Chlorophyll *a*, nutrients and phytoplanktonic community in a continental ecosystem highly influenced by marine waters

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Abstract

The Júcar River, characterized by a very irregular freshwater flow, discharges into the Spanish Mediterranean coastal waters. However, the flow at its mouth is usually insignificant due to the overexploitation of upstream waters, except during intense rain periods. The presence or absence of freshwater generates two different scenarios/conditions in this ecosystem.

The vertical gradient of salinity due to freshwater flows generates differences in nutrient content and phytoplankton composition and abundance along the water column. Nutrients, in general, increase their concentration in the superficial layers due to the river inputs. However, orthosilicic acid, nitrite and nitrate decrease with depth and when getting closer to the river mouth.

The main freshwater phytoplankton groups are Bacillarophyceae, Chlorophyceae and Cyanobacteria. Whether freshwater flow does not reach the estuary, the water column is almost entirely formed by marine water. Under this condition, nutrient concentrations are lower, and Cryptophyceae, Prymnesiophyceae, Dinophyceae and picocyanobacteria dominate phytoplankton composition.

This paper presents the results of two campaigns with different environmental conditions (with and without freshwater flow respectively). Physicochemical and biological variables were studied at three stations placed along the estuary.

Keywords: Nutrient, Phytoplankton community composition, Stratified Estuary, Freshwater flow, Mediterranean Sea.

1. Introduction

Estuaries in the Mediterranean Sea are usually highly stratified, with a salt wedge that is clearly distinguishable from the continental freshwater (Dyer, 1991). The halocline is located between two layers, and due to this fact, this area presents strong and highly variable gradients of physical and chemical properties (Cauwet, 1991; Fucks et al., 1991; Legovic et al., 1994, 1996; Zutic and Legovic, 1987). Some published examples are the Ebro Estuary, Spain (Sierra et al., 2002) and those of Croatia Karstic eastern Adriatic coast (Zutic and Legovic, 1987).

Continental waters usually have a much higher nutrient content than marine waters, and salinity variations are accompanied by a minor inverse variation in nutrient content. However, nutrient behaviour in an estuary is not only defined by the physical mixture but also by several biological and chemical processes (advection/settling, flocculation/disaggregation, adsorption/desorption, production/grazing and uptake/excretion) (Broche et al., 1998). An entrainment process usually occurs, that increases the salinity in the surface layers of the river. This increase in salinity influences phytoplankton composition, production and mortality (Muylaert and Sabbe, 1999). Furthermore, in highly stratified estuaries, saline intrusion may be enhanced if the river flow is reduced by human use. This increases the thickness and length of the salt wedge and decreases the size and influence of the river plume. These flow variations can produce changes in the ecosystem, nutrient levels and primary production (Alexander et al., 1996; Mallin et al., 1993).

The saline shock, availability of nutrients, light, temperature and zooplankton consumption are the factors that mainly affect the phytoplankton biomass distribution in the estuary (Shiah et al., 1996). Naudin et al. (1997) found that the decrease in salinity caused by freshwater entering the marine environment interrupts primary production and hinders its renewal during prevailing river influence. This process can be attributed to saline shock, which affects river organisms and is not counterbalanced by the proliferation of brackish/marine species. Phytoplankton production is at the base of important interactions that link environmental forces and aquatic ecosystems. The response of phytoplankton to physical and chemical factors in the surrounding environment (salinity, temperature, light, nutrients, water dynamics, and configuration of the water basin, to name a few) thus affects the structure and function of the entire planktonic community and makes this a central topic of research (Burić et al., 2007).

The aim of this paper is to study two different environmental conditions that take place in the last kilometres of the stratified Júcar Estuary in the Mediterranean Sea, where it has been verified that these two alternative scenarios are repeated through time (Falco et al., 2007; González et al., 2007; Romero et al.,

2007). In the first case, the river flow is continuous and significant and in the second one, the freshwater input is lower and discontinuous, due to surplus irrigation water: How these conditions are expected to affect phytoplankton composition?.

2. Materials and Methods

2.1. Study site

The Júcar River ends in Cullera Bay (Valencia, Spain) on the western Mediterranean coast (Fig. 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 23,777 inhabitants (INE, 2011), although in summer the seasonal population reaches 100,000 people, with more than 400,000 visitors.

