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Changes in Phytoplankton Population along the Saline Gradient of the Júcar Estuary and Plume

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ABSTRACT

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This paper presents the results of phytoplankton counts carried out with epifluorescence at five sampling stations: two in the Júcar River Estuary and the other three in the region of freshwater influence of the Júcar River. From June 2002 to July 2003, nine sampling campaigns were carried out as a part of the EU's ECOSUD project. Two of these campaigns (the 2nd and 8th) were selected for analysis. These sampling campaigns represent two different conditions: in the 2nd campaign the discharge of the Júcar River was almost null, while in the 8th campaign it was significantly higher.

Along the salinity gradient, as the influence of fresh water and nutrient loads decreases, a decrease in the population density of eukaryotic phytoplankton was observed. Typical freshwater phytoplankton groups (colonial cyanobacteria and chlorophyceae) clearly decrease in density and percentage as salinity increases. In general, picocyanobacteria exhibit the opposite behavior. The behavior pattern of groups with species adapted to fresh water and seawater is less clear. The density of these groups (diatoms and prymnesiophytes) is highest in the salt-wedge area due to nutrient accumulation. However, the densities are generally higher at the freshwater stations than in the marine environment. The vertical distribution at the estuarine stations shows clear density maximums in the interface area, which seems to have two causes: the retention of senescent phytoplankton affected by saline shock in this quiescent area and the growth of phytoplankton that exploit the accumulated nutrients.

ADDITIONAL INDEX WORDS: *Stratified estuary, saline shock, residence time, phytoplankton interface accumulation, epifluorescence, phytoplankton counts.*

INTRODUCTION

In coastal microtidal environments, estuaries are usually highly stratified, with a salt wedge that is clearly distinguishable from the freshwater (DYER, 1991). The halocline is located between these two layers. This area presents strong and highly variable gradients of physical and chemical properties (CAUWET, 1991; FUKS *et al.*, 1991; LEGOVIC *et al.*, 1994, 1996; ZUTIC and LEGOVIC, 1987). The entrainment process increases the salinity in the surface layers of the river and in the river plume. This increase in salinity influences phytoplankton species' composition, production and mortality (MUYLAERT and SABBE, 1999).

Although the structures of estuarine areas and estuarine plumes are different, both usually consist of a surface layer of freshwater or brackish water lying on top of a denser saltwater layer. Salinity variations are accompanied by inverse variation in nutrient content.

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 estuary. These thin layers have also been observed in a wide variety of marine systems, including estuaries (DONAGHAY *et al.*, 1992), coastal shelves (COWLES and DESIDERIO, 1993), fjords (ALLDREDGE *et al.*, 2002) and open-ocean waters (BJORNSEN and NIELSEN, 1991).

Saline shock, the availability of nutrients, light, temperature and zooplankton consumption are the factors that most affect the biomass distribution in the estuary and river plume (SHIAH *et al.*, 1996).

Some authors present evidence that saline shock can mask the effect of high nutrient availability in the area of intermediate salinity levels. For example, NAUDIN *et al.* (1997, 2001) found that a decrease in salinity caused by fresh water entering the marine environment interrupts primary production and hinders its renewal throughout the time in which fluvial influence dominates.

This may be applicable to the Júcar Estuary and plume. This work analyzes the distribution of phytoplankton groups along the salinity gradient of the Júcar Estuary and plume.

This study is part of the European ECOSUD project "Estuaries and Coastal Areas. Basis and Tools for a More Sustainable Development." This article is based on fieldwork carried out in the Júcar River outlet and Cullera Bay.

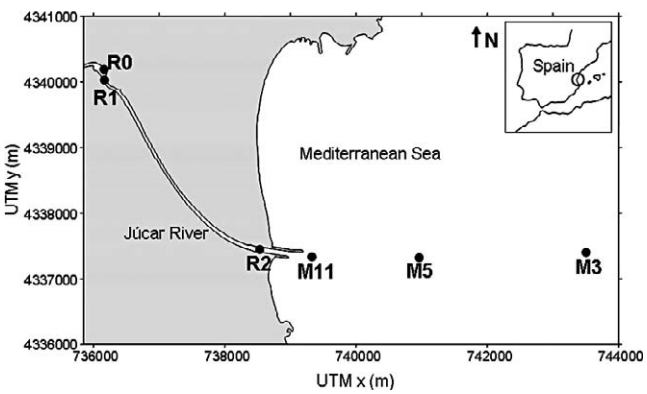

Figure 1. Júcar River and Cullera Bay on the northwestern Mediterranean coast. Location of sampling stations. Marquesa weir is located between stations R0 and R1.

