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Periphytic diatom community in a Mediterranean salt wedge estuary: the Ebro Estuary (NE Iberian Peninsula)

LAIA ROVIRA*, ROSA TROBAJO, CARLES IBÁÑEZ

IRTA – Aquatic Ecosystems, Carretera Poble Nou KM 5.5, 43540, St. Carles de la Ràpita, Catalonia, Spain.

The Ebro River discharge is the main factor controlling hydrological dynamics in the Ebro microtidal salt wedge estuary. The aim of this study was to describe the species composition of the periphytic diatom communities, and to elucidate the main environmental factors affecting them. Samples of periphytic diatoms were collected at 8 sites along the estuary in October 2007 and January 2008. The diatom community was sampled both from natural and artificial substrata. Water depth, velocity, pH, dissolved oxygen, temperature, conductivity, total chlorophyll and water chlorophyll *a*, total periphytic chlorophyll, dissolved nutrients ($P\text{-}PO_4^{3-}$, $N\text{-}NO_3^-$, $N\text{-}NO_2^-$, $N\text{-}NH_4^+$, $Si\text{-}SiO_4^{4-}$), total dissolved nitrogen and total dissolved phosphorous were determined in each campaign. Altogether, more than 120 taxa of diatoms were identified. The most abundant genera were *Cocconeis*, *Amphora*, *Navicula* and *Tabularia*. The variability of the diatom community was analyzed with multivariate analysis methods. Water stratification affected diatom community in both the horizontal and the vertical gradient, according mainly to salinity, dissolved oxygen and nutrient concentration differences.

Key words: Diatom, estuary, salt wedge, periphyton, distribution, taxonomic composition, Ebro, Mediterranean, Spain

Introduction

Diatoms are valuable indicators of ecological quality: they respond directly and sensitively to many physical, chemical and biological changes in aquatic environment. They are found in almost all aquatic habitats and their high contribution to primary production has been pointed out by several authors. They also have among the shortest generation times of all biological indicators, allowing them to respond rapidly to environmental changes and to provide early warning of potential changes in nutrient status for different water bodies (ROTT 1991, STEVENSON and PAN 1999).

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* Corresponding author: laia.rovira@irta.cat

Despite the ecological importance of diatoms as a periphytic component, the knowledge of lentic communities is less than the knowledge of lotic periphytic communities. The scarcity of diatom studies in estuarine and transitional water bodies may be caused by the complexity of these systems (with high fluctuations among environmental parameters), but also due to the taxonomical difficulty of identifying estuarine diatom flora (TROBAJO et al. 2004). However, this fluctuating dynamics is interesting to study because it may help us to understand the responses of diatoms to environmental gradients. Nowadays these studies are focused mainly in tidal estuaries and there is a lack of knowledge of periphytic communities in stratified estuaries (with small tidal effect).

The Ebro Estuary is classified as a Mediterranean salt wedge or highly stratified estuary (IBÁÑEZ et al. 1997), with two completely different water layers and low tidal range (around 20 cm). The Ebro River discharge is the main factor that affects the dynamics of the salt wedge, but other factors like the topography of the estuarine bed must be also considered. The river discharge has been highly regulated by dams built since the 1960s, which decreased the annual Ebro River discharge and, therefore, increased the presence of the salt wedge. This stratification in two water layers has an ecological significance since biological communities in the estuary will receive either freshwater or saline water, depending on the dynamics of the salt wedge. The Ebro River has also great socioeconomic importance since it is the largest river in Spain and its flow is the water source for irrigation in the Ebro basin (IBÁÑEZ et al. 1996).

Although during the last 20 years a number or research projects concerned with the Ebro River and its estuary have produced a large amount of ecological and hydrological data of these systems (MUÑOZ and PRAT 1989, GUILLÉN and PALANQUES 1992, IBÁÑEZ et al. 1999, SIERRA et al. 2002, 2004, FALCÓ et al. 2006), there have been no studies on its periphytic diatom communities.

The aim of this preliminary study was to carry out the first description of the periphytic diatom community in the Ebro Estuary and to explore the main factors that may affect their distribution. This is an initial analysis as a part of a broader study of diatom community of Mediterranean estuarine systems.

Materials and methods

Study site

The Ebro River is the largest river in Spain. Its estuary covers an approximate area of 7 km^2 and it is considered a »micro-tidal salt wedge estuary«. Tides have little or no influence because of the low tidal range (around 20 cm), the Ebro River discharge being the main factor controlling the hydrological dynamics of this transitional system.

The Ebro Estuary is 30 km long with a mean depth of 6.8 m and a mean width of 237 m. The microtidal range (around 20 cm), favours the stratification of the water column and the existence of a salt wedge, with a maximum intrusion into the Ebro river channel of 32 km. This salt wedge disappears when the river flow is above 400 m^{3}s^{-1}. When the river discharge is between 400 and 300 m^{3}s^{-1}, the salt wedge can occupy the last 5 km of the estuary, but with discharges lower than 300 m^{3}s^{-1} the salt wedge advances quickly up to 18 km from the mouth. When the river discharge is less than 100 m^{3}s^{-1} , the salt wedge reaches its maximum extent (i.e. 32 km from the mouth) (IBÁÑEZ et al. 1997).

Sampling

Eight sampling points were established along the estuary (Fig. 1). The distance between sampling points was approximately 5 km. The first point was above the maximum extent of the salt wedge (E-3), thus it did not receive saline water, and the last point was located at the river mouth.

Every site was sampled in October 2007 and January 2008. Water depth, temperature, electrical conductivity (EC_{25}), dissolved oxygen (DO_2) and pH were measured in situ in all sampling sites with an YSI 556 multiprobe. Flow direction and velocity were also measured using a Braystoke BFM 001 current flow meter. Irradiance was measured with a QSP-2100 Submersible Scalar PAR Sensor.

