Phytoplankton composition in shallow water ecosystems: influence of environmental gradients and nutrient availability

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Abstract

Environmental gradients caused by hydrological changes, whether natural or man-induced, affect the planktonic taxonomic and functional composition in shallow water ecosystems. In this sense, our aim was to find out the main variables or variable ratios that are the driving forces of the major phytoplankton taxonomic groups in Mediterranean coastal lagoons. For this purpose, 11 waterbodies were choosen, 6 of them in Empordà wetlands and 5 in the Doñana National Park, looking for representative conditions along a gradient of salinity and nutrient content. Two surveys were conducted in each waterbody (January and May 2007), after that the phytoplankton composition (chemotaxonomic composition) and also several physical and chemical environmental variables were analyzed. Two methodological approaches were used and compared: a first approach with a log (x+1) variable transformation and a second one where variables were transformed using the compositional data analysis (CoDa) techniques introduced by Aitchison (1986). When the *raw* data approach and the *CoDa* approach analysis (RDA) were compared, the results obtained were similar with regard to explained variance and also interpretation. The *CoDa* approach was characterized by the introduction of a new environmental driving factor, the bacterial biomass, whose ratio with the peptidase enzyme activity became significant. In this sense, the *CoDa* approach contributed to improving the biological interpretation of the phytoplankton distribution based on the *raw* analysis.

1. Introduction

Location and anthropogenic impact, but mainly hydrology, confer to shallow lagoons a high heterogeneity, especially concerning nutrient concentrations and organism abundance. Time and space variability makes it difficult to analyse organism abundance, diversity and composition in such ecosystems where many environmental factors co-occur and co-vary. At the same time, in order to understand the ecological processes governing shallow lagoons, a comparison between ecosystems, including time series and/or different communities, is often required.

In the specific case of shallow lagoons, the most relevant environmental factors determining changes in the phytoplankton community composition include the chemical characteristics of the water (i.e. nutrient content, conductivity, pH), its physical properties (temperature, turnover time) as well as the interaction of phytoplankton with the community structure and the functioning of other organisms (bacterioplankton, zooplankton). Most of these variables are significantly interrelated and, at the same time, show very different range values that may obscure the understanding of common multivariable analyses. More clarifying results can be obtained working with ratios among different variables, which would permit a visualization of system processes. In this sense, the ratio N:P has traditionally been used to determine if organisms and nutrients of one ecosystem follow the Redfield ratio (Redfield, 1934; Falkowski and Davis, 2004; van der Molen and Perissinotto, 2011). However, many other relationships among variables can elucidate results which could be difficult to demonstrate when working with raw variables. For example, the inorganic:organic nitrogen ratio has been described as a key factor in certain algal blooms (Glibert *et al.*, 2007) and has also been suggested as a determining factor of mixotrophic strategies (López-Flores *et al.*, 2006). The ratio between peptidase and phosphatase enzyme activities has been also described as more informative than the plain enzymatic activities (Sala *et al.*, 2001). On the other hand, physical variables also become more interesting when ratios are used. For example, López-Flores *et al*. (2009) found that the relationship between the lagoon surface and the catchment area became a driving force determining the heterotrophic-autotrophic contribution to the plankton community.

Although ratios produce interesting results, they are difficult to process statistically. For example, they cannot be included in some multivariable statistical analyses commonly used by ecologists, such as redundancy detrended analysis (RDA) or canonical

correlation analysis (CCA). However, compositional data analysis (CoDa) techniques introduced by Aitchison (1986) allow a correct mathematical transformation of ratio and percentages for use in multivariable or other statistical analyses.

The objective of this manuscript was to find out the main variables or variable ratios that are the driving forces of the major phytoplankton taxonomic groups in Mediterranean coastal lagoons. The study included the analysis of eleven lagoons from two coastal Mediterranean sites (Doñana National Park and the Empordà Wetlands), where the phytoplankton community composition and a complete set of environmental parameters were measured. In order to achieve the objective, two methodological approaches were used and compared: a first approach with a log (x+1) variable transformation and a second one where variables were transformed using the compositional data analysis (CoDa) techniques introduced by Aitchison (1986).

