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Annual trend patterns of phytoplankton species abundance belie homogeneous taxonomical group responses to climate in the NE Atlantic upwelling

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ABSTRACT

Phytoplankton is a sentinel of marine ecosystem change. Composed by many species with different life-history strategies, it rapidly responds to environment changes. An analysis of the abundance of 54 phytoplankton species in Galicia (NW Spain) between 1989 and 2008 to determine the main components of temporal variability in relation to climate and upwelling showed that most of this variability was stochastic, as seasonality and long term trends contributed to relatively small fractions of the series. In general, trends appeared as non linear, and species clustered in 4 groups according to the trend pattern but there was no defined pattern for diatoms, dinoflagellates or other groups. While, in general, total abundance increased, no clear trend was found for 23 species, 14 species decreased, 4 species increased during the early 1990s, and only 13 species showed a general increase through the series. In contrast, series of local environmental conditions (temperature, stratification, nutrients) and climate-related variables (atmospheric pressure indices, upwelling winds) showed a high fraction of their variability in deterministic seasonality and trends. As a result, each species responded independently to environmental and climate variability, measured by generalized additive models. Most species showed a positive relationship with nutrient concentrations but only a few showed a direct relationship with stratification and upwelling. Climate variables had only measurable effects on some species but no common response emerged. Because its adaptation to frequent disturbances, phytoplankton communities in upwelling ecosystems appear less sensitive to changes in regional climate than other communities characterized by short and well defined productive periods.

1. Introduction

Phytoplankton is considered a good indicator of the impact of the changing climate on aquatic ecosystems because, being the first trophic level, it receives the direct influence of environmental changes affecting the input of new energy and nutrients. Formed by small cells with generation times of days, phytoplankton responds rapidly to changes in the availability of nutrients and light. Marine phytoplankton is composed of many species with different strategies (Margalef, 1978; Smayda et al., 2004). For instance, diatoms are in general well adapted to turbulent, nutrient-rich environments where the nutrients are provided by advection of deep waters. In contrast, flagellated species, as dinoflagellates, have a competitive advantage in nutrient-poor, stratified environments, where motility and mixotrophy facilitates the acquisition of nutrients for growth. Diatoms are also associated to the export of organic matter in the form of large particles contributing to the biological sequestration of carbon in sediments, while the dominance of flagellates result in low export of organic particles and implied that most of the produced carbon would be respired in the water column (Legendre and Rassoulzadegan, 1996). Individual species of diatoms and dinoflagellates have a large variety of forms but at the same time these forms are characteristic for each group, thus phytoplankton cells and colonies can be easily assigned to one of these groups after a minimum training (Thomas, 1997). Distinctive photosynthetic pigments were also characteristic of diatoms, dinoflagellates and other taxonomical groups of phytoplankton,

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thus facilitating their quantification (Mackey et al., 1996; Latasa, 2014). For these reasons, the dichotomy diatoms vs. flagellates has been useful, mainly for biogeochemical studies. In this way, many studies described (Leterme et al., 2005; Bode et al., 2009; Hinder et al., 2012) and modeled (e.g. Thomas et al., 2012) the changes in the relative abundance of diatoms and dinoflagellates as a response to changes in climate, notable those induced by warming leading to the stratification of the surface ocean and thus a reduction in the input of nutrients from deep waters. The implicit assumption of these studies is that there were common functional responses of species in this classification. The response of individual species, however, can be very different even within the same taxonomic group as shown at seasonal (Nogueira et al., 2000) and annual time-scales (Tunin-Ley et al., 2009). Therefore, the final composition of the phytoplankton community after gradual climate changes would depend on how each individual species integrates all the environmental perturbations occurring at small temporal scales.

Upwelling ecosystems are characterized by high nutrient inputs from deep waters by vertical advection that are rapidly transformed in high amounts of phytoplankton biomass and cell numbers. As most coastal upwelling ecosystems display discontinuous events of advection (upwelling) and relaxation (downwelling), the phytoplankton populations are adapted to frequent perturbations in their nutrient and light fields and thus different species assemblages can be found in the different regimes. This variety of phytoplankton assemblages allows for the study of the performance of the different species in a natural laboratory of changing environmental conditions. Changes in the composition of the phytoplankton assemblages in upwelling ecosystems have been studied mainly in relation to the seasonal succession (Casas et al., 1999; Nogueira et al., 2000; Tilstone et al., 2000; Varela et al., 2001; Bode et al., 2005; Anderson et al., 2008; Hansen et al., 2014) and also at large palaeoceanographic scales (Esparza-Alvarez et al., 2007; Abrantes et al., 2011). However, there are less studies analyzing changes in the observed individual phytoplankton species abundance or biomass in relation to the present change in climate conditions in upwelling ecosystems (Tont, 1987; Du et al., 2014), and most studies were limited to one or a few species (Leterme et al., 2005; Pérez et al., 2010) or years (Silva et al., 2009). For instance, in the upwelling region of the NE Atlantic (Galicia) the analysis of multiannual time series of phytoplankton abundance revealed contrasting changes in the abundance of diatoms and dinoflagellates between coastal and open ocean areas. Warming of the surface ocean waters off the continental shelf has been related to a decrease in the abundance of diatoms (Bode et al., 2009) and a weakening of upwelling during some years has been related to the increase in dinoflagellates in protected bays and rias (Pérez et al., 2010) but not in open shelf waters (Bode et al., 2009). These differences were observed at relatively small spatial scales and suggest the prevalence of local over climatic factors in structuring phytoplankton communities. They also call for an investigation of the response of individual species to environmental drivers, as the separation in diatoms and dinoflagellates does not explain sufficiently the observed changes. In contrast, climate was claimed as the main influence on the relative dominance of these phytoplankton groups in studies considering large latitudinal scales in the N Atlantic (Leterme et al., 2005; Hinder et al., 2012), although these studies did not specifically include upwelling ecosystems.

The objective of this study is to identify phytoplankton species showing similar long-term abundance trends in relation to climate and local environmental drivers in shelf waters affected by upwelling in the coast of Galicia (NW Spain). For this purpose time series of phytoplankton species abundance and oceanographic variables covering the last two decades were analyzed.

2. Materials and methods

2.1. Phytoplankton

Samples of water and phytoplankton were taken at Station 2 of A Coruna (Spain) of the RADIALES time series project (http://www.seriestemporales-ieo.com/). This shelf station (80 m depth) was sampled from May 1989 to December 2008 at approximately monthly intervals (Fig. 1). Water samples (50–100 ml) were taken with Niskin bottles at 5 depth levels in the euphotic zone, preserved with Lugol's solution and kept in darkness until phytoplankton identification and counting using the Utermohl's technique (Varela, 1982; Casas et al., 1999). Depending on phytoplankton concentration 10–25 ml of sample were allowed to settle in the Utermohl chamber for up to 24 h. Observation of samples was carried out using a Nikon Diaphot TMD (until May 1997) or Nikon Eclipse TE3000 inverted microscopes with Nomarsky phase contrast system. Magnification powers of ×100, ×200 and ×400 were used, according to the size of organisms. The entire slide was examined at ×100 to account for large species while only transects or smaller areas were examined at higher magnification. At least 250 cells were counted for each sample. Species nomenclature was validated according to the World Register of Marine Species (WoRMS, 2015). All counts and species identification were made by the same expert (M.V.). In this study, only 54 phytoplankton species determined with certainty were employed. Most of the species were diatoms but dinoflagellates, chrysophtes, cryptopythes, prymnesiophytes and euglenophytes were also recorded. Cell counts were integrated in the euphotic layer and the results expressed in cells m^{-2}.

