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Picocyanobacteria distribution in the Ebro Estuary (Spain)

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The stratified estuary of the Ebro River is located on the Mediterranean coast of Spain. From samples obtained in 6 sampling campaigns between July 1999 and February 2000 from the last 18 km of Ebro River, the abundance of picocyanobacteria was estimated by epifluorescence microscopy. The abundance of picocyanobacteria reached 93.7 x10^6 cells L^{-1} in the river mouth (station R1) below the surface, in October 1999. In deeper layers of salt wedge area stations (near the river mouth), we could observe higher concentrations of marine cyanobacteria. This study offers comments about the abundance and distribution in cyanobacteria.

Key words: Phytoplankton, picoplankton, cyanobacteria, abundance, Ebro, estuary, Spain.

Introduction

Estuaries in areas with a small tidal range are usually highly stratified, with a low salt wedge. In recent years picoplankton (0.2–2 μm), composed of minute chroococcoid cyanobacteria and eukaryotic phytoplankton, have received attention in estuarine phytoplankton studies (RAY et al. 1989, IRIARTE 1993, SIN et al. 2000).

The significant contribution of picophytoplankton, of at least 10 %, to total global aquatic net primary productivity (RAVEN 1998) confirms their importance as an integral component of the microbial food web (SIEBURTH et al. 1978, CALLIERI and STOCKNER, 2002). The planktonic ciliates can probably exploit the autotrophic picoplankton encountered in oceanic waters (CHRISTAKI et al. 1999).

The succession dynamics of phytoplankton in the lower part of Ebro River have been extensively studied by SABATER and MUÑOZ (1990) who found 11 taxa of Cyanobacteria but no minute chroococcoid algae. Other investigations concerning phytoplankton were performed in the Ebro delta (COMIN 1984) and bays (LÓPEZ and ARTÉ 1973, DELGADO 1987, DELGADO et al. 1990, 1995, 1996).

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In the framework of the PIONEER European Project in the Ebro river estuary, phytoplankton monitoring was performed at several points covering the water column. The results presented in this paper are part of a more extensive study on phytoplankton ecology in the lower Ebro River and the aim of this work was to establish the abundance and distribution of picocyanobacteria. The main physical-chemical variables during the period are also presented.

Material and methods

Study area

The Ebro River has a drainage area covering 88,835 km^2 of the NE part of the Iberian Peninsula. This is one of the main rivers in Spain, with a length of 960 km, and an important flow (IBÁÑEZ et al. 1999). Our study area comprises the lower part of the river, approximately the last 15 km from Isla de Gracia to its mouth in the Mediterranean Sea (Fig. 1) with mean values of depth and width of 6.8 and 237 m respectively. The Ebro delta is classified as a »salt wedge estuary« or type 4 of the Hansen-Rattray classification (IBAÑEZ et al. 1997). It has a strong and clearly marked halocline because of its insignificant tide regime and its circulation is affected by the river discharges primarily. The annual discharge (mean = 424 m^3 s^{-1}) distribution is smoothed by the presence of about 170 dams according to IBÁÑEZ et al. (1999). These dams condition the suspended inert solids concentration (usually low) and the phytoplankton populations. In particular, the Mequinenza and Riba-Roja dams located 100 km upstream of the mouth, have an important regulatory effect on the discharges on the delta. A mean annual discharge of 10000 Hm^3 (variations between 5000 and 14000 Hm^3) is estimated.

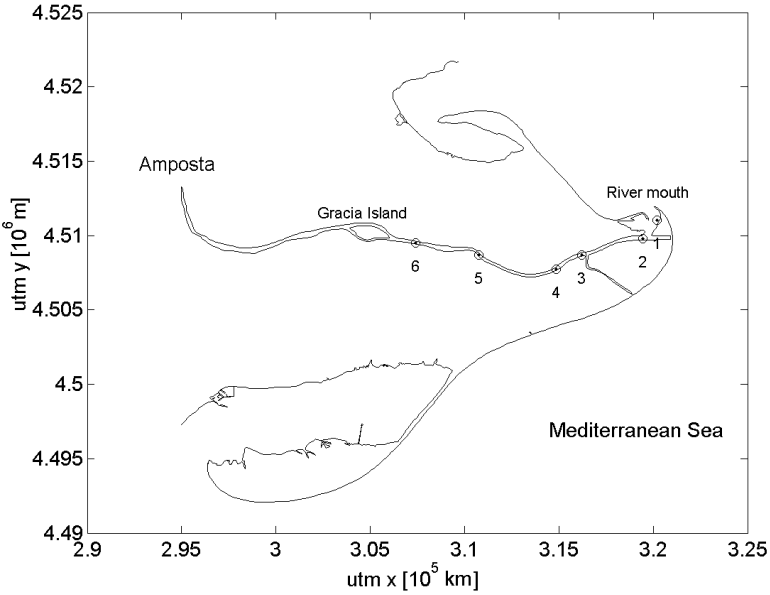

Fig. 1. Map of the lower Ebro River showing the sampling stations.