Periphytic samples were collected from two different substrata: natural substratum (mainly macrophytes *Potamogeton pectinatus* and *Ceratophyllum* sp., but also wood debris where macrophytes were not available) and from artificial substrata (fired clay bricks, Fig. 2), to avoid variability due to substratum. A known area of periphyton (4 cm^2) was scraped with a brush from the artificial substrata in each replicate. Three fragments from natural substrata were included in each replicate. In order to study the effects of the salt wedge on diatom communities, bricks were placed in the superficial water layer (0.5 m water depth) and in the deep-water layer (which ranged from 2–8 m). Bricks were considered the most appropriate artificial substratum due to their resistance to high flows and to the sudden variations of flows that characterize the lower Ebro River. Unfortunately, due to this flow dynamics, in some sites, artificial substrata were not encountered. Two replicates from superficial and deep water bricks (when they were available) were processed. Two replicates from natural superficial substrata were also processed.

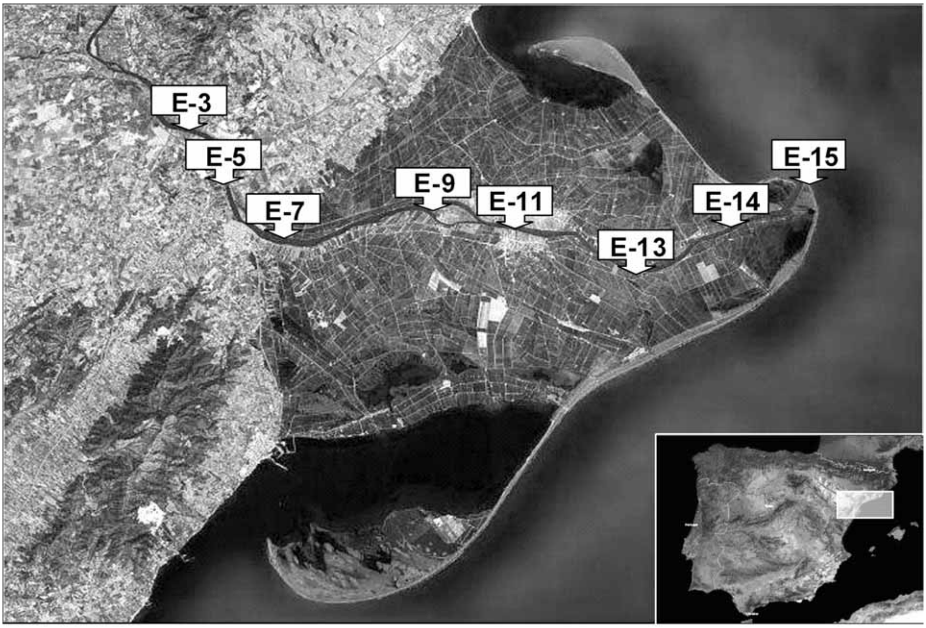

Fig. 1. Ebro estuary map showing the sampling points.

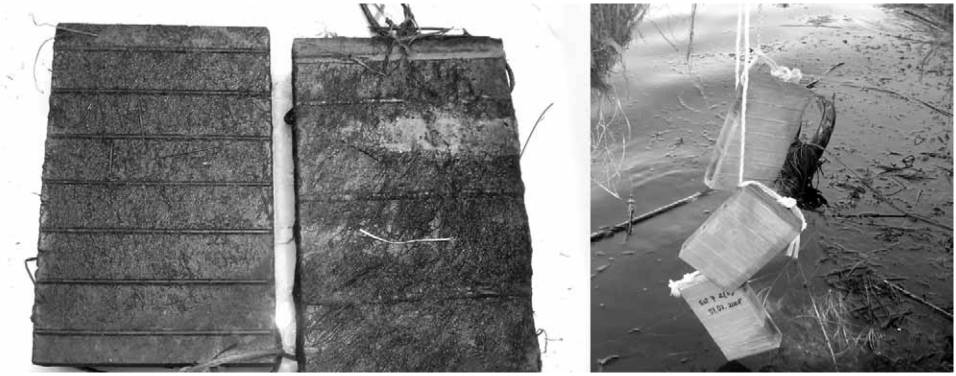

Fig. 2. Artificial substrata (fired clay bricks) used for diatom colonization both in superficial and deep-water layers.

Diatom identification and valve counting

The periphytic samples were cleaned of organic material using distilled water, H_2SO_4 and KNO_3 (Hustedt 1930). Clean valves were permanently mounted in Naphrax® (refractive index 1.74). The permanent slides were examined using a LEICA DMI 3000B light microscope equipped with differential interference contrast (DIC) with a 100 times oil immersion objective (n.a.=1.40). For the scanning electron microscopy (SEM) observations, the cleaned material was gold coated and studied under a JEOL – 6400 SEM.

The relative abundance of species was determined by counting a minimum of 400 frustules in each substratum replicate. Identification of diatoms to species level was based mainly on appropriate keys (Krammer and Lange-Bertalot 1991a, b; 1997a, b; Witkowski et al. 2000; Lange-Bertalot 2001).

Nutrient and chlorophyll analysis

Analysis of dissolved inorganic nutrients: silicate (Si-SiO_4^{4-}), nitrate (N-NO_3^-), nitrite (N-NO_2^-), phosphate (P-PO_4^{3-}); total dissolved nitrogen (TDN) and total dissolved phosphorus (TDP) were measured following Grasshoff et al. (1999), while ammonium (N-NH_4^+) was measured following the method proposed by the equipment manufacturer, ALLIANCE INSTRUMENTS, SA.

Total periphytic chlorophyll and water chlorophyll were extracted using 90% acetone and measured with a spectrophotometer using Jeffrey and Humphrey expressions (Rowan 1989).

