Chlorophyll *a* and Phytoplankton Maximum at the Halocline of Ebro River Estuary

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ABSTRACT

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The Ebro River flows into the Mediterranean coast of Spain and its last stretch behaves most of the time as a highly stratified estuary. Four field campaigns were carried out during years 1999-2000 to study water quality within the estuary. In this paper, the results of two of these field campaigns are shown. These results are based on the development of a new sampling technique, which allows obtaining samples at the halocline with a high resolution. As a consequence, concentration peaks for different nutrients (ammonium, phosphorus, and orthosilicic acid), chlorophyll *a* and some phytoplankton groups could be observed at the interface area. In the summer samplings, chlorophyll *a* peaks showed two different patterns at the halocline along the estuary. At the stations located close to the estuary head, these peaks were always found in the shallower zone of the interface, above of the observed peaks of ammonium, soluble reactive phosphorus (SRP) and orthosilicic acid. This chlorophyll accumulation in the shallower zone seems to come from surface layer phytoplankton settling, being temporally retained there due to the sharp increase of fluid density. The second pattern is observed close to the mouth, where chlorophyll *a* peaks spatially coincide with those of nutrients or even are located below these, suggesting a growing zone due to nutrient abundance. Phytoplankton counts confirm this hypothesis since peaks of multicellular prokaryotes, diatoms and chlorophyceae are observed at the halocline, with greater densities in the freshwater layer than in the saltwater one, indicating that this accumulation comes from the surface layer. Nevertheless other groups such as unicellular prokaryotes and cryptophyceae presenting larger concentration at the halocline deeper layer than in the shallower one seem to employ the interface as a proliferation zone taking advantage of the more favourable nutritive conditions due to mineralization occurring there.

ADDITIONAL INDEX WORDS: *Nutrient, stratified-estuary, freshwater-seawater interface.*

INTRODUCTION

In coastal microtidal environments, estuaries are usually highly stratified, with a salt wedge clearly distinguished from freshwater (DYER, 1991). Between these two layers is placed the halocline, which presents strong and highly variable gradients of physical and chemical properties (ZUTIC & LEGOVIC, 1987; CAUWET, 1991; FUKS *et al*., 1991; LEGOVIC *et al*., 1994; 1996). These factors directly or indirectly influence phytoplankton species composition, production and mortality (MUYLAERt & SABBE, 1999). Several authors have found thin layers with high chlorophyll concentrations (DENANT *et al*., 1991) and high phytoplankton densities (VILICIC *et al*., 1989, 1999) around the saltwater-freshwater interface of this type of estuaries. These thin layers have also been observed in a wide variety of marine systems including estuaries (DONAGHAY *et al*., 1992), coastal shelves (COWLES & DESIDERIO, 1993), fjords (ALLDREDGE *et al*., 2002) and open ocean waters (BJORNSEN & NIELSEN, 1991). These areas show an intense biological activity and can have an enormous impact on the biological and chemical dynamics of the marine pelagic zone (ALLDREDGE *et al*., 2002), although it has not been quantified yet. These layers may undergo elevated levels of nutrient uptake, increased intensity of competition and predation, higher accumulation of chemical wastes and toxins and higher levels of microbial degradation and remineralization, found in the vicinities of the interface (MASON *et al*., 1993; DONAGHAY & OSBORN, 1997; COWLES *et al*., 1998).

This may be applicable to the Ebro estuary, but there is limited information on its phytoplanktonic communities. MUÑOZ (1989) described them during an annual cycle but it worked only with surface water samples, concluding that alternation between diatoms and chlorophyceae is the characteristic feature of phytoplankton in the Ebro lower course.

The main objective of this work is to analyze the vertical distribution of chlorophyll *a* and phytoplankton along the Ebro estuary, focusing on the freshwater-saltwater interface.