STUDY AREA

The Júcar River ends in Cullera Bay (Valencia, Spain) on the western Mediterranean coast (Figure 1). The study area has a temperate climate with summer drought, known as a thermo-Mediterranean dry climate (CSIC, 1995).

Cullera Bay is one of the most popular tourist destinations on the Spanish Mediterranean coast. It has a permanent population of 21,372 inhabitants (INE, 2004), but in the summer the seasonal population reaches 100,000 people, with more than 400,000 visitors.

The Júcar River is approximately 427.5 km long and its basin area is about 21,578.5 km^2. The last stretch of the Júcar River is navigable and is the only inland port of the region of Valencia. The available water resources are mainly used for the population's supply, industry and crop watering (the latter accounting for 77% of total consumption).

The Júcar River is highly influenced by human activities. The reservoirs built in the river basin drastically modify the natural flow regime. At the Cullera observation station, located approximately 7.5 km from the river mouth (longitude 732619, latitude 4341460 in UTM coordinates), the registered flow is normally close to 0 m^3/s, except during rain events. The Marquesa weir, located 3 km upstream from the river mouth (longitude 736075, latitude 4339958 in UTM coordinates), prevents seawater intrusion and the underground salinization of farmland.

MATERIAL AND METHODS

Nine sampling campaigns were carried out between June 2002 and July 2003. This paper focuses on phytoplankton population differences between summer and spring conditions (the 2nd campaign in July 2002 and the 8th campaign in April 2003). These campaigns represent two different conditions of the system. The Júcar River is overexploited and most of the time the volume of flow at the river mouth is almost negligible. Under normal conditions, the final section of the river (after the Marquesa weir) only receives freshwater released from the weir and from small channel discharges. These were the conditions for the campaign carried out in July. Only in extremely rainy periods is there significant discharge through the Marquesa weir. These were the conditions for the campaign carried out in April (when the volume of flow ranged from 4 m^3/s to 20 m^3/s).

Six fixed stations were chosen for this study (Figure 1). Three of them were located in the estuary and the other three were along a cross-shore transect in Cullera Bay. At Station R0, located upstream from the Marquesa weir, a surface-water sample was taken for a reference value. At the other five stations, water samples were taken at different depths. Samples at depths of more than 1.00 m were taken by a hose connected to a vacuum pump. Water samples taken at depths of less than 1.00 m were collected with a high-precision sampling device in the uppermost layer. This device, called SWAS (surface water sampler), had previously been developed for sampling in the Rhone River (NAUDIN *et al.*, 1997, 2001). The Environmental Impact Assessment Group (Technical University of Valencia) and the Maritime Engineering Laboratory (Technical University of Catalonia) adapted it to the conditions of the system (ROMERO, 2004).

At each sampling station, the water was collected in plastic bottles, refrigerated and carried to the laboratory (always within 12 hours of being collected). The samples stored in glass bottles were treated in situ with glutaraldehyde (a final concentration of 2%) as indicated by SOURNIA (1978) for phytoplankton analyses.

Salinity was measured with a Grundy Environmental Systems Inc. 6230 N induction conductimeter calibrated with the appropriate standards (I.A.P.S.O. Standard Seawater, Ocean Scientific International Ltd., K15 = 0.99986, S = 34.995‰). The trichromatic method was used to determine chlorophyll-*a* based on spectrophotometry (APHA, 1998).

Phytoplankton analyses were carried out by filtering 10 ml samples through a 0.2 μm membrane filter and drying the filtered material. Salt was then removed by adding 5 ml of distilled water and again filtering and drying the samples. The material on the filter was then dehydrated by washing successively with 50%, 80%, 90% and 99% aqueous ethanol. Each dried filter was placed on a drop of immersion oil in the center of a slide and two more drops were added on the top side of the filter. Finally, a coverglass was placed on top of the filter (FOURNIER, 1978). Algal counts were carried out by epifluorescence microscopy (VARGO, 1978) using a Nikon Optiphot microscope with a 100× oil-immersion objective. A minimum of 300 cells was counted and at least 100 cells of the most abundant species or genera were counted with an error under 20% (LUND *et al.*, 1958).