2. Methods

2.1. Study sites

2.1.1. Empordà lagoons

The Empordà wetlands include a group of Mediterranean coastal lagoons and salt marshes, with variable depths (average depth of 0.60 m and maximum depth close to 2 m), located in the NE Iberian Peninsula (Figure 1). They show a typical Mediterranean hydrologic regime, which is greatly affected by the proximity of the sea (Quintana *et al.*, 1998). The hydrology of this area depends mainly on sudden and irregular intrusions during sea storms and intense rainfall. Despite sea storms, rainfall or the entry of fresh water from rivers, the marshes are confined (lack of water supply) for long periods of time and tend towards desiccation (López-Flores *et al.*, 2006; López-Flores *et al.*, 2009; Badosa *et al.*, 2006; Brucet *et al.*, 2005). In this study, three salt marshes (Turies, Litoral and Fra Ramon) and three freshwater lagoons (Ter Vell, Basses d'en Coll and Ànser) were sampled.
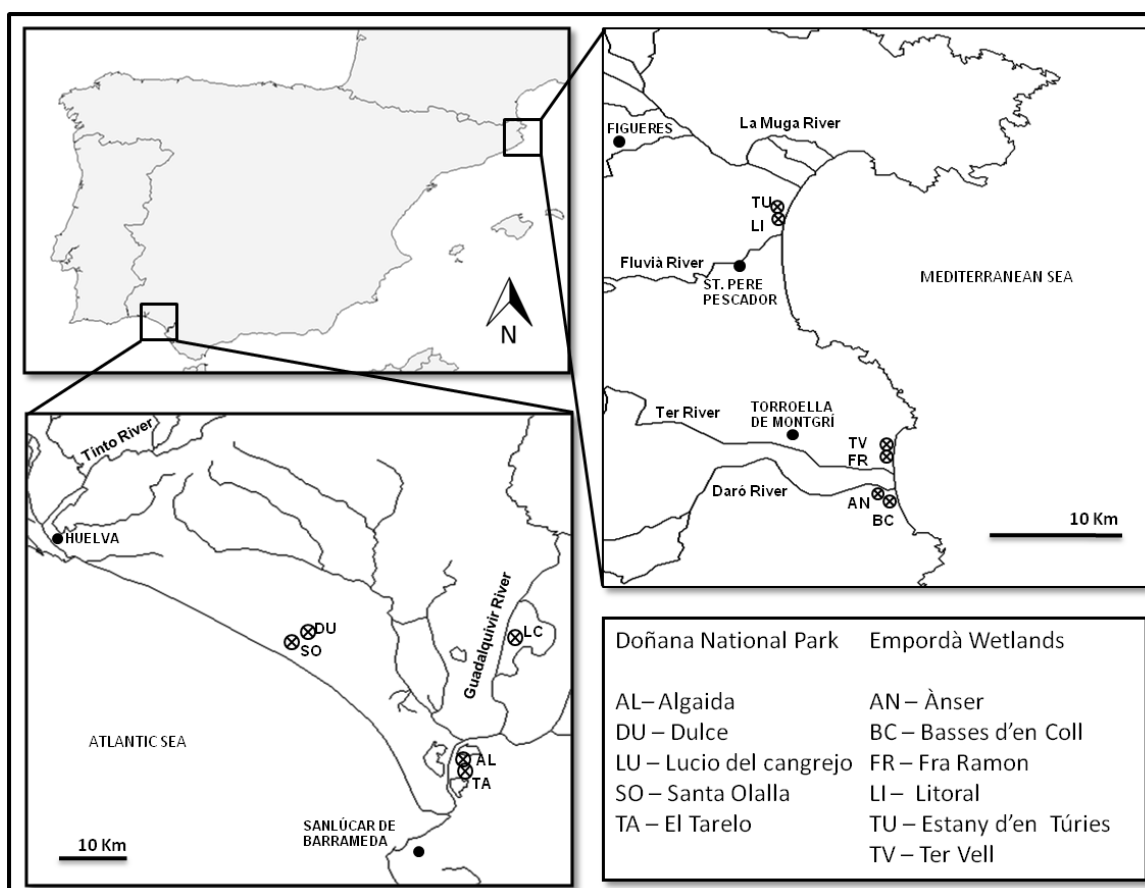

Figure 1. Location of the two study areas. Five and six sample sites were sampled twice in a year in Doñana National Park and in Empordà Wetlands, respectively.

