Hydrographical forcing and phytoplankton variability in two semi-enclosed estuarine bays

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Abstract

Alfacs and Fangar (North East of the Iberian Peninsula) are two embayments of the Ebre Delta complex with typical Mediterranean characteristics. Both are subject to the same meteorological forcing and receive similar freshwater inputs from irrigation drainage channels. However the basin volume in Alfacs is about ten times larger than in Fangar. We studied the temporal patterns of series of chlorophyll a and phytoplankton counts sampled between 1990 and 2003 from two depths of a fixed station in each bay, and related them to the variability of environmental variables (water temperature, salinity, and stratification). A principal component analysis performed on the correlation matrix among the (log-transformed) abundance data of the most frequent taxa revealed three main trends of variability. The first principal component (PC1) indicated a gradient of marine (more important in Alfacs) versus freshwater (particularly in Fangar) influence. PC2 reflected the seasonal cycle of phytoplankton in Alfacs, characterized by the dominance of a diatom assemblage typical of Mediterranean coastal waters in autumn and a group of dinoflagellates, including toxic taxa, in winter–early spring. PC3 expressed mainly the seasonal changes in Fangar and opposed a mixed phytoplankton group, including mostly dinoflagellates, with population maxima between May and October, to dinoflagellates of the winter group. Empirical Mode Decomposition was applied to the environmental variables and to the principal components in order to analyze the temporal structure of the data. All the series presented strong seasonal modes; an index based on phase shift between pairs of series revealed correlations between some of the principal components and environmental variables (temperature and salinity in Alfacs and temperature, salinity, and stratification in Fangar). Water temperature showed a slight increasing trend along the sampling period. Between 1997 and 2003, some phytoplankton taxa also presented a weak increasing trend, particularly in the bottom samples of Fangar. This finding does not indicate a direct relationship between phytoplankton variability and the actual magnitudes of temperature or salinity. Rather, these environmental variables should be considered here as proxies of the seasonal behavior of a complex of environmental and biotic factors. Differences among the seasonal patterns of phytoplankton variability in Alfacs and Fangar could be attributed to the lower residence times of the water in Fangar, which resulted in a stronger hydrological control of phytoplankton abundance and composition.

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1. Introduction

Estuarine environments, at the boundary between freshwater and marine ecosystems, sustain high biological productivity ecosystems and provide important resources and socio-economical services. Estuaries and, in general, coastal ecosystems are subjected to numerous natural and anthropogenic stresses, acting on a variety of spatio-temporal scales. Local processes include nutrient inputs from land, which will vary as a function of land and water uses. On a large scale, estuarine and coastal waters may be particularly susceptible to the effects of climate change, which could act not only through increasing temperatures, but also through other mechanisms such as alterations of sea level or precipitation patterns on land. The effects of environmental changes include shifts in algal community, food web structure, major nutrient cycles and carbon export (Short and Neckles, 1999; Le Quéré et al., 2007; Ji et al., 2007; Noiri et al., 2005; Sarmiento et al., 1998).

To assess the relative impact of local-scale and climatic forcing on a particular ecosystem, it is necessary to know the main mechanisms and physical variables that drive its behavior. In open waters of temperate areas, much of the variability is imposed directly or
phytoplankton populations may also occur as localized phenomena in nearshore waters present complex seasonal patterns, with large variability both across and within ecosystems (Cloern and Jassby, 2009). In addition to seasonally occurring, somewhat predictable, variability both across and within ecosystems (Cloern and Jassby, 2009). One of the main features triggered by the annual cycle of the phytoplankton community in the Ebre Delta is the senescence of primary producers, which is a key process that directly affects the phytoplankton and their relationship with physical forcing. As other estuaries, the bays of Alfacs and Fangar are very heterogeneous and dynamic systems; their water circulation patterns are complex and subject to changes at a wide range of temporal scales (including daily, seasonal and multiannual). Due to this variability, and in spite of the lack of a strongly dominant process of physical forcing. As other estuaries, the bays of Alfacs and Fangar are very heterogeneous and dynamic systems; their water circulation patterns are complex and subject to changes at a wide range of temporal scales (including daily, seasonal and multiannual). Due to this variability, and in spite of the lack of a strongly dominant process of physical forcing. As other estuaries, the bays of Alfacs and Fangar are very heterogeneous and dynamic systems; their water circulation patterns are complex and subject to changes at a wide range of temporal scales (including daily, seasonal and multiannual). Due to this variability, and in spite of the lack of a strongly dominant process of physical forcing.

