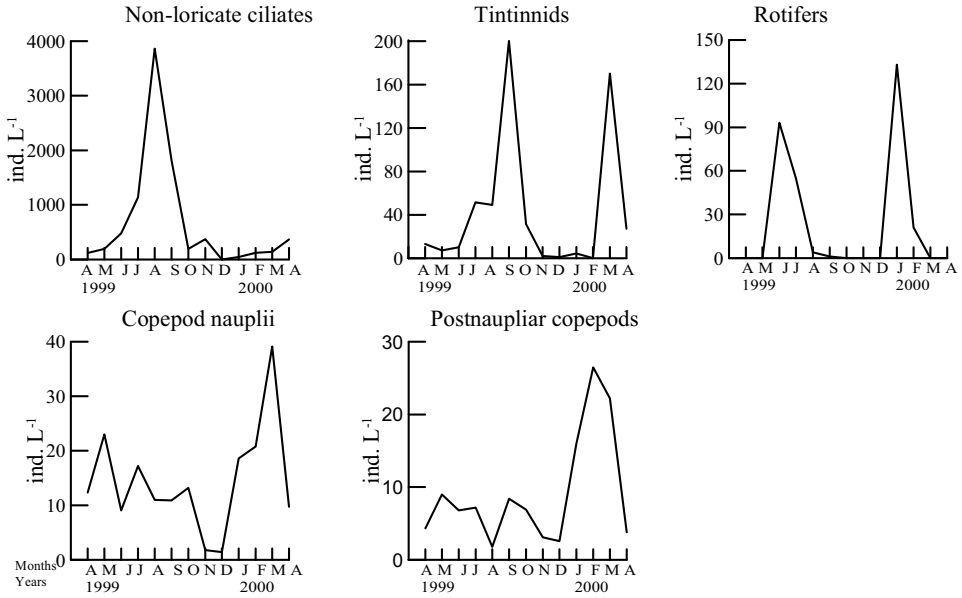


Fig. 3. Monthly variation of physical-chemical parameters, and phytoplankton and zooplankton abundances. Values are water column means.

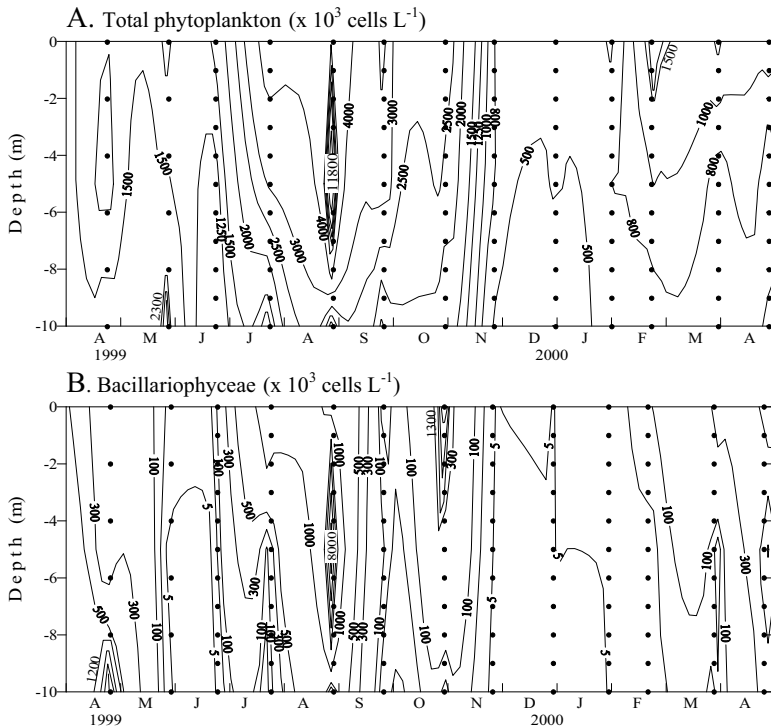


Fig. 4. Distribution of total phytoplankton (A) and diatoms (B).

Table 2. List of diatom taxa according to their occurrence in total number of samples (≥ 10 , 2–9 and ≤ 1 % occurrence in all samples), and monthly distribution of their mean abundance in the lower Neretva River estuary. Abundances are presented as follows: r = $< 10^{-1}$; 1 = 10^{-1} – 10^{-2} ; 2 = 10^{-2} – 10^{-3} ; 3 = 10^{-3} – 10^{-4} ; 4 = 10^{-4} – 10^{-5} ; 5 = 10^{-5} – 10^{-6} ; 6 = 10^{-6} – 10^{-7} .

