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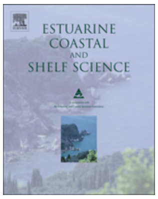

# Spatio-temporal structure of diatom assemblages in a temperate estuary. A STATICO analysis

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## ABSTRACT

This study examines the spatio-temporal structure of diatom assemblages in a temperate estuary (Ria de Aveiro, Western Portugal). Eighteen monthly surveys were conducted, from January 2002 to June 2003, at three sampling sites (at both high and low tide) along the estuarine salinity gradient. The relationship of diatom assemblages and environmental variables was analysed using the STATICO method, which has been designed for the simultaneous analysis of paired ecological tables. This method allowed examination of the stable part of the environment-diatom relationship, and also the variations of this relationship through time. The interstructure factor map showed that the relationship between the 11 environmental variables and the abundance of the 231 diatom species considered was strongest in the months May and September 2002 and January, February and May 2003. The stable part of the species–environment relationships mainly consisted of a combined phosphate, chlorophyll *a* and salinity gradient linked to a freshwater-marine species gradient. A more pronounced gradient was observed in January, February and May 2003. Diatom assemblages showed clear longitudinal patterns due to the presence of both marine and freshwater components. May and September 2002 had the least structured gradients with marine-estuarine species appearing in the freshwater side of the gradient. The most complete gradient in February 2003 could be considered, in terms of bio-ecological categories, as the most structured period of the year, with a combination of strong marine influence in the lower zone and freshwater influence in the upper. The best-structured gradients were during periods of a diatom bloom. Stable diatom assemblages (with a strong structure and a good fit between the diatoms and environment) are described and characterized. This study shows the efficiency of the STATICO analysis. The inclusion of space-time data analysis tools in ecological studies may therefore improve the knowledge of the dynamics of species–environmental assemblages.

## 1. Introduction

Various studies on phytoplankton communities in estuaries have concluded that diatoms are the most important taxonomic groups, either in terms of abundance or in terms of diversity or both (Trigueros and Orive, 2001; Lemaire et al., 2002; Adolf et al., 2006; Gameiro et al., 2007). Diatoms can survive in systems with a high turbidity and a short water retention time (Lionard et al., 2008). These communities are composed of dynamic multi-species assemblages characterized by high diversity and rapid successional shifts in species composition in response to environmental changes. Identifying the ecological variables that regulate the seasonal succession of diatom communities is essential to understand the consequences of eutrophication and climate change. Beyond that, phytoplankton composition and abundance are intimately linked to higher trophic levels through grazing by herbivores and cascading effects on ecosystem trophodynamics (Mallin and Paerl, 1994; Pinckney et al., 1998; Urrutxurtu et al., 2003).

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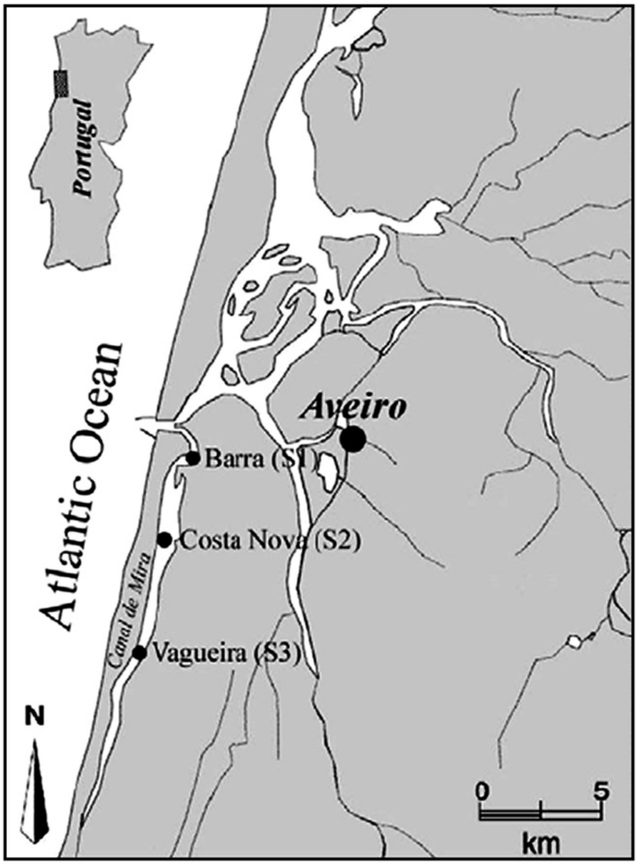

**Fig. 1.** Map of Canal de Mira – Ria de Aveiro and the study area with location of the 3 sampling sites (Resende et al., 2005).

In many temperate estuaries and coastal areas, seasonal patterns of phytoplankton community-composition are characterized by a spring diatom bloom (Pinckney et al., 1998; Domingues et al., 2005; Domingues and Galvão, 2007) or a late autumn/winter-spring diatom bloom (Adolf et al., 2006; Lopes et al., 2007). Diatom abundances usually decreases in the summer, due to Nitrogen (N) and/or Silicates (Si) limitation (Kocum et al., 2002; Domingues et al., 2005; Domingues and Galvão, 2007), and pelagic and benthic grazing (Vaquer et al., 1996; Domingues et al., 2005; Domingues and Galvão, 2007).