In order to sort out climate change effects from direct anthropogenic impacts on phytoplankton variability, it is necessary to understand the mechanisms underlying phytoplankton population changes. With this aim, the present work will explore the patterns of phytoplankton variability and their relationship with physical forcing in the bays of Alfacs and Fangar. The aim of this work is to characterize the patterns of phytoplankton variability and their relationship with physical forcing. These bays present typical shallow coastal ecosystem (SCes) characteristics that are influenced both by land inputs and exchanges with the sea. Seawater enters the bays from the Mediterranean Sea and freshwater comes mainly from discharge channels collecting irrigation water from the rice fields and draining into the bays. Until 2001, these channels were closed in winter, approximately from November to March, and open during the rest of the year. Starting in 2001, the channels are only closed from January to March (Serra et al., 2007). Their mean flow is 13 m$^3$ s$^{-1}$ at Alfacs and 10 m$^3$ s$^{-1}$ at Fangar (Camp and Delgado, 1987). The Chl a concentrations in the bays are high compared with those typical of coastal waters of the Mediterranean (López and Arté, 1973; Delgado, 1987). During 1982–1986, the annual mean Chl a concentrations of Alfacs (3.20 g l$^{-1}$) and Fangar (3.44 g l$^{-1}$) were one order of magnitude higher than those in the neighboring open sea (Delgado, 1987). This relatively high phytoplankton biomass is the main cause of the success of shellfish aquaculture in the bays (López and Arté, 1973).

The data used for this work were collected by IRTA-Santi Carles de la Ràpita (Institute for Food and Agricultural Research and Technology, named “Centre Nacional d’Agricultura” before 1999) from 1990 to 2003 as a component of the monitoring program on water quality of the shellfish growing areas in Catalonia. Temperature, salinity and chlorophyll for Alfacs were taken also during 2004, for but for the sake of simplicity, we will use 2003 when referring to the end of all the time series. The sampling sites were located in the center of the bays (40°N 46.5688°E 44.8611°) in Alfacs (Solé et al., 2009) and at (40°36'33''N, 0°39'22''E) in Fangar. Water samples were taken weekly from 0.5 m depth (surface) with a Niskin bottle or with plastic containers and from 0.5 m above the bottom, at about 5.5 m depth in Alfacs and 3.5 m...
in Fangar (bottom), with a bottom sampling bottle. Water salinity and temperature were measured by means of a portable sonde (WTW instruments). A stratification index was calculated using the Brunt–Väisäla frequency (Eq. (1), where $\rho$ is density, $g$ is acceleration of gravity and $z$ is depth) with the measured temperature and pressure at surface and depth.

$$N = -\sqrt{\frac{g \Delta \rho}{\rho \Delta z}}$$

Between 1990 and 1995, Chl $a$ was measured filtering 500 ml of water on Whatman GF/F filters that were placed on acetone 90% for 6–10 h. The Chl $a$ concentration of the extract was determined, after centrifugation, from trichromatic absorbance readings (Jeffrey and Humphrey, 1975) by means of a Shimadzu UV240 spectrophotometer. After 2000, Chl $a$ was estimated using a TURNER fluorometer. The conversion factor for the in vivo measurements is calculated by the linear regression of the in vivo readings versus measured Chl $a$ concentrations in acetone extracts for the same sample. For this purpose one integrated sample at the central station of each bay was analyzed every week. Water samples for phytoplankton examination were fixed with formalin solution (1% final concentration) and stored in hermetically closed bottles (Delgado et al., 2004). Phytoplankton cells were counted by means of Nikon or Leica DM-IL inverted microscopes (Utermöhl, 1958); settling chambers of 25 ml were used until 1995; thereafter, 50 ml chambers of better optical quality were adopted. The entire bottom of the chamber was scanned at 63–100× magnification to enumerate the larger organisms, and one transect at 200–400× magnification was examined to count the small, more abundant organisms. When possible, mainly in the case of large diatoms and dinoflagellates, the cells were classified down to the level of species or genus. Coccolithophores were not intensively studied, (they were not specially abundant) and only Syracosphaera pulchra was routinely identified. Small flagellates were not quantified.

The studied phytoplankton time-series covers the period from 1990 to 2003. However, there are some intervals without measurements. These gaps are sporadic and affect some of the taxa. Therefore, in order to obtain a long but consistent data set we had to discard some species.

Climatologies of the environmental (temperature, salinity and stratification) and biological data sets were calculated by pooling the data corresponding to each week of the year and calculating the mean for each week.

The linear temporal trends of the environmental and phytoplankton variables were calculated as the regression lines of their weekly anomalies (the difference between the value for a particular week and the corresponding global mean) with respect to time. Only slopes (as determined by the $t$ test) were considered. Due to the methodological changes between 1994 and 1995 and the interruption between 1996 and 1997, only the last part of the series (between 1997 and 2003) was used for the phytoplankton variables (diatoms, dinoflagellates and principal components). All the variable anomalies presented unimodal histograms, although only a few of them could be considered as normally distributed according to the Wilk–Shapiro test. In this context, we considered as significant those regressions of anomalies with respect to time in which both Spearman’s $\rho$ was significant and the 95% confidence interval of the regression slope did not include 0. Chl $a$ was excluded from the regression analysis because

![Fig. 1. Map of the study zone.](image-url)
of the large interval of missing data and the short time span of available measurements with each method (acetone extracts or in vivo). However, the medians of the 1990–1995 and 2000–2004 subseries were compared by means of the Mann–Whitney U test.