Data analysis

Diatom community variation along the Ebro estuary was analysed with a correspondence analysis (CA) using CANOCO 4.5 version with diatom relative abundance. To avoid the effect of rare species, only diatom species with a relative abundance (RA > 0.2%) and present in more than 10% of the samples (total number of samples = 32) were included in the analysis. Relative abundance data was square-root transformed in order to reduce the effect of highly variable population densities on ordination scores. Relationships between

CA dimensions and environmental parameters were determined with Pearson correlations using the SPSS 15.0 software for Windows. Only correlations with $P < 0.05$ were considered. Environmental data (except pH, water depth and water velocity) were logarithmically transformed before analysis.

Results

Physical and chemical parameters

The average values for the water physicochemical parameters measured in October 2007 and January 2008 at each sampling point are shown (Tab. 1).

Mean monthly river discharge was 175.95 $m^3 s^{-1}$ in October and 110.41 $m^3 s^{-1}$ in January.

In both sampling periods the salt wedge was detected up to the E-9 site (Fig. 1). In October we found and sampled the limit of the salt wedge, whereas in January the salt wedge was detected further upstream (between E-7 and E-9 deep water layers). The superficial layer was in both sampling periods oligohaline; salinity ranged approximately from 0.70 to near 3.00, whereas in the deep-water layer salinity ranged from oligohaline (0.70–0.80) in E-3, E-5 and E-7 to euhaline (36.00–37.00) in E-9, E-11, E-13, E-14 and E-15.

Irradiance values were 37 $\mu E\ m^{-2} s^{-1}$ at 6 m depth and 1104 $\mu E\ m^{-2} s^{-1}$ at surface (October); in January values ranged from 44 $\mu E\ m^{-2} s^{-1}$ at 7 m depth to 1407 $\mu E\ m^{-2} s^{-1}$ at surface.

Dissolved oxygen concentration (DO_2) was lower in October. In both sampling periods, DO_2 concentration was lower in the salt wedge than in the superficial freshwater layer and E-9D showed the lowest DO_2 concentrations among all sampling points.

Nutrients were usually higher in the freshwater layer than in the salt wedge. It should be noted that for both sampling periods, E-9D presented the highest nutrient concentrations among all the salt wedge sites (except in the case of $N\text{-}NO_3^-$ in October 2007) and the lowest values for pH and DO_2 among all sampling points (considering both freshwater layer and salt wedge). E-9D also showed the highest values of conductivity, $P\text{-}PO_4^{3-}$, TDP and $N\text{-}NH_4^+$ among all sampling points in October.

Chlorophyll

The highest values of both water chlorophyll *a* and total water chlorophyll concentrations (Tab. 2) were found in the sampling points where the salt wedge was present (E-9, E-11, E-13, E-14 and E-15). The average minimum water chlorophyll *a* values were 0.87 $\mu g\ L^{-1}$ in the freshwater layer and 0.79 $\mu g\ L^{-1}$ in the salt wedge, both in January. Minimum water total chlorophyll values were 1.10 $\mu g\ L^{-1}$ in the freshwater layer and 1.24 $\mu g\ L^{-1}$ in the salt wedge, both in January. Periphytic total chlorophyll ($a + b + c$) was always higher in superficial samples than in the deep ones, independently of the presence of the salt wedge (Tab. 2).

Diatom community

Altogether, 122 diatom species were identified in the 32 analysed samples. The most abundant genera (considering all species) were *Cocconeis* (24%), *Navicula* (21%), *Nitzschia* (17%) and *Tabularia* (11%). *Navicula* was the genus with the highest number of taxa

Tab. 1. Water physicochemical parameters measured in October 2007 and January 2008. The negatives values in water velocity mean that water flowed in the opposite direction to river flow. S – superficial water layer, D – deep water layer

Sampling point	Water depth (m)	Temp (°C)	pH	DO$_2$ (mg/L)	Salinity (psu)	Conductivity (µS/cm)	P-PO$_4^{3-}$ (mg/L)	TDP (mg/L)	N-NH4 (mg/L)	N-NO2 (mg/L)	N-NO$_3^-$ (mg/L)	TDN (mg/L)	Si-SiO$_4^{4-}$ (mg/L)	Water velocity (m/s)
October 2007														
E-3S	0.2	22.8	8.2	7.1	0.72	1375.0	0.028	0.067	0.086	0.014	1.79	2.46	0.85	0.34
E-3D	1.0	22.8	8.2	6.9	0.72	1374.4	0.029	0.070	0.028	0.014	1.63	2.40	0.65	0.16
E-5S	0.2	22.9	8.3	7.9	0.72	1373.7	0.041	0.077	0.070	0.014	1.75	2.35	0.76	0.17
E-5D	7.0	22.8	8.2	7.0	0.72	1367.9	0.028	0.066	0.075	0.013	1.73	2.50	0.79	0.12
E-7S	0.2	22.9	8.3	8.1	0.72	1373.8	0.021	0.052	0.127	0.011	1.68	2.38	1.31	0.19
E-7D	3.0	22.9	8.3	8.1	0.72	1372.6	0.024	0.058	0.061	0.011	1.70	2.42	1.04	0.17
E-9S	0.2	23.0	8.4	8.9	0.73	1408.0	0.022	0.050	0.075	0.012	1.75	2.34	1.24	0.28
E-9D	4.0	23.7	8.0	2.2	35.41	52248.4	0.049	0.095	0.295	0.014	0.04	0.40	0.91	–0.03
E-11S	0.2	23.2	8.4	8.9	1.09	2053.9	0.022	0.049	0.081	0.012	1.65	2.34	1.87	0.31
E-11D	6.0	21.5	8.3	5.5	35.80	50474.1	0.013	0.057	0.080	0.002	0.05	0.19	0.22	–0.07
E-13S	0.2	22.8	8.3	8.6	1.59	2917.8	0.017	0.044	0.031	0.013	1.77	2.26	2.61	0.39
E-13D	7.0	22.3	8.3	6.4	36.25	51887.9	0.006	0.042	0.017	0.000	0.03	0.16	0.08	–0.05
E-14S	0.2	22.5	8.4	9.1	1.98	3577.3	0.013	0.043	0.073	0.012	1.68	2.56	2.52	0.45
E-14D	6.8	22.1	8.4	7.8	36.42	51823.3	0.003	0.039	0.024	0.000	0.02	0.14	0.63	–0.14
E-15S	0.2	22.3	8.3	8.5	2.89	5074.8	0.016	0.047	0.047	0.012	1.56	2.08	2.08	0.47
E-15D	4.5	21.9	8.4	8.2	36.00	51139.7	0.003	0.037	0.022	0.001	0.06	0.12	0.52	–0.23