Resende et al. (2005) described for Ria de Aveiro (Western Portuguese Atlantic Coast) a diatom composition that resembled other European temperate estuaries but found no seasonal pattern of diatom density. Salinity and temperature were described as the environmental drivers of diatoms' distribution and composition patterns. Canonical Correspondence Analysis (CCA, ter Braak, 1986) was used by Resende et al. (2005) to identify the environmental variables governing the composition and structure of diatom assemblages (together with the study of ecological preferences). The CCA is a global analysis that does not consider the *a priori* information on time or space, information being presented *a posteriori*, when the results are displayed. Therefore a mixture of space-time effects is produced, showing evidence of the strongest effects compared to the weaker ones. Multitable analyses focus on the identification of spatial or temporal structures and the permanence of these structures in time or space. They thus represent a good alternative to CCA and to other methods that do not take into account the temporal variations of environmental factors and biological communities and the effect of the former on the latter (Carassou and Ponton, 2007).

In this study the main spatial structure of diatom assemblages and its temporal changes, in terms of bio-ecological categories and of their relation to some environmental variables, were studied using the STATICO method (Simier et al., 1999; Thioulouse et al., 2004) since it performs a simultaneous analysis of a sequence of paired ecological tables. The method has been used to obtain a clear representation of diatom-environment relationship, its evolution in time and to characterize the typology of the studied stations according to this temporal evolution. The efficiency of this methodology was demonstrated by Carassou and Ponton (2007) in a study with the spatio-temporal structure of pelagic larval and juvenile fish assemblages in coastal areas of New Caledonia (Southwest Pacific) and by Simier et al. (2006) on fish assemblages from the Gambia River. The present work aims at: (1) describing diatom dynamics in a temperate estuary; (2) determining the applicability of the STATICO method to diatom estuarine dynamics and (3) comparing the advantages of STATICO over the classical CCA technique.

## 2. Material and methods

### 2.1. Study area and sampling sites

Ria de Aveiro is located in the Northwest coast of Portugal (40°38' N and 8°44' W). Canal de Mira is an elongated shallow arm that can be considered a small estuary in itself (Resende et al., 2005). Three sites were sampled in Canal de Mira (Fig. 1): S1 – Barra (40°38' N; 08°44' W), S2 – Costa Nova (40°36' N; 08°44' W) and S3 – Vagueira (40°33' N; 08°45' W). Sampling took place monthly, in sub-surface water, always at new moon periods and at both low and high tide, from January 2002 to June 2003. A detailed description of the sampling sites is given by Resende et al. (2005).

**Table 1**
Ranges of environmental parameters during the study period, in the three sampling places.

| | Barra (S1) | | | Costa Nova (S2) | | | Vagueira (S3) | | |
|---|---|---|---|---|---|---|---|---|---|
| | Min | Max | Average ± SD | Min | Max | Average ± SD | Min | Max | Average ± SD |
| Sal ($g\ l^{-1}$) | 17.6 | 36.9 | 31.9 ± 4.5 | 1.0 | 36.7 | 24.8 ± 11.0 | 0.0 | 33.7 | 15.1 ± 10.8 |
| T (°C) | 11.2 | 19.7 | 15.7 ± 2.2 | 9.5 | 20.7 | 15.7 ± 2.6 | 10.2 | 21.8 | 16.6 ± 3.3 |
| pH | 6.39 | 8.31 | 8.0 ± 0.4 | 6.83 | 8.30 | 8.0 ± 0.3 | 7.50 | 8.45 | 8.0 ± 0.2 |
| $DO_2$ (% sat) | 66.0 | 171.2 | 97.0 ± 21.2 | 66.0 | 115.2 | 85.7 ± 13.7 | 50.3 | 152.3 | 86.0 ± 22.0 |
| $NO_3^-$ ($mg\ N\ l^{-1}$) | 0.002 | 0.388 | 0.113 ± 0.095 | 0.002 | 1.079 | 0.233 ± 0.282 | 0.002 | 1.498 | 0.303 ± 0.374 |
| $NO_2^-$ ($mg\ N\ l^{-1}$) | ND | 0.011 | 0.004 ± 0.003 | ND | 0.024 | 0.006 ± 0.005 | ND | 0.026 | 0.009 ± 0.008 |
| $NH_4^+$($mg\ N\ l^{-1}$) | 0.003 | 0.444 | 0.060 ± 0.088 | ND | 0.146 | 0.042 ± 0.031 | ND | 0.132 | 0.043 ± 0.035 |
| $PO_4^{3-}$($mg\ P\ l^{-1}$) | 0.001 | 0.227 | 0.036 ± 0.051 | 0.003 | 0.150 | 0.038 ± 0.040 | 0.011 | 0.168 | 0.055 ± 0.042 |
| N:P | 0.6 | 212.0 | 16.5 ± 35.4 | 0.9 | 148.9 | 16.0 ± 26.2 | 0.5 | 23.5 | 6.5 ± 5.1 |
| Chl *a* ($\mu g\ l^{-1}$) | 0.53 | 33.38 | 5.02 ± 7.13 | ND | 14.27 | 4.09 ± 3.46 | ND | 22.96 | 6.41 ± 5.17 |

ND – undetermined value. Sal – salinity; T – water temperature; $DO_2$ – dissolved oxygen; $NO_3^-$ – nitrate; $NO_2^-$ – nitrite; $NH_4^+$ – ammonium; $PO_4^{3-}$ – phosphate; N:P – N:P ratio; Chl *a* – chlorophyll *a*.

