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Phytoplankton community structure defined by key environmental variables in Tagus estuary, Portugal

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Abstract
In this work we analyze environmental (physical and chemical) and biological (phytoplankton) data obtained along Tagus estuary during three surveys, carried out in productive period (May / June / July) at ebb tide. The main objective of this study was to identify the key environmental factors affecting phytoplankton structure in the estuary. BIOENV analysis revealed that, in study period, temperature, salinity, silicate and total phosphorus were the variables that best explained the phytoplankton spatial pattern in the estuary (Spearman correlation, p=0.803). A generalized linear model (GLM) also identified salinity, silicate and phosphate as having a high explanatory power (63%) of phytoplankton abundance. These selected nutrients appear to be consistent with the requirements of the dominant phytoplankton group, Bacciliariophyceae. Apparently, phytoplankton community is adapted to fluctuations in light intensity, as suspended particulate matter did not come out as a key factor in shaping phytoplankton structure along Tagus estuary.

Keywords: Phytoplankton structure, nutrients, salinity, spatial scale, productive period, estuaries.

1. Introduction

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Phytoplankton composition is considered as a natural bioindicator because of its complex and rapid responses to fluctuations of environmental conditions (Livingston, 2001). The main environmental factors recognized as controlling community structure of phytoplankton are physical, (mixing of water masses, light, temperature, turbulence and salinity) and chemical (nutrients). Regulation of phytoplankton in estuarine systems is more complex due to the interaction of freshwater inputs and tidal energy (Alpine and Cloern, 1992). Further, the increasing human population densities and industrial development have contributed to the eutrophication of these ecosystems.

Tagus estuary, the major Portuguese estuarine system, represents an important ecological system, still subjected to considerable urban and industrial pressures. Regarding phytoplankton community of Tagus estuary, Cabrita and Moita (1995) conducted studies on biomass (chlorophyll $a$), Cabeçadas (1999) investigated in lower estuary photosynthetic response of phytoplankton and Oliveira (2000, 2003) studied algal composition and abundance along the estuary. More recently Gameiro et al. (2004) focused on the annual variability of phytoplankton composition and biomass, as well as physical and chemical parameters, in the restricted upper estuary. A screening model developed by Ferreira et al. (2005) derived some general features relating phytoplankton species composition to hydrology of a number of estuarine systems including Tagus estuary, giving an indication of the respective potential biodiversity. On the basis of physical, chemical and phytoplankton biomass a recent study, carried out in Tagus estuary, allowed distinct habitats to be identified in winter and summer. In the latter period the increment of productivity was particularly noticeable in mid-estuary region (Brogueira and Cabeçadas, 2006).

Environmental and phytoplankton data presented in this work were obtained during three surveys along the Tagus estuary carried out in the productive period, providing an
opportunity to explore potential reasons for differences in community composition. Specifically, the aim of this study is to evaluate the phytoplankton dynamics and the environmental variability along Tagus estuary and investigate the main environmental factors affecting phytoplankton structure.

2. Materials and methods

2.1 Area description and sampling

Tagus estuary is one of the largest estuaries in the western European coast, covering an area of 320 km$^2$. The estuary is meso-tidal and its shallow upper part is occupied by extensive tidal flats and salt marshes (Fig. 1). The main freshwater source is Tagus river, having an annual average flow of approximately 400 m$^3$/s, although its discharge varies greatly from summer to winter between approximately 30 m$^3$/s in a dry summer and 2000 m$^3$/s in a wet winter (SNIRH, 2006). Tagus river is the main nutrient source to the estuary, but Sorraia and Trancão rivers also represent significant nutrient contributors to the system. Although organic loadings to the estuary have been reduced in recent years, partially treated or untreated effluents from greater Lisbon still enter the estuary, particularly from southern shoreline, contributing approximately 30% for total organic nitrogen and 10% for nitrate (IST, Maretac, 2002).

A total number of 19 stations were sampled in July 2001, May 2002 and June 2003 along Tagus estuary (Fig. 1), always during ebb tide. Surface water samples were collected with a Niskin bottle for measurement of dissolved oxygen (DO), nutrients (nitrate+nitrite referred as NO$_3^-$, ammonium (NH$_4^+$), phosphate (PO$_4^{3-}$), silicate (Si(OH)$_4$), total nitrogen (TN), total phosphorus (TP), suspended particulate matter (SPM), and phytoplankton identification and abundance determination.
2.2. Physical and chemical measurements

Temperature (T) and salinity (S) were measured in situ utilizing an Aanderaa probe (CTD sensor), calibrated for salinity with an Autosal salinometer. Samples for DO determination were collected and analyzed according to Winkler method as described by Aminot and Chaussepied (1983). Water samples for nutrient determinations were immediately filtered through AcetatePlus MSI (MicroSeparation Inc.) filters and placed in a freezer. Samples for TN and TP measurements were preserved with sulfuric acid (4M) and maintained at 4ºC until analysis (maximum 48h).