| Taxa | Year/Month/Date | % | '99 | | | | | | | | | | | | '00 | | | |
|---|-----------------|---|-----|----|----|----|----|----|----|----|----|----|----|----|-----|--|--|--|
| | | | A | M | J | J | A | S | O | N | D | J | F | M | A | | | |
| | | | 21 | 25 | 21 | 21 | 26 | 24 | 28 | 25 | 29 | 31 | 22 | 30 | 27 | | | |
| | $\geq 10\%$ | | | | | | | | | | | | | | | | | |
| <i>Amphora ostrearia</i> Brébisson | 22 | 1 | 0 | 0 | 0 | 0 | 0 | r | 1 | 2 | 2 | 2 | 1 | 1 | | | | |
| <i>Asterionella formosa</i> Hassall | 18 | 3 | 3 | 0 | 2 | 0 | 0 | 2 | r | 0 | 2 | 2 | 2 | 2 | | | | |
| <i>Asterionellopsis glacialis</i> (Castracane) Round | 11 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 3 | 0 | 0 | | | | |
| <i>Cerataulina pelagica</i> (Cleve) Hendey | 43 | 4 | 0 | 1 | 3 | 3 | 0 | 3 | 0 | 1 | 2 | 2 | 2 | 1 | | | | |
| <i>Chaetoceros contortus</i> Schütt | 20 | 4 | 2 | 4 | 0 | 2 | 0 | 0 | 0 | 2 | 2 | 2 | 2 | 0 | | | | |
| <i>Chaetoceros curvisetus</i> Cleve | 11 | 4 | 0 | 0 | 0 | 1 | 0 | 2 | 0 | 2 | 1 | 1 | 0 | 0 | | | | |
| <i>Chaetoceros</i> sp. | 20 | 3 | 2 | 0 | 0 | 2 | 0 | 0 | 0 | 1 | 2 | 2 | 3 | 2 | | | | |
| <i>Cocconeis scutellum</i> Ehrenberg | 11 | 0 | 0 | 2 | 0 | 0 | 2 | 1 | 1 | 2 | 0 | 1 | 2 | 1 | | | | |
| <i>Cyclotella</i> sp. | 15 | 0 | 0 | 0 | 3 | 0 | 2 | 3 | 2 | 2 | 2 | 3 | 0 | 0 | | | | |
| <i>Diploneis bombus</i> (Ehrenberg) Cleve | 14 | 1 | 2 | 0 | 0 | 0 | 0 | 1 | 1 | 1 | 2 | 2 | 1 | 0 | | | | |
| <i>Guinardia flaccida</i> (Castracane) Peragallo | 32 | 2 | 2 | 2 | 2 | 4 | 0 | 0 | 0 | 1 | 1 | 1 | 2 | 2 | | | | |
| <i>Guinardia striata</i> (Stolterfoth) Hasle | 25 | 2 | 2 | 0 | 3 | 3 | 0 | 2 | 0 | 0 | 1 | 2 | 2 | r | | | | |
| <i>Hemiaulus hauckii</i> Grunow ex Van Heurck | 36 | 4 | 2 | 2 | 2 | 2 | 1 | 0 | 0 | 0 | 2 | 2 | 2 | 2 | | | | |
| <i>Leptocylindrus danicus</i> Cleve | 30 | 4 | 0 | 2 | 2 | 2 | 2 | 4 | 0 | r | 2 | 2 | 2 | 0 | | | | |
| <i>Leptocylindrus minimus</i> Gran | 17 | 3 | 0 | 0 | 0 | 0 | 0 | 5 | 0 | 2 | 1 | 2 | 1 | 0 | | | | |
| <i>Licmophora flabellata</i> C. Agardh | 18 | 1 | 2 | 2 | 1 | 1 | 0 | 2 | 1 | 1 | 1 | 1 | 1 | 2 | | | | |
| <i>Navicula</i> spp. | 11 | 2 | 2 | 0 | 1 | 1 | 0 | 1 | 0 | 1 | 0 | r | 2 | 0 | | | | |
| Naviculoid cells | 49 | 1 | 3 | 2 | 3 | 2 | 2 | 2 | 1 | 1 | 2 | 2 | 1 | 2 | | | | |
| <i>Nitzschia incerta</i> (Grunow) M. Peragallo | 16 | 1 | 2 | r | 1 | r | 0 | 1 | 1 | 1 | 1 | 1 | 1 | r | | | | |
| <i>Nitzschia longissima</i> (Brébisson) Ralfs | 55 | 0 | 0 | 0 | 4 | 6 | 4 | 4 | 2 | 1 | 3 | 3 | 2 | 0 | | | | |
| <i>Pleurosigma angulatum</i> (Queckett) W. Smith | 42 | 1 | 2 | 1 | 2 | 1 | 1 | 1 | 1 | 1 | 2 | 2 | 1 | 2 | | | | |
| <i>Pseudo-nitzschia delicatissima</i> (Cleve) Heiden | 50 | 2 | 0 | 2 | 4 | 3 | 1 | 3 | 0 | 1 | 2 | 2 | 2 | 3 | | | | |
| <i>Pseudo-nitzschia</i> sp. | 27 | 0 | 0 | 1 | 2 | 4 | 0 | 4 | 0 | 0 | 2 | 2 | 0 | 2 | | | | |
| <i>Proboscia alata</i> (Brightwell) B. G. Sundström | 47 | 2 | 3 | 2 | 3 | 3 | 1 | 1 | 1 | 0 | 1 | 1 | 2 | 1 | | | | |
| <i>Proboscia indica</i> (H. Peragallo) Hernández-Becerril | 11 | 0 | 0 | r | 0 | 2 | 0 | 0 | 0 | 0 | 0 | r | 1 | 1 | | | | |
| <i>Rhizosolenia imbricata</i> Brightwell | 36 | 2 | 2 | 1 | 1 | 2 | 1 | 0 | 0 | 1 | 2 | 2 | r | 1 | | | | |
| <i>Skeletonema marinoi</i> Sarno & Zingone | 10 | 3 | 3 | 0 | 0 | 0 | 0 | 5 | 0 | 0 | 0 | 0 | 0 | 0 | | | | |
| <i>Synedra</i> sp. 1 | 33 | 1 | 0 | 2 | 1 | 2 | 1 | 1 | 1 | 1 | 1 | 1 | 2 | 1 | | | | |
| <i>Thalassionema nitzschioides</i> (Grunow) Mereschkowsky | 48 | 2 | 2 | 0 | 0 | 1 | 1 | 2 | 1 | 2 | 2 | 2 | 2 | 3 | | | | |
| <i>Thalassiosira angulata</i> (Gregory) Hasle | 11 | 0 | 0 | 0 | 1 | 2 | 1 | 1 | 0 | r | 0 | 1 | 3 | 0 | | | | |
| <i>Thalassiosira</i> sp. | 30 | 5 | 3 | 2 | 0 | 0 | 0 | 0 | 0 | 0 | r | 4 | 4 | 5 | | | | |
| Unidentified pennate diatoms | 25 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 2 | 1 | 2 | 2 | 2 | | | | |

Table 2 (continued).