In the Ebro delta, rice crops and cultivated fields alternate with coastal lagoons. The region has a dry Mediterranean climate with a mean annual temperature around 16–17 °C, with around 550 mm of precipitation per year (MARTÍNEZ et al. 1999).

Sampling and measurements

Water samples were taken from the water column at six sampling stations (Fig. 1) in July and October 1999 and February 2000, below the scope of the PIONEER European Project. At each station approximately 10 water samples at different depths were taken. Four points were located at fixed depths (surface, 1.5, 3 and 4.5 m depth) and 6 points at the freshwater-seawater interface depth, which varied depending on the sampling station.

In order to locate the freshwater-seawater interface a multiparametric sounding Hydrolab Surveyor 3 was used. This sounding records a vertical profile each 0.10 m accounting for the following parameters: depth, conductivity, temperature and pH. Samples were taken using a pump whose hose was located at the depth selected by Hydrolab. Salinity was measured with a Grundy Environmental Systems Inc. 6230 N induction conductometer.

Phytoplankton samples for qualitative and quantitative analyses were collected with bottles at each depth. The material was fixed *in situ* with glutaraldehyde (2% final concentration) according to SOURNIA (1978). A slide was prepared from each sample by filtering 10 or 20 ml of water, depending on phytoplankton concentration, onto 0.2 μm (pore size) Millipore membrane filters. The cell counts were made by epifluorescence microscopy (VARGO 1978) using a Nikon Optiphot microscope equipped with a mercury lamp, using 100x oil-immersion objective. Under blue light excitation, cyanobacterial cells fluoresced yellow-orange. A minimum of 300 cells were counted and at least 100 cells of the species more abundant were counted with an error lower to 20% (LUND et al. 1958).

Results

Hydrobiological data

The water temperature varied between 26.5 °C to 27.2 in July, 21.9 to 23.4 in October and between 10.6 to 12.3°C in February (Fig. 2B). Salinity in the estuary ranged from <0.03 at the head to 36.5 close to the river mouth, saline stratification on the water column was observed almost for every sampling day in all the campaigns. Figure 2A shows position of the interface in July, October and February. Only on February 3, 2000, stratification was not found, due to the high discharge of the river which was over 300 m^3s^{-1}, pushing the salt wedge out of the estuary. During winter, when the Ebro river flow is large, the lowest salinity water was recorded at the mouth and during summer the value was 10.3 just in the boundary of the river water and the mixing layer. This salinity distribution reflects the distance to which the saline wedge can penetrate due to the local sources of freshwater. The pH values were generally within the range 8–8.3, which is lightly alkaline. In the whole estuary, the maximal values of salinity were found in the lower layer (37.16 < salinity < 29.74), while the lower values were found in the superficial layer (4.89 < salinity < 2.85).