2.1.2. Doñana lagoons

The Doñana National Park is a protected area formed by a heterogeneous group of ecosystems and located in the SW of the Iberian Peninsula (Figure 1). Flooded areas are under tidal and fluvial influence (Guadalquivir River). The salt marshes, which follow a seasonal flooding pattern, become dry during summer. However, there are many lagoons where water remains throughout the year (Serrano, 2006; Serrano *et al.*, 1999; Reyes *et al.*, 2008; Díaz-Delgado, 2010). In this study two peridunal lagoons (la Dulce and Santa Olalla), two salt marshes (Lucio de Cangrejo and Algaida) and one agricultural-shocked freshwater lagoon (el Tarelo) were sampled.

2.2. Sampling and analysis

Samples were taken twice in a year, in January and May 2007, at a central point of each basin at a depth of 15-30 cm. Three replicates of every measure and sample were done. Temperature and electrical conductivity were measured *in situ*. Filtered samples (Whatman GF/F) were frozen for later analysis of NH_4^+, NO_2^-, NO_3^- and soluble reactive phosphate (SRP). Unfiltered samples were either frozen for later analysis of total nitrogen and total phosphorus or refrigerated for total organic carbon analysis (TOC). Nutrient analyses followed Grasshoff *et al*. (1983) and total organic carbon was measured using a TOC analyser. The organic nitrogen (ON) and phosphorous (OP) were calculated from the difference between the total and the inorganic nutrient. Phytoplankton pigments were analysed and used to calculate the contribution of chemotaxonomic phytoplankton classes following López-Flores *et al*. (2006) and by means of the Chemtax program (Mackey *et al.* 1996)**.** The extracellular enzyme activities of leucine-aminopeptidase (EC 3.4.11.1) and phosphatase (EC 3.1.3.1–2) were measured spectrofluorometrically by incubating the samples with artificial substrate analogues, following Romaní and Sabater (1999). Bacterioplankton biovolume was calculated with a FACSCalibur (Becton & Dickinson) flow cytometer, following the methods described in López-Flores *et al*. (2009). After that, biovolume was transformed to biomass (carbon content) using the equation described by Loferer-Krößbacher *et al*. (1998).

2.3. Data transformation and statistical analyses

In order to quantify the influence of the distinct sources of variability on phytoplankton pigment classes, we carried out a redundancy detrended analysis (RDA). All canonical

axes were used to evaluate the significant variables under analysis by means of a Monte Carlo test (1000 permutations). RDA tests were performed using version 4.5 of CANOCO (ter Braak and Smilauer, 2002). The correlations were carried out using PASW Statistic[®] for Windows 18.0.0 (SPSS, Chicago, Illinois).

Two data matrices were used. One included the contribution of phytoplankton taxonomic groups to total chlorophyll, and a second one included the environmental variables that may affect phytoplankton variability between lagoons and sampling time. The environmental matrix included physical data (temperature), chemical data (conductivity, soluble reactive phosphorous (SRP), nitrite (NO_2^-), nitrate (NO_3^-), ammonia ($NH4^+$), organic nitrogen (ON), organic phosphorous- (OP), dissolved organic carbon (DOC) and particulate organic carbon (POC)) and the activities of the extracellular enzymes phosphatase and leucine-aminopeptidase (PHO, PEP and PEP/BB, peptidase per bacterial biomass). Although these latter variables are biological, they were included within the environmental matrix as indicators of either phosphorus or nitrogen limitation of the plankton community.

Data were examined using two approaches. First, RDA was done with raw data, previously log (x+1) transformed (from here on out the *raw data* approach). Then, a second RDA was performed (from here on out the *CoDa* approach). Here, the biological data were log (x+1) transformed and centred (centred log-ratio) and the environmental matrix was transformed using log-contrasts or balances (Egozcue *et al.*, 2003; Egozcue and Pawlowsky-Glahn, 2005).