Study area

The Ebro River has an estimated length of 928 km and is one of the largest rivers in Spain. The study area at the present work focuses on the river estuary, placed inside the Delta that the river forms on the Mediterranean Sea coast (figure 1). The Ebro estuary is considered as a "salt wedge estuary" or type 4 of the Hansen-Rattray classification (IBAÑEZ *et al*., 1997). Since the estuary is located in a microtidal area (with maximum range for the astronomical tide of 0.25 m) it has a strong and clearly marked halocline whose dynamics is mainly driven by river discharge. The mean annual discharge is about 424 m^3/s, which is close to the critical discharge determining the formation and rupture of the salt wedge (IBAÑEZ, 1993). IBAÑEZ *et al*. (1997) found a high correlation between river discharge and salt wedge depth. However, the extension of the salt wedge is not linearly related to river discharge due to the irregular bed topography of the estuary. This bottom irregularity retains the salt wedge on a few shallow points for a wide range of discharges.

Material and methods

Four sampling campaigns in the Ebro estuary and plume were performed in the frame of PIONEER research project, funded by European Union. Such campaigns were carried out during a whole year, one per season (since April 1999 to February 2000). In this work data from spring and summer campaigns (April and July 1999) are analyzed.
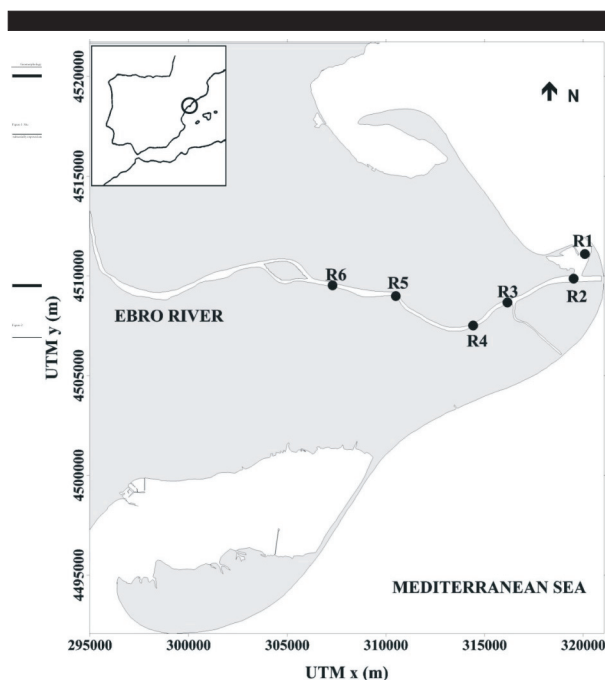
Figure 1. Delta of the Ebro river, on the north-western Mediterranean coast. Locations of sampling stations.

Water samples were taken at different stations located on the river as shown in figure 1. During the first sampling campaign (April 1999) samples were collected at different depths on the water column taking as many samples as possible in the vicinities of the halocline. In order to find the location of the freshwater-seawater interface a multiparametric probe Hydrolab Surveyor 3 was used, giving data of conductivity. This allowed fixing the interface position at depths where the largest conductivity gradients were observed. Samples were then taken at those depths of high salinity gradient employing a pump. Once the obtained samples were analyzed, it could be seen that the higher values of some nutrients (SRP and ammonium) along the water column were reached at the interface. These results compel a modification of the sampling technique in order to maximize the number of samples at the halocline zone. As a consequence, a new sampling device named SWIS (Salt-Wedge Interface System) was developed, which allows performing a more-intensive and detailed sampling of this area. With this new equipment it was possible to take 6 samples simultaneously in the freshwater-seawater interface area (at +30, +20, +10, 0, -10 and -20 cm from the interface point indicated by conductivity measurements). The device consists on six teflon tubes, with an internal diameter of 6 mm, which join a metallic structure that can be adapted to fit the Hydrolab probe. The tubes are connected to a vacuum system which is operated from the surface in order to collect the samples.

Nutrient analyses were performed with an Alliance Instruments Evolution II autoanalyzer. The employed methods are described by TREGUER and LE CORRE (1975), considering PARSONS *et al*. (1984) and KIRKWOOD *et al*. (1991). The optimisation of the equipment was done following COAKLEY (1981).

Salinity was measured with a Grundy Environmental Systems Inc. 6230 N induction conductimeter, calibrated with the suitable standards (I.A.P.S.O. Standard Seawater, Ocean Scientific International Ltd, K15= 0.99986, S= 34.995‰).

On the determination of chlorophyll *a* the trichromatic method was used, based on spectrophotometry (APHA, 1998).