Nutrient analysis was carried out using a TRAACS autoanalyser following colorimetric techniques outlined by the manufacturer. Estimated precision (10 replicates) are the following: 2.6% for NO$_3$, 2.9% for PO$_4$, 0.2% for Si(OH)$_4$, 2.0% for NH$_4$ at middle scale concentrations. Accuracy of nutrient measurements was maintained by using CSK Standards (WAKO, Japan). TN and TP were determined, after simultaneous oxidation according to the ISO methodology (1997) based on Koroleff method (1983), also automatically. SPM was determined gravimetrically by filtering water samples through pre-combusted Whatman GF/F filters and drying at 70 ºC.

2.3. Phytoplankton

Phytoplankton samples (500 mL) were preserved immediately after collection with acidified Lugol. Identification was performed using an Axioscop Zeiss microscope with phase contrast. Counting was done according to Utermohl (1958) and Lund et al. (1958) with an Invertoskop Zeiss IM 25. Species richness (S), expressed as species number per sample (Pielou, 1975), was estimated considering all species identified during the study period. Species diversity was calculated according to Shannon and Wiener (1963).
\( H' = -\sum p_i \log p_i, \quad p_i = N_i / N, \quad N_i \) number of cells of species \( i \) and \( N \) total number of cells/sample) and Evenness \( (J') \) as \( H' / \log S \) (Pielou, 1975), using \( \log_2 \) in both formulations.

### 2.4 Statistical Analysis

#### 2.4.1 Multivariate Analysis

Multivariate statistical analysis was applied to environmental and phytoplankton data in order to identify spatial trends or discrete groups and correlations between environmental and phytoplankton variables. These analyses were undertaken using PRIMER (v.6) software (Plymouth Routines in Multivariate Ecological Research, Plymouth Marine Laboratory, Plymouth, U.K; Clarke and Gorley, 2006).

**ANOSIM test**

The analysis of similarity, ANOSIM (Clarke, and Green, 1988; Clarke 1993) was performed to test statistical differences in environmental and phytoplankton data among sampling months and sampling stations. We used a two-way crossed test, based on Bray-Curtis similarities (phytoplankton abundances, fourth-root transformed) and Euclidean distance matrix (environmental data, \( \log(x+1) \) transformed) as our study focused on a fixed set of sites, sampled at several occasions.

**PCA, MDS and BIOENV analysis**

Environmental data (\( \log(X+1) \) transformed) was handled using correlation-based Principal Component Analysis (PCA), on the basis of standard Euclidean distance between samples to define their dissimilarity.

Multidimensional scaling, MDS, was applied to phytoplankton abundance (4th root transformed). Similarities between species are obtained by Bray-Curtis similarity coefficient and the corresponding rank similarities used to construct MDS.
configuration. Sampling points are arranged in a continuum such that points emerging together correspond to sites in which species composition is similar, and points which are far apart correspond to sites that are dissimilar. Stress levels of MDS representation less than 0.1 indicate good representation of the data. Also on the basis of Bray-Curtis similarities, the similarity percentages analysis (SIMPER) was applied to phytoplankton species abundance, in order to allow the separation of every two groups of stations according to phytoplankton species.

Further, patterns in community structure identified by MDS analyses were linked to environmental variables (based on Euclidean similarity index) by using the BIOENV method. This procedure allows identifying the environmental variables (individual or combined) that “best match” the patterns of community structure.

2.4.2. **Univariate Analysis**

Generalized Linear Model (GLM) was also applied to further explore the relationship between the environmental variables and phytoplankton abundance. This model, adequate for data with counts, uses the method of least squares, by linking the binary response to the explanatory covariates through the probability of either outcome, which does vary continuously from 0 to 1. The transformed probability is then modeled with an ordinary polynomial function, linear in the explanatory variables. This univariate analysis was carried out using Brodgar Software (2006).