| Taxa | Year/Month/Date | % | '99 | | | | | | | | '00 | | | | |
|---|-----------------|---|-----|----|----|----|----|----|----|----|-----|----|----|----|----|
| | | | A | M | J | J | A | S | O | N | D | J | F | M | A |
| | | | 21 | 25 | 21 | 21 | 26 | 24 | 28 | 25 | 29 | 31 | 22 | 30 | 27 |
| 2-9% | | | | | | | | | | | | | | | |
| <i>Achnanthes longipes</i> Agardh | | 2 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | r | 0 | 0 |
| <i>Amphora</i> sp. | | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | r | 1 | 1 | 0 | 0 |
| <i>Asterolampra marylandica</i> Ehrenberg | | 2 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| <i>Auricula insecta</i> (Grunow) A. Schmidt | | 2 | 0 | 0 | 2 | 0 | 0 | 0 | 2 | 0 | 0 | 0 | 0 | 0 | 0 |
| <i>Bacteriastrum hyalinum</i> Lauder | | 3 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 0 |
| <i>Campylodiscus</i> sp. | | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 0 |
| <i>Chaetoceros affinis</i> Lauder | | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2 | r | 0 |
| <i>Chaetoceros atlanticus</i> Cleve | | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 1 | 1 | 0 |
| <i>Chaetoceros brevis</i> Schütt | | 3 | 0 | 0 | 0 | 0 | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| <i>Chaetoceros convolutus</i> Castracane | | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 1 | 0 | 0 |
| <i>Chaetoceros danicus</i> Cleve | | 7 | 2 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 1 | 1 | 0 |
| <i>Chaetoceros decipiens</i> Cleve | | 8 | 0 | 0 | 1 | 2 | 0 | 0 | 0 | 1 | r | 2 | 2 | 0 | 2 |
| <i>Chaetoceros delicatulus</i> C. E. H. Ostefeld | | 3 | 2 | 0 | 0 | 4 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| <i>Chaetoceros diversus</i> Cleve | | 2 | 0 | 0 | 1 | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| <i>Chaetoceros perpusillus</i> Cleve | | 3 | 0 | 0 | 0 | 0 | 4 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| <i>Chaetoceros simplex</i> Ostefeld | | 2 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 0 |
| <i>Chaetoceros tetrastichon</i> Cleve | | 2 | 0 | 0 | 0 | 0 | 0 | 0 | r | 0 | 0 | 0 | 1 | 0 | 0 |
| <i>Chaetoceros wighamii</i> Brightwell | | 2 | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 |
| <i>Coscinodiscus</i> sp. (24-30 µm) | | 5 | 0 | 0 | r | 0 | 0 | r | 2 | 1 | 0 | 0 | 0 | 0 | 0 |
| <i>Coscinodiscus stellatus</i> F. Kitton | | 3 | 1 | 0 | 0 | 0 | 0 | 0 | 1 | r | 0 | 0 | 0 | 0 | 0 |
| <i>Cylindrotheca closterium</i> (Ehrenberg) W. Smith | | 5 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 1 | 0 | 1 | 1 | 0 | 1 |
| <i>Cymbella</i> sp. | | 4 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2 | r | r | 0 | 0 |
| <i>Dactyliosolen fragilissimus</i> (Bergon) Hasle | | 5 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 1 | 0 |
| <i>Detonula pumila</i> (Castracane) Gran | | 2 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| <i>Diatoma tenue</i> C. Agardh | | 2 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| <i>Diploneis crabro</i> Ehrenberg | | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | r | 0 | 1 | 1 | 0 | 0 |
| <i>Eucampia cornuta</i> (Cleve) Grunow | | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 1 | 0 |
| <i>Eunotia</i> sp. | | 2 | 0 | 0 | r | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 |
| <i>Fragilaria crotonensis</i> Kitton | | 4 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 |
| <i>Grammatophora oceanica</i> Ehrenberg | | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 |
| <i>Guinardia delicatula</i> (Cleve) Hasle | | 8 | 0 | 0 | 2 | 0 | 0 | 0 | 2 | 0 | 1 | 1 | 1 | 2 | 1 |
| <i>Gyrosigma balticum</i> (Ehrenberg) Rabenhorst | | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | r | r | 0 | 1 |
| <i>Hemiaulus sinensis</i> Greville | | 5 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 0 |
| <i>Lauderia annulata</i> Cleve | | 5 | 2 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 1 | 2 | 0 |
| <i>Licmophora paradoxa</i> (Lyngbye) C. Agardh | | 3 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 |
| <i>Licmophora</i> sp. | | 8 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 1 | 1 | r | 1 |
| <i>Lioloma pacificum</i> (E. Cupp) Hasle | | 5 | 1 | 0 | 0 | 0 | r | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 0 |
| <i>Melosira numuloides</i> C. Agardh | | 3 | 0 | 0 | 2 | 0 | 1 | 0 | 0 | r | 0 | 0 | 0 | 0 | 0 |

Table 2 (continued).

| Year/Month/Date | | '99 | | | | | | | | | | '00 | | | |
|--|---|-----|----|----|----|----|----|----|----|----|----|-----|----|----|--|
| | | A | M | J | J | A | S | O | N | D | J | F | M | A | |
| Taxa | % | 21 | 25 | 21 | 21 | 26 | 24 | 28 | 25 | 29 | 31 | 22 | 30 | 27 | |
| 2–9% | | | | | | | | | | | | | | | |
| <i>Navicula distans</i> (W. Smith) Ralfs | 2 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | r | r | 0 | 0 | |
| <i>Nitzschia lorenziana</i> Grunow | 2 | r | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | |
| <i>Nitzschia</i> sp. | 2 | 1 | 0 | 0 | 0 | 0 | r | 0 | 0 | 0 | 0 | 0 | 0 | 0 | |
| <i>Odontella mobiliensis</i> (J. W. Bailey) Grunow | 4 | 0 | 0 | 0 | 0 | 0 | 0 | r | 0 | 0 | 1 | 1 | r | 0 | |
| <i>Paralia sulcata</i> (Ehrenberg) Cleve | 7 | 2 | 1 | 0 | 0 | 0 | 0 | 1 | 0 | 2 | 2 | 2 | 2 | 0 | |
| <i>Pleurosigma formosum</i> W. Smith | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | |
| <i>Pleurosigma macrum</i> W. Smith | 5 | 0 | 0 | 0 | 0 | 2 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | |
| <i>Pseudosolenia calcar-avis</i> (Schultze) Sundström | 3 | 2 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | |
| <i>Striatella interrupta</i> (Ehrenberg) Heiberg | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | |
| <i>Striatella unipunctata</i> (Lyngbye) C. Agardh | 2 | 3 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | |
| <i>Synedra</i> sp. 2 | 2 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | r | 0 | |
| <i>Tetracyclus excentricus</i> (Ehrenberg) D. M. Williams | 4 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 1 | 1 | 0 | 0 | |
| <i>Tropidoneis</i> sp. | 2 | 0 | 0 | 0 | 0 | 0 | 0 | r | 1 | r | 0 | 0 | 0 | 0 | |
| <i>Ulnaria acus</i> (Kützing) M. Aboal | 5 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | r | 1 | 1 | |
| ≤1% | | | | | | | | | | | | | | | |
| <i>Ardissonea crystallina</i> (C. Agardh) Grunow | 1 | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | |
| <i>Asterionella bleakeleyi</i> W. Smith | 1 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | |
| <i>Bacillaria paradoxa</i> J. F. Gmelin | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | |
| <i>Chaetoceros lorenzianus</i> Grunow | 1 | 0 | 0 | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | |
| <i>Chaetoceros rostratus</i> Lauder | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | |
| <i>Entomoneis paludosa</i> (W. Smith) Reimer | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | |
| <i>Grammatophora marina</i> (Lyngbye) Kützing | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | |
| <i>Navicula cancellata</i> Donkin | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 0 | 0 | |
| <i>Neocalyptrella robusta</i> (G. Norman ex Ralfs) Hernández-Becerril & Meave del Castillo | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | r | 0 | 0 | 0 | 0 | |
| <i>Pleurosigma elongatum</i> W. Smith | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | r | 0 | 0 | |
| <i>Psammodictyon panduriforme</i> (Gregory) Meunier | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | |
| <i>Synedra superba</i> Kützing | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | r | r | 0 | 0 | |