2.2. Oceanographic and climatic variables

In each sampling, temperature, salinity, chlorophyll-fluorescence and irradiance were measured with a Seabird-25 CTD. Moreover, additional water samples from the Niskin bottles were taken for the determination of dissolved nutrients and

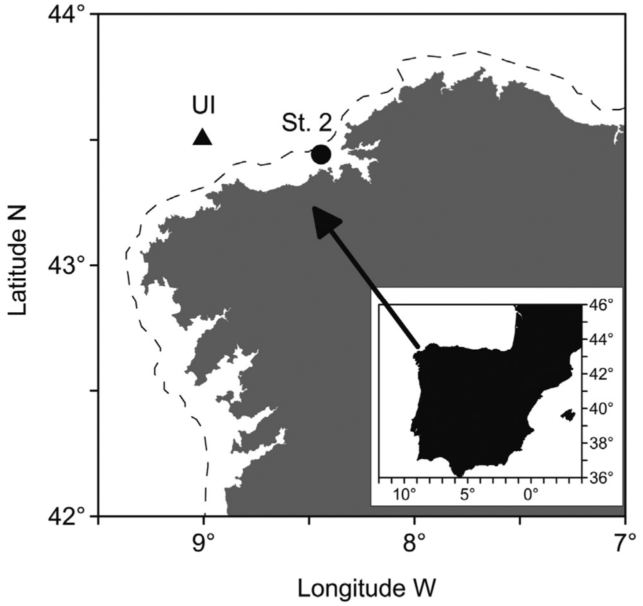


Fig. 1. Location of the sampling site (St. 2) and the centre of the cell of 1° × 1° used for computing the upwelling strength (UI).

chlorophyll. The methods used to determine these variables and detailed information on these data can be found in Casas et al. (1999). Briefly, dissolved nutrients (in this study: nitrate, phosphate and total reactive silica) were determined by flow-segmented colorimetric analysis (Grasshoff et al., 1983) and chlorophyll-a (Chla) by fluorimetric analysis of acetonic extracts of phytoplankton collected on 0.8 μm pore size membrane filters (until 1992) or glass-fiber GF/F type filters (from 1993 onwards). Specific calibrations were performed to ensure the continuity of the chlorophyll series when changing from the filter fluorometer method (Parsons et al., 1984) to the spectrofluorimetric technique (Neveux and Panouse, 1987) after 2001.

Local oceanographic conditions at the time of sampling were summarized by the values of surface temperature (SST, averaged between 0 and 5 m depth), the difference in temperature (dt) and density (dst) between the surface and 40 m depth (indices of stratification), and the nitrate (NO3), phosphate (PO4) and silicate (Si) concentrations integrated in the upper 40 m (average depth of the euphotic zone as determined with the irradiance sensor of the CTD).

The upwelling strength (UI) was estimated by the Ekman transport ($m^3\ s^{-1}\ km^{-1}$) and computed from the values of atmospheric pressure at the sea level for a 1° × 1° cell centered at 43.5°N, 9°W (González-Nuevo et al., 2014). In this study we used monthly averages of the index provided by the Instituto Español de Oceanografia, Spain (http://www.indicedeafloramiento.ieo.es; downloaded on June 2011).

General climatic conditions in the study region were represented by the dominant atmospheric pressure anomalies (Hurrell and Dickson, 2004): the North Atlantic Oscillation (NAO) and the Eastern Atlantic Pattern (EA). Monthly values of these indices were obtained from the Climatic Prediction Center of the National Oceanographic and Atmospheric Administration, USA (http://www.cpc.ncep.noaa.gov/; downloaded on June 2011).

2.3. Statistical procedures

To identify the main seasonal blooms and significant long-term changes in trends, each time series of phytoplankton abundance y(t) was decomposed in the additive form $y(t) = T(t) + S(t) + R(t)$, where T(t), S(t) and R(t) denote the long-term trend, the seasonal and the remainder components, respectively. Gaps in the monthly series represented a small fraction of the whole observed sequences (19 out of 236) and were overcome by subsequent local smoothing. Local linear regression -LLR- (Fan and Gijbels, 1996) was sequentially used as a smoothing procedure on the series to obtain nonparametric approximations of these components. Given a series of abundance records $y(t)$ at the time points $t = t_1,\ldots,t_N$, the LLR estimator $\hat{y}(t_0)$ at an arbitrary point t_0 is obtained fitting a straight line in a local neighborhood of t_0. The size of the neighborhood is determined by a nonnegative number b_N (bandwidth or smoothing parameter) and the local fit is performed weighting the contribution of every time point t_i according to a kernel function K. Bandwidth b_N plays a main role by controlling how strong the smoothing level of the time series is. As b_N gets larger the smoothing gets stronger. In fact, by working on time series, local linear smoothers can be seen as a low-pass filter allowing to pass the low-frequency signals and attenuating signals with frequencies higher than a cutoff frequency controlled by the bandwidth. For instance, a Gaussian kernel and a bandwidth (in months) $b_N = 5$ lead to local fits where observations farther than one year have a poor contribution, and therefore annual and shorter cycles are filtered out in the estimation process. Taking $b_N = 15$ months means to obtain local averages on 3-year periods approximately, and so multiannual oscillation effects are also removed. All time series were subjected to an initial exploratory analysis including local linear fittings with bandwidths $b_N = 2.5$, 5, 10, and 15 months.

The estimation procedure works sequentially as follows. First, the long-term trend component $T(t)$ of each log-transformed time series is approximated by a local linear smoother $\hat{T}(t)$ using a large bandwidth to cancel short cycle effects. Then, the detrended series $y(t) - \hat{T}(t)$ are split into subseries of length 12 covering annual periods on the observation interval. Based on all the annual curves, an estimator $\hat{S}(t)$ of the seasonal component is obtained by LLR smoothing with bandwidth optimized by a proper plug-in procedure (Ruppert et al., 1995). The estimated trend and seasonal components are then subtracted from the original series to obtain the remainder component $\hat{R}(t) = y(t) - \hat{T}(t) - \hat{S}(t)$. Finally, an autoregressive and moving-average (ARMA) model is fitted to the remainder component in order to determine a possible decomposition in ARMA component and residual term ("white noise").

Dissimilarity-based hierarchical clustering was performed to group the phytoplankton species into homogeneous clusters according to the temporal patterns of their abundances. If homogeneous groups of species are identified, then a few representative series would be useful to describe the temporal dynamics of all analyzed species. The based-dissimilarity clustering procedures consist in computing an initial pairwise dissimilarity matrix, $D = (d_{i,j})$, with $d_{i,j}$ denoting the distance between the i-th and j-th series, and then developing a standard cluster algorithm starting from the matrix D. In this point, a key issue is how dissimilarities $d_{i,j}$ are measured. Since our clustering purpose is aimed to identify similar dynamic behaviors, a conventional dissimilarity such as the Euclidean distance could lead to unsuitable results due to the interdependence relationship between values is ignored. To overcome this drawback, a functional approach is considered. Specifically, each smoothed series is treated as a functional data and a semi-metric distance between curves is then assessed. A range of families of semi-metrics have been provided in the literature, including those based on functional principal components analysis, on successive derivatives, on functional partial least squares and so on (see Ferraty and Vieu, 2006). The choice among one or the other can be drawn by the shape of curves, exogenous information or the goal of the analysis. In this work, we follow the partial least squares approach (MPLSR), which has recently gained popularity in the functional data literature (Delaigle and Hall, 2012). The approach MPLSR (multivariate partial least squares regression) computes a simultaneous decomposition of predictors and responses in such a way that the covariance between both sets of variables is maximized. MPLSR has shown a good performance in analysis of a large number of highly multivariate and complex data sets, particularly in presence of multicollinearity, as in the case of functional data (Preda and Saporta, 2007). Based on these nice properties, the functional MPLSR semi-metric introduced by Ferraty and Vieu (2006) has been selected to perform clustering on smoothed versions of the standardized time series. As the number of underlying clusters was unknown, agglomerative hierarchical clustering was performed to obtain a sequence of nested cluster solutions. The optimal partition of clusters was determined using the Average Silhouette Width (ASW, Kaufman and Rousseeuw, 1990). The ASW coefficient for a particular partition provides an overall measure of how well objects have been split: the larger ASW, the better the split. In order to support the heterogeneity of the obtained cluster solution, the equality of mean curves for the groups forming the cluster partition was checked using a functional k-sample test (Estévez and Vilar, 2013).