**Table 2**
List of the codes and habitat affinity of diatoms species that stood out in the compromise factor map (see Fig. 3b): (a) For Brackish species (B) and Freshwater species (F); (b) For Marine species (M). [adapted from Resende et al. (2005)].

| Code | Taxa | Habitat |
|---|---|---|
| (a) | | |
| BAPA | *Bacillaria paxillifer* | B |
| FAPY | *Fallacia pygmaea* | B |
| GYFA | *Gyrosigma fasciola* | B |
| MENU | *Melosira numuloides* | B |
| NZBR | *Nitzschia brevissima* | B |
| NZSG | *Nitzschia sigma* | B |
| PASU | *Paralia sulcata* | B |
| STSP | *Stauroneis specula* | B |
| TYAC | *Tryblionella acuminata* | B |
| TYAP | *Tryblionella apiculata* | B |
| AUGR | *Aulacoseira granulata* | F |
| CYME | *Cyclotella meneghiniana* | F |
| NARA | *Navicula radiosa* | F |
| NACA | *Navicula capitata* | F |
| NZCL | *Nitzschia clausii* | F |
| RHAB | *Rhoicosphenia abbreviata* | F |
| SUBR | *Surirella brebissonii* | F |
| SYPU | *Synedra pulchella* | F |
| STPH | *Stauroneis phoenicenteron* | F |
| TAFE | *Tabellaria fenestrata* | F |
| (b) | | |
| ACLO | *Achnanthes longipes* | M |
| AMCO | *Amphora commutata* | M |
| ANEX | *Anorthoneis excentrica* | M |
| ATSE | *Actinoptychus senarius* | M |
| BIAL | *Biddulphia alternans* | M |
| CODI | *Cocconeis disculus* | M |
| COPS | *Cocconeis pseudomarginata* | M |
| COSC | *Cocconeis scutellum* | M |
| DIMI | *Dimeregramma minor* | M |
| DPDY | *Diploneis didyma* | M |
| GRMA | *Grammatophora marina* | M |
| GROC | *Grammatophora oceanica* | M |
| LIGD | *Licmophora grandis* | M |
| LYAB | *Lyrella abrupta* | M |
| ODMO | *Odontella mobiliensis* | M |
| OPPA | *Opephora pacifica* | M |
| PEMO | *Petroneis monilifera* | M |
| PLEL | *Pleurosigma elongatum* | M |
| PLNO | *Pleurosigma normanii* | M |
| PLST | *Plagiogramma staurophorum* | M |
| RHAD | *Rhabdonema adriaticum* | M |
| TEAM | *Terpsinoë americana* | M |
| THWS | *Thalassiosira weissflogii* | M |

### 2.2. Environmental data

In total, 108 samples were collected between January 2002 and June 2003: 36 in Barra (S1), 36 in Costa Nova (S2) and 36 in Vagueira (S3). At each site, pH, salinity, water temperature (°C) and dissolved oxygen (% sat) were measured, *in situ*, with a WTW MultiLine P4 portable meter. Water samples for chemical analyses and chlorophyll *a* quantification were collected and immediately stored in the dark and at low temperature (4 °C), until further processing was possible. At the laboratory, these water samples were filtered through GF/C filters (1.2 µm pore diameter) for quantification of photosynthetic pigments. Filtrates were used for the determination of nutrient contents (Resende et al., 2005). Chlorophyll *a* concentration was determined spectrophotometrically at 665 and 750 nm, before and after acidification (Strickland and Parsons, 1972). Nitrate and nitrite concentrations were determined using sodium salicilate and sulfanilic acid and α–naphtylamine method respectively (Rodier, 1984). Ammonium concentration was determined by the indophenol blue technique following the recommendations and procedures of Hall and Lucas (1981). Phosphate in the form of orthophosphate was determined using the stannous chloride method (APHA, 1992). The N: P ratio and distance to the mouth of the estuary were also considered in following analyses. The ranges of environmental parameters during the study period, in the three sampling places are presented in Table 1. A more detailed description can be found in Resende et al. (2005).

### 2.3. Biological data

During the eighteen months' study period (from January 2002 to June 2003) samples for taxonomic and quantitative study were collected with a glass bottle (1 l capacity) at the water subsurface and immediately preserved with Lugol 1% (iodine/iodide potassium) (Resende et al., 2005). A total of 231 species were identified (Resende et al., 2005) in this study using the standard floras of Peragallo and Peragallo (1897–1908); Germain (1981); Hustedt (1985); Krammer and Lange-Bertalot (1986, 1988, 1991a,b); Round et al., (1990); Sims (1996); Tomas (1996) and Witkowski et al. (2000).

### 2.4. Data analysis

Data were organized in two series of tables: one for the 11 environmental variables and the other one for 231 diatoms species abundances. Each pair of tables corresponded to the three sites at two tidal conditions, which means six rows (sampling sites) per table. Species abundance was changed to $\log(x+1)$ prior to calculations (Legendre and Legendre, 1979), to minimize the dominant effect of exceptional catches and environmental data were normalized to homogenize the table.