Phytoplankton analyses were carried out filtering 10 ml samples through a membrane filter of 0.2μm until dry. Then salt was removed adding 5ml of distilled water and the samples were filtered once again until dry. After this, the material on the filter was dehydrated by washing successively with 50, 80, 90

and 99 per cent aqueous ethanol. Each dried filter was placed onto a drop of immersion oil in the centre of a slide and 2 more drops were added on the top side of the filter. Finally, a coverglass was placed on the top of the filter (FOURNIER, 1978). Algal counts were made by epifluorescence microscopy (VARGO, 1978) with a Nikon Optiphot microscope, using a 100× oil-immersion objective. A minimum of 300 cells was counted and at least 100 cells of the species or genera more abundant were counted with an error lower than 20% (LUND *et al*., 1958).

RESULTS AND DISCUSSION

Since the second field campaign (July 1999) the new sampling method was employed allowing a more vertical spatial resolution of water sampling at the interface. In figure 2a, vertical profiles recorded on 7th July 1999 at four stations (the two closest and the two more distant to river mouth) can be observed. The dotted line corresponds to salinity profiles, showing an interface with strong gradients. This interface is deeper at the stations close to the estuary head (R5 and R6) than those located close to the estuary mouth (R1 and R2). Moreover, the interface width is smaller at stations closer to the estuary head. In the surface layer, salinity at stations R1 and R2 is higher than at R5 and R6 probably due to entrainment of saltwater from bottom layer (DYER, 1997). The bottom layer shows an almost homogeneous structure with salinity about 36‰.

In the same figure, vertical profiles of ammonium can be observed, showing the presence of sharp peaks at stations R1 and R2 and a small peak at station R5. These peaks are a consequence of organic matter mineralization (ZUTIC and LEGOVIC, 1987; VILICIC *et al*. 1989; SEMPERE and CAUWET, 1995) settling from the surface layer due to saline shock undergone by freshwater phytoplankton. KINNE (1971) identified a critical salinity boundary at 4-7 PSU as a region of great physiological stress for freshwater phytoplankton. At station R6 the increase in ammonium concentration is so significant that masks the presence of peaks at the interface. Regarding the horizontal direction, an increase of ammonium concentration in the bottom layer from the river mouth to the estuary head can be observed. This increase is due to the accumulation process of materials settling from surface layer as a consequence of the aforementioned saline shock, being dragged for the salt wedge motion towards the estuary head. This differential accumulation of organic matter obviously affects ammonium concentration because of its decomposition and subsequent release of this composite. This process also involves other nutrients and for this reason some authors consider this type of estuary as a nutrient trap (REDFIELD *et al*., 1963; DARNELL and SONIAT, 1981). Organic matter remineralization implies oxygen consumption, leading to a decrease of its concentration in these areas. A clear hypoxia has been observed in the salt wedge at stations located in the estuary head (FALCO, 2003).