RESULTS AND DISCUSSION

This paper analyzes the results of counting phytoplankton with epifluorescence and chlorophyll-*a* measurements in the final stretch of the Júcar River and the area of influence of its plume. In this study, two different conditions were found. The first is with significant water flow (4–20 m^3/s) through the Marquesa weir and the second is when the estuarine area only receives water released from the weir and from irrigation channels.

Table 1. *Results obtained at station R0.*

Date	Salinity (‰)	Colonial cyanobacteria (10^6 cell/l)	Picocyano-bacteria (10^6 cell/l)	Chlorophyceae (10^6 cell/l)	Diatoms (10^6 cell/l)	Prymnesio-phytes (10^6 cell/l)	Cryptophyceae (10^6 cell/l)	Eukaryotes (10^6 cell/l)	Prokaryotes (10^6 cell/l)
July 2002	<2.85	17.87	—	19.36	18.61	0.25	4.96	45.66	17.87
April 2003	<2.85	8.38	—	4.56	5.31	2.42	—	12.75	8.38

Clear differences exist in the initial conditions of the river phytoplankton before it drifts into the estuary zone. The sample counts (Table 1) at R0 (station located before the Marquesa weir) show (according to ROMERO *et al.*, 2007) that the phytoplankton community exhibits a lower growth rate, thus reducing the biomass, due to a shorter residence time caused by significant water flow (ENGELHARDT *et al.*, 2004; SHIAH *et al.*, 1996; SNOW *et al.*, 2000). Cellular densities are clearly lower in April (8th campaign) than in July (2nd campaign), with the number of eukaryotes and prokaryotes 2 to 3.6 times higher in July (Table 1). Another clear difference in the composition of the population is a significant presence of prymnesiophytes in April and cryptophyceae in July.

Two groups are distinguished by analyzing the behavior of prokaryotes along the saline gradient: colonial cyanobacteria, which are typical of fresh water, and picocyanobacteria, which are more common in seawater but are also found in fresh and brackish water.

Colonial Cyanobacteria

These organisms show a clear difference between the two scenarios. In July, saline concentrations in the study area are higher due to lower water flow. Because of these higher salinity values, the colonial cyanobacteria at Station R1 are already affected by saline shock, which causes senescence and later sedimentation.

As detected in a previous study in the Ebro River Estuary (FALCO *et al.*, 2006), during this sedimentation process the cells of affected organisms are held in the halocline (a calm zone) where they accumulate, producing vertical distribution peaks (Figure 2).

Surface-water dragging contributes some residual cells (probably senescent), which only reach the point closest to the surface at R2. No significant quantities of these organisms were detected in the rest of the area (Figure 3).

The results of the 8th campaign showed a broader distribution due to the greater continental influence. However, no significant qualitative differences were found with respect to the previous campaign. Colonial cyanobacteria dominated the freshwater layers in the estuary surface zone.

Again, in this case, saline shock clearly affected them, producing senescence and sedimentation with a relative low rise in salinity. In fact, no significant amount of cells was found in R1 at −0.5 m. This relatively low rise in salinity between