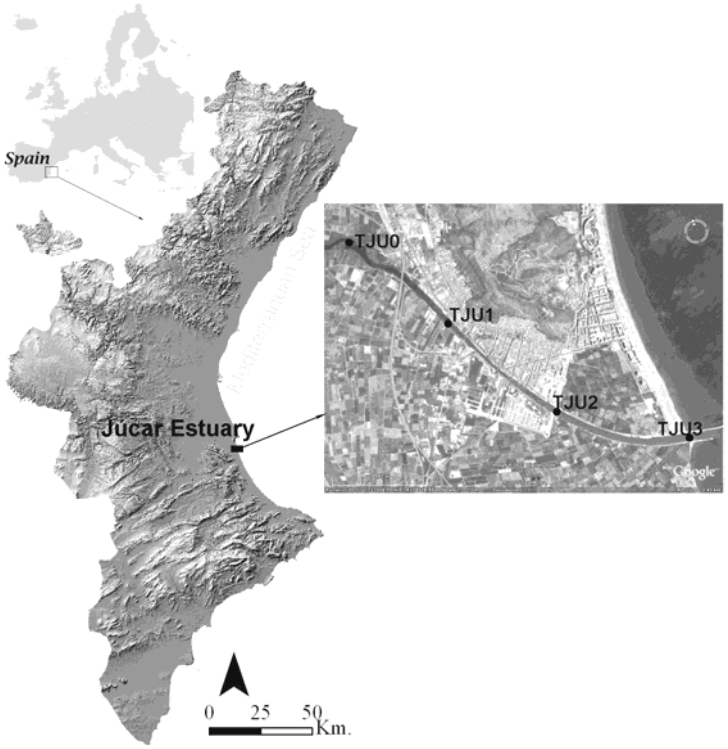

Fig. 1. Júcar Estuary, on the northwestern Mediterranean coast and location of sampling stations.

The Júcar River is approximately 427.5 km long and its basin area is about 21,578 km^2. The last stretch of the Júcar River is navigable and it has the only inland port of the region of Valencia. The available water resources are mainly used for the population supply, industry and crop watering.

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, the registered flow is usually close to 0 $m^3 s^{-1}$, except during rain events. The freshwater outflow of the Júcar River follows a typical Mediterranean pattern (Vidal-Abarca and Suárez, 2007), with relatively high flows from October to May and lower rates during the summer. The Marquesa Weir, located 3 km upstream from the river mouth, prevents seawater intrusion and the underground salinization of farmland.

The Júcar River water presents high levels of nutrient concentrations due to the intensive agricultural exploitation of the river drainage basin, the use of the river water for irrigation and the generous use of pesticides and fertilizers. Partially treated domestic and industrial wastewater from riverbank towns and industries is also discharged into the river, resulting in the eutrophication of the lower course of the Júcar.

2.2. Sampling

Two different sampling campaigns were carried out in the 8th November 2007 and the 14th May 2008. These campaigns represent the system under two different sets of conditions. The flow of the Júcar River is overexploited and it is usually negligible at the river mouth. Only in exceptionally rainy periods there is a significant discharge through the Cullera Weir (Romero et al., 2007) and these were the conditions during the campaign carried out in November 2007 (first scenario). Under normal climatic environmental conditions, the final stretch of the river (after the Cullera Weir) only receives freshwater coming from the weir and small channel discharges (Romero et al., 2007) which are the conditions during the campaign carried out in May 2008 (second scenario).

For this study, three fixed stations were chosen in the estuary (Fig. 1). At these stations (TJU1, TJU2 and TJU3), water samples were taken at different depths based on the halocline position. Furthermore an additional station was located upstream of the Marquesa Weir (TJU0) with the aim of obtaining reference values. Thus, only one surface water–sample, representative of the whole water column due to its shallowness, was taken in this station.

At each sampling station a multiparametric probe Turo T-611 was used in order to establish the depth of the freshwater-saltwater interface, as the position at which observed conductivity gradients were the largest. Then water samples were taken using a Density Interface Water Sampler (DIWS) and Superficial Water Sampler (SWAS). A detailed description of these devices can be consulted in Mösso et al. (2008).

Water samples were collected in 2 L polyethylene bottles. A subsample of 250 mL was removed for phytoplankton cell counts. They were kept refrigerated until arrival at the laboratory, which never took

longer than 12 hours.

2.3. Analytical techniques

At the laboratory, the water samples were divided into several equal parts, following the conservation procedures suggested by APHA (1998). The samples were filtered through 0.45 μm cellulose acetate membrane filters (Millipore HAWP) for nutrient and chlorophyll *a* analyses. For the analysis of nutrients (ammonium, nitrite, nitrate, soluble reactive phosphorus (SRP) and orthosilicic acid) an Alliance Instruments Integral Futura air-segmented continuous-flow auto-analyzer was used. The methodology described by Treguer and Le Corre (1975) was followed, considering the remarks by Parsons et al. (1984) and Kirwood et al. (1991). The equipment optimization was carried out following the theories of Coakley (1981).

Salinity was measured using an induction conductimeter (Salinometer Portasal Guideline 8410A) gauged with the appropriate patterns (I.A.P.S.O. Standard Seawater, Ocean Scientific International, Ltd, K15= 0,99986, S= 34,995‰).

Chlorophyll *a* content was determined with the thricromatic method based on visible spectroscopy (APHA, 1998), using Jeffrey and Humprey (1975) equations to obtain the concentration. Pigment extraction was performed with acetone 90%. The Pigment Diversity Index (Margalef Index) was also determined based on the absorbance at 430 and 665 nm (Margalef, 1965).