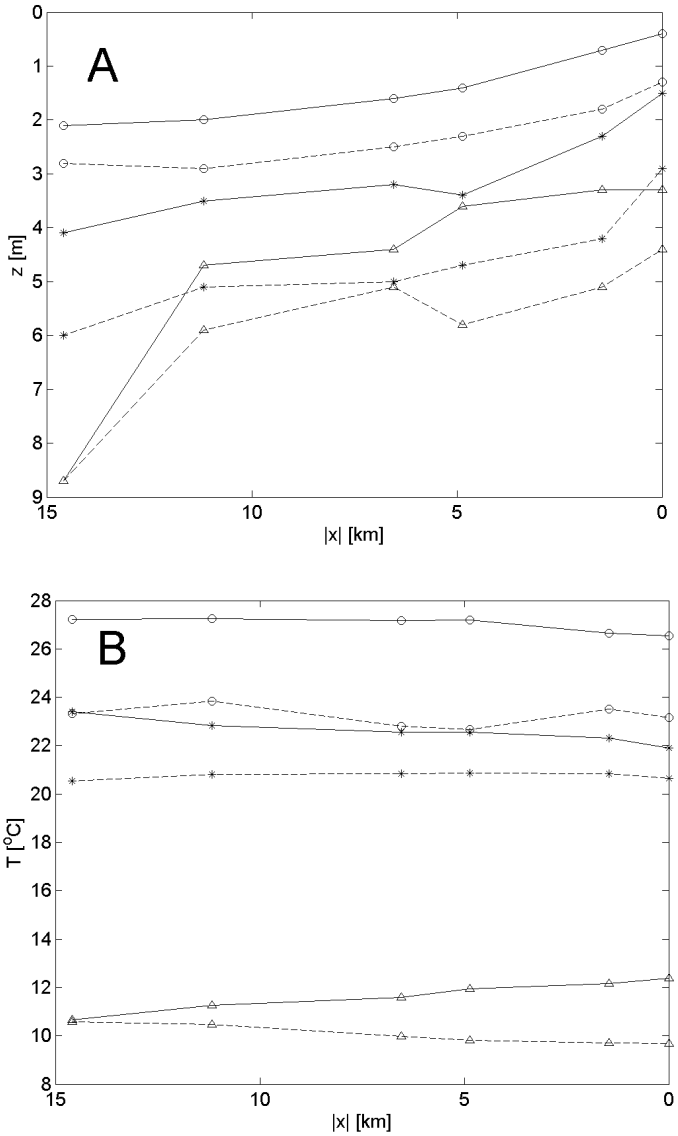

Fig. 2. A) Beginning (-) and end (—) of the interface in the last 15 km of the Ebro River from July (o), October (*) and February 5(Δ), note that the saline wedge was not found in February 3. B) Maximum and minimum temperatures from the water column in the same period, lower temperatures were measured in saline water (37.16 < salinity < 29.74), while highest were in the superficial river water (4.89 < salinity < 2.85).

Phytoplankton abundance and composition

Clear differences in the phytoplankton vertical distribution have been detected in the interface area and also in the salt wedge at the Ebro river estuary. The distribution of phytoplankton cells was highly dynamic during this study period.

The phytoplankton community was composed of about 300 taxa consisting mainly of diatoms and green algae (PÉREZ et al. in preparation). The total phytoplankton counts by epifluorescence microscopy indicate that the maximal recorded values were $53.6x10^6$ cells L^{-1} in July, $106.5x10^6$ cell L^{-1} in October and $29.0x10^6$ cell l^{-1} in February 2000 (Fig. 3). The highest values of total phytoplankton agree with the maximum abundance of picocyanobacteria (Figs. 3, 5).

A picocyanobacteria population with a species very probably belonging to *Cyanobium* sp. was dominant in all samples with high salinity with a direct relationship proportional to 10^6 (Fig. 4); they fluoresced yellow-orange and were ~0.8–1.6 μm in diameter. These marine picoplanktic cyanobacteria reached their highest concentrations in the deepest zone near the river mouth with $93.7x10^6$ cells L^{-1} (station R1, Fig. 1) at a depth of 3 m on October 6, 1999 (Fig. 5).

Discussion

The distribution of picocyanobacteria in the Ebro Estuary was heterogeneous during this study period and dependent on two mean factors: river discharge, regulated by several dams, and salt water entry from Mediterranean Sea.

IBAÑEZ et al. (1997) defined the stratification and affirmed that the salt wedge of the Ebro delta is pushed out and the estuary becomes a river with a flow higher than 300 m^3s^{-1}.

The recorded densities of picocyanobacteria are similar to those of other estuarine Mediterranean environments (Tab. 1). In July 1996 JACQUET et al. (1998) recorded $43x10^6$ cells L^{-1} on average and densities up to $70x10^6$ cells L^{-1} in the Bay of Villefranche-sur-mer. Similar and higher concentrations were observed in Thau lagoon, NW Mediterranean Sea, (VAQUER et al. 1996) and Varano lagoon, Adriatic Sea (CAROPPO 2000) (Tab. 1).