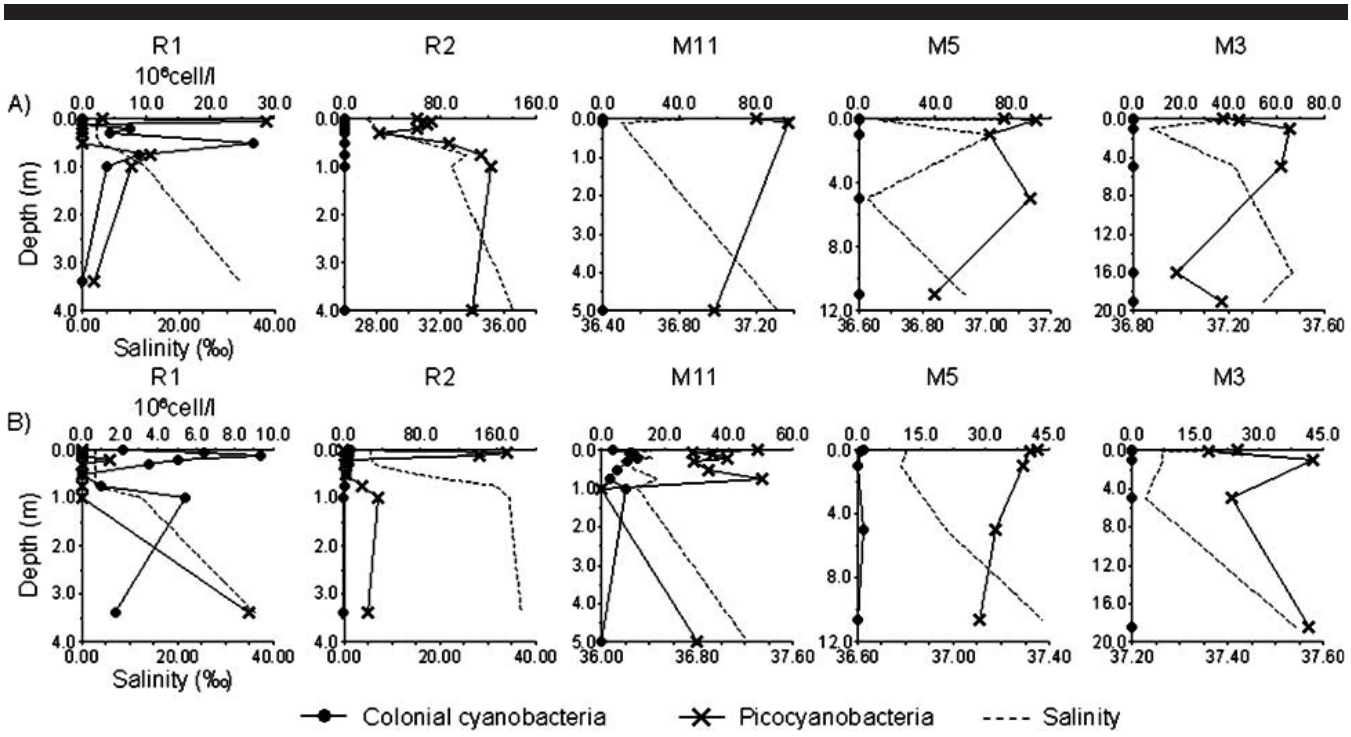

Figure 2. Vertical profiles of salinity, colonial cyanobacteria and picocyanobacteria at Stations R1, R2, M11, M5 and M3 on July 2002 (A) and April 2003 (B).

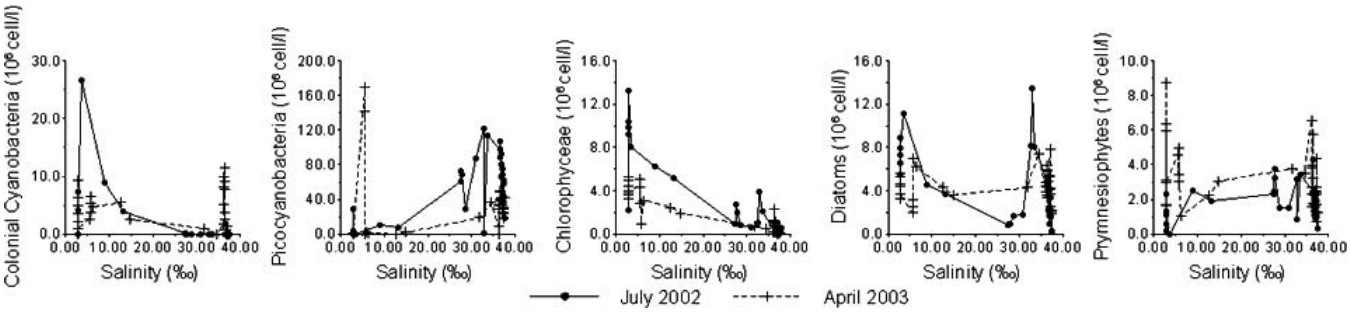

Figure 3. Variations in phytoplankton species with salinity in July 2002 and April 2003.

0.1 and 0.5 m leads to a gradual decrease in population, reaching its minimum at 0.5 m.

Further down in the water column, the process of sedimentation of senescent cells and their retention in the halocline produces a secondary maximum in R1 at −1.0 m. The cyanobacteria concentration in the salt wedge is lower, probably because most of the material that reaches this zone has already decomposed. This behavior is similar to that observed in the Ebro River Estuary (FALCO, 2003). At Stations R2 and M11, the pattern of vertical distribution is similar to that of R1, with subsurface and interface peaks that can be attributed to the same causes as in R1.

These organisms appear in significant quantities in the surface layer of M5 because senescent organisms are dragged by plume waters. These senescent cells are responsible for the secondary peak at a depth of 5 m at this station.