In order to analyze the phytoplankton communities, the epifluorescence microscopic count method was used (Pachés, 2010; Pachés et al., 2012). Samples contained in 250 ml glass bottles were fixed with glutaraldehyde until reaching a final concentration of 2%. Samples were filtered with Millipore GTTP membranes (pore size 0.2 μm). Finally, a cover glass was placed on top of the filter (Fournier, 1978). The counts were performed by epifluorescence microscopy (Vargo, 1978) with a Leica DM 2500, using the 100x-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)

2.4. Statistical techniques

Relationships between the phytoplankton composition and environmental factors were assessed using redundancy analysis (RDA), which is a linear method of direct ordination (ter Braak, 1994; Lepš and Šmilauer, 2003). For the analysis, the main phytoplankton classes identified in the samples were selected as variables. All data (phytoplankton composition and environmental data) were log(x+1)-transformed to

stabilize variance and reduce the influence of dominant classes on the ordination. RDA was performed using CANOCO for Windows 4.0, with forward selection to identify the environmental variables that best explained the phytoplankton species composition. The significance of the variables and the first ordination axis was determined using Monte Carlo permutation testing, implemented in CANOCO (Lepš and Šmilauer, 2003).

3. Results

3.1. River Flow, Temperature, Salinity and pH.

According to data provided by the Júcar River Hydrographical Confederation (Fig. 2), at the Cullera checkpoint in November 2007 campaign the mean daily flow was 4.5 m^3s^{-1}, while in May 2008 no river flow was detected. Furthermore, it was checked that the highest river flows in 2007 and 2008 were detected in September and October which are the months with the highest rainfall periods (Fig. 2).

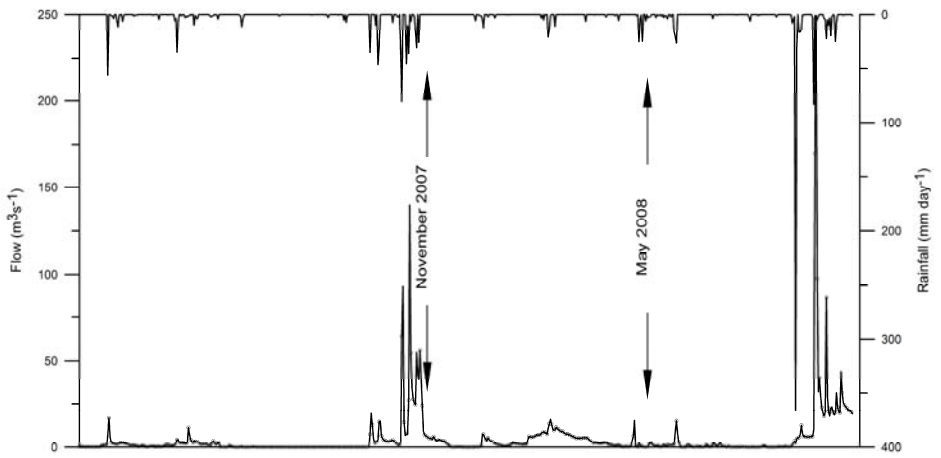

Fig. 2. Daily average Júcar flow levels and precipitation from 1st January 2007 to 12th November 2008 (lower line: flow; upper line: rainfall).

Fig. 3 shows the salinity and temperature vertical profiles at the three sampling stations of estuary (TJU1, TJU2 and TJU3) in both scenarios. This figure shows a sharp halocline dividing the water column into upper freshwater/brackish and lower marine waters. In the November sampling campaign, the halocline is placed at approximately 1 meter in TJU1 and TJU2, and 0.20 m in TJU3. The salinity over the halocline (freshwater) is < 5, while the salinity down the halocline (marine water) ranges from 35 to37.

However, in the second sampling campaign (May 2008) there is no virtually freshwater layer, only the superficial water samples taken at TJU1 and TJU2 stations exhibit lower salinity values.