Unidentified phytoflagellates and diatoms represented the major fraction (>55%) of total phytoplankton abundance (Tab. 3), during all the year. Their mean abundance varied less than one order of magnitude (Fig. 3), and maximum was observed in August 1999. Diatoms contributed to the total phytoplankton abundance from 0.20 to 42.10%. With the exception of dinoflagel-

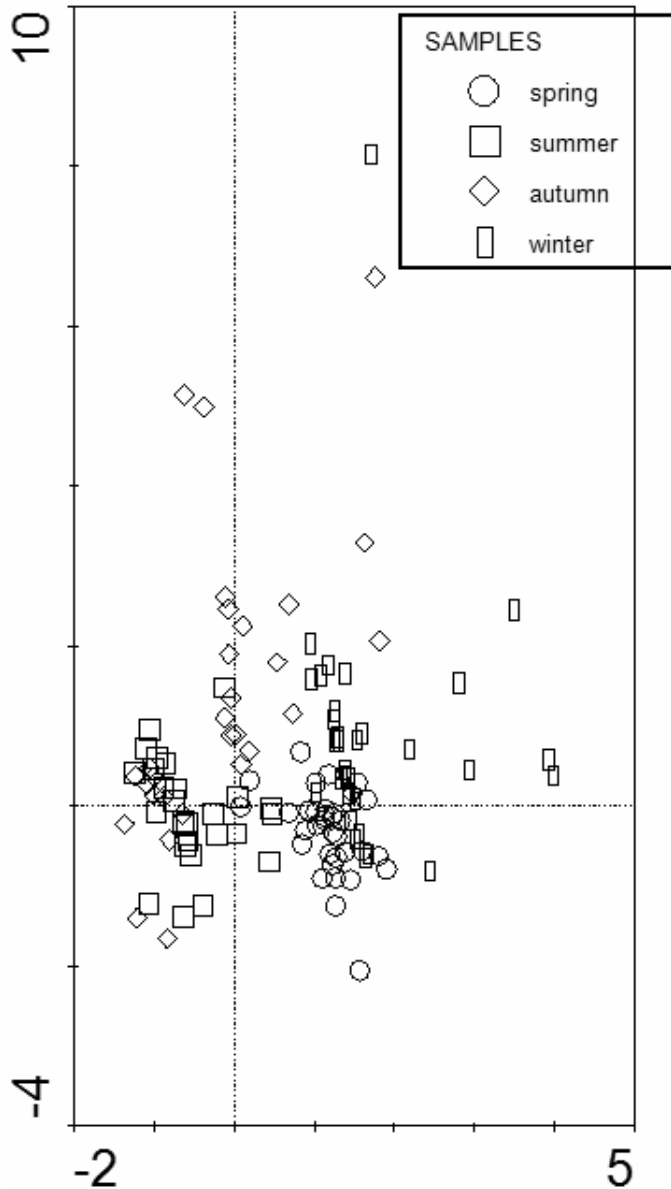


Fig. 5. CCA ordination of samples. Samples are separated according to seasons.

delicatulus, *Pseudo-nitzschia delicatissima*, and *Nitzschia longissima*, with maxima of 19.9, 2.0, and 1.0×10^5 cells L^{-1} respectively.

N. longissima dominated the total diatom abundance (98.08%; max. 7.9×10^6 cells L^{-1} at 5 m depth) in August 1999. Its abundance was higher (up to 6.8×10^4 cells L^{-1}) throughout September and October 1999. With the exception of *N. longissima*, only *Guinardia flaccida* and *Pseudo-nitzschia* sp. exceeded 10^4 cells L^{-1} in August 1999.

Skeletonema marinoi and *Leptocylindrus minimus* were the most abundant species in October 1999, exceeding 10^5 cells L^{-1} and contributing 83.61 % to the total diatom abundance.

From November 1999 to January 2000, *N. longissima* was the most abundant diatom ($0.2\text{--}4.0 \times 10^3$ cells L^{-1}). In February 2000, *Thalassiosira* sp. started to increase again, attaining concentrations between 0.1 and 3.3×10^5 cells L^{-1} in February–April 2000.