In the case of SRP (figure 2b) a similar pattern for vertical distribution is observed, with peaks appearing at the interface of the salt wedge (stations R1, R2 and R5) due to remineralization of organic matter accumulated there. Again at R6 station there is not concentration peak for the same reason that in the case of ammonium. In horizontal direction an increase of SRP concentration can be observed in the bottom layer towards the estuary head such as in the ammonium case, but in the surface layer the two stations more distant to the estuary mouth present similar concentrations with values lower than the two stations closer to the mouth. Therefore, it seems that saline shock not only produces senescence/settlement of microorganisms but these are also partially mineralized in the surface layer giving rise to SRP concentration increase. Obviously this process is more easily detected at those stations where the saltwater influence is higher (the closest to the river mouth) so that saline shock has an impact on larger planktonic populations. Orthosilicic acid presents a similar distribution pattern to SRP. The only difference occurs at the interface of station R6, where
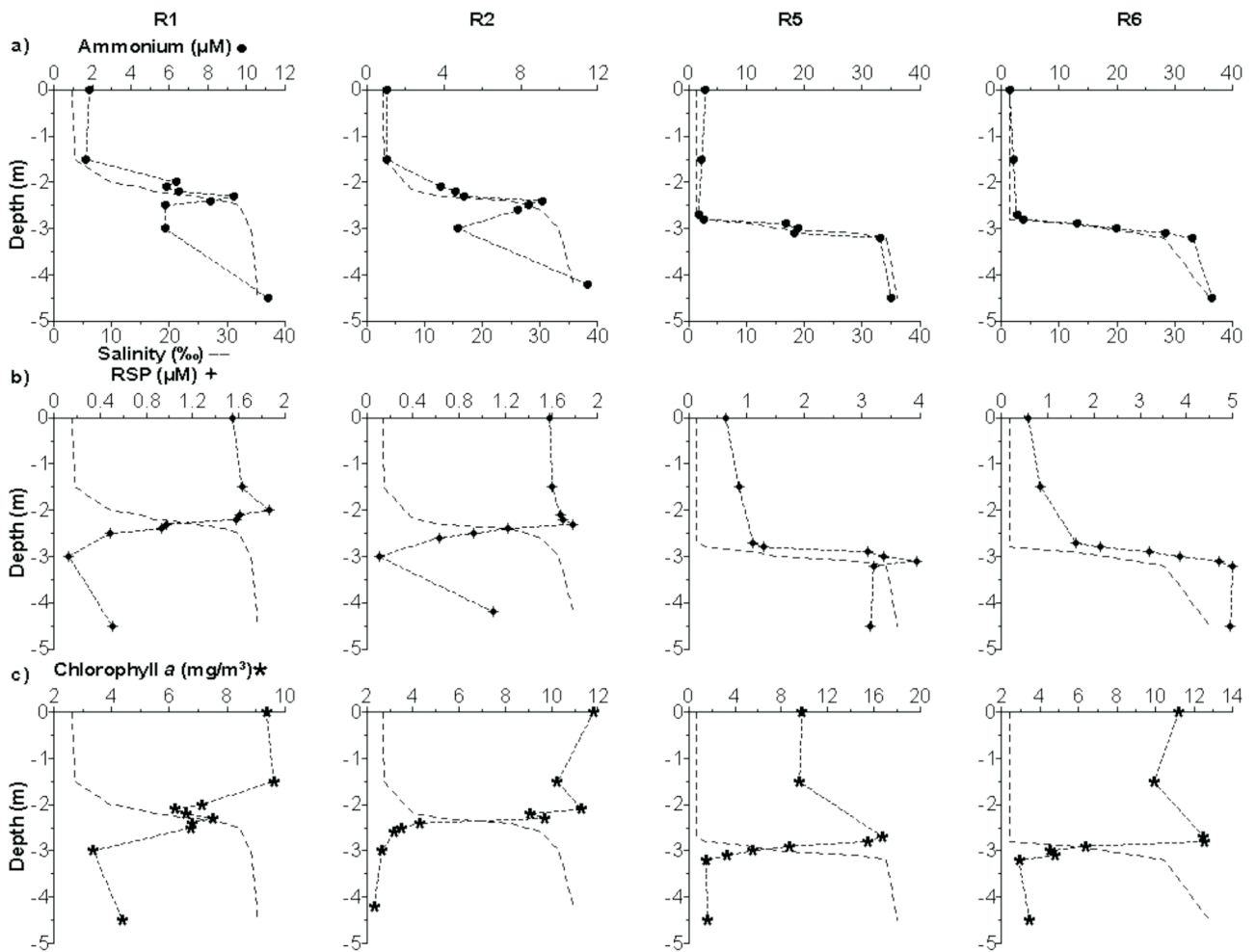
Figure 2. Vertical profiles of a) ammonium, b) RSP and c) chlorophyll *a* at stations R1, R2, R5 and R6 on 7 July 1999. Salinity is presented as a dotted line to denote the interface.

the concentration peak is also observed because it is not masked by the increase of this nutrient at the salt wedge (FALCO, 2003). Finally, the vertical profiles of chlorophyll *a* (figure 2c) show higher concentrations in the surface layer than in the bottom one as well as that at the interface there are peaks. At stations closest to the estuary head (R6 and R5) the peak is located in the shallower part of the interface and above of ammonium, SRP and orthosilicic acid peaks. At station R2 the chlorophyll *a* peak is located above the ammonium one, below the SRP peak and at the same level that the orthosilicic acid peak. Finally, at station R1, the closest to the river mouth, the chlorophyll *a* peak coincides with the ammonium one, located below the SRP and orthosilicic acid peaks. This suggest two different patterns in the peak distribution, since at the stations closest to the estuary head the chlorophyll *a* accumulation apparently comes from phytoplankton settling from surface layer, which can be temporarily retained at the interface. This is probably due to the sudden increase of fluid density acting as a filter, leading to a decrease of particle settling velocity and its temporal retention. At the stations closest to the estuary mouth the chlorophyll *a* peak is located below nutrient peak probably indicating a growing zone due to nutrient abundance.