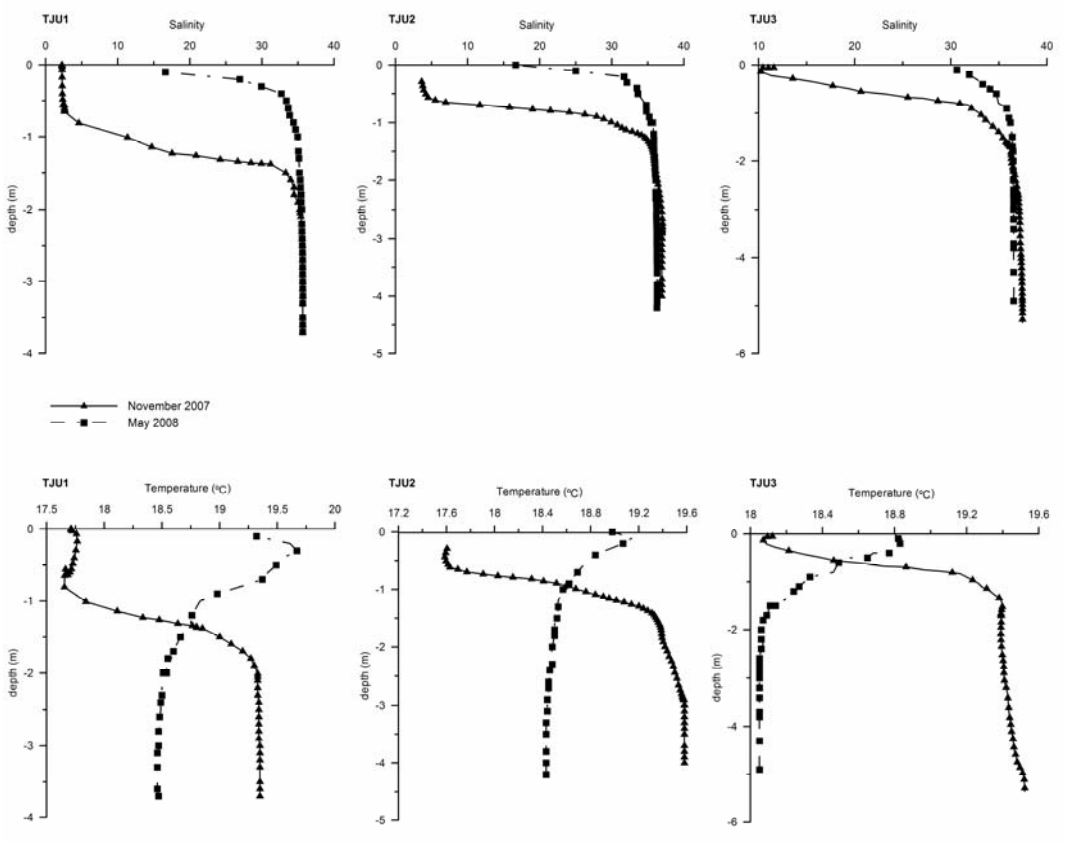

Fig. 3. Vertical salinity and temperature profiles at stations TJU1, TJU2 and TJU3.

It can be observed an inverse behaviour in temperature stratification. In May, the highest values are reached in the superficial layer due to the warming for the solar radiation. However in November (Fig. 3) the water coming from the river is colder and, since freshwater floats on the top of estuarine water (warmer), the latter remains in the bottom.

The pH ranges from 7.18 to 7.95 in November with peaks at intermediate deeps, meanwhile in May the values varies from 7.80 to 8.22.

3.2. Nutrients

Nutrient concentrations found in TJU0 (reference station) are different in each sampling campaign (Table 1). In November, soluble reactive phosphorus (SRP) and ammonium are those with higher values, while nitrate, orthosilicic acid and chlorophyll *a* are higher in May. Only nitrite has similar values in both campaigns.

	Chlorophyll *a* ($\mu g\ L^{-1}$)	Pigment Diversity Index	pH	Salinity ($g\ kg^{-1}$)	SRP ($\mu mol\ L^{-1}$)	Ammonium ($\mu mol\ L^{-1}$)	Nitrite ($\mu mol\ L^{-1}$)	Nitrate ($\mu mol\ L^{-1}$)	Orthosilicic acid ($\mu mol\ L^{-1}$)
Nov-07	11.34	3.72	7.49	< 5	2.8	10.5	5.81	620.5	131.3
May-08	52.94	2.44	8.22	< 5	0.66	0.4	6.45	1347.1	442.3

Table 1 Results obtained at station TJU0 (surface).

Fig. 4 and 5 show the nutrient variations with regard to the salinity in each one of the sampling stations for both campaigns. In November campaign all nutrient concentrations in the superficial water layer of TJU1 and TJU0 are roughly the same, since this superficial freshwater layer comes from the weir when a given flow exists. However, in May the ammonium and SRP concentration increase with respect to those found in TJU0. The rest of nutrients are kept similar while chlorophyll *a* values are lower. This confirms the absence of freshwater flow coming from the weir.

In the estuary, as it was expected, nutrient concentrations decrease when salinity increases (Fig. 4 and Fig. 5). This pattern occurs in both sampling campaigns as getting closer to the river mouth (decrease from TJU1 to TJU3) and with increasing depth. These variations found are largely due to the different initial concentrations and flow. However, these decreases regarding salinity are not linear in all cases except for orthosilicic acid, nitrite and nitrate. For these parameters it has been included in Figure 4 and 5 the linear regression analysis with salinity, since the best adjustments are obtained in the three stations and in both sampling campaigns. Moreover, these nutrients show a statistical significant correlation with salinity (α 0.01). At large salinity values, ammonium and SRP may have enhancement values in some cases.