Higher densities were observed in summer of 1995 in the Central Baltic Sea by ALBERTANO et al. (1997), in July and August 1995. Lower and higher densities were also observed in San Francisco Bay (NING et al. 2000) (Tab. 1). On the other hand, low concentrations or no cells were recorded at the surface upstream of the Ebro estuary (Fig. 5). At the deepest points (salt wedge area), we can observe the highest concentrations of this marine picoplanktic cyanobacteria in the stations located near the mouth

The traditional cyanoprokaryotic genus *Synechococcus* Nägeli includes several species, as picoplanktic organisms live as solitary cells (KOMÁREK and ANAGNOSTIDIS 1998, KOMÁREK et al. 1999). In according with KOMÁREK (1996), picoplanktic cyanoprokaryotic organisms are very simple and their identification on the basis of morphological features alone is impossible. The picoplanktic species are usually designated in almost all papers as »*Synechococcus* sp.« and probably the oceanic picoplanktic populations described by several authors, belong to the genus *Cyanobium* sp., and it is necessary to make DNA analyses to identify the species with security (KOMÁREK 1996, KOMÄREK et al. 1999). In addition, KOMÁREK (1996) postulated that the taxonomic classification of simple picoplanktic cyanobacteria has to be based on a combination of cytomorphological, ecophysiological and molecular-biological criteria.

Temperature, light and inorganic nutrients availability are the main factors that control picophytoplankton distribution, biomass and growth, and the relative abundances of each picophytoplankton group. Differences in temperature tolerance for example, may be the key factor explaining the distributional pattern of the different picophytoplankton groups (AGAWIN et al. 1998).

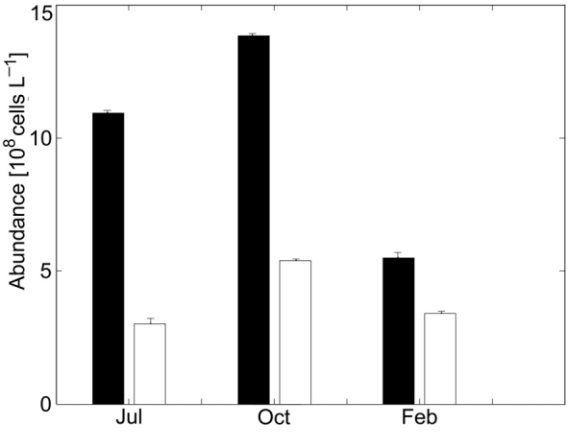

Fig. 3. Abundance of total phytoplankton (solid) and the total picocyanobacteria (white) in July, October and February, in the Ebro Estuary.

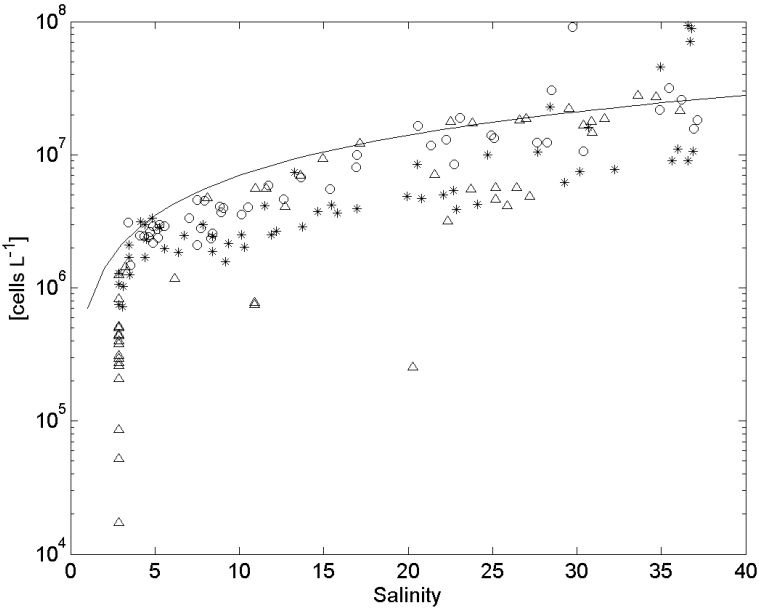

Fig. 4. The relationship between picocyanobacteria abundance and salinity in July (o), October (*) and February (Δ) in the Ebro Estuary.