The common structure between environmental and species abundances tables and the stability of this structure over the sampling period were assessed by STATICO method (Simier et al., 1999; Thioulouse et al., 2004). The STATICO method proceeds in three stages: (1) the first stage consists in analysing each table by a one-table method (normed PCA of the environmental variables and centered PCA of the species data); (2) each pair of tables is linked by the Co-inertia analysis (Dolédec and Chessel, 1994) which provides an average image of the co-structure (species-variables); (3) Partial Triadic Analysis (Thioulouse and Chessel, 1987) is finally used to analyze this sequence. It is a three-step procedure, namely the interstructure, the compromise and the intrastructure analyses. STATICO also enables to plot the projection of the sampling sites of each original table on the compromise axes (of the PCA factor map), in terms of species abundances and environmental factors structures. Hence, it is possible to discuss the correlation between species distribution and environmental factors. Calculations and graphs were done using ADE-4 software (Thioulouse et al., 1997). This software is available free of charge at the following Internet address: http://pbil.univ-lyon1.fr/ADE-4.

## 3. Results

### 3.1. Interstructure

The interstructure factor map of the STATICO analysis, based on the 11 environmental variables and on the abundances of the 231 diatom species, showed that the relationship between environmental variables and diatoms appeared to be stronger in September 2002 (with the longest arrow) followed, in decrease order of importance in the compromise, by May 2003, May 2002, January 2003 and February 2003 (meaning that the compromise will be more influenced by these dates) (Fig. 2). The remaining sampling dates presented short arrows, which means that the corresponding tables are less structured and that their importance in the compromise will be lower. The first two axes represented, respectively, 21% and 10% of the total variability (Fig. 2b).

### 3.2. Compromise

The first axis was clearly dominant, and accounted for 78% of the explained variance in contrast with the second axis which accounted for 13% of the explained variance and was much less significant (Fig. 3c). Temperature and N: P ratio presented a weak representation on this factorial plan. For the factor map of the environmental variables (Fig. 3a), the first axis describes a salinity gradient, with high values of dissolved oxygen, pH and salinity (positively correlated) on the left side and high values of chlorophyll *a*, phosphate, nitrite, nitrate and low salinity waters (positively correlated) on the right side of the factorial plan. This opposition linked the "marine side" to the "freshwater side" (Fig. 3b), with the majority of marine species on the left quadrants and the majority of freshwater species on the right quadrants of the ordination. Diatom species were therefore ranked according to their salinity affinities. As a result, the stable part of the species–environment relationships mainly consisted of a combined phosphate, chlorophyll *a* and salinity gradient linked to a freshwater-marine species gradient (Table 1 and Fig. 3).

### 3.3. Trajectories

The trajectories maps focused on May and September 2002 and January, February and May 2003, when the observed co-structure between environment and diatom abundances was the most significant (see Fig. 2). For each sampling month, the projection on the compromise axes of the environmental variables (Fig. 4a) and of the species (Fig. 4b) allowed to visualize the relationships between environmental factors and species abundances and distribution. In general, the trajectories factor maps indicated that the relationships between the most abundant species in the assemblages and environmental factors differed between sampling months (Fig. 4). May 2003 presented a performance best approximated to January and February 2003, contrasting with May and September 2002. In January and February 2003, salinity, pH, dissolved oxygen and temperature appeared positively correlated on the left side of axis 1, along which diatom species appeared to distribute. Salinity, pH, dissolved oxygen and temperature were less correlated during May and September 2002 (at the same time as the distribution of diatom species abundances were most correlated with axis 2). The trajectories factor maps showed a different gradient, the line bisecting the origin on the plan 1–2, with a contrast between brackish, freshwater and marine species (Figs 4a, b).

The co-structure graphics (divided according to sampling dates) clearly showed the dynamics of diatom species–environment relationships and highlighted differences between sites (Fig. 5). Whatever the date, the species points (circles) were more stable than the environmental points. This expresses the steady establishment of the diatom assemblages, in spite of the high environmental variability (salinity in particular). The ends of the arrows (environmental variables) had comparatively different values (generally presented separated) and simultaneously close diatom abundances (the circles were always in the same area). Indeed, from the species point of view, the sites were regularly projected on the right-hand side of the first axis, characterized by the highest diatom abundances (see Fig. 3b).

In general, site one was the one that often presented the shortest arrow, which means that the environmental factors explained well the distribution of species for that site. At this site, located at the mouth of estuary, at low tide, and in particular for May 2002 and September 2002 the arrows were mostly short. This means a higher correlation between the distribution of diatom abundances and the environment, under the direct influence of high values of phosphate, ammonium and chlorophyll *a*. On the other hand, and notwithstanding the strong dispersion of the environmental points and a poor fit between the diatoms and environment (long arrows), sites 1LT (station 1 low tide), 1HT (station 1 high tide) and 2HT (station 2 high tide) were regularly grouped together, as well as sites 2LT (station 2 low tide), 3LT (station 3 low tide) and 3HT