3. Results

3.1. Environmental regularities

In the Empordà wetlands, the lagoons with a higher proportion of freshwater (lower conductivity) were also the ones with a higher (NH_4^+) + (NO_2^-) + (NO_3^-) :SRP and (NH_4^+) + (NO_2^-) + (NO_3^-):DOC ratios (Table 1). That pattern was observed both in winter and in summer. In contrast, in the saltiest lagoons in summer (Litoral, Fra Ramon, Ter Vell and Túries), and also in the most confined lagoon in winter (Litoral) low proportions of (NH_4^+) + (NO_2^-) + (NO_3^-), from here on out DIN (dissolved inorganic nitrogen), were registered, coinciding with high values of the enzyme activity ratio, PEP:PHO.

	code	T (ºC)	Conductivity (mS/cm)	DIN:SRP	DIN:DOC	PEP:PHO
Empordà Wetlands						
Winter						
Ànser	AN1	10,9	1,4	91,4	39,9	12,5
Basses d'en Coll	BC1	12,4	1,7	147,9	26,9	8,3
Fra Ramon	FR1	13,5	25,0	9,6	0,14	8,7
Litoral	LI1	10,1	17,1	0,3	0,02	160,0
Ter Vell	TV1	12,5	4,5	37,8	1,14	4,4
Turies	TU1	8,1	17,6	5,3	0,05	8,2
Summer						
Ànser	AN2	21,0	1,2	56,3	11,08	5,1
Basses d'en Coll	BC2	19,0	2,3	33,8	4,12	6,7
Fra Ramon	FR2	29,9	23,1	0,3	0,01	9,4
Litoral	LI2	20,1	37,0	0,1	0,03	21,3
Ter Vell	TV2	24,7	5,4	0,7	0,16	8,4
Turies	TU2	21,1	31,3	0,8	0,142	13,6
Doñana National Park						
Winter						
Algaida	AL1	8,2	28,3	54,1	12,37	0,6
La Dulce	DU1	8,5	1,0	13,5	0,07	0,4
Lucio cangrejo	LU1	7,5	7,2	24,9	1,83	3,6
Santa Olalla	SO1	9,7	3,7	128,2	0,74	1,9
Tarelo	TA1	12,0	16,1	40,3	6,66	2,0
Summer						
Algaida	AL2	24,4	24,7	0,9	0,33	3,2
La Dulce	DU2	17,9	0,8	10,8	0,11	0,8
Lucio cangrejo	LU2	21,7	4,8	33,7	0,62	1,1
Santa Olalla	SO2	19,4	2,8	3,5	0,27	2,2
Tarelo	TA2	22,2	14,7	69,4	0,18	6,3

Table 1. Main environmental variables and ratios classified by sample site and sample period.

In the Doñana lagoons there was no regularity between the decrease in salt content and the increase in the DIN:SRP or DIN:DOC concentration. In general, in this wetland, conductivity was higher in winter than in summer. The highest enzyme activity ratio was registered in Tarelo during the summer, but all the values observed in these wetlands were lower than those found in the Empordà lagoons.

3.2. Major factors affecting the phytoplankton community composition

Both RDA yielded similar results. In the first case, when raw variables were used (Figure 2), four environmental variables (conductivity, PHO, OP and NO_3^-) contributed significantly ($p<0.1$) to the distribution of phytoplankton groups, explaining 47.7% of the variation of the data set. The first two axes of the RDA explained 70.4% and 23.4% of the species–environment relationship. In the second RDA conducted, appropriate transformations of CoDA were applied. Once more, conductivity was significant, as

were three more variables, the enzyme activity ratio (PEP:PHO), the DIN:DOC ratio and the PEP:BB ratio ($p<0.1$). In this second solution, significant variables explained 51.1% of the variation of the taxonomic data set (Figure 3). The first two axes of the RDA explained 62.8 % and 21.6% of the species–environment relationship.