To determine the significant relationships between phytoplankton and environmental series we focused on the representative abundance series within each cluster. The representative pattern of a particular group was determined by selecting the series whose average dissimilarity to all the objects in the group is minimal, commonly referred as “medoid” in clustering. This way, the representative element for each group is necessarily a member of dataset, i.e. a particular species, and the temporal dependence structure is preserved. Regarding the environmental variables, redundancy is also expected due to the large number of considered variables, and a smaller set of non-redundant variables was identified from the correlation matrix. This selection was preferred from that of a principal components analysis that required seven components to explain a high fraction of the environmental data variability. As in the case of the abundances series, temporal variability in monthly mean values for each environmental series was sequentially decomposed in trend, seasonal and remainder components by using again LLR-based approximations. In all cases ARMA structures were fit to the estimated remainder components to obtain white noise free of temporal dependence. Based on these residual sequences, the relationships between abundance data and environmental variables were investigated by using linear regression and also nonparametric additive regression models in the form $y(t)=f_1(x_1(t))+f_2(x_2(t))+\ldots+f_r(x_r(t))+\varepsilon(t)$, where $y(t)$ is a selected phytoplankton species, $x_1, x_2, \ldots, x_r$ denote the environmental variables, $f_1, f_2, \ldots, f_r$ are arbitrary smooth functions estimated from the data and $\varepsilon(t)$ are residuals having constant variance and zero-mean.

All statistical analyses were performed by using some specific libraries and self-programmed code implemented in the R language (R Core Team, 2014), which is available upon request.

3. Results

3.1. Phytoplankton species clusters

The statistical analysis produced 4 clusters of species series with similar characteristics in the trends (Fig. 2). Cluster 1 included 23 species characterized in general by large abundances (>59% of total abundance) with some interannual variability but no overall trend (Table 1). The diatom *Nitzchia longissima* was the representative species (medoid) for this cluster that included also 4 dinoflagellates, 1 chrysophyte (*Distephanus speculum*) and the euglenophyte *Eutreptiella* sp. (see Supplementary Table A1 for detailed species information). Cluster 2 was formed by species accounting for 14% of total abundance and showing a marked decrease in abundance during the 1990's but more stable distributions thereafter. The cluster included 10 diatoms, 2 dinoflagellates, the chrysophyte *Solenicola setigera* and the prymnesiophyte *Phaeocystis pouchetii*, with the diatom *Thalassiosira antarctica* being the representative species. The species in Cluster 3 (9% of total abundance) showed an increase in their distributions in early 2000's, as exemplified by small species of the diatom genus *Chaetoceros*. This cluster included 7 diatoms, 5 dinoflagellates and the chrysophyte *Dictyocha fibula*. Last cluster (Cluster 4) was formed by species showing a marked increase in abundance in the early 1990's followed by a period of relative stability and divergent responses (either increases, decreases or no changes) in the last decade (Fig. 2). This cluster was best characterized by the trend of cryptophytes but also included the dinoflagellates *Heterocapsa niei* and *Torodinium robustum* and the

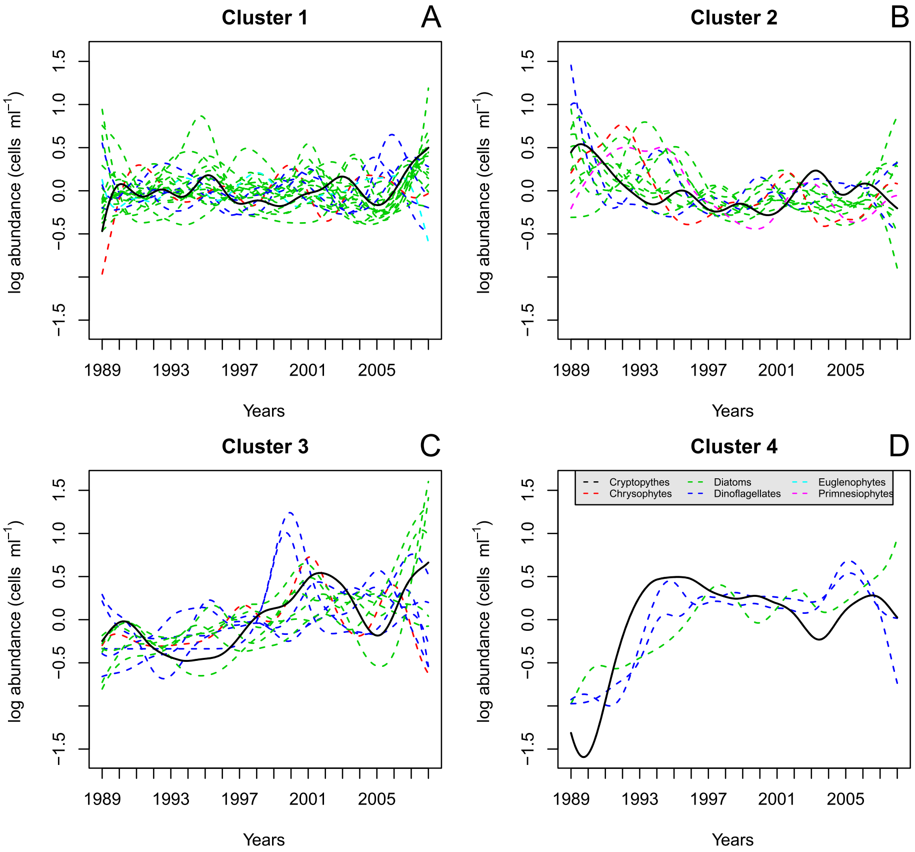


Fig. 2. Long-term trends: Four-cluster solution from the hierarchical clustering of LLR estimators of the standardized abundance (dashed curves) based on a high amount of smoothing (b = 10). Solid lines identify the medoids within each group. Cluster 1, B: Cluster 2, C: Cluster 3, D: Cluster 4. Color of lines indicates the taxonomic group of each species (cryptophytes; chrysophytes; diatoms; dinoflagellates; euglenophytes; prymnesiophytes).

Table 1
Characteristics of species clusters. % total: percent of total phytoplankton abundance; mean ± se: cell abundance (cell ml^{-1}); pattern species: taxa with trend in the medoid of the cluster trends. The number of taxa of diatoms, dinoflagellates, cryptophytes, chrysophytes, prymnesiophytes and euglenophytes in each cluster is also indicated (the complete composition of clusters appears in Supplementary Table A1).

	Cluster			
	1	2	3	4
% total	59.15	13.79	9.00	18.07
Mean ± se (cell ml^{-1})	14.55 ± 8.44	5.57 ± 2.15	3.91 ± 1.37	25.55 ± 23.10
Pattern species	*Nitzschia longissima*	*Thalassiosira antarctica*	*Chaetoceros* spp.	*Cryptophyceae*
Diatoms	17	10	7	1
Dinoflagellates	4	2	5	2
Cryptophytes	0	0	0	1
Chrysophytes	1	1	1	0
Prymnesiophytes	0	1	0	0
Euglenophytes	1	0	0	0

diatom *Navicula transitans*. Despite the low number of species, this cluster accounted for 18% of total abundance. It must be noted that all clusters included diatom and dinoflagellate species.

The trend in total abundance followed closely that of *Nitzschia longissima*, the representative species of Cluster 1, while it was much less coincident with the representative species of the other clusters (Fig. 3).

3.2. Environmental variability

Most of the environmental variables considered were significantly correlated (Table 2). Therefore a subset was selected for subsequent analysis. Changes in the structure of the water column were summarized by dt, as it was correlated with SST and dst, and only NO_3 (as an indicator of fertilization) and Si (particularly important for diatoms) were retained. Climatic indices were independent and UI was only correlated with EA. Chlorophyll concentration, an indicator of phytoplankton biomass was uncorrelated with most environmental variables, except with UI. Although not exactly an environmental variable independent of the phytoplankton abundance, Chla was employed in the additive models along with the selected variables to measure the contribution of each phytoplankton species to the variability of total phytoplankton biomass.