Tab. 1. continued

Sampling point	Water depth (m)	Temp (°C)	pH	DO_2 (mg/L)	Salinity (psu)	Conductivity (µS/cm)	$P\text{-}PO_4^{3-}$ (mg/L)	TDP (mg/L)	N-NH4 (mg/L)	N-NO2 (mg/L)	$N\text{-}NO_3^-$ (mg/L)	TDN (mg/L)	$Si\text{-}SiO_4^{4-}$ (mg/L)	Water velocity (m/s)
January 2008														
E-3S	0.2	11.0	7.8	13.7	0.76	1105.9	0.050	0.064	0.024	0.019	3.49	3.77	0.99	0.27
E-3D	3.0	11.0	7.9	13.2	0.76	1105.6	0.038	0.061	0.024	0.018	3.51	3.75	1.06	0.18
E-5S	0.2	11.7	7.9	13.7	0.74	1093.9	0.029	0.055	0.104	0.018	3.54	4.07	0.87	0.12
E-5D	7.0	11.4	7.9	13.7	0.75	1098.5	0.030	0.046	0.045	0.017	3.50	3.87	0.80	0.10
E-7S	0.2	11.6	8.0	16.5	0.78	1142.9	0.021	0.036	0.022	0.017	3.34	3.70	0.92	0.15
E-7D	3.0	11.4	8.0	14.8	0.79	1156.4	0.015	0.038	0.019	0.017	3.46	3.74	1.10	0.11
E-9S	0.2	11.7	8.0	17.7	0.91	1335.4	0.027	0.045	0.007	0.021	3.53	3.93	1.34	0.27
E-9D	5.0	13.3	7.9	9.0	36.36	42631.2	0.029	0.054	0.089	0.015	0.13	0.27	0.45	0.00
E-11S	0.2	11.9	8.1	19.1	1.34	1932.9	0.020	0.045	0.027	0.022	3.69	3.86	1.05	0.29
E-11D	6.0	13.3	8.0	11.3	36.60	42892.7	0.009	0.020	0.042	0.008	0.09	0.14	0.16	0.00
E-13S	0.2	11.8	8.0	15.6	1.63	2322.8	0.026	0.050	0.031	0.023	3.45	3.81	0.77	0.35
E-13D	7.0	13.2	8.0	10.4	36.78	43053.6	0.004	0.015	0.038	0.006	0.09	0.13	0.10	–0.03
E-14S	0.2	11.8	8.1	20.5	1.84	2604.0	0.031	0.048	0.040	0.023	3.42	3.78	0.92	0.65
E-14D	8.0	13.3	8.0	10.6	37.34	43688.1	0.002	0.015	0.036	0.004	0.11	0.16	0.08	–0.06
E-15S	0.2	11.5	8.0	13.6	2.57	3547.3	0.037	0.051	0.049	0.023	3.24	3.70	0.66	0.55
E-15D	4.0	13.3	8.0	10.4	37.36	43776.1	0.001	0.012	0.029	0.003	0.08	0.14	0.05	–0.09

Tab. 2. Mean values of total periphytic chlorophyll, water chlorophyll *a* and total water chlorophyll measured in October 2007 and January 2008. The range values (minimum – maximum) for each sample are represented in brackets. Chl – chlorophyll, S – superficial water layer, D – deep water layer