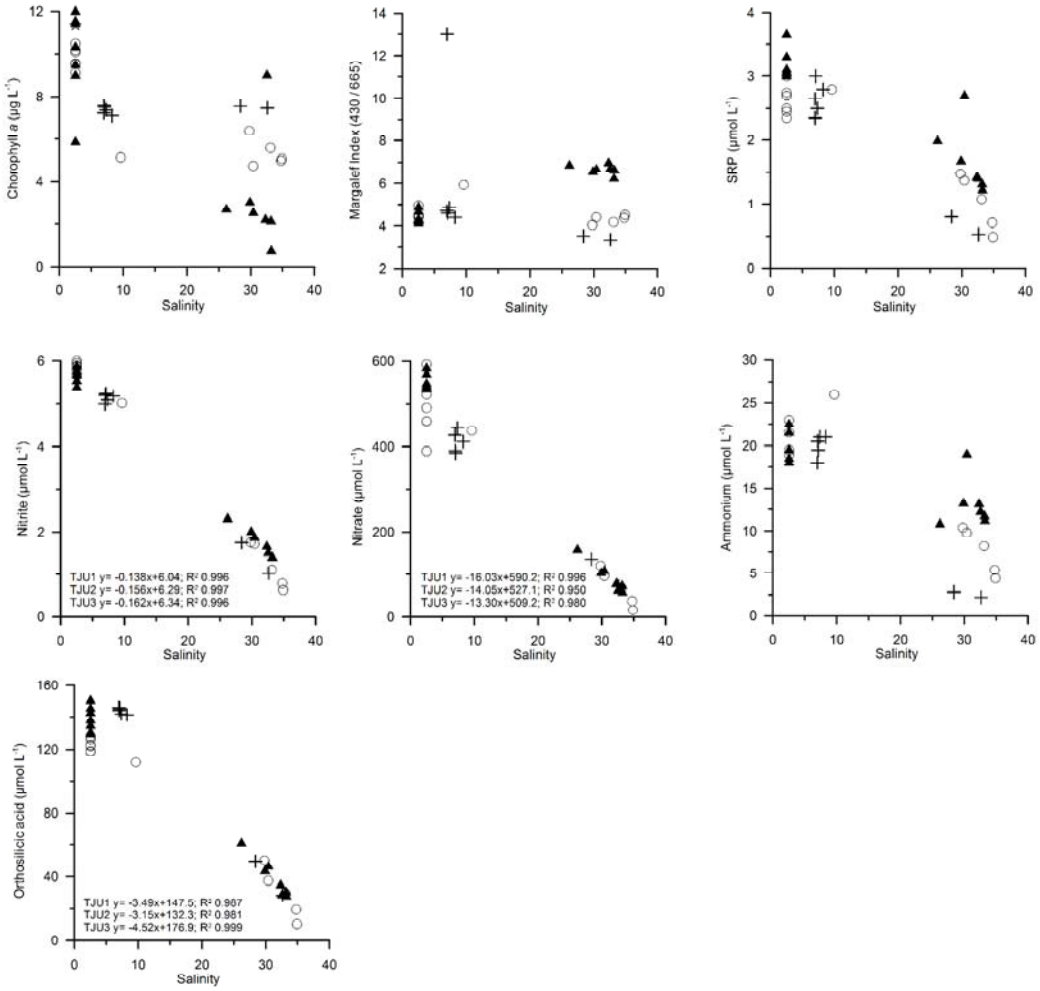

Fig. 4. Variations in chlorophyll a, Pigment diversity index (Margalef Index), SRP, ammonium, nitrite, nitrate and orthosilicic acid as salinity changes on November 2007. Key: (▲) TJU1 samples station (●); TJU2 samples station; (+) TJU3 samples station.