The phytoplankton data were analyzed by means of a Principal Component Analysis (PCA). The aim of PCA is to reduce the dimensionality of a data set and provide a new set of uncorrelated variables, named Principal Components (PC), which are a linear combination of the source data and are ordered so that the successive components explain decreasing proportions of the variance present in the original variables (Legendre and Legendre, 1998).

The weights of the variables or loadings reflect the relative importance of a variable within a principal component. PCA allows to summarize in a few principal components a maximized proportion of the information contained in the original data set. As the analysis is based on the correlation or covariance matrix among all the original variables, it is also useful to extract unbiased trends from data with a large associated error, as happens with phytoplankton counts (Estrada, 1979; Delgado, 1987).

The PCA was performed on the correlation matrix among the log-transformed abundances of selected taxa from a pooled data set including the surface and bottom samples of Alfacs and Fangar. We considered a total of 36 taxa that were present in more than 10% of the samples of either Alfacs or Fangar. The exceptions were *D. caudata* and *Scenedesmus* sp. These taxa were found in less than 10% of the samples of both bays, but were included because of their interest (*D. caudata* is

![Fig. 2. Environmental and biological data from Alfacs. Black dots: data at 0.5 m depth; grey circles: data at 5.5 m depth. From top to bottom: A) temperature (°C), B) salinity, C) stratification (s−1), D) Chl a (µg l−1), E) diatoms (log(cell l−1)), F) dinoflagellates (log(cell l−1)).]
a producer of Diarrhetic Shellfish Poisoning (DSP) and *Scenedesmus* sp. is an indicator of freshwater. The number of samples was 1103 (572 from Alfacs and 531 from Fangar). Separate analyses including the whole series or the segments between 1990 and 1994 and 1997 and 2003 (before and after the changes in counting method) gave consistent results with respect to the distribution of the taxa in the component space; for simplicity, only the whole series was considered in this work.

Grossman et al. (1991) concluded that the eigenvalue methodologies, such as PCA, can be used to detect patterns if the number of samples is at least three times the number of descriptors. In our case, the data set was 1103 samples × 36 descriptors (the taxa), with a ratio of about 30 to 1, indicating that our PCA should be stable.

In order to give a precise quantification of the timing and period of the temporal fluctuations of the environmental and biological variables, we used Empirical Mode Decomposition, a recently developed method for time series analysis (Huang et al., 1998) that is particularly well-suited for the study of phytoplankton data sets, which are typically non-linear and non-stationary.

The first step of the EMD is the decomposition of a time series into Intrinsic Mode Functions (IMF) that have well behaved Hilbert transforms and permit the calculation of instantaneous frequencies. The IMFs satisfy two conditions (Huang et al., 1998): (1) The number of extrema and the number of zero crossings must be either equal or differ at most by one and (2) at any point, the mean value of the envelope defined by the local maxima and the envelope defined by...
the local minima is zero. The sum of all IMFs retrieves the original data.

The EMD methodology (Huang et al., 1998) was applied to the time series of temperature, salinity, stratification and to principal components 1 to 3 from the Alfacs and Fangar PCAs.

The degree of synchronization between the IMFs was assessed by means of an index, the quantity \( \phi \) defined in Solé et al. (2009):

\[
\delta_{nm}(t) = \cos(\Delta \theta_{nm}(t)) = Re\left(e^{i\Delta \theta_{nm}(t)}\right)
\]

being \( \Delta \theta_{nm}(t) \) the phase difference between two IMF \( c_n \) and \( c_m \). The variance of \( \delta \) is indicative of the regularity of the phase difference between the IMF that are being compared. We will call this quantity \( \delta \) index.

\[
\delta \text{ index} = \text{var}[\delta_{nm}]
\]

If the \( \delta \) index is 0 both series will have a constant phase shift, while if it is high (up to a maximum of 1) they will be strongly decorrelated. We used \( \text{var}[\delta_{nm}] = 0.25 \) (a slightly lower value than the 0.30 adopted by Solé et al. (2007)) as an operational threshold to select pairs of IMF with a sufficiently constant phase shift.

3. Results

3.1. Environmental variables

The time series of temperature showed a strong seasonal cycle, while the fluctuations of salinity and stratification were more irregular. The dinoflagellates of Fangar seemed to present a well-marked seasonal signal. As can be seen in the figures, there were differences between the two bays concerning the fluctuations of salinity, stratification and dinoflagellate abundance. Examination of graphs (data not shown) of the weekly seasonal variability of the variables indicated that none of the series seemed to present any consistent changes in the yearly timing of maxima and minima.

The temperature climatologies (Figs. 4 and 5) of both Alfacs and Fangar were very similar. The highest temperatures occurred in August with weekly means of 28 °C (although in some occasions temperatures could reach 30 °C). The lowest temperatures, with means of 10 °C and extremes of 6 °C were observed in January. The deep layer was warmer than the shallow layer during the fall and winter months and colder during spring and winter. Bottom salinity ranged between 31 and 36 in the shallow layers of Alfacs, with the higher salinities during the closed channel period. At Fangar, which is the smallest bay, the inputs of freshwater had a greater effect and higher salinities during the closed channel period. The seasonal climatology of stratification (Figs. 4 and 5) reflected the differences between the salinity of the upper and lower water layers and showed a marked increase in April–May. The lowest average stratification was found between January and May in Alfacs and between February and May in Fangar.