In both solutions, the first axis was closely and indirectly correlated with the conductivity. In the second RDA, moreover, the first axis was correlated directly with the ratio PEP:BB. The taxonomic groups showed a similar response to this axis in both analyses. Chlorophytes and cryptophytes dominated the community when the conductivity was low and the PEP:BB was high, while the other taxonomic groups dominated in the opposite situation.
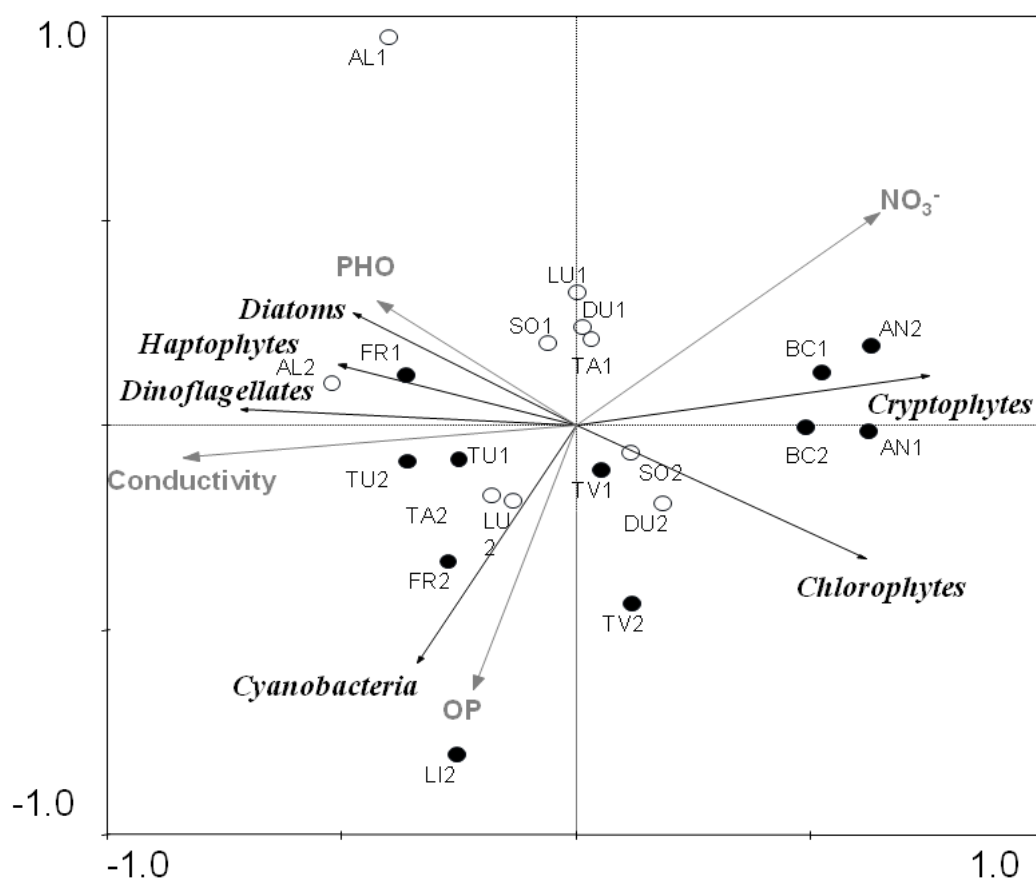