According to Indicator Species Analysis, indicators of brackish layer were: *Asterionella formosa*, naviculoid cells, *Cyclotella* sp., *Licmophora flabellata*, *Ulnaria acus* and *Fragilaria crotonensis*. No statistically significant indicator values were recorded for taxa in marine layer. Nine, mostly rare (in ≤ 1 % of all samples), taxa were only found in brackish layer (*Asterionella bleakeleyi*, *Bacillaria paradoxa*, *Entomoneis paludosa*, *Eunotia* sp., *Grammatophora marina*, *G. oceanica*, *Licmophora* sp., *Pleurosigma elongatum* and *Synedra superba*). In total, 27 taxa exclusively occurred in marine layer (*Amphora* sp., *Asterolampra marylandica*, *Bacteriastrium hyalinum*, *Chaetoceros affinis*, *Ch. atlanticus*, *Ch. contortus*, *Ch. convolutus*, *Ch. lorenzianus*, *Ch. rostratus*, *Ch. simplex*, *Ch. tetrastichon*, *Ch. wighamii*, *Coscinodiscus stellatus*, *Cylindrotheca closterium*, *Diploneis crabro*, *Licmophora paradoxa*, *Lioloma pacificum*, *Navicula* spp., *Neocalyptrella robusta*, *Nitzschia* sp., *Odontella mobiliensis*, *Pleurosigma formosum*, *Psammодиctyon panduriforme*, *Pseudosolenia calcar-avis*, *Dactyliosolen fragilissimus*, *Striatella interrupta* and *Tetracyclus excentricus*). Anosim analysis revealed that Global test R reached low value (0.193) which indicated no difference between two layers based on diatom taxa abundances ($P < 0.005$).

Small zooplankton taxa

There were high densities of non-loricate ciliates from June to September 1999 (Fig. 3), with highest mean water column in August 1999. Among tintinnids, *Tintinnopsis minuta* was the most abundant species, reaching 1,008 ind. L^{-1} (99.5 % of total tintinnids) in March 2000. A dense population of *T. fracta* (5–385 ind. L^{-1} , mean 211 ind. L^{-1}) occurred in September 1999.

There were two peaks of rotifers over the year. The first was in June 1999 (244 ind. L^{-1} at 4 m depth). The second peak was observed in January 2000 due to high concentrations of *Synchaeta neapolitanum* (496 ind. L^{-1} at 4 m depth). *S. curvata* (range for the water column 3–56 ind. L^{-1}) was abundant in February 2000.

The lowest abundance of copepod nauplii was in December 1999. The maximum, 66 ind. L^{-1} , was found near the bottom in March 2000. January to March 2000 featured high densities of postnauplii (maximum 40 ind. L^{-1} in March 2000). *Euterpina acutifrons* accounted for about two-thirds of copepods at this time.

Relationships between planktonic diatoms and environmental parameters

Eigenvalues from the CCA analysis for the first four axes were 0.858, 0.643, 0.179 and 0.068 (Fig. 6). The species–environmental correlation for the first and second axes was 0.958 and 0.857, respectively. The first two axes explain 85.1 % of variance of species–environment relationship. Temperature ($F = 14.717$, $P = 0.002$), salinity ($F = 7.363$, $P = 0.002$), and TIN ($F = 6.829$, $P = 0.002$) were the most important factors influencing diatom community structure in the lower estuary, followed by SiO_4 and depth. PO_4 was not significant. For example, *Proboscia alata*, *Pleurosigma macrum* and *Nitzschia longissima*, mostly inhabiting marine layer, were associated to higher salinity and temperature values (Fig. 7). In the case of salinity, the taxa *Ulnaria acus*, *Asterionella formosa*, *Cyclotella* sp. and *Licmophora* sp., were associated with lower salinities, at the lower right quadrant. The TIN vector is found within the same quadrant as the most taxa included. The taxa *Leptocylindrus minimus*, *L. danicus* and *Skeletonema marinoi*, among others, were the most abundant in the period October–November, when TIN was high. In addition,

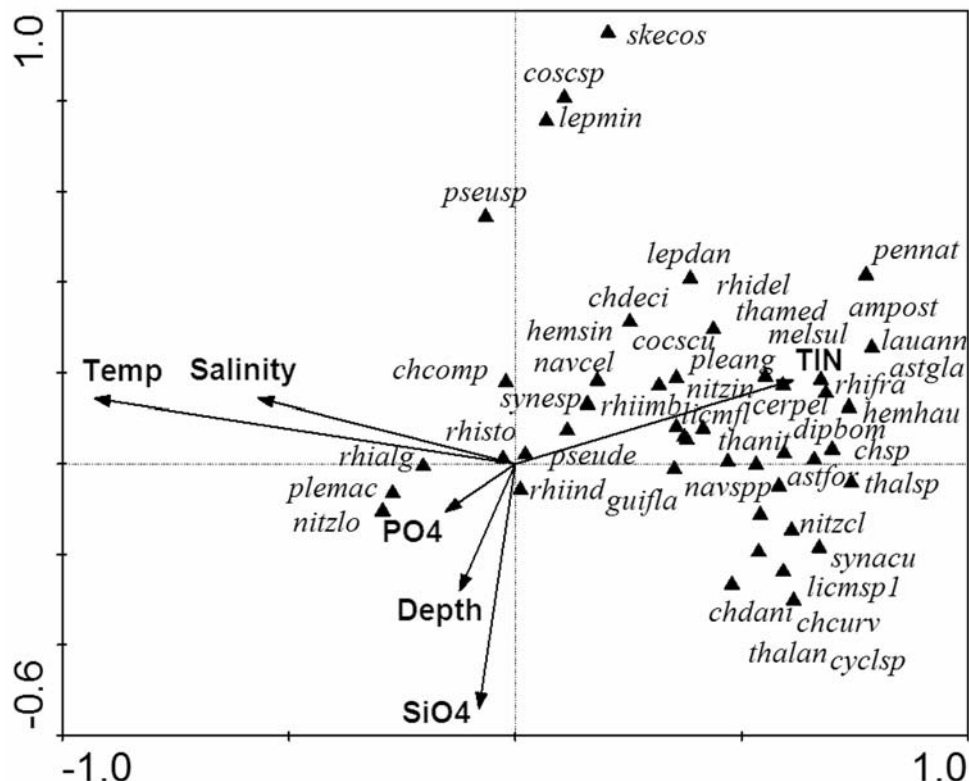


Fig. 6. CCA biplot showing diatom taxa (triangle) and vectors of the six environmental variables (arrows) based on 124 samples. A dataset of 45 diatom taxa found in more than 5% of the total number of samples was selected. Codes for diatom taxa are:

| | | | | | |
|---------|---|--|--------|---|---------------------------------------|
| ampost | = | <i>Amphora ostrearia</i> | navspp | = | <i>Navicula</i> spp. |
| astfor | = | <i>Asterionella formosa</i> | nitzcl | = | <i>Cylindrotheca closterium</i> |
| astgla | = | <i>Asterionellopsis glacialis</i> | nitzin | = | <i>Nitzschia incerta</i> |
| cerpel | = | <i>Cerataulina pelagica</i> | nitzlo | = | <i>Nitzschia longissima</i> |
| chcomp | = | <i>Chaetoceros contortus</i> | pennat | = | unidentified pennate diatoms |
| chcurv | = | <i>Chaetoceros curvisetus</i> | pleang | = | <i>Pleurosigma angulatum</i> |
| chdani | = | <i>Chaetoceros danicus</i> | plemac | = | <i>Pleurosigma macrum</i> |
| chdeci | = | <i>Chaetoceros decipiens</i> | pseude | = | <i>Pseudo-nitzschia delicatissima</i> |
| chsp | = | <i>Chaetoceros</i> sp. | pseusp | = | <i>Pseudo-nitzschia</i> sp. |
| cocscu | = | <i>Cocconeis scutellum</i> | rhialg | = | <i>Proboscia alata</i> |
| cocscsp | = | <i>Coscinodiscus</i> sp. (24–30 μ m) | rhidel | = | <i>Guinardia delicatula</i> |
| cyclsp | = | <i>Cyclotella</i> sp. | rhifra | = | <i>Dactyliosolen fragilissimus</i> |
| dipbom | = | <i>Diploneis bombus</i> | rhiimb | = | <i>Rhizosolenia imbricata</i> |
| guifla | = | <i>Guinardia flaccida</i> | rhiind | = | <i>Proboscia indica</i> |
| hemhau | = | <i>Hemiaulus hauckii</i> | rhisto | = | <i>Guinardia striata</i> |
| hemsin | = | <i>Hemiaulus sinensis</i> | skecos | = | <i>Skeletonema marinoi</i> |
| lauann | = | <i>Lauderia annulata</i> | synacu | = | <i>Ulnaria acus</i> |
| lepdan | = | <i>Leptocylindrus danicus</i> | synesp | = | <i>Synedra</i> sp. 1 |
| lepmin | = | <i>Leptocylindrus minimus</i> | thalan | = | <i>Thalassiosira angulata</i> |
| licmfl | = | <i>Licmophora flabellata</i> | thalps | = | <i>Thalassiosira</i> sp. |
| melsul | = | <i>Paralia sulcata</i> | thamed | = | <i>Lioloma pacificum</i> |
| navcel | = | naviculoid cells | thanit | = | <i>Thalassionema nitzschioides</i> |

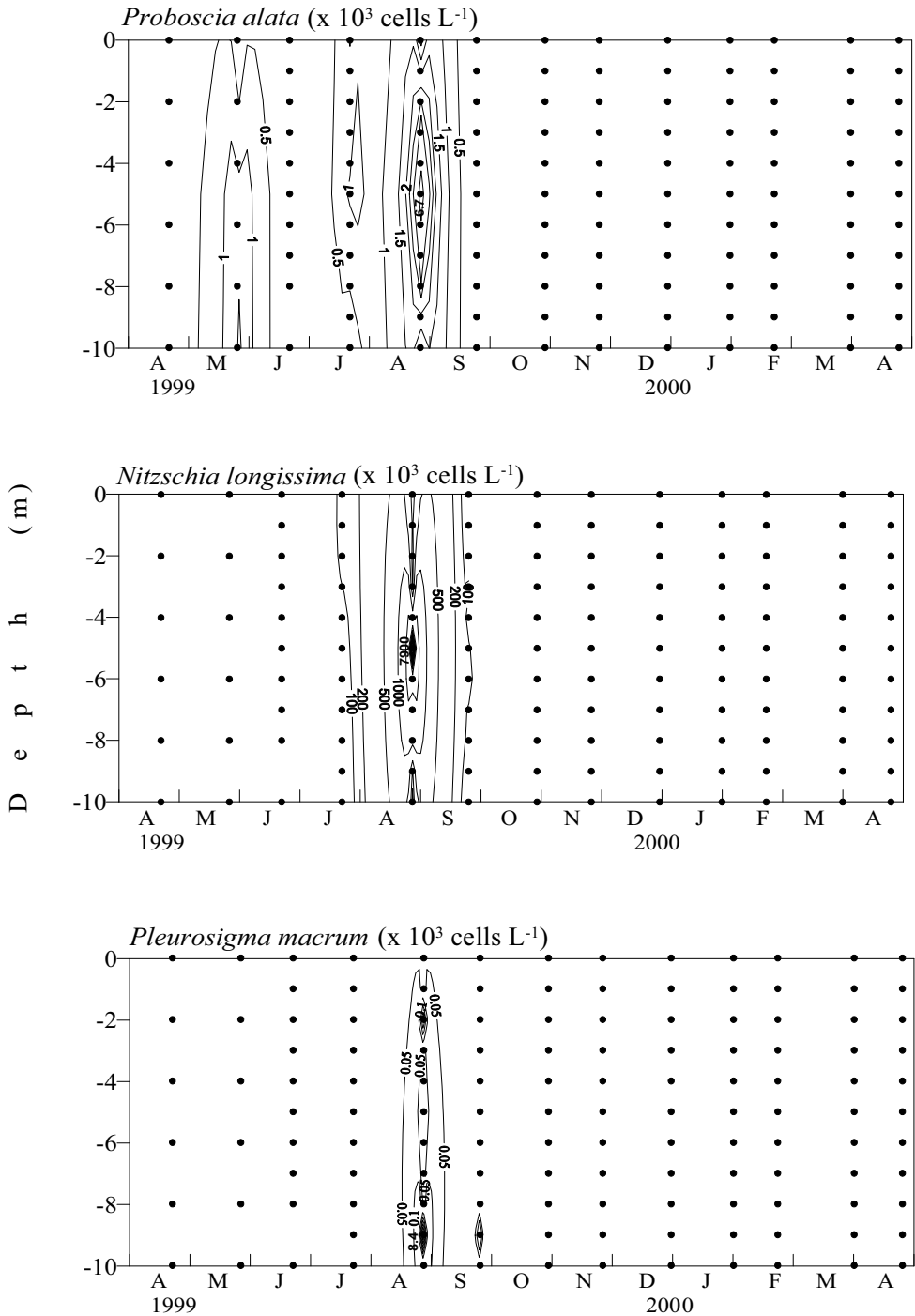


Fig. 7. Distribution of taxa associated with higher salinity and temperature values (cf. CCA multivariate analysis).