The time series of temperature anomalies (Figs. 6 and 7) presented a slight positive slope, while those of salinity and stratification did not show any statistically significant trend except for the bottom salinity samples of Alfacs (Table 1).

3.2. Dynamics and composition of phytoplankton

3.2.1. Principal Component Analysis

The first three principal components (PCs) of the analysis of the pooled Alfacs and Fangar data were considered for further

![Graphs showing temperature, salinity, stratification, and chlorophyll data over time.](image)
Diatoms and dinoflagellates from stratification (1/s) from 5.5 m depth. In the bottom graph, grey circles – linear correlation coefficient of Chl a concentration with diatoms (r = 0.39, significant, for both Alfacs and Fangar) than with dinoflagellates (r = 0.12 for Alfacs and 0.17 for Fangar, also significant values). Chl a peaked in October in Alfacs, while Fangar presented high concentrations from July to November. The lower Chl a values for both bays occurred during late winter and early spring.

The median values of Chl a were 3 (Alfacs) and 2.7 (Fangar) mg m\(^{-2}\) for the period 1990–1995 and 3.8 (Alfacs) and 2.7 (Fangar) mg m\(^{-2}\) for 2000–2004. The difference was significant for Alfacs (p = 0.0002) and not significant in Fangar (p = 0.39) according to the Mann–Whitney U test for a 0.05 significance level. The linear temporal trends of the anomalies of bottom diatoms and dinoflagellates of Fangar were also positive for the 1997–2003. However, the magnitude of the changes was less than 0.05 (log total cells l\(^{-1}\) year\(^{-1}\)) in all cases. None of the series of yearly minimum and maximum values presented statistically significant trends (data not shown).

The time series of the scores of the three first Principal Components can be found in Figs. 9–10. In both bays, the amplitude of the fluctuations of some principal components tended to be somewhat higher from examination. The amounts of variance explained by these components were, respectively 14.95%, 7.84% and 6.85%. These relatively low values are typical of PCAs of phytoplankton data sets with a high number of variables (Legendre and Legendre, 1998).

The position of the taxa vectors in component space, based on their correlations (or loadings) with the first principal components is shown in Fig. 8. The species that contribute the most to the values (or scores) or each component are those with the highest loadings. The first principal component (PC1) presents positive loadings with most of the species, which is a common finding in phytoplankton PCAs. This reflects the common response of most taxa to general conditions favoring phytoplankton growth. The taxa with the highest loadings included dinoflagellates like (the numbers in parentheses refer to Fig. 8) Ceratium furca (3), Prorocentrum minimum (13) and Prorocentrum micans (12). Among the diatoms, the highest positive loadings corresponded to Centric diatoms (20), Cerataulina pelagica (21) and Thalassiosira nitzschioides (31). The only negative loadings correspond to Chaetoceros spp. large (22), Thalassiosira spp. (32) and Scenedesmus spp. (35).

The highest positive loadings on the second principal component (PC2) were found by a group of three diatoms: T. nitzschioides (31), Liloa pacifica (27) and Cylindrotheca closterium (24), accompanied by the heterotrophic ebridian Heteromastus filiformis (34). A group of dinoflagellates with D. sp. (6), A. minutum (2), and Karolodinium spp. (10) presented the most negative loadings. The third principal component opposed a mixed group with high positive loadings, including Eutreptiella sp. (33) and the dinoflagellates (Peridinium quinquecorne (15), Akashiwo sanguinea (1) and Protoperidinium spp. (16) to dinoflagellates like Karolodinium spp. (10), C. furca (3), Ceratium fusus (4), P. micans (12) and Karenia spp. (9), and the benthic diatom Pleurosigma sp. (28).

### 3.2.2. Seasonal and interannual variability

The seasonality of the phytoplankton counts was very different in the two bays (Figs. 4 and 5). In Alfacs the diatom peak occurred in the fall, during the months of September and October, for both the shallow and the deep layers. The cell abundances at the bottom layer were 3–5 times smaller than those at surface. On the other hand, the peak of diatom abundance in Fangar was found during summer, and the shallow layer concentrations were only slightly higher than those of the bottom waters. Dinoflagellates were about one order of magnitude less abundant than diatoms; they reached their maximum during the first months of each year in Alfacs, but peaked in July in Fangar (Figs. 4 and 5). Cell numbers were similar in shallow and deep layers, although on some occasions the deep concentrations in Alfacs were higher than those at the surface. The Chl a followed the diatom trend, as reflected in the higher linear correlation coefficient of Chl a concentration with diatoms (r = 0.39, significant, for both Alfacs and Fangar) than with dinoflagellates (r = 0.12 for Alfacs and 0.17 for Fangar, also significant values). Chl a peaked in October in Alfacs, while Fangar presented high concentrations from July to November. The lower Chl a values for both bays occurred during late winter and early spring.