Picocyanobacteria

Although there tend to be more of these organisms in sea ecosystems—especially in oligotrophic waters, where they are a fundamental constituent of phytoplankton—they are also present in fresh and brackish water.

The data analyses for both campaigns seem to indicate the existence of two different populations. The first, typical of brackish water, may grow in the surface layer of the estuary, making use of the available nutrients.

The lack of a significant number of these organisms in R0 (Table 1) and the fact that their concentrations are higher in the surface layer of R2 than in that of R1 in April (Figures 2 and 3), together with high concentrations in the surface layers of M11, indicate that this is an opportunistic population adapted to a wide range of salt concentrations and high luminosity conditions. However, their sudden disappearance in lower layers shows that they are incapable of competing when salinity reaches a stable value.

The second population seems to be strictly marine. It is abundant in the salt wedge, where it reaches its highest concentrations, and takes advantage of interface nutrients to proliferate there, as in the Ebro River Estuary (FALCO, 2003) and the Krka Estuary (VILICIC *et al.*, 1989, 1999). Moreover, a high density of picocyanobacteria was present in the entire water column at the marine stations (M5 and M3).

Chlorophyceae

Most species of chlorophyceae live in fresh water, although some of them are adapted to seawater and live in littoral ecosystems. Most of the species collected during the field campaigns are typical of freshwater, especially when the population reached higher densities.

These populations are affected by saline shock and suffer the abovementioned processes of senescence and sedimentation, in which they accumulate in the interface because they are held in their descent (FALCO *et al.*, 2006).

The peaks in the area of intermediate salinity (Figure 3) correspond to these accumulations of senescent cells in the interface. In April, the accumulation area was in the upper part of the interface, which is why these peaks corresponded to salinity levels under 10‰, whereas in July they corresponded to the lowest part of the interface, so salinity levels were higher (peaks of around 27 and 33‰).

A peak at higher salinity was also observed during the 8th campaign at M11 at −1 m. This was due to the sedimentation/accumulation process, since these are freshwater forms that occur at the point where the peaks of colonial cyanobacteria were observed. Neither of these peaks produced by accumulation is shown in the chlorophyll distribution profile, which suggests that the cells accumulated there are senescent.

Diatoms

The behavior of diatoms along the salinity gradient is determined by saline shock in freshwater communities, which produces senescence and sedimentation, thus reducing their population (Figure 3). Because of this perturbation, the diatom population takes a long time to recover through the growth of new species adapted to the new conditions, regardless of the availability of nutrients. This phenomenon had already been detected by other authors (FALCO, 2003; NAUDIN *et al.*, 1997, 2001). The peaks present where salinity increases (R1 at −0.5 m in July and R2 at −0.2 m and −0.3 m in April) match points at the beginning of the interface where senescent cells that are settling accumulate. The maximum of the 2nd campaign, which occurs at a salinity level of 32‰, is due to the proliferation of a chaetoceros population

in the R1 salt wedge that takes advantage of the nutrients accumulated there.

Prymnesiophytes

Prymnesiophytes behave differently in the two scenarios analyzed due to different representations in the initial population (Figure 3).

Like diatoms and picocyanobacteria, prymnesiophytes are a ubiquitous group. There are freshwater forms (which suffer salinity shock due to entrainment) and forms adapted to saline conditions (which proliferate by taking advantage of nutrients provided directly or indirectly by the senescence and mineralization processes).

Consequently, values are higher at each end of the salinity gradient and lower in the area of intermediate salinity. In this intermediate area, there are also peaks due to the processes of sedimentation and accumulation of senescent cells in the interface and/or the proliferation of marine forms that use the accumulated nutrients.

Unfortunately, the counting technique used (epifluorescence) cannot differentiate between the two populations.

CONCLUSIONS

Water residence time affects phytoplankton biomass (chlorophyll-*a* and phytoplankton counts), since it increases as residence time increases.

There are clear differences in the behavior of the different phytoplankton groups. The groups that are mainly freshwater forms, such as chlorophyceae and colonial cyanobacteria, decrease their cellular density and percentage in the phytoplankton population as salinity increases, although the retention of senescent cells in the sedimentation process at the salt-wedge interface produces peaks at intermediate salinity levels.

The phytoplankton groups with freshwater and saline forms have higher values at each end of the salinity gradient. They have lower levels at intermediate salinity values because they are unable to compensate for the losses caused by saline shock. They may present peaks at intermediate salinity levels because they proliferate in the salt-wedge interface by using the nutrients accumulated in this area.

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