Cellular concentrations of different phytoplanktonic groups for the same day are shown in figure 3. Multicellular prokaryotes (figure 3a) present a higher density in the surface layer than in the bottom one, where practically they are not detected indicating that this is a freshwater species. At all the stations these peaks are observed at the interface produced by

settling of these multicellular cyanobacteria that remain retained there. At the halocline the cells die due to osmotic shock leading to a decrease of cellular density in the bottom layer. On the other hand, a decrease of their density in the upper layer is observed towards the estuary mouth, owing to saline stress induced by entrainment. This shock causes the cell senescence and their settling.

Peaks appearing at the interface deeper part, as for unicellular cyanobacteria, are due to saltwater species growing as a consequence of higher nutrient concentrations. A decrease of cell density towards the estuary head can be observed in the bottom layer probably due to less light penetration (interface located at more depth) and hypoxia. It is interesting to note one group of eukaryotes named cryptophyceae that has a peculiar behaviour (figure 3c). There are both freshwater and saltwater species. At the four stations there are peaks at the interface. At R1 and R2 these peaks are absolute maximums and the cell concentrations in the surface and bottom layers are significantly much lower than at the halocline. At the estuary head (station R6) there is also a peak at the halocline, but cell densities in both the surface and bottom layers are higher. At station R5 an intermediate behaviour is observed with an absolute maximum at the interface but cell densities similar to this maximum in both layers. This distribution suggests that close to the river mouth saltwater cryptophyceae grow at the interface, taking advantage of higher nutrient concentrations, while in the estuary head these peaks are due to freshwater cryptophyceae (dying from saline shock and settling) as well as saltwater ones
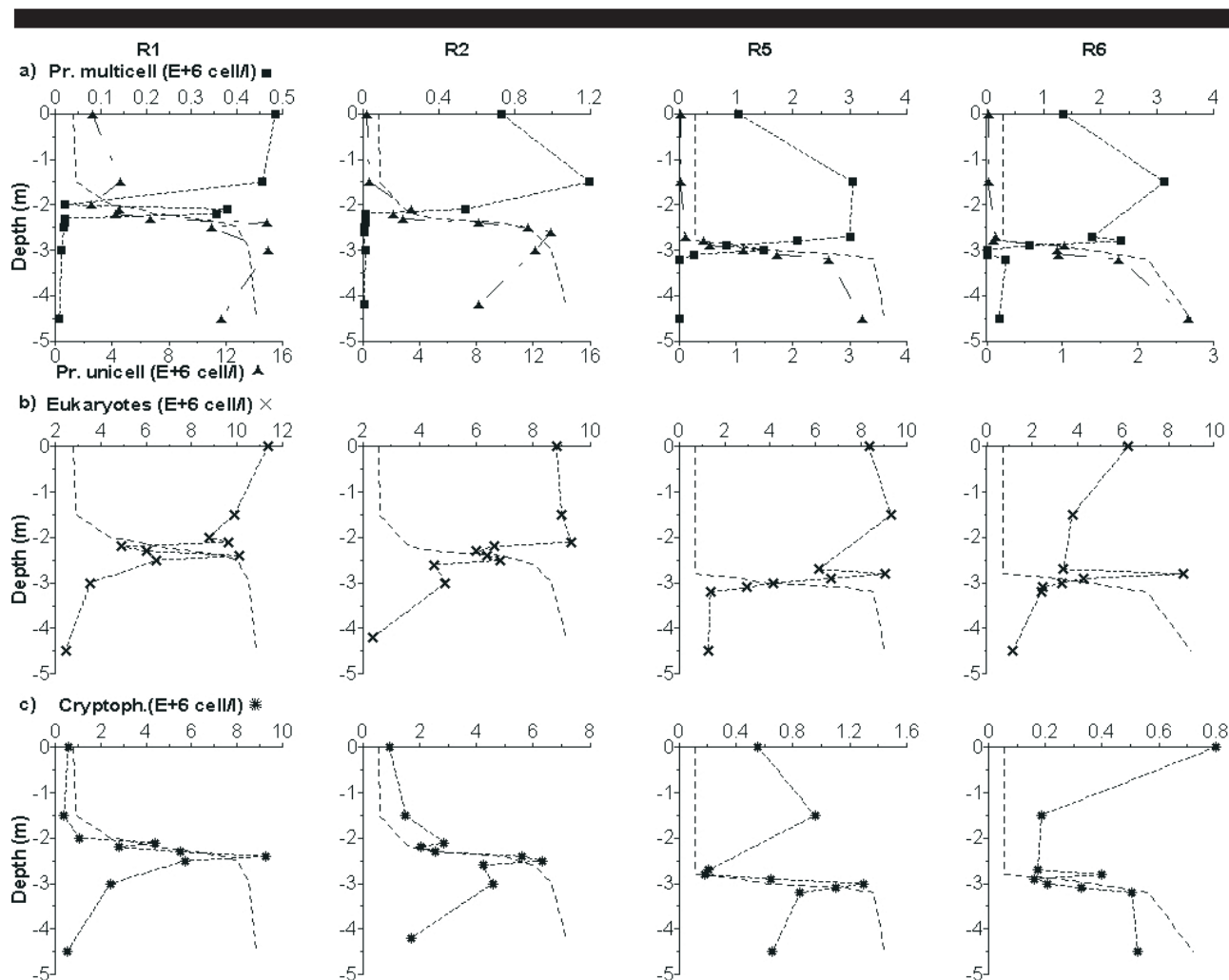
Figure 3. Vertical profiles of a) multicellular and unicellular prokaryotes, b) eukaryotes and c) cryptophyceae at stations R1, R2, R5 and R6 on 7 July 1999. Salinity is presented as a dotted line to denote the interface.