The median values of Chl a were 3 (Alfacs) and 2.7 (Fangar) mg m\(^{-2}\) for the period 1990–1995 and 3.8 (Alfacs) and 2.7 (Fangar) mg m\(^{-2}\) for 2000–2004. The difference was significant for Alfacs (p = 0.0002) and not significant in Fangar (p = 0.39) according to the Mann–Whitney U test for a 0.05 significance level. The linear temporal trends of the anomalies of bottom diatoms and dinoflagellates of Fangar were also positive for the 1997–2003. However, the magnitude of the changes was less than 0.05 (log total cells l\(^{-1}\) year\(^{-1}\)) in all cases. None of the series of yearly minimum and maximum values presented statistically significant trends (data not shown).

The time series of the scores of the three first Principal Components can be found in Figs. 9–10. In both bays, the amplitude of the fluctuations of some principal components tended to be somewhat higher from
As will be discussed later, this finding may be an artifact due to changes in the counting method. All components presented somewhat marked seasonal variations, but the patterns were different in Alfacs and Fangar. The most regular fluctuations were shown by PC2 in Alfacs and by PC3 in Fangar, especially after 1995. According to the climatologies (Fig. 11), PC1 maxima tended to occur in November in Alfacs and between May and September in Fangar. PC2 presented a maximum in September and a minimum in March–May in Alfacs. In Fangar, PC2 was weakly positive during most of the year and had a minimum in June–July. PC3 tended to be highest between May and October in both bays, but the shape of the distribution was different in Alfacs and Fangar. With the exception of PC2 in Fangar, all PCs were significantly correlated with Chl a concentration (data not shown). The highest linear correlation coefficients with Chl a were shown by PC1 in Alfacs ($r = 0.46$), followed by PC2 in Alfacs, and PC1 and PC3 in Fangar ($r = 0.26$ for all of them).
In both bays, the interannual variability of the phytoplankton variables was relatively small with respect to the seasonal fluctuations, as can be seen by comparing the ranges of the annual means of the variables (shown in Fig. 12 for the period 1997–2003) with the range of the seasonal maxima and minima (Figs. 4, 5 and 11).

3.3. Empirical Mode Decomposition

The application of Empirical Mode Decomposition to the time series of temperature, salinity, stratification, and the three first principal components gave from 7 to 9 IMFs with frequencies ranging from 0.02 week$^{-1}$ to 0.001 week$^{-1}$ for each series. For simplicity, only
4. Discussion

4.1. Environmental variables

The linear regression of weekly temperature anomalies showed a marginally significant positive slope for both surface and bottom samples of both bays. Solé et al. (2009) also reported a small increasing trend for monthly temperature anomalies in Alfacs; however, in their case, the slope was non-significant. Although these observations must be interpreted with caution, given the low correlation coefficients and the short time span of the measurements, they are likely to be associated with the general warming of air temperatures detected in the region (Martín-Vide, 2005). Salinity presented a weak significant increasing trend in the bottom layer of Alfacs. The magnitude of the trend is similar to that detected in coastal waters of Mallorca (0.017 and 0.031 per year at 10 and 50 m depth (Vargas-Yáñez et al., 2007)). The mechanisms accounting for the salinity increase of marine waters are likely to imply circulation changes rather than direct effects of warming or evaporation (Vargas-Yáñez et al., 2007). Similarly, the trends in the bays may be related to changes in the estuarine circulation in response to effects such as the increased temperature (and decreased density) mainly affecting the shallow waters. This increasing trend of salinity contrasts with the changes rather than direct effects of warming or evaporation (Vargas-Yáñez et al., 2007)). The mechanisms accounting for the salinity increase of marine waters are likely to imply circulation changes rather than direct effects of warming or evaporation (Vargas-Yáñez et al., 2007).
tendency to a change in timing along the studied period. Interestingly, the lowest salinities and highest stratification values occurred in Fangar between October and December during the closed channel period, a feature that did not change (data not shown) if the climatology was performed only for the years before 2001, when the ecological freshwater flow between October and November had not been established yet. This observation may be linked to interaction between estuarine circulation and higher surface water densities due to low autumn–winter temperature (Camp, 1994). In addition, freshwater is allowed into Fangar during these months in connection with hunting and fishing activities. Unfortunately, no good data appear to be available on the actual freshwater fluxes. The input of underground freshwater into both bays, with significant implications for both nutrient fluxes and water column stability (Llebot et al., 2010), is poorly known. The underground flux and the channel discharge in Alfacs have been estimated, respectively, by means of a salinity balance (Camp, 1994) and an approximation based on the irrigated area (Camp, 1994; Farnós et al., 2007). A hydrodynamic 3D model implemented by Llebot et al. (2010) corroborated that the calculated magnitudes are realistic. Similar flux estimates are not available for Fangar, which does not yet have a hydrodynamic model.