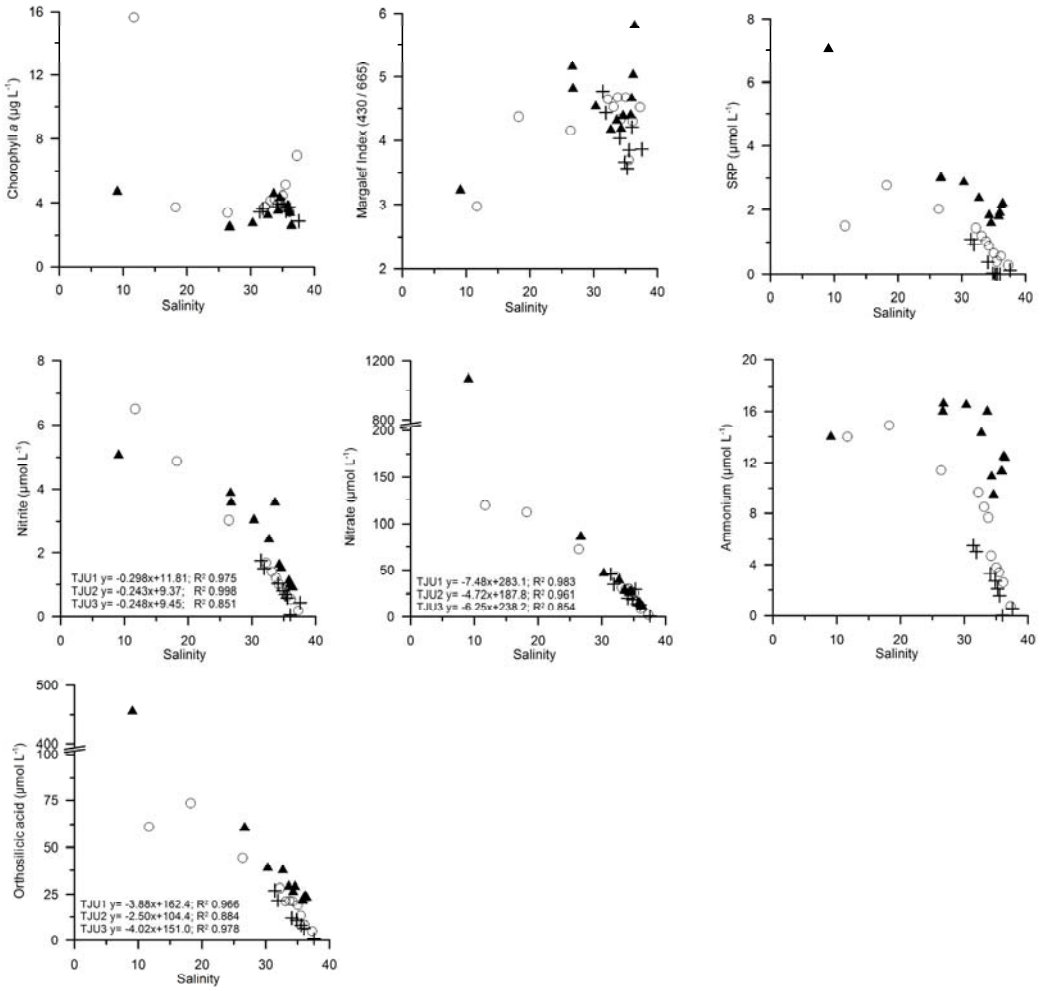

Fig. 5. Variations in chlorophyll a, Pigment diversity index (Margalef Index), SRP, ammonium, nitrite, nitrate and orthosilicic acid as salinity changes on May 2008. Key: (▲) TJU1 samples station (●); TJU2 samples station; (+) TJU3 samples station.

3.3. Phytoplankton composition and abundance

In Fig. 6A, phytoplankton community composition along the salinity gradient in November campaign is shown (salinity < 5- 34.88).The main phytoplankton groups are Bacillarophyceae, Chlorophyceae, and Cyanobacteria. However high percentages of Dinophyceae are reached in low salinity values placed close to the weir samples. In these samples it is also observed a decrease in Cyanobacteria:picocyanobacteria ratio probably because picocyanobacteria concentrations increase. Furthermore, in deeper water samples and therefore with higher salinity values the Cryptophyceae and Prymnesiophyceae abundance percentages increase.

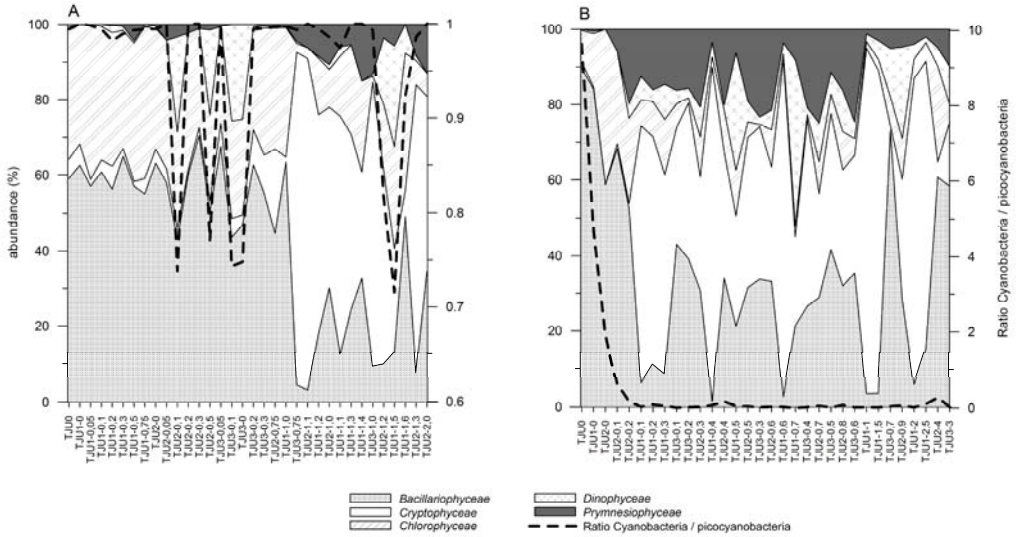

Fig. 6. Relative frequency of each phytoplankton class and ratio Cyanobacteria:picocyanobacteria in: (**A**) November 2007 campaign and (**B**) May 2008 campaign.

Phytoplankton composition in May (Fig. 6B) varies with regard to the previous campaign. Cryptophyceae, Prymnesiophyceae, and Dinophyceae raise their abundance percentage while the Cyanobacteria:picocianobacteria ratio decreases. The Bacillarophyceae group, which might be of marine origin, is still present in these samples although their rates have been reduced compared to those percentages obtained in November.

Fig. 7 shows the vertical abundance distribution of Bacillarophyceae, Chlorophyceae and Cyanobacteria in November. It is important to highlight that although other taxonomical phytoplankton groups have been identified, these are those with the greatest cell densities in freshwater layers in November campaign. In this figure, it can be observed the increase cell densities at depths of few centimetres (especially in TJU1) and also at intermediate depths in the water column.