The selected variables showed in general weak trends during the period considered (Fig. 4). The trends accounted for only 3.99%, 45.43%, 30.90%, 61.96%, 95.86%, 90.96% and 15.54% of total variance of dt, Chla, NO_3, Si, NAO, EA and UI, respectively. Thermal stratification was almost unchanged during the study and only NAO displayed a clear negative trend. Trends in nutrients included periodic

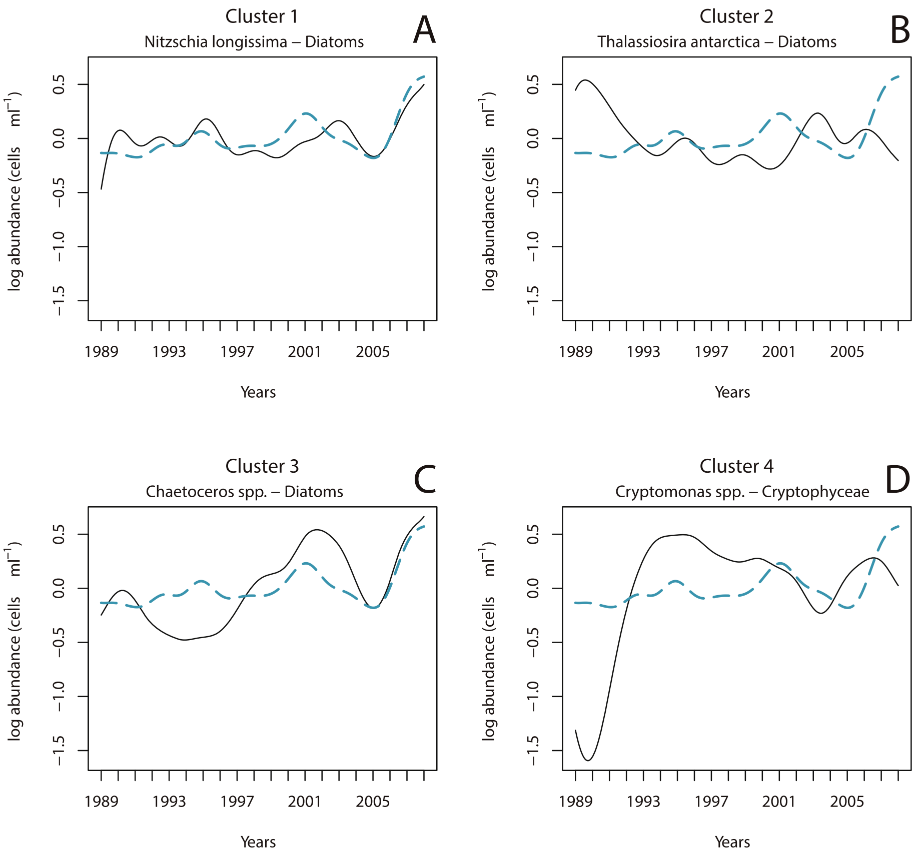


Fig. 3. Representation of the cluster medoids along with the trend for total abundance (dashed line). A: Cluster 1, B: Cluster 2, C: Cluster 3, D: Cluster 4.

Table 2
Correlations (Pearson's r) between environmental variables. Significant ($p < 0.05$) values appear in boldface. SST: sea surface temperature; dt: difference in temperature between 5 and 40 m depth; dst: difference in density (σ_t) between 5 and 40 m depth; Chla: chlorophyll-a; NO3: nitrate; PO4: phosphate; Si: silicate; NAO: North Atlantic Oscillation; EA: Eastern Atlantic Pattern; UI: upwelling strength.

	SST	dt	dst	Chla	NO3	PO4	Si	NAO	EA	UI
SST	1.000	–	–	–	–	–	–	–	–	–
dt	**−0.777**	1.000	–	–	–	–	–	–	–	–
dst	**0.554**	**−0.665**	1.000	–	–	–	–	–	–	–
Chla	0.069	**−0.280**	0.099	1.000	–	–	–	–	–	–
NO3	**−0.419**	**0.282**	**−0.134**	−0.113	1.000	–	–	–	–	–
PO4	**−0.300**	**0.123**	**−0.143**	−0.074	**0.654**	1.000	–	–	–	–
Si	**−0.125**	**0.213**	−0.090	−0.146	**0.501**	**0.417**	1.000	–	–	–
NAO	**−0.168**	0.047	−0.014	−0.106	0.140	0.138	−0.051	1.000	–	–
EA	**0.205**	−0.056	0.121	−0.088	**−0.272**	**−0.216**	−0.010	0.068	1.000	–
UI	**0.123**	**−0.441**	**0.160**	**0.293**	−0.045	0.097	**−0.273**	0.123	**−0.443**	1.000

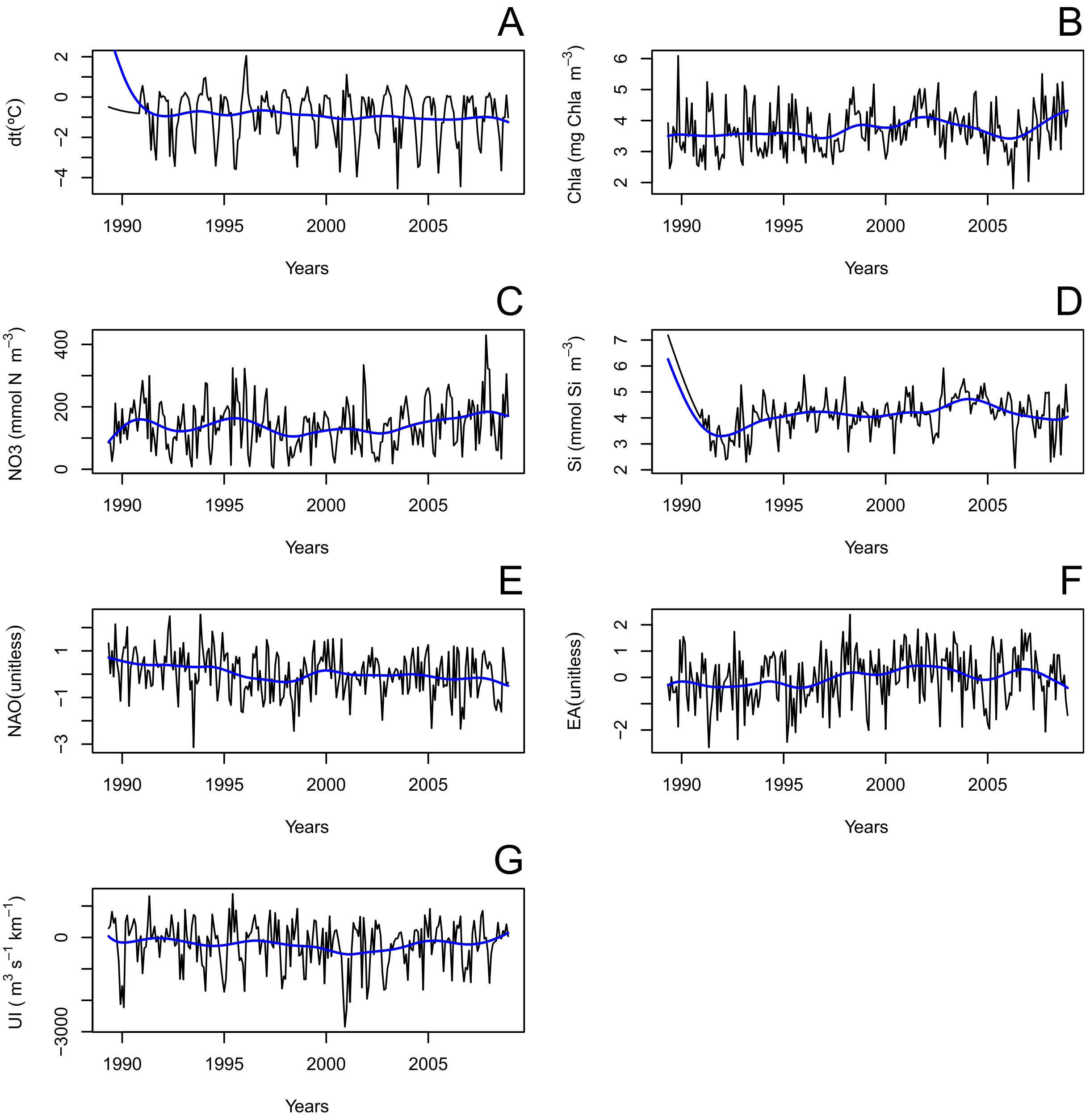


Fig. 4. Series and estimated trends ($b = 10$) of environmental variables (A: dt, B: Chla, C: NO3, D: Si, E: NAO, F: EA, G: UI) selected after correlation analysis (Table 2).