![Fig. 9. Scores of the three first principal components (PCs) for the 0.5 m depth samples of Alfacs. From top to bottom: A) PC1, B) PC2 and C) PC3.](image1)

![Fig. 10. Scores of the three first principal components (PCs) for the 0.5 m depth samples of Fangar. From top to bottom: A) PC1, B) PC2 and C) PC3.](image2)

4.2. Dynamics and composition of phytoplankton

The lack of differences in the median concentrations of Chl α in Fangar between the 1990–1995 and 2000–2004 subseries suggests that there were no trends in overall phytoplankton biomass during the studied period. In Alfacs, the differences between the two periods are significantly different, showing an increase of the Chl α concentration during the second period. However, these data must be interpreted with caution, due to the change in measurement method that took place in 2000.

The positive trends detected in diatoms, dinoflagellates and principal components for some of the sample subsets (Table 1) suggest that there may have been a slight increase in the abundance of the taxa associated positively with the principal components, but they were too small to be relevant.

Most phytoplankton taxa encountered in this study could be found in both Alfacs and Fangar, but some of them (like Karlodinium spp. and T. nitzschioides) showed striking differences in mean abundance between the two bays (see Table 3). An interesting feature was the qualitative and quantitative differences in the seasonality of diatoms and in particular of dinoflagellates between Alfacs and Fangar. In Alfacs, diatoms peaked in October–November, while dinoflagellates were more abundant between December and April (Fig. 4). In contrast, in Fangar, both diatoms and dinoflagellates were more abundant during the summer (Fig. 5). However, the overall behavior of dinoflagellates in Fangar masks the occurrence of two assemblages that dominate the seasonal cycle and tend to proliferate at different times of the year (summer or winter), as will be discussed below, in connection with the PCA results.

The differences in phytoplankton composition between Alfacs and Fangar are well reflected in the ordination of samples in relationship with PC1. As can be seen in Fig. 15, the samples from Fangar tend to be concentrated in the negative side, while those of Alfacs also occupy the positive side. In phytoplankton analyses in general, most taxa are correlated with one of the sides (the positive one in our case) of the first PC, reflecting that many taxa tend to respond in the same way to favorable sets of environmental conditions (in a spring bloom, for example). In our case, there is also a sizable group of species negatively correlated with PC1 indicating that they favored under different conditions than those of the positive side. The occurrence of organisms like Scenedesmus, P. quinquecornis and Eutreptiella in the negative side (Fig. 8) indicates the relationship of the positive-negative direction of PC1 with a high–low freshwater influence trend. The concentration of the Fangar samples in the negative side of PC1 expresses this trend. It must be noted that “freshwater influence” as used here is not the same as salinity; although the climatology of PC1 is comparable to that of salinity in Fangar, it is quite different in Alfacs, suggesting that the PC1 trend incorporates other ecological effects. The positive correlation of PC1 with Chl a (with a significant value of 0.46) indicates that the high Chl a situations are not associated with a strong freshwater influence. This finding may be explained by the low water residence times associated with situations of large freshwater input in Fangar, which has a smaller basin volume than Alfacs (see Section 4.3). The low water residence times do not allow enough time for the development of large phytoplankton populations.

The obvious change of pattern shown by all series of PC scores between 1994 and 1995 can be accounted for by the changes in the counting method introduced in 1995. The higher settled cell numbers are mainly due to the change in measurement method that took place in 2000.

As can be seen in the series and climatologies of PC2 and PC3 (Figs. 9, 10, 11 and 15), the seasonal fluctuations of PC2 are more regular and
have larger amplitudes in Alfacs than Fangar, while the opposite happens with those of PC3. Both PC2 of Alfacs and PC3 of Fangar (Fig. 11), show a peak in August–September, coinciding with the seasonal water temperature maximum. These observations suggest that PC2 and PC3 mainly express the seasonal cycles in Alfacs and Fangar, respectively, although, of course, each of them also fluctuates in the other bay. The group of diatoms positively correlated with PC2, *C. closterium*, *L. pacificum* and *T. nitzschioides*, which form a consistent cluster of taxa with high positive loadings on PC2, moderately positive on PC1 and close to 0 on PC3, are typical of autumn phytoplankton blooms in the Catalan coast (Margalef, 1969); the prevalence of *T. nitzschioides* in Alfacs (Table 3) responds to the marine character of this bay. The late winter–early spring negative values of PC2 in Alfacs are related to the proliferation of a group of dinoflagellates including *D. sacculus*, *A. minutum*, *Karlodinium* spp. and *Scrippsiella* sp. Two of these taxa, *A. minutum* and *Karlodinium* sp., in addition to others such as *C. furca* and *C. fusus*, generally also with winter maxima, are typical of the winter assemblage of Fangar, as shown by their negative correlation with PC3. In contrast with PC2, the positive side of PC3 includes mainly dinoflagellates (one of them, the brackish water species *P. quinquecorne*) with maxima in late spring and summer; the co-occurrence of other indicators of freshwater influence (like *Eutreptiella* sp.) may be related to the presence of these maxima during a period of relatively high freshwater inputs. The positive correlation of Chl a with PC2 in Alfacs and PC3 in Fangar indicates that the higher phytoplankton biomasses are associated with the assemblages characterizing the positive extreme of these PCs, the autumn blooming diatoms in Alfacs and the dinoflagellate-dominated group in Fangar.