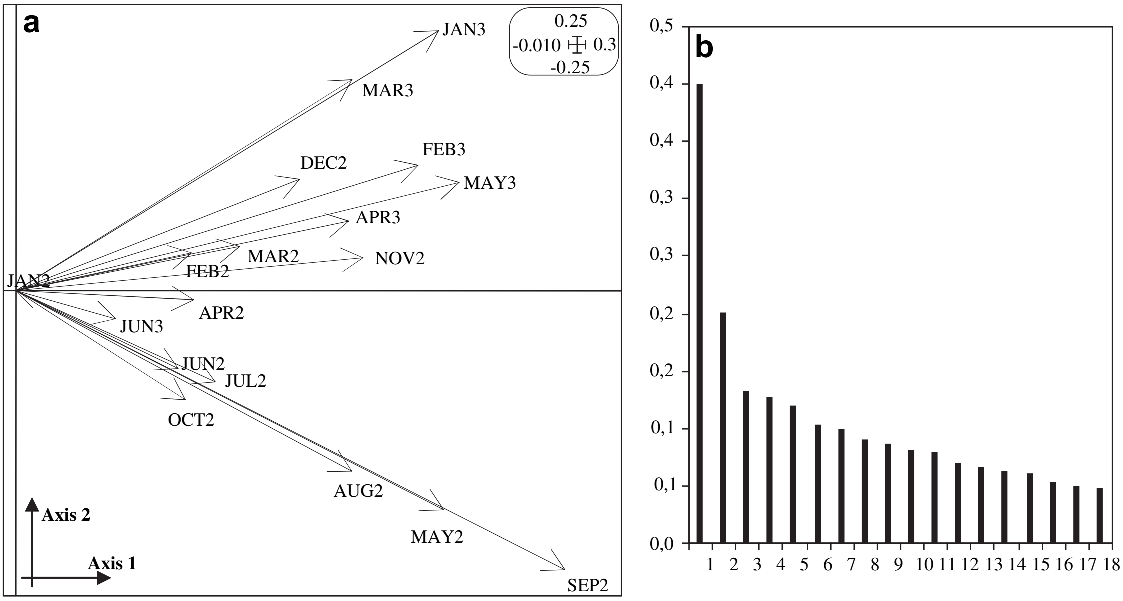

**Fig. 2.** Interstructure factor map of the STATICO analysis on the Ria de Aveiro data. (a) This map shows the importance of each sampling date in the compromise. Each date is identified by the three first letters of the month followed by a number: 2 for the year 2002 and 3 for the year 2003 (e.g. JAN2 – January 2002). Axis 1 the first principal component; Axis 2 the second principal component. The scale of the graph is given in the rounded box (upper right side). (b) Eigenvalues bar plot of the interstructure.