Coscinodiscus sp. (24–30 µm), *Leptocylindrus minimus* and *Skeletonema marinoi* occur in the quadrant opposite to the SiO₄ vector. The taxa *Proboscia indica*, *Guinardia striata* and *Pseudo-nitzschia delicatissima*, among others, are principally situated in the centre of the plot, which means that they were not strongly influenced by any of considered variables.

Discussion

In contrast to the oligotrophic freshwater lakes of the upper Neretva estuary (Jasprica & Hafner 2005, Jasprica et al. 2005), the present data define the water of the lower estuary as mesotrophic. This may be explained by the intensive anthropogenic activities carried on in the lower estuary and its surrounding area, especially over the past 50 years.

Estuarine circulation has a strong influence on the distribution of hydrographic parameters. The highest concentrations of most nutrients measured in this study were in the summer-autumn period. Late autumn and winter concentrations are controlled to a large degree by the increased freshwater discharge that accompanies the heavier rainfall of November–December (Katušin 2000, 2001). In this regard, the current data are consistent with those of Štambuk-Giljanović (2003).

NH₄ made up the highest proportion of TIN. This might be explained either by reduction of NO₃ under hypoxic conditions in the bottom layer or by heterotrophic processes in the estuarine sediments (Nowicki & Nixon 1985, Abril et al. 2000). The PO₄ maximum can be attributed to excretion by the dense population of non-loricate ciliates in August 1999. Peaks of PO₄ coincided with those of SiO₄ and TIN in November 1999, indicating a possible nutrient enrichment due to terrigenous inputs. TIN and PO₄, even at their minimum concentrations, were sufficient to satisfy the feeding requirements of phytoplankton (see Kohl & Niklisch 1988, Sommer 1993). SiO₄ in the layer below the pycnocline, however, was insufficient for phytoplankton nutrition in December 1999 and January 2000.

The summer SiO₄ peak coincided with a bloom composed mainly of *Nitzschia longissima*, *Guinardia flaccida*, and *Pseudo-nitzschia* sp. These taxa are typical of the summer bloom of southern Adriatic coastal waters (cf. Jasprica & Carić 2001). On the contrary, the rapid decrease of SiO₄ in October 1999 coincided with a bloom of *Skeletonema marinoi*. Different pattern of SiO₄ and diatoms abundance may be explained by monthly sampling design. These findings were confirmed by the CCA multivariate analysis. *S. marinoi* (formerly named as *S. costatum*) is an abundant winter-spring species in western Adriatic coastal waters (Totti & Artegani 2001, Caroppo et al. 1999) and in the eastern Adriatic Krka estuary (Cetinić et al. 2006). Borkman & Smayda (2009) showed that *Skeletonema* blooms may occur different times during the year in Narragansett Bay (Rhode Island, USA) and that they are related to weather patterns connected with the North Atlantic Oscillation. *Skeletonema* generally prefers eutrophic waters (Viličić et al. 2009 and references therein), in which their higher growth and nitrate uptake rates allow them to out-compete many diatoms (Liu et al. 2005).

Nine diatom taxa (9% of the total number of taxa) occurred only in brackish layer. These diatoms mostly belonged to the group of rare taxa and, among other diatoms (i.e. *Ulnaria acus* and *Licmophora* sp.), were present in winter, in correspondence to low salinity and temperature values. This was confirmed by CCA analysis. Some of these taxa originated in the upper Neretva River and its tributaries (Jasprica & Hafner 2005). Similarly, taxa found only in marine layer had low frequency of occurrence (< 10%), while they were found in more than one season. This may explain why no significant differences, in terms of diatom taxa abundances, between brackish and marine layers have been noted. In fact, the importance of depth is relatively reduced when compared with the other selected variables.

The diatom community in the Neretva estuary is typical coastal marine community under the influence of freshwater inflow. Diatom taxa found in this study resembled those found in three eastern Adriatic estuaries: the Ombla (Carić & Jasprica 2004), the Zrmanja (Burić et al. 2005), and the Krka (Cetinić et al. 2006). Similar diatom assemblages, especially in terms of the most abundant taxa, are found in parts of the estuaries which were directly under the marine influence, e.g. in shallow Urdaibai estuary (Trigueros & Orive 2001), or in Bay of Brest (Del Amo et al. 1997), and in the other temperate coastal areas (Ignatiades 1969, García-Soto et al. 1990, Kemp et al. 2000, Baytut et al. 2010).

Nitzschia, *Pseudo-nitzschia*, *Leptocylindrus*, and *Chaetoceros* genera included the most abundant taxa and remain convincing indicators of the influx of nutrients in the Adriatic Sea. Some of these (e.g. *Chaetoceros*) are typical of nutrient rich waters (Jasprica & Carić 1994, Aizawa et al. 2005, Viličić et al. 2009). However, many of major diatom taxa found in the Neretva estuary have been previously characterized as euryhaline, such as *Thalassionema nitzschioides*, *Thalassiosira* sp., *Rhizosolenia imbricata* and others (Braarud 1951).

Some diatom taxa (*Thalassionema nitzschioides*, *Leptocylindrus danicus*, and *Pseudo-nitzschia* spp.) dominated the diatom community both in the Krka and Zrmanja estuaries (Burić et al. 2005). Cetinić et al. (2006) concluded that *Pseudo-nitzschia*, an abundant summer genus in all three eastern Adriatic estuaries, is an indicator of an elevated degree of eutrophication. Additionally, a bloom of *Pseudo-nitzschia* spp. was noted during mid-winter in the Zrmanja estuary (Burić et al. 2008). Viličić et al. (1995 a, 2009) reported that *Pseudo-nitzschia* species are present the entire year and are generally considered to be dominant in the phytoplankton of the Adriatic Sea, but the actual species composition and species succession remain to be elucidated.