It is important to point out that *D. sacculus* and *A. minutum* are respectively DSP and PSP (Paralytic Shellfish Poisoning) producers.

Fig. 12. Variation of the annual means of the phytoplankton variables for the years 1997 to 2003. The central mark is the median, the edges of the box are the 25th and 75th percentiles, the whiskers extend to the most extreme data points not considered outliers, and outliers are plotted individually. AS: Alfacs Surface; AB: Alfacs Bottom; FS: Fangar Surface; FB: Fangar Bottom.
while *Karlodinium* species produce fish-killing toxins. *Karlodinium* was formerly identified as *Gyrodinium corsicum* (Delgado and Alcaraz, 1999). Later studies of Alfacs strains indicated that there were, in fact, two *Karlodinium* species, *K. veneficum* and *K. armiger* (Garcés et al., 2006), both of them toxic. It is also interesting to note that *D. caudata*, another DSP producer, appears on the positive side of PC2, indicating that it tends to proliferate in autumn rather than in late winter to spring like *D. sacculus*. Another potentially toxic (ASP, Amnesic Shellfish Poisoning, producer) taxon, *Pseudo-nitzschia* spp., does not show any significant association with PC2 and is only weakly correlated with PC3. This finding may be explained because *Pseudo-nitzschia* spp. represents a mixture of species blooming at different times of the year (Quijano-Sheggia et al., 2008).

The interest of multivariate methods such as PCA is not only theoretical. The seasonal patterns shown by the PCs are more regular than those of the individual taxa (data not shown) and of the taxonomically-based functional groups (diatoms or dinoflagellates) to which they belong (compare the diatoms in Fig. 2 with PC2 in Fig. 9, and the dinoflagellates in Fig. 3 with PC3 in Fig. 10). The use of PCs or similar multivariate methods enhances our predictive power with respect to the potential appearance of noxious taxa and can help to improve the management of aquaculture resources.
4.3. Coupling between environmental and biological variables

The EMD analysis allowed us to establish the frequency and amplitude of the seasonal fluctuations with quantitative criteria. The method also allowed us to analyze the timing and variability of the biological variables in relationship with the physical forcing. This point has a particular importance in order to propose typical coupled physical–biological scenarios for the behavior of the two bays. The definition of these scenarios should be a starting point to implement numerical models of the bay ecosystems.

One of the main objectives of this paper was to find relationships between the main climatic and physical forcings and the ecology of Alfacs and Fangar. In both bays, we found significant couplings between trends of phytoplankton variability expressed by the first PCs, temperature and salinity (Table 2). However, as can be seen in Fig. 14, the phase relationships which were very regular for periods like 1991 to 1995 or 1997 to 2001, often broke down near the extremes of the series. This observation was probably an artifact of the method, as shown by repeated EMD analyses with slightly different parameters. Another common drawback with EMD decomposition is that it can produce IMFs with mixed frequencies. This was the cause of the decreased amplitude of Alfacs surface temperature between 2001 and 2003 (Fig. 14). This type of problem can be monitored by means of the Hilbert spectrum (data not shown) of the variable (Huang et al., 1998), but limits the interpretation of the IMFs. EMD has the important advantage over more traditional techniques that it can be applied to nonlinear and non-stationary data, but has rarely been used with ecological data. Further applications of the method should help to explore its potential.

The interpretation of the coupling between the environmental and biological variables must take into account that, rather than to direct
effects of temperature of salinity, the association of these variables with phytoplankton is likely to be mediated by other environmental and biotic factors (like nutrient availability due to freshwater input or predator abundance) that have themselves a seasonal behavior and that are therefore also associated with the seasonal changes of temperature or salinity. The seasonal cycle of phytoplankton biomass is therefore also associated with the seasonal changes of temperature or salinity. The association of these variables with phytoplankton may therefore be mediated by environmental effects of temperature or salinity, the association of these variables with phytoplankton seasonality at the land–sea interface is driven by more than a few climatic factors. In the Ebre Delta basins, tidal currents are relatively weak, but freshwater discharges, both from human-controlled channels and from underground sources, affect not only the stability of the water column, but also represent an important source of nutrients, both inorganic and organic (Llobet et al., 2010).