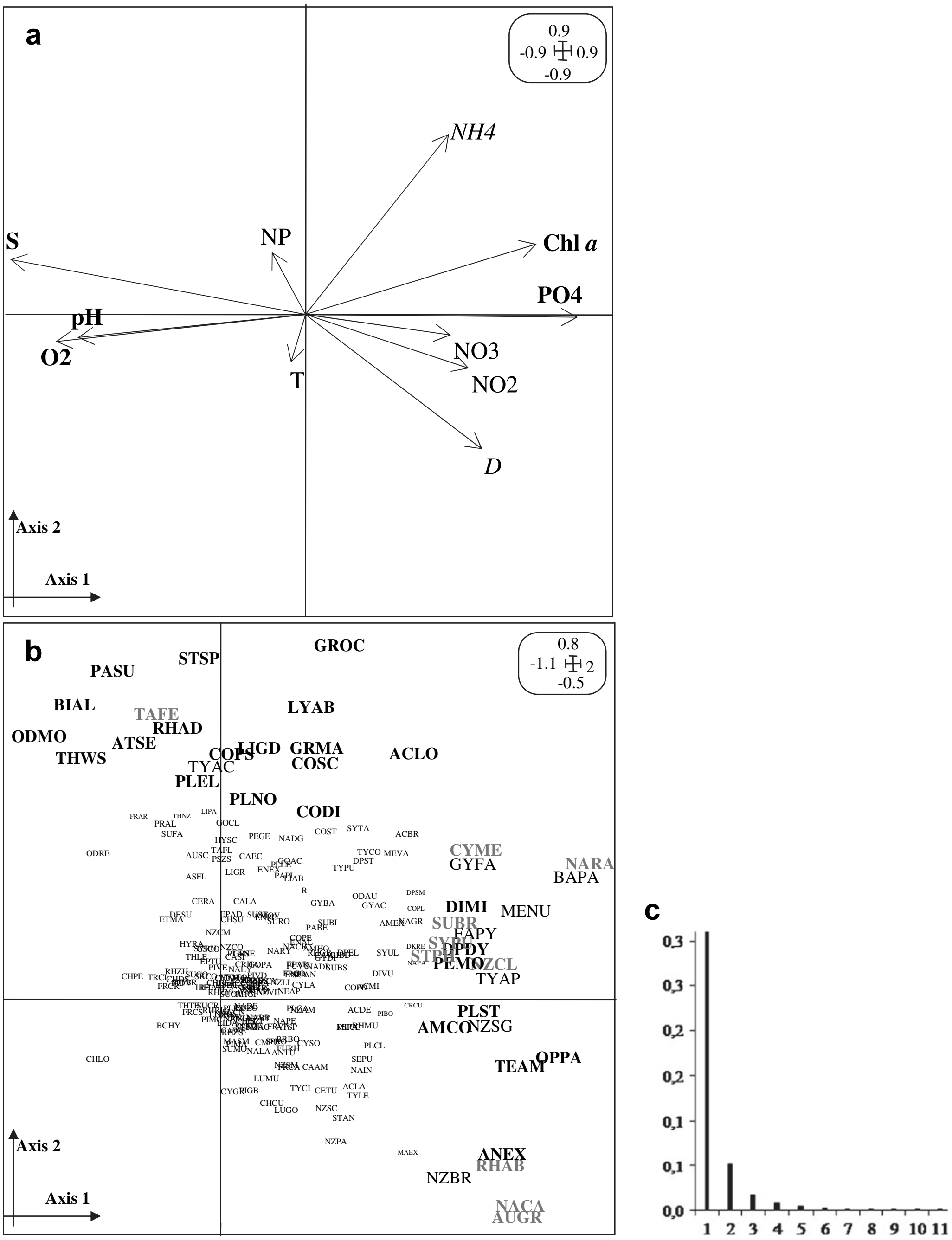

**Fig. 3.** Compromise factor maps of the STATICO analysis. These maps show the stable part of the diatoms–environment relationships: (a) Environmental variables projected on the first factorial plan. See Table 1 for environmental codes; D – distance to the mouth of the estuary. (b) Diatom abundances projected on the same factorial plan. The labels of most abundance diatom species and thus of importance in these graphics, were with big size letter for clarity. Different gray levels are used to distinguish marine species from freshwater species. See Table 2 for species affinities and codes. Axis 1 the first principal component; Axis 2 the second principal component. The scale of the graph is given in the rounded box (upper right side). Note for different scales. (c) Eigenvalues bar plot of the compromise.

(station 3 high tide) although the latter expressed a better consensus in the relationship species–environment. In fact, this was most clear for January 2003 and February 2003. Specifically the environmental points (end of arrows) for the first group (sites 1LT, 1HT and 2HT) were located on the left-hand side of the first axis corresponding to saline waters with the warmest temperatures, higher values of pH and dissolved oxygen. On the contrary, environmental points (end of arrows) corresponding to the aggregation of sites 2LT, 3LT and 3HT (freshwater stations) were located on the right-hand side of the first axis, which means higher nutrient concentrations (ammonium, phosphate, nitrite, nitrate) and chlorophyll *a* (opposed to salinity). Besides this, for these ones the arrows were mostly short when compared to the other group and in particular for February 2003. For that case, this revealed a strong structure, which means a best fit between the diatoms (corresponding to a majority to freshwater and/or brackish affinities) and environment (short arrows).

## 4. Discussion

This study has focused on the months with the highest contribution to the co-structure between environmental factors

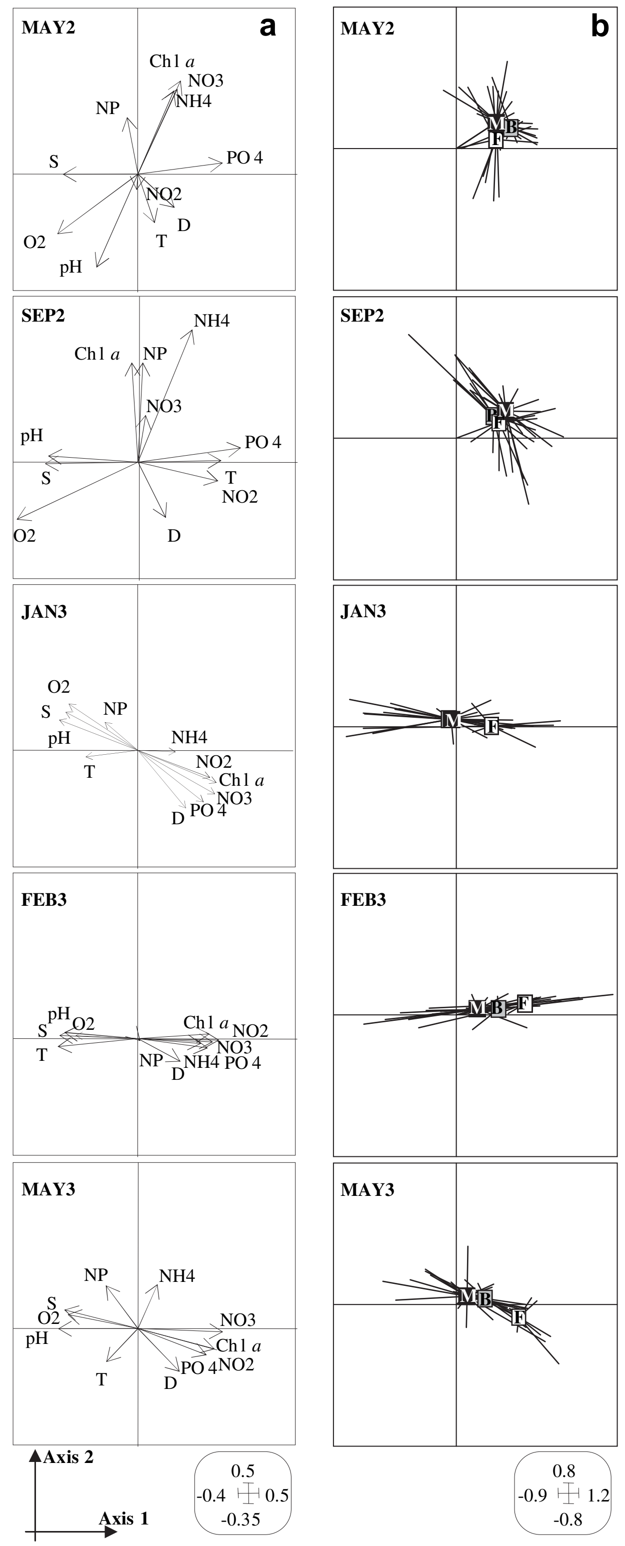

**Fig. 4.** Trajectories factor plots of the STATICO analysis: (a) Projection of the environmental variables on the first factorial plan. See Table 1 for environmental codes; D – distance to the mouth of the estuary. (b) Projection of the average positions of diatom habitat affinities (only for species that stood out on the compromise diagram). See Table 2 for species habitat affinities. Graphs are given only for the five dates that showed the highest contribution to the co-structure between environmental factors and diatom abundance. See Fig. 3 for legend of months. Axis 1 the first principal component; Axis 2 the second principal component. The scales for axes are given in the rounded box. Note for different scales.