oscillations every 3–5 yr, with a net increase in nitrate and a decrease in silicate in the last decade. The upwelling intensity decreased slightly with oscillations during the 1990's but increased after 2000 to reach the values recorded at the beginning of the observation period. Average chlorophyll remained almost constant until mid 1990's and then displayed an oscillation to reach a maximum in 2002 and a relative minimum in 2006, followed by a continuous increase in recent years.

3.3. *Relationships between phytoplankton groups and the environment*

A nonparametric additive regression model fitted to total abundance of phytoplankton showed a significant effect of chlorophyll and nitrate (Fig. 5). In the case of Chla the relationship was positive and almost linear, thus confirming that the abundance of the selected 54 species was representative of the whole phytoplankton community. In the case of nitrate, its effect on the abundance was positive but only at low concentration values, suggesting the modulating effect of other variables not considered at high concentrations.

Additive models fitted for the different species time series showed a large variety of results and only 10 series could not be adjusted (Table 3, see also supplementary Table A1). Chlorophyll was the most frequent variable selected for the individual species models in all trend clusters (up to 40% of species in Cluster 1), followed by nitrate (33% of species in Cluster 4) and silicate (20% in Cluster 2). In contrast, the climatic indices were correlated with only a few species: NAO was selected for up to 5% of species in Clusters 1 and 2 (but not for species in Clusters 3 and 4) and EA for up to 8% of species in Clusters 1, 2 and 3 (but not for Cluster 4). Upwelling intensity was significant for up to 20% of species in all clusters.

The variety of results in the individual species revealed that similar trends can result from different combinations of environmental variables. This was illustrated by the models for the representative species of the trend of each cluster (medoid) and those of the species with larger abundance values in the cluster (Fig. 6). In the case of cluster 1 the model for the medoid *Nitszchia longissima* included a negative and linear relationship with thermal stratification and the converse with nitrate with a direct impact in the chlorophyll concentration. However, the most abundant species in this cluster (*Chaetoceros socialis*, see Table A1) is better explained by the combined effect of positive values of EA and UI, modulated by increasing amounts of nitrate, particularly at low concentrations. This species also contributed to Chla but only at high concentration values. This difference in responses is more evident in the case of cluster 2. The medoid of this cluster (*Thalassiosira antarctica*) only displayed a positive (but non linear) relationship with Chla (Fig. 6). In contrast, the dominant *Pseudoniszchia pungens* was favored by a moderate increase of stratification but negatively affected by intense stratification. In addition, high nitrate and low silicate concentrations contributed to the increase in abundance of this species.

Table 3
Frequency (%) of species time series in each cluster with significative relationships with environmental variables in the additive models. Variable names as in Table 2.

	Cluster			
	1	2	3	4
dt	10.0	4.0	7.1	16.7
Chla	40.0	36.0	35.7	16.7
NO3	20.0	24.0	28.6	33.3
Si	7.5	20.0	7.1	16.7
NAO	5.0	4.0	0.0	0.0
EA	7.5	8.0	7.1	0.0
UI	10.0	4.0	14.3	16.7

For clusters 3 and 4 the medoid species was also the dominant. *Chaetoceros* spp. contributed almost linearly to Chla and was positively related to nitrate concentrations but only up to an upper concentration limit (Fig. 6). Cryptophytes were among the few series that showed a significant correlation with the upwelling intensity, in this case linear and negative.

4. Discussion

The analysis of individual long term trends revealed that the abundance of phytoplankton showed largely species-specific independent responses. Trends of similar statistical shapes were observed in species of very different taxonomical and functional groups. This result is in apparent contradiction with the expected response of phytoplankton according to their functional group, at least with the changes observed in other regions of the N Atlantic (Leterme et al., 2005; Hinder et al., 2012; Hernández-Fariñas et al., 2014). However, previous studies in the Galician shelf already noticed that the trends observed in the relative proportion of diatoms vs. dinoflagellates changed from oceanic to coastal waters (Bode et al., 2009).

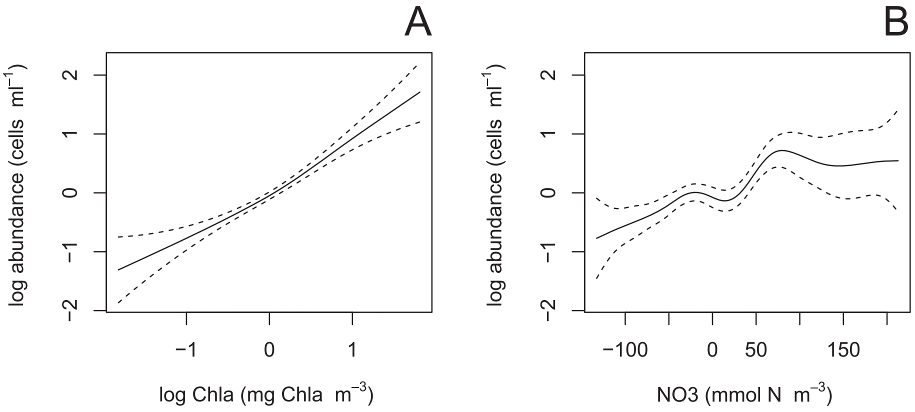
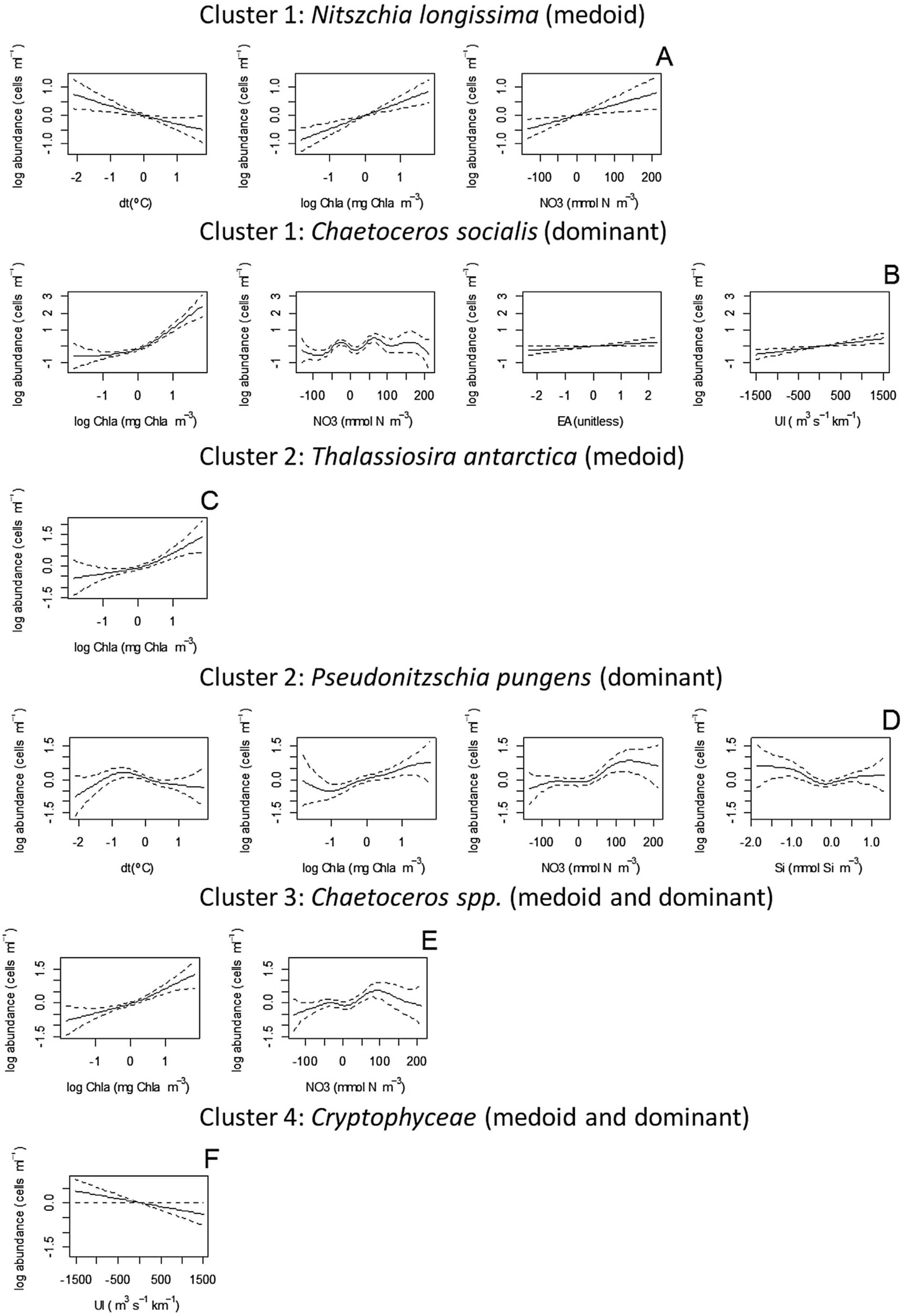