Figure 2. Ordination triplot based on the redundancy analysis (RDA) of phytoplankton data and environmental variable of the studied lagoons. Only significant variables were represented ($p<0.1$). The arrow lengths are proportional to the individual variables influence in the ordination. Filled circles represent Empordà Wetlands sample sites and empty circle represent Doñana National Park sample site.
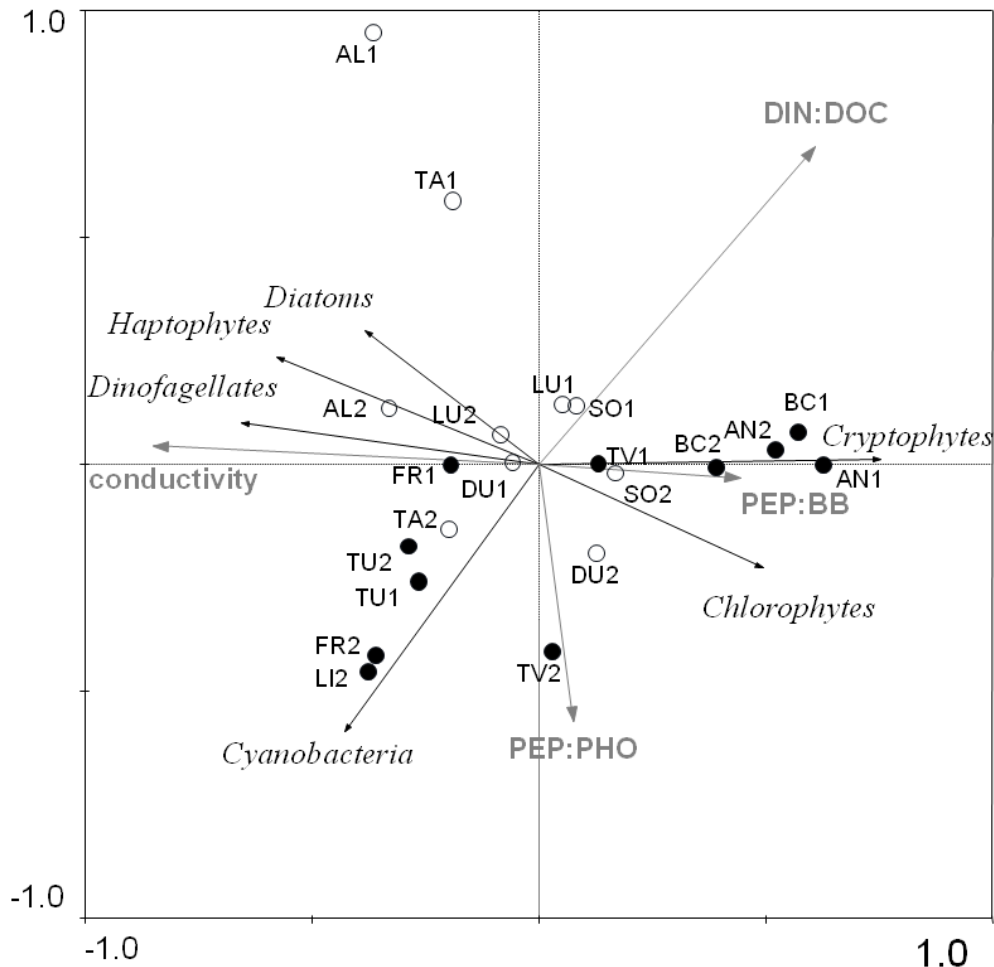
Figure 3. Ordination triplot based on the redundancy analysis (RDA) of phytoplankton data (clr transformed) and environmental variable ratios (log-contrasts) of the studied lagoons. Only significant variables were represented ($p<0.1$). The arrow lengths are proportional to the individual variables influence in the ordination. Filled circles represent Empordà Wetlands sample sites and empty circle represent Doñana National Park sample site.

The second axis was related with nutrient availability in both solutions. In the first RDA, the OP was the variable most correlated with this axis, while in the second one the second axis was mainly correlated with the enzyme activity ratio (PEP:PHO), whose relationship with the axis was indirect. The NO_3^- concentration and the PHO were correlated with both axes, as was the ratio DIN:DOC in the second analysis. In regard to taxonomic groups, cyanobacteria were the taxon most related with the second axis and

its high relative abundance coincided with the high OP concentration and the low NO_3^- availability, and according to the second analysis, favoured by a high PEP:PHO ratio. Diatoms and chlorophytes were correlated with the axis in an opposite position, indirectly and directly, respectively. Finally, dinoflagellates, haptophytes and cryptophytes showed weak and similar correlations with this second axis.

	Conductivity		NO_3^-		OP		PHO		
	r	p	r	p	r	p	r	p	n
Conductivity (mS/cm)	1	-	-0,303	n.s.	0,480*	0,028	0,063	n.s.	21
Nitrate (NO_3^- - µM)	-0,303	n.s.	1	-	-0,115	n.s.	-0,218	n.s.	21
Organic phosphorous (OP -µM)	0,480*	0,028	-0,115	n.s.	1	-	0,067	n.s.	21
Phosphatase (PHO - µM/h)	0,063	n.s.	-0,218	n.s.	0,067	n.s.	1	-	21
Temperature (° C)	0,143	n.s.	-0,291	n.s.	0,540*	0,012	-0,091	n.s.	21
Ammonia (NH_4^+ - µM)	-0,417	n.s.	0,466*	0,033	0,058	n.s.	0,274	n.s.	21
Nitrite (NO_2^- - µM)	0,045	n.s.	0,670*	<0,01	0,108	n.s.	0,165	n.s.	21
Soluble Reactive Phosphorous (SRP - µM)	0,247	n.s.	0,203	n.s.	0,559*	<0,01	-0,159	n.s.	21
Organic Nitrogen (ON - µM)	0,372	n.s.	-0,374	n.s.	0,546*	<0,01	0,544*	0,011	21
Dissolved Organic Carbon (DOC - µM)	0,122	n.s.	-0,331	n.s.	0,424	0,055	0,230	n.s.	21
Particulated Organic Carbon (POC - µM)	0,306	n.s.	-0,212	n.s.	0,724*	<0,01	0,107	n.s.	21
Peptidase (PEP - µM/h)	0,558*	<0,01	-0,364	n.s.	0,687*	<0,01	0,584*	<0,01	21
Bacterial Biomass (BB - mg C/ L)	0,570*	<0,01	-0,054	n.s.	0,839*	<0,01	0,270	n.s.	21