and diatom abundances (May and September 2002 and January, February and May 2003). The relationship between environmental variables and diatoms was strongest in May 2002, September 2002, January 2003, February 2003 and May 2003 while the remaining sampling dates are less structured. This result revealed by the interstructure was failed to be achieved by CCA analysis in Resende et al. (2005). However, these sampling months corresponded to an increase of diversity from April 2002 to June 2002 (afterwards species diversity dropped in the summer months, when community was dominated by *Pseudo-nitzschia seriata* accounting for 73% of the diatom community), January 2003 (an increase of diversity was verified and the community was dominated by *Aulacoseira granulata* and *Tryblionella apiculata*), and the registered higher diatoms densities in September 2002 ($5.6 \times 10^5$ cells $l^{-1}$) (Resende et al., 2005). The winter dominance by *A. granulata*, reaching maximal abundances (in the most downstream areas) is a common feature (Muylaert et al., 2000) and the interstructure was able to reveal ecological structure where CCA was inefficient.

In the Ria de Aveiro estuary, the simultaneous analysis of diatom abundances and environmental variables has emphasized the preponderant role of salinity in the spatio-temporal structuring of diatom assemblages. The main spatial structure was a longitudinal gradient from marine to freshwater assemblages. The most abundant diatoms (marine species *Paralia sulcata*, *Biddulphia alternans*, *Odontella mobiliensis*, *Thalassiosira weissflogii*, *Actinoptychus senarius*, *Rhabdonema adriaticum*, *Stauroneis spicula*, *Pleurosigma elongatum*, *Cocconeis pseudomarginata* and freshwater species *Cyclotella meneghiniana*, *Navicula radiosa*, *Surirella brebissonii*, *Synedra pulchella*, *Stauroneis phoenicenteron*, *Nitzschia clausii*, *Rhoicosphenia abbreviata*, *Navicula capitata* and *Aulacoseira granulata*) were associated with different environmental variables. As a result, the stable part of the species–environment relationships mainly consisted of a combined phosphate, chlorophyll *a* and salinity gradient linked to a freshwater-marine species gradient. The marine component of the community was associated with saline waters, high values of pH, dissolved oxygen and low phosphates, while the freshwater component was characteristic of low saline waters and high concentrations of phosphates. Although this salinity gradient is relatively stable in time and space, changes in river discharge and marine waters intrusion induce variations in its position through the course of the year (Soetaert and Herman, 1995; Muylaert et al., 2000). McLusky (1993) claimed that the use of a fixed reference frame to determine spatial variation in a spatio-temporal data set in an estuarine environment will fail to capture all spatially structured variation because longitudinal estuarine gradients vary overtime, what Martin (2003) also designates as the contamination of the spatial data with temporal effects.

The water characteristics of Ria de Aveiro indicates that during low freshwater flow, in late spring and summer months, there is enhanced salinity intrusion upstream estuary. On the other hand, increased precipitation promoted higher freshwater flows moving the salinity intrusion seawards (Lopes et al., 2007). External forcing features (meteorological events, river discharge, and nutrient loading) are major determinants of ecosystem response (Pinckney et al., 1998) and seasonal patterns are strongly influenced by freshwater flow (Adolf et al., 2006). The river discharge (Si loading, and other nutrients) of unusual rainy periods may contribute to a prolonged supply of Si into the system. This river discharge is important, determining diatom variability (Adolf et al., 2006; Gameiro et al., 2007; Lopes et al., 2007) over these periods. On the other hand increases in discharge during late summer were shown to result in washout of phytoplankton from the freshwater tidal reaches of an estuary (Muylaert et al., 2000;