The effects of environmental forcing (including temperature, mixed layer depth and freshwater fluxes) on the dynamics of two phytoplankton functional groups with basic properties of diatoms and dinoflagellates have been explored for Alfacs by means of a zero-dimensional ecological model (Llobet et al., 2010). The simulations showed that the magnitude of the exchanges with the sea and the nutrients, including dissolved organic phosphorus (Loureiro et al., 2009), provided by freshwater, are essential to explain the primary production observed in Alfacs, and could influence basic features of the abundance and seasonal variability of the two groups. Based on their model, Llobet et al. (2010) suggested that nitrogen was the most limiting nutrient during late spring and summer, while phosphorus was most limiting in the fall and early spring. During winter, sediment resuspension could lead to phosphorus release and to short periods of limitation basic properties of phytoplankton. For example, it appears that dinoflagellates have a relatively higher P demand than diatoms (Estrella et al., 2003). Therefore, winter sediment resuspension events could favor proliferations of dinoflagellates such as *A. minutum*, a producer of paralytic shellfish toxins that tends to bloom in late winter to early spring, or of *Karenia* spp., which blooms at the beginning of winter and can cause water discoloration and death of bivalves and fish. It must be noted, however, that many HAB producers, including dinoflagellates, do not thrive this seasonal variability, PC2 for Alfacs and PC3 for Fangar, peak in February–March, while the magnitude of the exchanges with the sea and the nutrients, including dissolved organic phosphorus (Loureiro et al., 2009), provided by freshwater, are essential to explain the primary production observed in Alfacs, and could influence basic features of the abundance and seasonal variability of the two groups. Based on their model, Llobet et al. (2010) suggested that nitrogen was the most limiting nutrient during late spring and summer, while phosphorus was most limiting in the fall and early spring. During winter, sediment resuspension could lead to phosphorus release and to short periods of limitation basic properties of phytoplankton. For example, it appears that dinoflagellates have a relatively higher P demand than diatoms (Estrella et al., 2003). Therefore, winter sediment resuspension events could favor proliferations of dinoflagellates such as *A. minutum*, a producer of paralytic shellfish toxins that tends to bloom in late winter to early spring, or of *Karenia* spp., which blooms at the beginning of winter and can cause water discoloration and death of bivalves and fish. It must be noted, however, that many HAB producers, including dinoflagellates,
are capable of mixotrophy, so that the relationships between nutrients and HABs are likely to be complex. In any case, nutrients alone are unlikely to explain the marked fluctuations of dinoflagellate numbers at Fangar, which show autumn and winter minima, at times when the proportion of freshwater, as reflected by the low salinities, should ensure a relatively good nutrient availability. Comparison of Fig. 5 (salinity (B), and dinoflagellates (E)) suggests that the decrease in dinoflagellates during the final months of the year might be related to the salinity differences between surface and bottom layers during these months. A likely possibility is that dinoflagellate growth is unable to cope with presumably vigorous estuarine circulation associated with the strong salinity gradient; this could also explain the concomitant (although less intense) decline of diatoms and Chl a.

Further studies of the hydrodynamics of the bays and their effect on the phytoplankton should be addressed to clarify these points.

One of the reasons for the different patterns of environmental variables such as salinity and stratification in Alfacs and Fangar may be the different sizes of the two basins (16×10^6 m^3 Fangar, 153×10^6 m Alfacs). A lower mass of water causes higher fluctuations of the physical variables such as temperature and salinity (Camp and Delgado, 1987). Preliminary calculations of the water residence time in Alfacs obtained with a hydrodynamical model (C.L., unpublished results) suggest that water residence times in Alfacs would range between about 10 days in the open channel period and 25 days when channels are closed. Assuming that the freshwater inputs are of the same order of magnitude in both bays, the resulting freshwater residence times for Fangar would be 5 times shorter than in Alfacs. These differences can account for the marine versus freshwater influence gradient in the phytoplankton composition detected by PC1. High advection rates in an estuary can lead to low productivity, even in conditions of high nutrient concentrations, if the dilution rates are higher than phytoplankton growth rates (Malone, 1977). The lower residence times relative to Alfacs can account for the lower Chl a concentration in Fangar, although it is clear that the residence time in this bay must be high enough to allow Chl a concentrations one order of magnitude higher than those of the neighboring sea (Delgado, 1987). The two bays also present marked differences in the seasonal distribution of freshwater discharges, as indicated by the comparison of the corresponding salinity and stratification climatologies. In correspondence with the higher nutrient content of freshwater, Fangar shows higher nutrient loads (Delgado and Camp, 1987) and concentrations than Alfacs. However, as noted in Section 4.2, the lower residence times of water in Fangar allow for only a partial consumption of these nutrients by phytoplankton, with the result that the build-up of phytoplankton biomass, as measured by Chl a concentrations, is comparable to that found in Alfacs. Other habitat differences between Alfacs and Fangar could include factors such as circulation patterns and turbulence intensity.

Altogether, the fluctuations of the Alfacs and Fangar phytoplankton can be characterized by a large seasonal component resulting from an integration of the meteorological cycle and of anthropogenic influences linked to it (opening and closing of the irrigation drainage channels). The weakness of the temporal trends, together with the relatively low range of interannual variability indicates that during the last few years, there have not been large disturbances affecting the bay ecosystems. However, long-term monitoring will be needed in order to ascertain the response of these ecosystems to continuing global change.
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