Fig. 5. Significant effect variables (A: chlorophyll; B: nitrate) in the generalized additive model (GAM) for the total abundance with their respectives point–wise 95% confidence bands (doted lines).



Cluster 1: *Nitszchia longissima* (medoid)

Cluster 1: *Chaetoceros socialis* (dominant)

Cluster 2: *Thalassiosira antarctica* (medoid)

Cluster 2: *Pseudonitzschia pungens* (dominant)

Cluster 3: *Chaetoceros spp.* (medoid and dominant)

Cluster 4: *Cryptophyceae* (medoid and dominant)

Fig. 6. Significant relationships with environmental variables of the additive models for species representative of the clusters (medoid and dominant species). A: Cluster 1 (medoid), B: Cluster 1 (dominant), C: Cluster 2 (medoid), D: Cluster 2 (dominant), E: Cluster 3 (medoid and dominant), F: Cluster 4 (medoid and dominant).

4.1. Species trends

The 54 species analyzed are only a subset of all phytoplankton cells described in this ecosystem and it can be argued that there were not representative of the whole phytoplankton community. Casas et al. (1999) reported 205 species or categories of phytoplankton cells in the coast of A Coruña but only 49 contributed more than 20% to total abundance and could be identified with certainty. Most of the remaining categories were undetermined species, generally of small size. Total abundance of the species selected in this study, however, explained a significant fraction of the chlorophyll concentration, a measure of phytoplankton biomass that takes into account all the phytoplankton types present. Indeed, the abundance of species of large size, either because their individual cells are large or because they form long chains and colonies, as those of the genus *Chaetoceros* showed the highest correlations with chlorophyll. These results agree with those of previous studies in this region demonstrating that large phytoplankton cells are the main drivers of change in phytoplankton biomass measured either as chlorophyll or carbon (Varela et al., 1991; Huete-Ortega et al., 2010).

The low coherence of clusters can be interpreted as a consequence of the mostly weak trends found in individual species. For instance, the average silhouette coefficient for all the 4 clusters obtained was 0.58 (ranging from 0.42 for cluster 3 to 0.80 for cluster 4). Alternative clustering procedures resulted in similar groups of species (not shown) but in all cases the trend cluster did not correspond with taxonomical groups. Even diatom species were not segregated from flagellated phytoplankton (as dinoflagellates, cryptophytes, chrysophytes or euglenophytes), indicating that there was not a common long-term trend of the studied phytoplankton community that could be ascribed to functional traits related to the ability of obtaining nutrients in stratified conditions. Our results agree with the analysis of optimum temperature and thermal niche width in a large number of phytoplankton strains that were not related at taxonomic groups above the genus level (Thomas et al., 2012). In addition, other studies have described phytoplankton dynamics as the result of species-specific life traits unrelated to major taxonomical groups (Reynolds, 1997; Nogueira et al., 2000; Alves De Souza et al., 2008). For instance, species rapidly responding to upwelling in Galicia (competitors) were generally r-strategists of low cellular size, with high nutrient absorption and growth rates, while stress-tolerant species dominated in non-upwelling, stratified waters and were generally k-strategists of large size, with low nutrient and light harvesting efficiencies (Nogueira et al., 2000). A third group of species (ruderals) were adapted to intermediate and highly-fluctuating environments. All groups included diatom and dinoflagellate species as well as species from other taxonomical entities, as the clusters found in the present study. Using the assignation of individual species to competitor, stress-tolerant or ruderal strategies in the analysis of Nogueira et al. (2000) the composition of the trend clusters in this study also indicated a large variability but some patterns emerged (Table 4). Half of the species of clusters 1 and 3 were classified as competitors while only a minor fraction of species in all clusters were stress-tolerant or ruderals. The latter were only codominant with competitors in cluster 2. Moreover, all the representative species of the trend clusters (medoid or dominant) were competitors. The species in Cluster 1, that was the main contributor to total phytoplankton abundance, showed no clear trend during the study. The representative species of this cluster (*N. longissima* and *C. socialis*) were typical species blooming in spring and after upwelling events (Casas et al., 1999; Nogueira et al., 2000), as most species of this cluster. These results support the major role of blooms as determinant factors of the structure of the phytoplankton communities in upwelling ecosystems at seasonal (Casas et al., 1999; Nogueira et al., 2000; Anderson et al., 2008; Silva et al., 2009) and also at interannual scales (Esparza-Álvarez et al., 2007; Bode et al., 2009; Silva et al., 2009; Du et al., 2014). Other competitors, as those characteristic of Cluster 2 (e.g. *T. antarctica* and *P. pungens*) showed a decrease during the 1990's but their numbers stabilized thereafter, while small *Chaetoceros* spp. cells showed a clear increase in the last decade, characteristic of species in Cluster 3. Finally, species in Cluster 4 (e.g. cryptophytes) showed trends that mirrored those of Cluster 2: with increases in the 1990's and subsequent stabilization. Examples of stress-tolerant species were *R. imbricata* and *D. acuminata* (Cluster 1), *P. sulcata* (Cluster 2), *G. spyrale* (Cluster 3) and *T. robustum* (Cluster 4), while ruderal species included *Chaetoceros affinis*, *S. trochoidea* and *Eutreptiella* sp. (Cluster 1), *Chaetoceros decipiens* and *L. annulata* (Cluster 2), *T. nitzschioides* (Cluster 3) and *H. niei* (Cluster 4).

Table 4
Relative proportion of species types in each cluster (percent of total number of species in the cluster) classified according to their life history strategies (competitors, ruderals or stress-tolerant). Individual species assignations to strategies can be found in Nogueira et al. (2000).