growing at this zone.

Comparing the eukaryote and chlorophyll *a* peaks a similar pattern between them can be observed at the halocline deeper part in the mouth stations but not at the halocline shallower part in the estuary head. This suggest that in the estuary head cells are senescent and as a consequence they decreased their cellular quota, while peaks in the estuary mouth correspond to growing cells taking advantage of nutrient accumulation.

SUMMARY AND CONCLUSIONS

Through a new sampling method, phytoplankton accumulation at the Ebro estuary interface could be verified observing chlorophyll *a* and phytoplanktonic cell peaks at most of the observation points along the estuary. These chlorophyll *a* peaks in the summer field campaign showed two different patterns in their spatial distribution along the estuary. One was observed close to the estuary head where the chlorophyll *a* peaks were always located at the interface shallower part and above of the peaks observed for ammonium, SRP and orthosilicic acid, suggesting that chlorophyll *a* accumulation was mainly promoted by phytoplankton settling from surface layer. The other pattern was observed at the stations closest to estuary mouth, where the chlorophyll *a* peaks were found at the interface deeper part, coinciding or even being below of the nutrient peaks, thus indicating the existence of a growing zone due to nutrient abundance.

Higher cell concentrations in the surface layer than in the bottom one were observed for multicellular prokaryotes. At all the stations peaks were observed at the interface as a consequence of freshwater phytoplankton retention, which is

settling from surface layer. Along the estuary a cell concentration decrease was observed seawards in the surface layer due to the salinity increase originated by entrainment.

The concentration of unicellular prokaryotes was higher in the bottom layer than in the upper one. In most of the observations they presented concentration peaks at the halocline deeper part, indicating the growing of such organisms. In the surface layer they only could be detected close to the river mouth where salinity presented higher values than in the estuary head due to entrainment. In the bottom layer a decrease of their density towards the estuary head was detected, probably due to the hypoxia occurring there together with the larger interface depth, which implies less light intensity.

Eukaryote phytoplanktonic community showed higher cell concentrations in the surface layer than in the bottom one. Moreover, practically at all the Ebro estuary concentration peaks were detected at the interface, which depending on the responsible species indicate accumulation due to settling or growing due to nutrient richness.

Cryptophyceae showed a singular behaviour since the saltwater species took advantage of interface as growing zone due to the great amount of nutrients existing there.

ACKNOWLEDGEMENTS

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