Sampling point	Water depth (m)	Total periphytic chl (µg/cm^2)		Water chl *a* (µg/L)		Total water chl (µg/L)	
October 2007							
E-3S	0.20	2.80	(0.88–4.72)	1.47	(1.26–1.90)	2.35	(1.31–2.45)
E-3D	1.00	0.23	(0.20–0.26)	1.83	(1.48–2.31)	3.34	(1.75–4.49)
E-5S	0.20	2.63	(1.89–3.39)	2.00	(1.70–2.32)	3.57	(2.18–4.15)
E-5D	7.00	0.27	(0.22–0.32)	2.21	(2.14–2.24)	3.82	(2.82–3.21)
E-7S	0.20	7.07	(4.88–9.25)	2.19	(2.12–2.26)	4.14	(0.00–3.16)
E-7D	3.00	–	–	2.25	(2.04–2.36)	3.79	(2.44–3.26)
E-9S	0.20	0.40	(0.28–0.51)	3.19	(3.07–3.38)	5.12	(3.54–4.48)
E-9D	4.00	0.28	(0.21–0.36)	2.69	(2.72–2.73)	4.19	(3.01–3.62)
E-11S	0.20	7.88	(4.61–11.15)	3.42	(3.30–3.57)	5.23	(3.75–5.58)
E-11D	6.00	–	–	3.23	(3.19–3.31)	4.66	(3.83–4.07)
E-13S	0.20	–	–	2.65	(2.16–3.42)	4.99	(2.49–6.42)
E-13D	7.00	1.95	(1.35–2.56)	5.13	(4.90–5.24)	7.65	(5.97–6.43)
E-14S	0.20	–	–	2.40	(1.92–2.67)	5.28	(2.39–5.42)
E-14D	6.80	–	–	5.41	(5.22–5.67)	8.32	(6.51–7.33)
E-15S	0.20	–	–	2.62	(2.46–2.79)	4.71	(3.35–4.46)
E-15D	4.50	0.85	(0.77–0.93)	3.79	(3.65–4.07)	5.71	(4.39–5.53)
January 2008							
E-3S	0.20	5.87	(5.40–6.33)	1.09	(0.81–1.31)	1.69	(0.87–2.93)
E-3D	3.00	0.24	–	1.37	(0.92–2.07)	1.43	(0.84–2.20)
E-5S	0.20	–	–	1.08	(0.68–1.55)	1.59	(0.74–2.80)
E-5D	7.00	–	–	1.10	(1.04–1.24)	1.24	(1.03–1.65)
E-7S	0.20	–	–	0.87	(0.70–1.00)	1.10	(0.70–1.77)
E-7D	3.00	–	–	0.79	(0.06–1.19)	2.13	(1.60–2.66)
E-9S	0.20	–	–	2.44	(2.16–2.69)	3.19	(2.49–3.64)
E-9D	5.00	–	–	1.17	(1.02–1.33)	1.91	(1.44–2.37)
E-11S	0.20	3.72	(2.04–5.40)	6.21	(6.00–6.44)	7.87	(7.61–8.13)
E-11D	6.00	0.55	(0.45–0.65)	1.43	(0.90–2.26)	1.84	(1.17–2.87)
E-13S	0.20	8.71	–	4.32	(4.16–4.52)	5.48	(5.09–5.74)
E-13D	7.00	0.68	(0.64–0.71)	1.78	(0.80–3.43)	2.75	(0.79–5.87)
E-14S	0.20	2.16	–	4.34	(4.00–4.65)	5.11	(4.61–5.48)
E-14D	8.00	–	–	1.55	(1.02–2.09)	2.85	(1.44–4.27)
E-15S	0.20	–	–	3.40	(3.28–3.51)	4.14	(4.09–4.19)
E-15D	4.00	–	–	1.14	(0.70–1.37)	1.39	(0.70–1.44)

(20), followed by *Nitzschia* (13). The 45 diatom taxa with a relative abundance > 0.2% and present in more than 10% of the samples are listed in table 3. Species richness and Shannon-Wiener diversity Index for natural and artificial samples are shown in table 4. No major differences were observed in diatom community diversity between substrata type.

Tab. 3. Diatom taxa of all samples (ranged alphabetically) with relative abundance (RA) > 0.2% and present in more than 10% of the Ebro Estuary samples.

Diatom taxa	% RA
Achnanthes amoena Hustedt	1.29
Achnanthes minutissima Kützing	1.26
Amphora aff. *helenensis* Giffen	0.35
Amphora inariensis Krammer	0.99
Amphora lybica Ehrenberg	3.55
Amphora ovalis (Kützing) Kützing	0.37
Amphora pediculus (Kützing) Grunow	6.51
Amphora sp.1	0.45
Bacillaria paradoxa Gmelin	3.75
Cocconeis pediculus Ehrenberg	0.47
Cocconeis placentula Ehrenberg	23.00
Cyclotella meneghiniana Kützing	0.48
Fallacia sp.1	3.33
Gomphonema cf. *Olivaceum* (Hornemann) Brébisson	0.33
Gomphonema clevei Fricke	0.29
Melosira varians Agardh	0.32
Navicula aff. *mollis* (W. Smith) Cleve	1.01
Navicula antonii Lange-Bertalot	1.40
Navicula capitatoradiata Germain	0.25
Navicula cryptotenella Lange-Bertalot	1.96
Navicula aff. *cryptotenelloides* Lange-Bertalot	0.50
Navicula gregaria Donkin	0.83
Navicula cf. *margalithii* (Lange-Bertalot)	0.91
Navicula perminuta Grunow	4.47
Navicula recens (Lange-Bertalot) Lange-Bertalot	2.76
Navicula cf. *subminuscula* Manguin	0.52
Navicula sp.1	0.57
Navicula tripunctata (O.F. Müller) Bory	0.36
Nitzschia amphibia Grunow	0.58
Nitzschia constricta (Kützing) Ralfs	0.41
Nitzschia dissipata (Kützing) Grunow	3.66
Nitzschia filiformis (W. Smith) Van Heurck	1.63
Nitzschia cf. *fonticola* (Grunow) Grunow	0.33
Nitzschia cf. *frustulum* (Kützing) Grunow	4.22
Nitzschia cf. *inconspicua* Grunow	2.89
Nitzschia microcephala Grunow	0.32
Nitzschia palea (Kützing) W. Smith	1.26
Nitzschia cf. *palea* (Kützing) W. Smith	1.36
Nitzschia prolongata Hustedt	0.67
Nitzschia cf. *sociabilis* Hustedt	0.26
Pleurosira laevis (Ehrenberg) Compère	0.61
Rhoicosphenia abbreviata (Agardh) Lange-Bertalot	5.86
Stephanodiscus aff. *alpinus* Hustedt	0.21
Synedra ulna Ehrenberg	0.33
Tabularia fasciculata (Agardh) Williams et Round	6.94

Diatom community changed along the horizontal and vertical gradients (Fig. 3). The diatom community of the natural substrata in October 2007 (Fig. 3A) was dominated by *Cocconeis placentula* Ehrenberg (Figs. 4A, B). *Bacillaria paradoxa* Gmelin (Fig. 4H) and *Tabularia fasciculata* (Agardh) Williams et Round (Figs. 4C, D) also appeared as abundant species in most sites (E-3 to E-13). There was a change in diatom community structure in E-14, *Navicula perminuta* Grunow (Fig. 4N) being the dominant species and *Rhoicosphenia abbreviata* (Agardh) Lange-Bertalot (Figs. 4F, G) abundant. Another change occurred in E-15, where *Fallacia* cf. *clepsidroides* Witkowski (Fig. 4J) and *Nitzschia* cf. *inconspicua* Grunow (Fig. 4K) dominated the sample. In January 2008 (Fig. 3B), the dia-

Tab. 4. Species richness and Shannon–Wiener Index for natural and artificial samples in October 2007 and January 2008.