Table 2. Pearson coefficients between the significant *raw* variables obtained by the RDA analysis and the rest of the *raw* variables.

Due to the covariance, several variables or ratios of variables were eliminated from the RDA. However, their relationship with the significant variables and ratios can help to explain how phytoplankton taxonomic groups respond to the environment. Tables 2 and 3 present the significant variables and ratios correlating with the rest of the variables in the analysis.

	Conductivity		PEP: PHO		NO2+NO3+NH4:DOC		PEP:BB		
	r	**p**	**r**	**p**	**r**	**p**	**r**	**p**	**n**
Conductivity	1	-	0,336	*n.s.*	-0,313	*n.s.*	-0,628**	<0,01	21
PEP: PHO	0,336	*n.s.*	1	-	-0,056	*n.s.*	-0,433*	0,05	21
NO2+NO3+NH4:DOC	-0,313	*n.s.*	-0,056	*n.s.*	1	-	-0,025	*n.s.*	21
PEP:BB	-0,628**	<0,01	-0,433*	0,05	-0,025	*n.s.*	1	-	21
Temperature	0,143	*n.s.*	0,343	*n.s.*	-0,435*	0,049	0,136	*n.s.*	21
PEP: NB	-0,486*	0,03	-0,537*	0,01	-0,005	*n.s.*	0,946**	<0,01	21
PHO: NB	-0,450*	0,04	-0,922**	<0,01	0,036	*n.s.*	0,726**	<0,01	21
PHO:BB	-0,532*	0,01	-0,905**	<0,01	0,027	*n.s.*	0,776**	<0,01	21
NO2+NO3+NH4:SRP	-0,433	0,05	-0,329	*n.s.*	0,741**	<0,01	0,091	*n.s.*	21
NO2+NO3:NH4	0,024	*n.s.*	0,162	*n.s.*	0,725**	<0,01	-0,148	*n.s.*	21
NO2+NO3+NH4:ON	-0,398	*n.s.*	-0,025	*n.s.*	0,937**	<0,01	0,022	*n.s.*	21
NO2:NO3	0,322	*n.s.*	-0,190	*n.s.*	-0,502*	0,02	0,119	*n.s.*	21
SRP:OP	-0,079	*n.s.*	0,081	*n.s.*	0,333	*n.s.*	0,042	*n.s.*	21
ON:DOC	0,267	*n.s.*	-0,085	*n.s.*	0,104	*n.s.*	-0,133	*n.s.*	21
ON:POC	-0,203	*n.s.*	-0,386	*n.s.*	0,247	*n.s.*	0,224	*n.s.*	21
OP:POC	-0,161	*n.s.*	-0,140	*n.s.*	0,332	*n.s.*	0,135	*n.s.*	21
OP:DOC	0,355	*n.s.*	0,493*	0,02	0,220	*n.s.*	-0,336	*n.s.*	21
DOC:POC	-0,297	*n.s.*	-0,338	*n.s.*	0,197	*n.s.*	0,266	*n.s.*	21

Table 3. Pearson coefficients between the significant environmental *ratios* obtained by the RDA analysis and the rest of the *ratios* calculated.

4. Discussion

The multivariable analysis of the phytoplankton community composition in eleven shallow lagoons shows that conductivity and nitrogen and phosphorus availability are the main factors determining variability among wetland lagoons. This was shown by both the *raw* data approach and the *CoDa* approach, although the latter provides more information for a better ecological interpretation.