The dominance of small phytoflagellates (< 15 µm), particularly in spring and summer, has been reported in coastal areas of the southern Adriatic Sea (Caroppo et al. 1999). A reliable identification of small phytoflagellates requires culture studies and analysis with electron microscope.

Considering the seasonal cycle, confirmed by CCA analysis, the winter-spring diatoms were composed of small, fast-growing taxa, mainly *Thalassiosira* sp. These are capable of exploiting nutrients at lower concentrations, owing to their relatively high surface/volume ratio and high reproductive rate. The summer community, on the other hand, was composed of larger diatoms with lower growth rates (*Nitzschia longissima* and *Pseudo-nitzschia* sp.). In autumn, diatoms made a smaller contribution to phytoplankton abundance, while dinoflagellates, with lower growth rates, flourished. In this study, a possible explanation for the bloom of the cyst-forming dinoflagellate *Scrippsiella trochoidea* observed in September 1999 may be the germination of cysts from bottom sediment resuspended during wind-induced autumn turn-over (Anderson & Wall 1978, Dale et al. 1999). On the contrary, in the Guadiana estuary of south-western Spain, the relative decrease in diatoms was attributed to the combination of light limitation, extremely low summer river discharge (Barbosa et al. 2010), and increased grazing (Domingues & Galvão 2007).

Although Jasprica (1989) observed lower diatom abundances in a reduced light regime in southern Adriatic bays, no relationship between the monthly pattern in transparency (Secchi disc) and the total diatom abundance in the Neretva estuary was noted. This was expected, due to diatom occurrence in the whole water column. Diatoms spend a substantial fraction of their time in dark layer of the water column, where they respire but do not photosynthesize, thus their growth is limited by light (Sverdrup 1953). In addition, some taxa are capable of vertical migration to take up nutrients trapped in a stratified water column and utilize them for growth under more favorable light condition in the euphotic layer (Moore & Villareal 1996).

Five environmental variables related to the distribution of diatom taxa. Of these, temperature, salinity and TIN were the most important factors influencing community structure. Jasprica & Carić (2001) found that, in southern Adriatic coastal waters, the highest number of diatom

taxa depended on temperature and the lowest on phosphate. In our case, it seemed PO_4 had no important effect. This was also found in a coastal area of western South Adriatic (Caroppo et al. 1999).

CCA showed that *Proboscia alata*, *Pleurosigma macrum* and *Nitzschia longissima* were positively correlated with temperature and salinity. These taxa reached their maximum abundance in summer when the river discharge was low (Jasprica & Hafner 2005, Jasprica et al. 2005), and salinity is higher. It has been observed that *Nitzschia longissima* regularly blooms in August in the southern Adriatic coastal area (Jasprica unpublished data) and occurs in aggregates during summer mucilage events (Najdek et al. 2005). This taxon was also the most abundant in two natural hyperhaline (salinity > 40) marine lakes along Croatia's Adriatic coast (Carić et al. 2008). Higher abundances of some other "neritic" taxa (*Proboscia indica* and *Guinardia striata*) also coincided with a decrease of freshwater discharge. This phenomenon has been documented for Adriatic estuaries as well as for other areas, including San Francisco Bay (Cloern et al. 1983), Chesapeake Bay (Marshall & Alden 1990, 1993), Boston Bay and near-shore Spencer Gulf, South Australia (Paxinos 2007), and the Suwannee River estuary (Quinlan & Philips 2007).

Autumn taxa *Leptocylindrus minimus* and *L. danicus*, among others, showed a positive correlation with TIN. Jasprica & Carić (2001) found *Leptocylindrus* spp. in two moderately eutrophic bays over the whole year with the highest abundance in spring. On the contrary, *Leptocylindrus* has been reported as "summer" genus from the French coasts (Gailhard et al. 2002), and eutrophic Black Sea coastal area (Baytut et al. 2010). They are common in most waters but rarely a dominant species (Hasle & Syversten 1996).

The continuous presence of *Licmophora flabellata* and, occasionally, of a few other benthic taxa (*Cymbella* sp., *Eunotia* sp., *Striatella* spp. etc.), underlines the importance of wind-induced sediment resuspension, currents, and, to low extent, tides. Bottom hypoxia caused by low freshwater discharge in summer-autumn, low seawater inflow, strong vertical stratification, and decomposition of organic matter was not reflected in the distribution of diatoms. This result agrees with the findings of Lashaway and Carrick (2010) from the Great Lakes (USA), where diatom growth and physiology did not differ significantly between hypoxic and oxic areas.

Ciliated protozoans dominated microzooplankton. Abundances of non-loricate ciliates increased with those of nanoplanktonic phytoflagellates. Bojanić et al. (2005) found high positive correlation between the densities of non-loricate ciliates and nano-/pico-phytoplankton in Kaštela Bay (Middle Adriatic Sea). Diatoms are usually regarded as the most important food for copepods (Raymont 1983). Viličić et al. (1995 b) pointed out that the sharp decline of chain-forming summer diatom blooms in the coastal southeastern Adriatic might have been owed partly to higher grazing of small copepods. However, most of small copepods are omnivorous, and opportunistic feeding is main characteristic of their ecology (Turner 2008). Furthermore, results presented for copepods in this study are not representative due to the methodology applied. Therefore, in our study it is not easy to evaluate the existing relationship between diatoms and copepods.

In summary, although a monthly sampling strategy does not offer fine-grained resolution of the annual phytoplankton cycle, the classic seasonal diatom blooms (spring and autumn) do occur in the Neretva estuary. An additional bloom, one with the highest diatom peak, occurs in summer. A main feature of diatom assemblages is the dominance of more than one species during the blooms. The reason may be found in the frequent and rapid changes in environmental conditions typical of estuarine ecosystem.

Results presented herein deal only with the lower estuary. Comparable research in other parts of the estuary will be required for a more complete understanding of this complex system. These studies must include other important abiotic (e.g. irradiance, water renewal time) and biotic (e.g. bacterioplankton) factors not addressed in the present work. From a merely scientific standpoint, it will be intriguing to enumerate the number of diatom taxa present in the estuary. More practi-

cally, the quantification of their contribution to the flow of energy and cycling of material in the Neretva estuary will be useful for a rational management of this important regional resource.

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