October 2007	Species richness	Shannon–Wiener Index
Natural samples	42	3.03
Artificial samples	34	3.22
January 2008	**Species richness**	**Shannon–Wiener Index**
Natural samples	40	3.61
Artificial samples	42	3.73

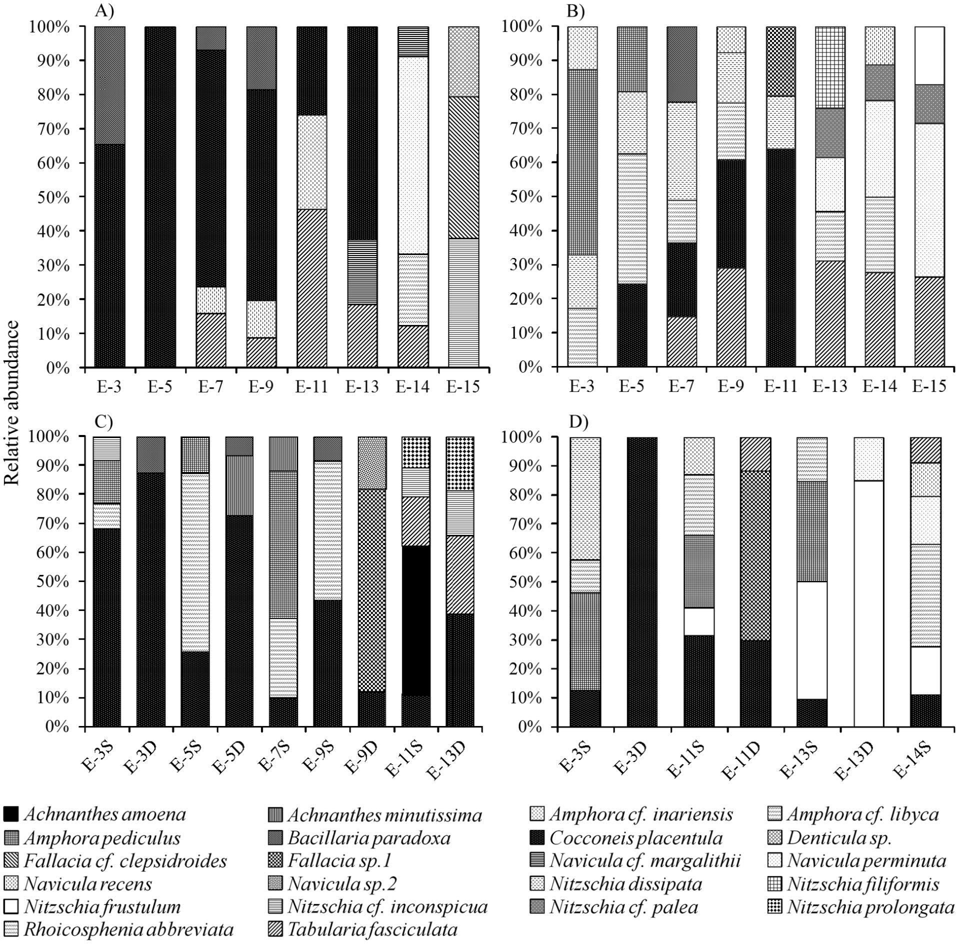

Fig. 3. Diatom community of the Ebro Estuary. A) natural substrata in October 2007, B) natural substrata in January 2008, C) artificial substrata in October 2007, D) artificial substrata in January 2008. Natural substrata were only collected at superficial level. S – superficial water layer, and D – deep water layer. Only diatom taxa with a relative abundance > 0.1 are shown