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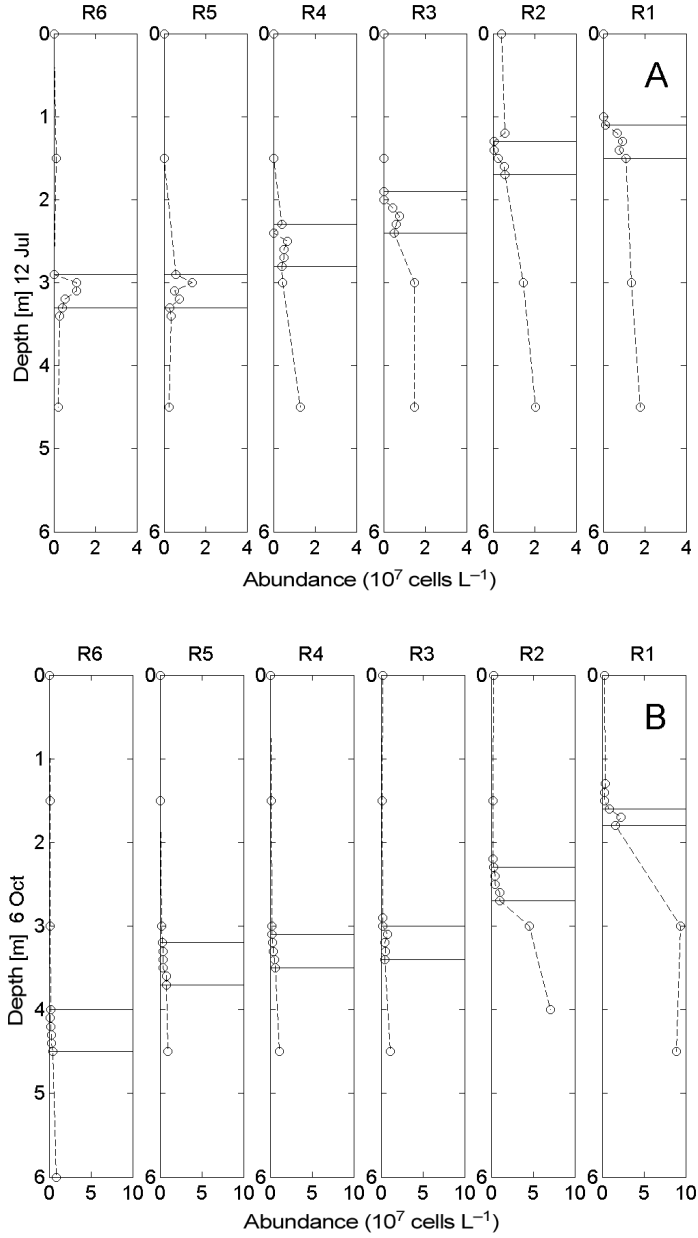

Fig. 5. Picocyanobacteria abundance from July (A) and October (B) in the R1 to R6 fixed stations. Horizontal lines represent the depth of the beginning and end of the salinity interface in each profile.

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Tab. 1. Picocyanobacteria abundances from different geographical locations. (DCM = deep chlorophyll maximum, PPP= picophytoplankton).

Site	Location	Abundance cells L^{-1}	Species	Depth	Date	Reference
Barcelona – Baleares Is.	NW Mediterranean Sea	1.7–13 $x10^6$ 4 – 175 $x10^6$	*Synechococcus* *Synechococcus*	surface DCM	summer 1995	AGAWIN and AGUSTI 1997
Bay of Villefranche-sur-mer	NW Mediterranean Sea	43 $x10^6$ (mean)	*Synechococcus*	surface	July 1996	JACQUET et al. 1998
Thau lagoon	NW Mediterranean	3.5 $x10^7$ (mean)	PPP	surface	Nov. 1991 – Febr. 1994	VAQUER et al. 1996
Varano lagoon	W Adriatic Sea	0.7 – 448.6 $x10^6$	PPP	surface and bottom	March 97 – Febr. 98	CAROPPO 2000
Baltic Sea	central area	4.3 – 6.7 $x10^8$ 1.8 – 59$x10^8$	*Synechococcus* *Synechococcus*	different depths different depths	July 1995 August 1995	ALBERTANO et al. 1997
San Francisco Bay	Pacific Ocean	0.046 – 5.2 $x10^8$	*Synechococcus*		April – August 1998	NING et al. 2000
Mauritanian coast (about 400 km offshore)	NE tropical Atlantic	2.5 – 4 $x10^8$	*Synechococcus*	0–30m	June 1992	MOREL 1997.
Central North Pacific	Central North Pacific	5.8 $x10^7$ (max.) 105–10^6	*Synechococcus* *Synechococcus*	20 m–near 42 °N surface – subtropical gyre	22 August – 28 October 1990	ISHIZAKA et al. 1994
Falkland Is. – British Is.	Atlantic Ocean	225 $x10^6$ (max.)	*Synechococcus*	surface (7m) – 40–50 °N	22 April – 26 May 1997	ZUBKOV et al. 2000

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