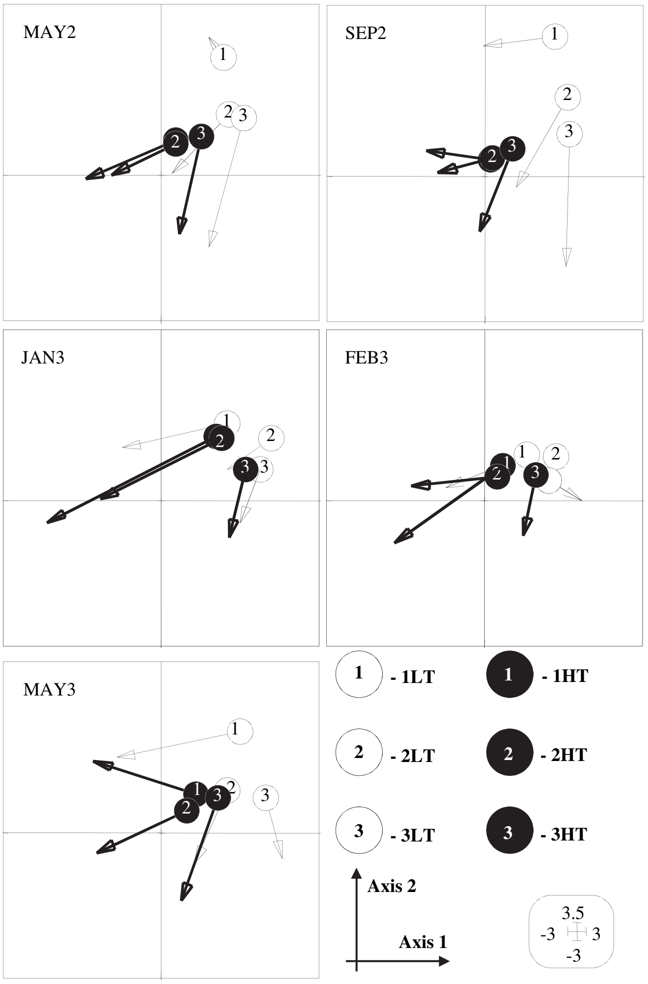

**Fig. 5.** Projection of the sampling sites on the compromise axes, in terms of both environmental and diatom structure. Graphs are given for each sampling date that showed the highest contribution to the co-structure between environmental factors and diatom abundance. Each site is represented by two points: one is the projection of the row of the diatoms table (circle: origin of arrows), and the other is the projection of the row of the environmental table (end of arrows). The length of the connecting line reveals the disagreement or the consensus between the two profiles (species–environment), i.e., the length of the line is proportional to the divergence between the datasets. When the datasets agree very strongly, the arrows will be short. Likewise, a long arrow demonstrates a locally weak relationship between the environment and diatoms features for that case. See Fig. 3 for legend of months. Axis 1 the first principal component; Axis 2 the second principal component. The scale of the graph is given in the rounded box.

Gameiro et al., 2004; Lionard et al., 2008). In Chesapeake Bay Adolf et al. (2006) reported phytoplankton composition forcing driven mainly by River flow (and its effect on spatial and temporal variability of light and nutrients). These authors found a high diversity of taxa in summer, and environmental forcing by extremes of freshwater flow that elicited a shift to diatoms. This complexity contrasts with other systems with relatively predictable spring diatom blooms. These stable ecosystems with good species evenness and diversity, with abundance patterns related to seasonal cycles contrasts with the unstable situations, in terms of spatial homogeneity and agreement with the environmental conditions.

The more pronounced gradients were observed in January, February and May 2003. Diatom assemblages showed clear longitudinal patterns due to the presence of both marine and freshwater components. In May and September 2002, least structured gradients, marine-estuarine species were in the “freshwater side” of the gradient. This strong longitudinal organization varied on a temporal scale. The most complete gradient in February 2003 could be considered, in terms of bio-ecological categories, as the more structured period of the year, with a combination of strong marine influence in the lower zone and freshwater influence in the upper zone. The best-structured gradients are periods of diatom blooms (abundance and/or diversity).

The dynamics observed in stations 2LT, 3LT and 3HT (at high tide but the furthest from the sea) in January and February 2003 and May 2003 can be explained by the river inputs of water and sediment containing dissolved and particulate nutrients which causes higher nutrient loadings to the estuary (that can be caused by highest freshwater flows) (Domingues and Galvão, 2007; Lopes et al., 2007). The January and February 2003 spatial homogeneity of the intermediate brackish (in low tide) and freshwater zones of the estuary and strong structure corresponded to a good fit between diatoms and environment. Stations 1LT, 1HT and 2HT (with higher marine affinities) presented the most heterogeneous pattern from both an environmental and diatoms point of view (independently from the sampling month). This high heterogeneity was associated with a poor fit between the species abundances and environment. These sites of the estuary were directly under the marine influence and consequently subject to both short-term ebb/flood changes and monthly variations, in particular spring differences. In the marine influenced zone of the estuary was associated a good fit between diatoms and the environment at low tide in May and September 2002. In a system with suggested phosphate limitation (Lopes et al., 2007), May and September 2002 were associated with high values of phosphate.

CCA is useful and effective evaluating the relationships between environmental variables and species distribution. Variability can however be masked or not shown in the first principal axes of CCA. STATICO graphical complementary plots revealed the co-structure between environmental variables and diatom species distribution. The application of this method proved to be well adapted in taking into account the spatio-temporal dynamics of both environmental factors and abundances of diatoms, and the relationships between these two datasets. This study corroborates the conclusions of Carassou and Ponton (2007) demonstrating the method’s ability to distinguish the environmental factors which have a general effect on species distribution from those which act only for a given period, location or condition.

## 5. Conclusions

Like many other temperate estuaries, nutrient enrichment of the catchment area of Ria de Aveiro has been responsible for cultural eutrophication which may induce alteration in phytoplankton assemblages (Lopes et al., 2007). Identifying the ecological variables that regulate phytoplankton dynamics is essential for understanding the consequences of eutrophication problems and biological response to climate change in estuaries. The earlier study of Resende et al. (2005), based on a CCA analysis, while correct in broadly characterizing the ecosystem, failed to capture variability associated with different strengths of environmental forcing. The results presented in the present study add new ecological information on diatom estuarine dynamics (relationships between diatoms and environmental parameters and their space-time structures) and are of importance for the understanding of estuarine ecosystems. In further studies the inclusion of physical parameters, e.g. water column depth, spring-neap cycle as a major driver of temporal variations and diel cycles, could be interesting to explore fully the role of the estuarine and coastal zones.

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