Cluster	Competitors	Ruderals	Stress-tolerant
1	50.0	25.0	25.0
2	40.0	40.0	20.0
3	50.0	25.0	25.0
4	33.3	33.3	33.3

These results suggest that competitor species, accounting for a large fraction of total abundance, are the main drivers of phytoplankton community at long time scales in this nutrient-rich region favored by coastal upwelling. These species dominate in all types of trends, as found in the present study but also were the main agents of seasonal succession (Casas et al., 1999; Nogueira et al., 2000). In contrast, ruderal and stress-tolerant species can be considered as the background flora filling the niche spaces open during the short transitional periods between blooms (Varela et al., 1991; Tilstone et al., 2000). As found for taxonomic groups, there was no clear separation of annual trend patterns according to these major life-traits thus suggesting that individual species performance at large time scales are tuned according to the response of other species in the community and other factors as grazing or parasites (Litchman et al., 2012). In this way, the successional sequence repeated seasonally or after every upwelling event (Casas et al., 1999; Nogueira et al., 2000) would not be translated at decadal time scales as the final composition of the community would depend on the realization of the ecological niche of each species in the presence of other species. In addition, the response to changing environmental conditions at these time scales would not only imply species replacement but also evolutionary changes (Thomas et al., 2012) that would be also affected by species interactions within the community (Litchman and Klausmeier, 2008; Litchman et al., 2012).

4.2. Environmental drivers

In contrast with other studies in non upwelling areas we did not find any major effect of the regional climate in the studied phytoplankton community. For instance, NAO and other climate indicators were identified as the major factors influencing the changes in the composition of the phytoplankton community in most of the N Atlantic (Leterme et al., 2005; Hernández-Fariñas et al., 2014). However, in our study NAO only explained part of the variability in 3 out of 54 species (Suppl. Table A1) and the climatic indices were not selected in the additive models explaining the abundance of the representative species of the trend clusters, with the only exception of the index EA that was positively related to *C. socialis* (Fig. 6). Previous studies in this upwelling ecosystem also noted the lack of a consistent effect of these indices over the abundance and biomass of plankton (Bode et al., 2009; Pérez et al., 2010) as a consequence of the differential effects on the surface ocean caused by the tri-polar distribution of the nodes of atmospheric pressure across the Atlantic (Visbeck et al., 2003). This feature implies that the changes in currents and upwelling induced by modifications in the wind curl in northern regions may be of opposite sign than those observed in southern regions, thus causing different changes in their ecosystems (Leterme et al., 2005; Pérez et al., 2010). During the period considered in this study NAO showed a small decreasing trend in the 1990's followed by a period of stability, while EA showed almost the opposite pattern. The effect of these major climatic modes on the local oceanography of the study area seemed also small as there were no clear trends in the intensity of upwelling (UI) nor in water column stratification (dt).

Because of the relative stability of regional climatic forcing factors between 1989 and 2008, the trends in abundance of phytoplankton species appeared related mainly with nutrient inputs (Table 3). Nutrients provided by upwelling and winter mixing would have different concentrations according to the origin of the deep water masses and the depth of the winter mixing in the region (Pérez et al., 2005). This would explain that variations in the UI series did not match those in nitrate or silicate (Fig. 4). Additional inputs of nutrients are provided by the atmosphere and by the land. Atmospheric inputs may be significant not only for oligotrophic regions of the ocean but also for coastal regions heavily urbanized (Howard et al., 2014). Nutrient inputs from the land are enhanced in periods of high precipitation by runoff, as observed in Galicia with silicate (Prego and Bao, 1997). Finally, coastal eutrophication is expected to be enhanced by the interaction of increasing anthropogenic nutrient discharges with oceanographic conditions imposed by warming (Statham, 2012). Other studies suggest an increase of coastal nutrients from remineralized organic matter that would explain the interannual changes observed in phytoplankton assemblages in Galicia (Pérez et al., 2010).

The phytoplankton species of the coastal upwelling ecosystem of Galicia showed interannual trends in abundance that were more related with the local availability of nutrients than with climatic or physical oceanographic properties. This feature contrasts with the effects observed at seasonal scales where phytoplankton communities changed following major life-traits related to the efficiency of nutrient uptake and growth in relation to stratification and oceanographic regimes (Nogueira et al., 2000; Edwards et al., 2013). In the absence of major changes in regional climate and upwelling dynamics non-upwelling nutrient input appears as a major driver of change in species abundance patterns for phytoplankton communities already adapted to high nutrients and frequent changes in light fields caused by turbulence and mixing as those living in upwelling ecosystems. This result implies that the predictions of the composition of phytoplankton communities in the future need to consider not only major changes in warming and stratification (for instance a reduction in upwelling) but also responses of individual species to the available nutrients. These responses are determined by trade-offs between functional traits and tolerance to relevant environmental variables (Edwards et al., 2013) and are predictable over major environmental gradients (e.g. Hinder et al., 2012) but they are not well described for fluctuating environments (Litchman and Klausmeier, 2008) as upwelling ecosystems.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.marenvres.2015.07.017.

References

Abrantes, F., Rodrigues, T., Montanari, B., Santos, C., Witt, L., Lopes, C., Voelker, A.H.L., 2011. Climate of the last millennium at the southern pole of the North Atlantic oscillation: an inner-shelf sediment record of flooding and upwelling. Clim. Res. 48, 261–280.

Alves-De-Souza, C., González, M.T., Iriarte, J.L., 2008. Functional groups in marine phytoplankton assemblages dominated by diatoms in fjords of southern Chile. J. Plankton Res. 30, 1233–1243.

Anderson, C.R., Siegel, D.A., Brzezinski, M.A., Guillocheau, N., 2008. Controls on temporal patterns in phytoplankton community structure in the Santa Barbara Channel, California. J. Geophys. Res. 113 http://dx.doi.org/10.1029/2007JC004321.

Bode, A., Alvarez-Ossorio, M.T., Cabanas, J.M., Miranda, A., Varela, M., 2009. Recent trends in plankton and upwelling intensity off Galicia (NW Spain). Prog. Ocean. 83, 342–350.

Bode, A., Alvarez-Ossorio, M.T., González, N., Lorenzo, J., Rodríguez, C., Varela, M., Varela, M.M., 2005. Seasonal variability of plankton blooms in the Ria de Ferrol (NW Spain): II. Plankton abundance, composition and biomass. Est. Coast. Shelf Sci. 63, 285–300.

Casas, B., Varela, M., Bode, A., 1999. Seasonal succession of phytoplankton species on the coast of A Coruña (Galicia, northwest Spain). Bol. Inst. Esp. Oceanogr. 15, 413–429.

Delaigle, A., Hall, P., 2012. Achieving near-perfect classification for functional data. J. R. Stat. Soc. B 74, 267–286.

Du, X., Peterson, W., O'Higgins, L., 2014. Interannual variations in phytoplankton community structure in the northern California current during the upwelling seasons of 2001–2010. Mar. Ecol. Prog. Ser. 519, 75–87.

Edwards, K.F., Litchman, E., Klausmeier, C.A., 2013. Functional traits explain phytoplankton community structure and seasonal dynamics in a marine ecosystem. Ecol. Lett. 16 (1), 56–63.

Esparza-Alvarez, M.A., Herguera, J.C., Lange, C., 2007. Last century patterns of sea surface temperatures and diatom (>38 μm) variability in the Southern California current. Mar. Micropaleontol. 64, 18–35.

Estévez, G., Vilar, J.A., 2013. Functional ANOVA starting from discrete data: an application to air quality data. Environ. Ecol. Stat. 20, 495–517.

Fan, J., Gijbels, I., 1996. Local Polynomial Modelling and its Applications. Chapman and Hall, London.

Ferraty, F., Vieu, P., 2006. NonParametric Functional Data Analysis. Theory and Practice. In: Springer Series in Statistics. Springer, New York.

Grasshoff, K., Ehrhardt, M., Kremling, K., 1983. Methods of Seawater Analysis. Verlag Chemie, Weinheim.

González-Nuevo, G., Gago, J., Cabanas, J.M., 2014. Upwelling index: a powerful tool for marine research in the NW Iberian upwelling system. J. Oper. Oceanogr. 7, 45–55.

Hansen, A., Ohde, T., Wasmund, N., 2014. Succession of micro- and nanoplankton groups in ageing upwelled waters off Namibia. Part B J. Mar. Syst. 140, 130–137.