In the *raw* data approach, a high abundance of cyanobacteria was observed when the organic phosphorous concentration was high and the inorganic nitrogen concentration was low. On the other hand, the *CoDa* approach showed a relationship between the cyanobacteria dominance of the community and the enzyme activity ratio (PEP:PHO) during inorganic nitrogen scarcity periods, reinforcing previous results (Nausch, 2000; Neddermann and Nausch, 2005). This correlation was hidden in the first approach; in fact, no correlation between cyanobacteria and the phosphatase activity was observed there, as their position on the two first axes of the plot was clearly orthogonal.

The negative relationship found between temperature and the ratio DIN:DOC can be related with the hydrological pattern, since in these ecosystems, water inputs, loaded with inorganic nitrogen, are most important during winter (Badosa *et al.*, 2006; López-Flores *et al.*, 2003). Moreover, during summer the decrease or the lack of flow (Serrano, 2006; Quintana *et al.*, 1998) and the sharp denitrification (Golterman, 2000) favour the DOC proportional increase. On the other hand, the increase in the enzymatic ratio coinciding with the OP:DOC ratio increase suggests a differential effort by the community to find nitrogen during periods of phosphorous availability (Francoeur and Wetzel, 2003; Nausch, 2000).

Conductivity was a main factor in the taxonomic group's distribution. Diatoms, haptophytes and dinoflagellates dominated in saltwater while chlorophytes and cryptophytes dominated in freshwater. Although these phytoplankton assemblages seem to be much less affected by nutrient availability (much lower variability within the second axes), the freshwater groups were also characterized by higher values of the peptidase:bacterial biomass ratio. Thus, the dominance of freshwater opportunistic organisms, chlorophytes and cryptophytes (Reynolds, 2006) coincided with situations of high efficiency of bacteria to recycle organic nitrogen. In this regard, some authors have previously described growth-promoting effects of bacteria on some phytoplankton organisms (Liu *et al.*, 2008) and also the ability of several cryptophyte species to

incorporate organic nitrogen in eutrophic lagoons (Hammer *et al.*, 2002), two behaviours that could support the coexistence of both trophic groups.

When the sample distributions were analysed, the importance of water circulation became relevant again. After the division between salt and freshwater sites, another division occurred. Samples from the Doñana and the Empordà wetlands were divided into two groups, according to their hydrological pattern. This division was easier to observe in the *CoDa* approach. Doñana is under tidal influence, so the saltwater inputs are mainly marine and periodical (Díaz-Delgado, 2010). On the other hand, the Empordà salt lagoons are fed, by both marine and freshwater, during autumn and spring seasons, but after that they are subjected to intense confinement, which increases their salt concentration, the main origin of their high conductivity (Quintana *et al.*, 1998). This difference in the saltwater origin explains why, during the summer, the community was dominated by diatoms in the Doñana sample sites and by dinoflagellates and cyanobacteria in the Empordà sample points. Diatoms have high growth rates (Stolte and Garcés, 2006) and often show a quick response to nutrient availability (Margalef, 1978), as for example, due to sea water inputs during tides. On the other hand, cyanobacteria and dinoflagellates have adapted to scarcity, either through their low growth (Stolte and Garcés, 2006) or their ability to incorporate atmospheric or organic nitrogen (Burkholder *et al.*, 2006) during confinement periods.

The role of some environmental variable ratios has been previously reported by several authors working in aquatic ecosystems (Barlett and Leff, 2010; Rubin and Leff, 2007; Kisand *et al.*, 2001; López-Flores *et al.*, 2009). The possibility of using ratios between environmental variables in the multivariable analyses is interesting because that allows us to work with relative values and to compare space, time and different communities in which variation ranges are wide. In this study, when the *raw* data variables and the *CoDa* approach analysis were compared, the results obtained were similar with regard to explained variance and also interpretation. The *CoDa* approach was characterized by the introduction of a new environmental driving factor, the bacterial biomass, whose combination with the peptidase enzyme activity became significant. In this sense, the *CoDa* approach contributed to improving the biological interpretation of the phytoplankton distribution based on the *raw* analysis.

5. Acknowledgements

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