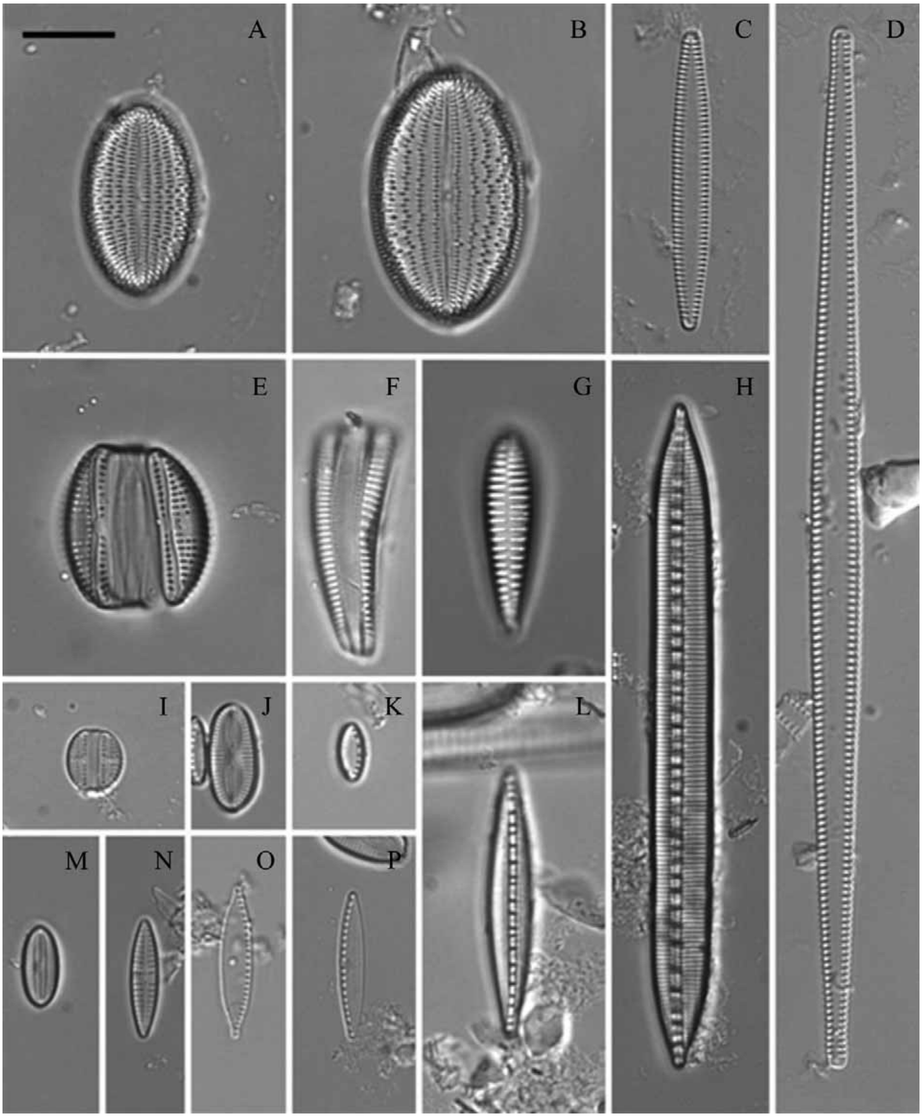

Fig. 4. Representative diatom taxa from the Ebro Estuary. **A, B**: – *Cocconeis placentula* Ehrenberg; **C, D** – *Tabularia fasciculata* (Agardh) Williams et Round; **E** – *Amphora libyca* Ehrenberg; **F, G** – *Rhoicosphenia abbreviata* (Agardh) Lange-Bertalot; **H** – *Bacillaria paradoxa* Gmelin; **I** – *Amphora pediculus* (Kützing) Grunow; **J** – *Fallacia* cf. *clepsidroides* Witkowski; **K** – *Nitzschia* cf. *inconspicua* Grunow; **L** – *Nitzschia dissipata* (Kützing) Grunow; **M** – *Fallacia* sp.1; **N** – *Navicula perminuta* Grunow; **O** – *Nitzschia* cf. *palea* (Kützing) W. Smith; **P** – *Nitzschia* cf. *frustulum* (Kützing) Grunow. Scale bar denotes 10 µm.

tom community was more diverse than in October. In this period a change in the diatom community along the estuary was also observed. In E-13, *Cocconeis placentula* and *Nitzschia dissipata* (Kützing) Grunow (Fig. 4L) drastically decreased and other diatom taxa became representative, like *Navicula perminuta*, *Nitzschia* cf. *palea* (Kützing) W. Smith (Fig. 4O) and *Nitzschia* cf. *frustulum* (Kützing) Grunow (Fig. 4P), among others.

The diatom community of the artificial substrata in October (Fig. 3C), showed a clear change in E-9D (the limit of the salt wedge), where C*occoneis placentula*, *Amphora libyca* Ehrenberg (Fig. 4E) and *Amphora pediculus* (Kützing) Grunow (Fig. 4I) decreased; and *Fallacia* sp.1 (Fig. 4M) clearly dominated the sample. Unfortunately, several artificial substrata could not be recovered in the January campaign and thus only few data were available (Fig. 3D).

Factors affecting diatom distribution

Results of the correspondence analysis (CA) show that the first dimension of the CA (DIM1) explained 24.6% of diatom community variability, and seems to differentiate the upstream points (with no salt wedge) from those closer to the sea (Fig. 5). This dimension was significantly and negatively correlated with phosphorous (TDP) and positively with water velocity and salinity (Tab. 5). The second CA dimension (DIM2) explained 13.5% of variation and differentiated E-9O (deep layer, October) from the rest of samples. It was significantly and positively correlated with salinity and water temperature; and negatively with oxygen (DO_2), and nitrogen ($N\text{-}NO_3^-$ and TDN).

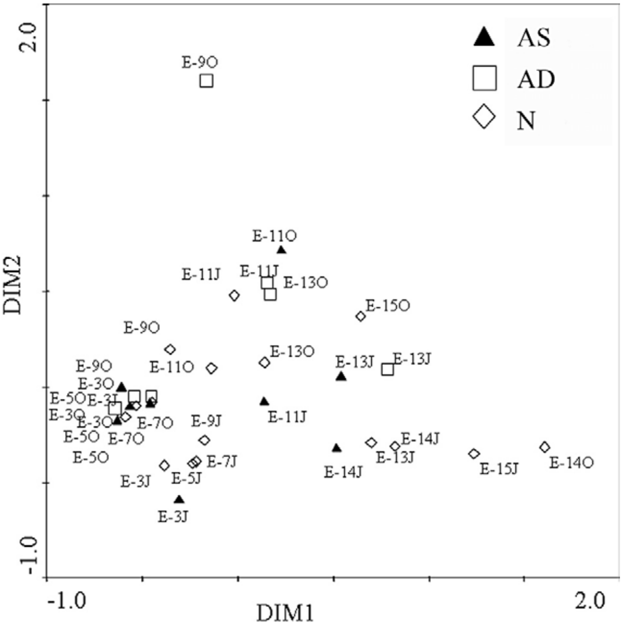

Fig. 5. Sample ordination in the plane defined by the two first CA dimensions. O = October, J = January. Only diatom taxa with a relative abundance (**RA**) > 0.2% and present in more than 10% of the samples are included in the analysis. **AS** – artificial superficial samples, **AD** – artificial deep samples, **N** – natural samples (only collected at superficial level).