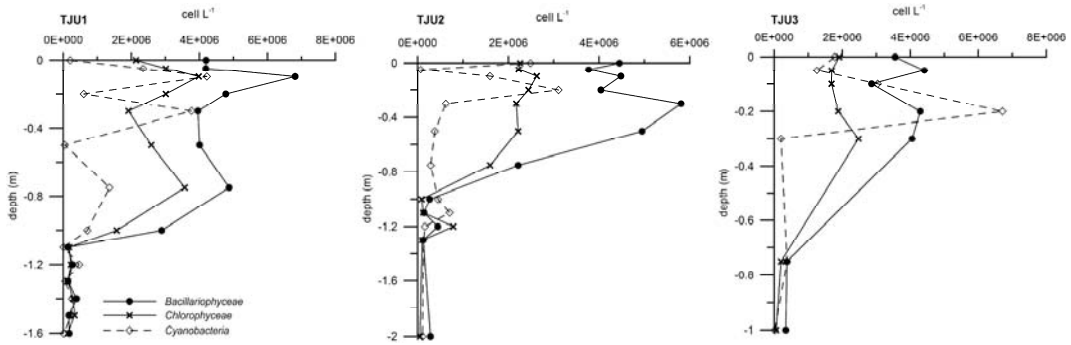

Fig. 7. Vertical profiles of Bacillarophyceae, Chlorophyceae and Cyanobacteria at stations TJU1, TJU2 and TJU3 on November 2007.

3.4. Environmental parameters and phytoplankton community

The results of the Redundancy Analysis (RDA) are displayed in Fig. 8. RDA with forward selection yielded four significant environmental variables explaining the variability in the phytoplankton composition: salinity (F-ratio = 26.08, P-value=0.0020), pH (F-ratio = 5.45, P-value=0.0020), nitrate (F-ratio = 3.38, P-value=0.008) and ammonium (F-ratio = 2.82, P-value=0.0280). The other environmental variables were not significant (P-value > 0.05).

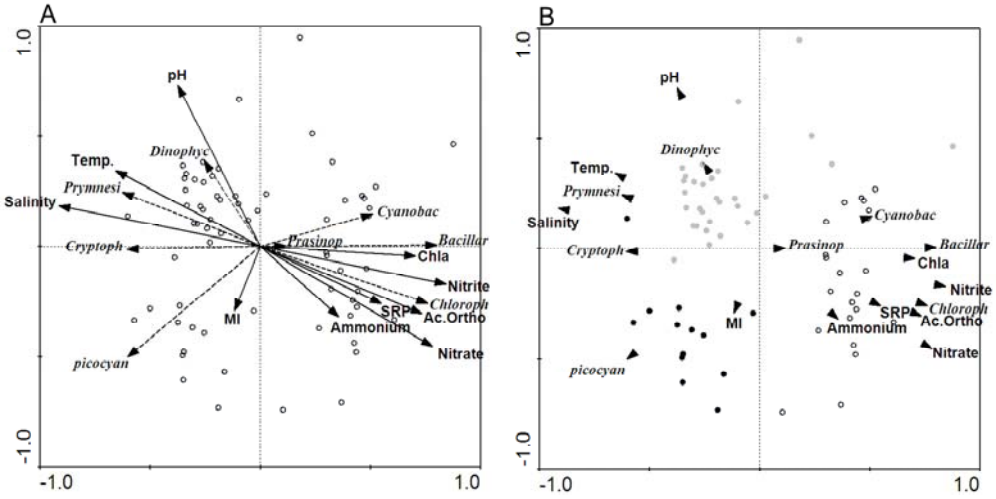

Fig. 8. (**A**) Redundance Analysis (RDA) graph showing phytoplankton with physicochemical parameters relation. Key: dashed arrows represent phytoplankton groups; solid arrows represent physicochemical variables. (**B**) Ordination samples in the RDA. Key: (●) 1st group, samples of deeper marine origin (November campaign); (o) 2nd group, freshwater samples (November campaign); (●) 3rd group, marine origin (May campaign).

In the RDA ordination (Fig. 8a) the first two axes explained 41.2% of the total variance in the phytoplankton composition. An 85.3% of this 41.2% is explained by the environmental variables. The first axe describes a gradient from higher salinity values towards higher nutrient concentration indicating a transition in the environmental conditions with and without freshwater flow. The second axe seems to be related to primary production. It describes a gradient from a high pH values towards high ammonium concentrations and Margalef Index values.

In general, the phytoplankton groups could be divided into two groups according to their correlation with the environmental factors. The first group is located in the right part of the graph (Fig. 8A) and it is formed by phytoplankton groups related to high nutrient concentration and low salinity values (Bacillarophyceae, Chlorophyceae and Cyanobacteria). The second one (in the left part of the graph) is related to high salinity and temperature values (Cryptophyceae, Prymnesiophyceae, Dinophyceae and picocyanobacteria).

In the Fig. 8B, the ordination of the samples in the diagram shows that samples could be divided in three main groups. The first group is formed by deepest depths water column samples of November campaign

(samples of deeper marine origin). The second group is composed by superficial water samples of November campaign (freshwater samples). Finally, the third group is formed by May samples campaign at the three stations (with marine origin throughout the column).

4. Discussion

According to the results obtained in this ecosystem the salinity gradient may be placed at greater or lesser depth depending on the freshwater flow. This was observed in previous works carried out by our research group for the European ECOSUD project (Estuaries and Coastal Areas. Basis and Tools for a More Sustainable Development) (Romero et al., 2007) and also in research projects performed in the Ebro Estuary (Sierra et al., 2002). Furthermore, the lack of a physical barrier that hampers the entrance of marine water leads to find inside the estuary marine water in the bottom and freshwater/brackish water in the upper part of the column.