Hernández-Fariñas, T., Soudant, D., Barillé, L., Belin, C., Lefebvre, A., Bacher, C., 2014. Temporal changes in the phytoplankton community along the French coast of the eastern English Channel and the southern Bight of the North Sea. ICES J. Mar. Sci. 71, 821–833.

Hinder, S.L., Hays, G.C., Edwards, M., Roberts, E., Walne, A.W., Gravenor, M.B., 2012. Changes in marine dinoflagellate and diatom abundance under climate change. Nat. Clim. Change 2, 271–275.

Howard, M.D.A., Sutula, M., Caron, D.A., Chao, Y., Farrara, J.D., Frenzel, H., Jones, B., Robertson, G., McLaughlin, K., Sengupta, A., 2014. Anthropogenic nutrient sources rival natural sources on small scales in the coastal waters of the Southern California Bight. Limnol. Oceanogr. 59 (1), 285–297.

Huete-Ortega, M., Marañón, E., Varela, M., Bode, A., 2010. General patterns in the size scaling of phytoplankton abundance in coastal waters during a 10-year time series. J. Plankton Res. 32, 1–14.

Hurrell, J.W., Dickson, R.R., 2004. Climate variability over the North Atlantic. In: Stenseth, N.C., Ottersen, G., Hurrell, J.W., Belgrano, A. (Eds.), Marine ecosystems and Climate Variation – the North Atlantic. Oxford University Press, Oxford, pp. 15–31.

Kaufman, L., Rousseeuw, P.J., 1990. Finding Groups in Data: an Introduction to Cluster Analysis. Wiley, New York.

Latasa, M., 2014. A simple method to increase sensitivity for RP-HPLC phytoplankton pigment analysis. Limnol. Oceanogr. Methods 12, 45–63.

Legendre, L., Rassoulzadegan, F., 1996. Food-web mediated export of biogenic carbon in oceans: hydrodynamic control. Mar. Ecol. Prog. Ser. 145 (1–3), 179–193.

Leterme, S.C., Edwards, M., Seuront, L., Attrill, M.J., Reid, P.C., John, A.W.G., 2005. Decadal basin-scale changes in diatoms, dinoflagellates, and phytoplankton color across the North Atlantic. Limnol. Oceanogr. 50, 1244–1253.

Litchman, E., Klausmeier, C.A., 2008. Trait-based community ecology of phytoplankton. Annu. Rev. Ecol. Evol. Syst. 39, 615–639.

Litchman, E., Edwards, K.F., Klausmeier, C.A., Thomas, M.K., 2012. Phytoplankton niches, traits and eco-evolutionary responses to global environmental change. Mar. Ecol. Prog. Ser. 470, 235–248.

Mackey, M.D., Mackey, D.J., Higgins, H.W., Wright, S.W., 1996. CHEMTAX – a program for estimating class abundances from chemical markers: application to HPLC measurements of phytoplankton. Mar. Ecol. Prog. Ser. 144, 265–283.

Margalef, R., 1978. Life forms of phytoplankton as survival alternatives in an unstable environment. Oceanol. Acta 1, 493–509.

Nogueira, E., Ibanez, F., Figueiras, F.G., 2000. Effect of meteorological and hydrographic disturbances on the microplankton community structure in the Ria de Vigo (NW Spain). Mar. Ecol. Prog. Ser. 203, 23–45.

Neveux, J., Panouse, M., 1987. Spectrofluorometric determination of chlorophylls and pheophytins. Arch. Hydrobiol. 109, 567–581.

Parsons, T.R., Maita, Y., Lalli, C.M., 1984. A Manual of Chemical and Biological Methods for Seawater Analysis. Pergamon Press, Oxford.

Pérez, F.F., Castro, C.G., Ríos, A.F., Fraga, F., 2005. Chemical properties of the deep winter mixed layer in the Northeast Atlantic (40–47°N). J. Mar. Syst. 54, 115–125.

Pérez, F.F., Padin, X.A., Pazos, Y., Gilcoto, M., Cabanas, M., Pardo, P.C., Doval, M.D., Farina-Bustos, L., 2010. Plankton response to weakening of the Iberian coastal upwelling. Glob. Change Biol. 16, 1258–1267.

Preda, C., Saporta, G., 2007. PCR and PLS for clusterwise regression on functional data. In: Brito, P., Cucumel, G., Bertrand, P., Carvalho, F. (Eds.), Selected Contributions in Data Analysis and Classification. Springer, Berlin, pp. 589–598.

Prego, R., Bao, R., 1997. Upwelling influence on the Galician coast: silicate in shelf water and underlying surface sediments. Cont. Shelf Res. 17, 307–318.

Reynolds, C.S., 1997. Vegetation processes in the pelagic: a model for ecosystem theory. In: Kinne, O. (Ed.), Excellence in Ecology. Ecology Institute, Oldendorf/Luhe.

Ruppert, D., Sheather, S.J., Wand, M.P., 1995. An effective bandwidth selector for local least squares regression. J. Am. Stat. Assoc. 90, 1257–1270.

Silva, A., Palma, S., Oliveira, P.B., Moita, M.T., 2009. Composition and interannual variability of phytoplankton in a coastal upwelling region (Lisbon Bay, Portugal). J. Sea Res. 62, 238–249.

Smayda, T.J., Borkman, D.G., Beaugrand, G., Belgrano, A., 2004. Responses of marine phytoplankton populations to fluctuations in marine climate. In: Stenseth, N.C., Ottersen, G., Hurrell, J.W., Belgrano, A. (Eds.), Marine ecosystems and Climate Variation – the North Atlantic. Oxford University Press, Oxford, pp. 49–59.

Statham, P.J., 2012. Nutrients in estuaries - an overview and the potential impacts of climate change. Sci. Total Environ. 434, 213–227.

Thomas, C.R., 1997. Identifying Marine Phytoplankton. Academic Press, San Diego, 858 pp.

Thomas, M.K., Kremer, C.T., Klausmeier, C.A., Litchman, E., 2012. A Global pattern of thermal adaptation in marine phytoplankton. Science 338, 1085–1088.

Tilstone, G.H., Míguez, B.M., Figueiras, F.G., Fermín, E.G., 2000. Diatom dynamics in a coastal ecosystem affected by upwelling: coupling between species, succession, estuarine circulation and biogeochemical processes. Mar. Ecol. Prog. Ser. 205, 23–41.

Tont, S.A., 1987. Variability of diatom species populations: from days to years. J. Mar. Res. 45, 985–1006.

Tunin-Ley, A., Ibañez, F., Labat, J.P., Zingone, A., Lemée, R., 2009. Phytoplankton biodiversity and NW Mediterranean Sea warming: changes in the dinoflagellate genus *Ceratium* in the 20th century. Mar. Ecol. Prog. Ser. 375, 85–99.

Varela, M., 1982. Composición y distribución del fitoplancton de las rías de Muros, Arosa y plataforma continental proxima en setiembre de 1978. Bol. Inst. Esp. Oceanogr. 7, 191–222.

Varela, M., Diaz Del Río, G., Alvarez Ossorio, M.T., Costas, E., 1991. Factors controlling phytoplankton size class distribution in the upwelling area of the Galician continental shelf (NW Spain). Sci. Mar. 55, 505–518.

Varela, M., Prego, R., Belzunce, M.J., Martín Salas, F., 2001. Inshore-offshore differences in seasonal variations of phytoplankton assemblages: the case of a Galician Ria Alta (Ria de A Coruña) and its adjacent shelf (NW of Spain). Cont. Shelf Res. 21, 1815–1838.

Visbeck, M., Chassignet, E.P., Curry, R.G., Delworth, T.L., Dickson, R.R., Krahmann, G., 2003. The Ocean's response to North Atlantic oscillation variability. In: Hurrell, J.W., Kushnir, Y., Ottersen, G., Vis, M. (Eds.), The North Atlantic Oscillation: Climatic Significance and Environmental Impact. American Geophysical Union, Washington DC, pp. 113–145.

WoRMS Editorial Board, 2015. World Register of Marine Species. Available from: http://www.marinespecies.org. at VLIZ. Date accessed 15.06.15.