Tab. 5. Pearson correlation coefficients between CA dimensions and environmental parameters. Only significant correlations at $P < 0.05$ and with a Pearson correlation coefficient higher than 0.4 are listed.

Environmental parameters	DIM 1	DIM 2
% explained variance	24.6	13.5
Salinity (ppt)	0.431	0.539
TDP (mg L)	–0.465	–
DO_2 (mg L)	–	–0.601
$N\text{-}NO_3^-$ (mg L)	–	–0.609
TDN (mg L)	–	–0.510
Water velocity (m s^{-1})	0.477	–
Water temperature (°C)	–	0.404

Discussion

Water chlorophyll values found in this study were lower than those reported in previous Ebro Estuary papers. The minimum total chlorophyll (*a* + *b* + *c*) values in July 1991 were 21.5 µg L^{-1} in the freshwater layer and 3.2 µg L^{-1} in the salt wedge (IBÁÑEZ et al. 1995). From 1989 to 1992 in the freshwater layers the minimum chlorophyll *a* values were 20 µg L^{-1} and 7 µg L^{-1} in the salt wedge (CASAMAYOR et al. 2001); and in July 1999 the minimum chlorophyll *a* values were around 9 µg L^{-1} in the freshwater layer and 2 µg L^{-1} in the salt wedge (FALCÓ et al. 2006). This decrease in phytoplankton has been attributed to a decrease in riverine phosphorous (IBÁÑEZ et al. 2008), which has allowed light to reach the salt wedge and probably has permitted periphytic communities to become established. In July 1989, the light intensity was practically zero at 4.8 m depth, below the interface (CASAMAYOR et al. 2001). In our study, the light reached the river bed (6 m depth in October and 7 m depth in January). In addition, light also reached the river bed in April 2008 (10 m) and July 2008 (7 m), thus the increase in water transparency was not a sampling period effect. However, due to the lower light intensity, the periphytic total chlorophyll concentrations in deep substrata were considerably lower than those found in superficial substrata.

This study allowed identification of the importance in terms of abundance of some periphytic diatom species in the Ebro Estuary, like *Cocconeis placentula,* and the need to study it at infraespecific level in further studies, because different varieties may have different ecological responses. Some of the most representative diatom species found in the Ebro estuary (*Cocconeis placentula* in freshwater layers, *Navicula perminuta* in saline layers, *Amphora pediculus* or *Rhoicosphenia abbreviata*) are not found in the same proportion and/or occurrence in other estuarine studies (MCINTIRE and OVERTON 1971, MOORE and MCINTIRE 1977, MCINTIRE 1978, UNDERWOOD 1994, NAYAR 2005). The Ebro Estuary has specific characteristics (high stratification, irregular and sudden salt wedge intrusions) that are not met in other estuarine systems studied (e.g. Atlantic estuaries, fiords), and these differences could explain differences in diatom flora.

The diatom community structure changed along the Ebro Estuary. A change in diatom community structure was observed in points closer to the sea (from E-13 to E-15) with higher salinity. In these points the marine and riverine influences can be both strong and they change depending on the river flow, sea storms and winds, resulting in a more dynamic situation at superficial layers. Fluctuating conditions have been previously reported as an important factor affecting diatom community structure, diversity and composition of such environments (SULLIVAN 1978, UNDERWOOD 1994, TROBAJO et al. 2004).

Another important change in the diatom community was found in E-9D in October. This point was the limit of the salt wedge, the interface between fresh and saline water. In this zone the water could remain still at the bottom of the river for a long period. It may well be that the decomposition of organic matter promoted the oxygen depletion found in October. However, the observed oxygen depletion did not reach anoxic conditions that were previously recorded (IBÁÑEZ et al. 1995, CASAMAYOR et al. 2001, FALCÓ et al. 2006). In January, due to the lower river flow, the limit of the salt wedge had to be further upstream (between E-7 and E-9), and unfortunately it was not sampled.

Artificial substrata should allow the comparison of diatom communities from superficial and deep layers, avoiding substratum variability. Several investigations had used artificial substrata to study periphytic diatom communities in estuaries and other transitional systems (MCINTIRE and OVERTON 1971; MCINTIRE 1973, 1978; MOORE and MCINTIRE 1977; LAI 2001; TROBAJO et al. 2004; NAYAR et al. 2005). The CA analysis did not clearly segregate the samples according to the type of substrata (natural vs. artificial). Therefore, it seems that the periphytic diatom community in the Ebro Estuary is more affected by the environmental conditions (mainly salinity, dissolved oxygen, nutrient concentrations) than by the substrata type. Similar results were found by SNOEIJS (1994) in the study of epiphytes from the Baltic Sea, indicating that epiphytic diatom community composition was more affected by environmental parameters than by macroalgal hosts.

The preliminary hypothesis would indicate that diatom community in the Ebro Estuary is determined by salt wedge intrusions, which cause high and irregular fluctuations of salinity and nutrient concentrations. The diatom community could be also affected by the water residence period, which could cause the oxygen decrease observed from the river mouth to the limit of the salt wedge. These initial results suggest that the factors affecting the diatom community and its distribution in the Ebro Estuary are salinity, phosphorous, nitrogen, oxygen, water temperature and water velocity. The results show a longitudinal variation correlated with salinity and inversely with phosphorous (TDP), and as we expected it could be related to the presence of the salt wedge, which causes a system shift. The highest water residence period was reached in the limit of the salt wedge intrusion. The particular conditions in this site (high salinity, low oxygen and nitrogen depletion) also affected diatom community composition and structure.

Therefore, it seems that the salt wedge dynamics (not only vertical and longitudinal salinity gradients but also the magnitude and frequency of salinity oscillations) have an influence on the periphytic diatom composition of the Ebro Estuary and have to be taken into account in further studies. Estuarine dynamics is different in more mixed estuaries where salinity gradients and salinity changes are more regular and predictable (due to tidal circulation).

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