As it is known, continental waters usually have higher nutrient loads than seawater (Bethoux et al., 1992; Le Pape et al., 1996; Moutin et al., 1998). Nutrient concentration variations in stratified estuaries are related to two main processes: variations in river inputs (quantity and quality of the waters) and variations due to internal biochemical processes (Romero et al., 2007). This system is basically determined for the fluvial discharges. Thus, a reduction of river flow could lead to significant effects in this ecosystem, nutrient levels and primary production (Alexander et al., 1996; Malin et al., 1993; Romero et al., 2007). Therefore, nitrate concentration shows the highest correlation to river contribution since it comes from the agriculture (Lidón et al. 1999; Ramos et al., 2002).

Higher nutrients concentration were found in TJU0 in May sampling campaign, mainly due to both, the longer residence time caused by the absence of water flow and the higher temperatures registered. Therefore, long residence time favours phytoplankton proliferation (chlorophyll *a* increase) and the reduction of preferential nutrients (ammonium and SRP) for their consumption.

Nutrients concentrations higher than expected at large salinity values (ammonium and SRP), especially striking in TJU1, are probably due to the death and subsequent mineralization processes of freshwater phytoplankton cells affected by saline shock (Falco, 2003; Falco et al., 2006; González del Rio et al., 2007). Clear differences in biomass and phytoplankton composition were determined in both campaigns. Higher biomass values are related to freshwater river flow mainly to the sub-superficial water layers. This is due to the higher nutrient content in this waters that enable higher phytoplankton cells abundance. However when

no freshwater flow exits biomass values are more homogeneous.

The phytoplankton composition is determined by the freshwater flow and may changes through the water column depending on the halocline depth. Freshwater origin groups dominate the composition in November in the superficial water layer. However, the peaks observed for Dinophyceae, (many of which are mixo or heterotrophic) linked to the decrease in Cyanobacteria:picocyanobacteria ratio, may be due to the benefit obtained by Dinophyceae when small-celled species increase (Suikkanen et al., 2007). The increase in picoplankton organism abundance may indicate that although these organisms are better adapted to oligotrophic conditions (Fogg, 1986) they are present in the estuary and growth fast under high nutrient load. Furthermore, lower down in the water column phytoplankton composition shifts towards Cryptophyceae and Prymnesiophyceae dominance. The results obtained in this study are in concordance with those achieved by Gonzalez del Rio et al., (2007) where groups with freshwater and saline forms showed the higher values in both ends of the salinity gradient. However in the other scenario studied since no freshwater influence exits phytoplankton composition is formed mainly of marine Cryptophyceae, Prymnesiophyceae, and Dinophyceae forms.

Cells abundance in superficial water layer is affected by the photoinhibition effect which reduce growth and photosynthesis rates in the superficial water layer. Therefore cells densities increase at depths of few centimetres (Marcoval et al., 2008). At intermediate depths in the water column, the sedimentation of phytoplankton senescent cells affected by saline shock yield a secondary abundance maximum in the halocline. This phenomenon has also been detected in previous studies in the Júcar Estuary (Falco et al., 2007; González del Río et al., 2007 and Romero et al., 2007) and in the Ebro River Estuary (Falco, 2003).

In May there are no differences in phytoplankton abundance probably due to the lack of halocline through the whole water column.

The variance in the phytoplankton composition data explained by the environmental variables in the RDA do not include other factors like grazing, sedimentation, light conditions, etc., that also affect phytoplankton population and should be taken into account in future researching projects (ter Braak and Smilauer, 1998).

Low values of the Margalef Index correspond to high productivity communities and therefore high photosynthetic activity (Margalef, 1965; Urrutia and Casamitjana, 1981). High productivity population (Margalef, 1965) may increase pH values. In addition mineralization of the organic matter affects the

physicochemical equilibrium of the carbonic-carbonate system triggering pH decreases, (Falco, 2003; Hinga, 2002; Macedo et al., 2001). These statements are in concordance with the results obtained in this study, since pH is related negatively to ammonium concentrations and Margalef Index, suggesting that phytoplankton affected by saline shock is being decomposed.

5. Conclusions

This study reveals that when a significant freshwater contribution from Júcar River exists estuarine ecosystem conditions change. Under these conditions, a salinity vertical gradient is observed, in which nutrient concentrations diminish when increasing the salinity and when approaching to the river mouth. These nutrient decreases are almost linear for orthosilicic acid, nitrite and nitrate.

In the superficial freshwater layer influenced by the river, phytoplankton biomass is higher and the most important phytoplankton groups that contribute to it are Bacillarophyceae, Chlorophyceae and Cyanobacteria. However, at the deepest layers where continental influence diminishes, the main phytoplankton groups are those of marine origin such as Cryptophyceae, Prymnesiophyceae and Dinophyceae. In addition, picocyanobacteria dominate over freshwater colonial cyanobacteria in higher salinity values.

For this estuary ecosystem the canonical correspondence analysis shows that salinity, ammonium, nitrate and pH are the environmental variables that mainly affect phytoplankton communities. Salinity and nitrate are related to freshwater inputs from the river (continental influence) affecting biomass and phytoplankton community composition.

To conclude, the phytoplankton community abundance and composition in Mediterranean ecosystem highly influenced by marine waters, seems to reflect the combination of freshwater inputs and the primary production mineralization process.

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