3. **Results**

Based on ANOSIM analysis no significant differences among sampling months was found, neither for most environmental data (p varying from -0.095 to 0.286, p>5%, except for DO and SPM) nor for phytoplankton data. Therefore, data was averaged over the three months, at each sampling site.
3.1. Environmental parameters

Spatial variations of environmental parameters along Tagus estuary during productive period are shown in Fig. 2. Salinity mean values decreased progressively from a maximum of 34.5 at St.1 in the estuary mouth to a minimum of 0.3 at St. 14, the most river-influenced site (Fig.2a). An opposite trend was observed in relation to water temperature ranging from 17ºC at St. 1 to 24-25 ºC at Sts. 12-14 (Fig. 2b).

The system was well oxygenated, as oxygen saturation levels remained above 90%, except at St. 15, nearby Montijo saltmarsh, where saturation decreased to 87%, corresponding to 6.5 mg/l, the minimum value found in the estuarine surface water (Fig. 2c).

Nutrient concentrations, corresponding mostly to moderate levels, decreased along the estuary from riverine to marine, influenced area, reflecting in general, the main nutrient discharge from Tagus river. NO$_3$ concentrations varied from 10 µmol/L at St.1 to 50-60 µmol/L at St.11-14 in the upper estuary (Fig. 2d). The same trend was observed for Si(OH)$_4$, ranging from 4 µmol/L to 60 µmol/L (Fig. 2e), and for PO$_4$, ranging from 1 µmol/L to 3-4 µmol/L (Fig. 2f). TN distribution also reflects the major input from Tagus river (maximum of 102 µmol/L at St. 14), as well as the influence of the extensive salt-marshes in the upper estuary (St. 8, 9 and 11) (Fig. 2g). TP also varied from minimum values of 2-3 µmol/L in the lower estuary (Sts. 1-3) to 6-7 µmol/L in the upper estuary (Sts. 13, 14) (Fig. 2h).

The existence of NH$_4$ point sources in the estuary is notorious, maximum value (60µmol/L) being found at St. 15 (nearby Montijo saltmarsh) (Fig. 2i). However, high values were also detected close to St. 16 (Seixal saltmarsh) and St. 4 (nearby Trancão river discharge), respectively 18 and 13 µmol/L.
SPM varied quite irregularly from 21 to 58 mg/L all over the estuary (Fig. 2j). Besides St. 4 and St. 5 located in the vicinity of Trancão river discharge, which showed the highest levels of SPM, sites at the left margin of the estuary (Sts 15, 16, 17, 18, 19) close to extensive saltmarsh zones, exhibited considerably high variability of suspended matter levels.

Concerning nutrient stoichiometry, it can be observed (Fig. 3) that in the lower/middle estuary (Sts. 1-8 and Sts. 15-19) N:P and Si:P values were, in general, lower than 16 and Si:N values lower than 1. By contrast, in the upper estuary (Sts. 9-14) N:P values were in general balanced, some Si:P were above 16, while Si:N were close or above 1.

3.2 Phytoplankton Community Structure

3.2.1 Composition and abundance

Mean phytoplankton abundance ranged from a minimum value of 78 cells/mL at St. 18 in the lower/middle estuary to a maximum of 1600 cells/mL at St. 11 in the upper estuary (Fig. 4a). Bacillariophyceae was the most important algal group accounting for 87% of the total abundance (Table 1). The algal maximum occurring at St. 11 was mostly due to proliferation of *Chaetoceros socialis* and *Melosira moniliformis* (Fig. 4b,c). Two other phytoplankton peaks were observed, one at St. 14 due to proliferation of *Stephanodiscus hantzschii* (Fig. 4d) and the other at St. 8, mainly as consequence of the development of *Chaetoceros socialis* and *Skeletonema costatum* (Fig. 4b,e). This last euryhaline species, adapted to a large salinity range, is even developed at the upper estuary, where the community was characterized by freshwater diatoms *Stephanodiscus hantzschii*, *Fragilaria crotonensis*, *Aulacoseira distans* and *A. granulata*. At lower and middle estuary other important Bacillariophyceae species were recorded, namely
Chlorophyceae attained 5% of the total algal abundance (Table 1). Its maximum was reached at St. 14, the most river-influenced site (salinity 0.30) being represented mainly by freshwater species (*Scenedesmus acuminatus*, *S. armatus*, *Monoraphidium contortum*, *Chlamydomonas* spp.). Cryptophyceae were important only at Sts. 4 and 6 (lower/middle estuary), accounting for 4% of the total algal abundance through proliferation of *Plagioselmis* sp. and *Hilea fusiformis*.

The oceanic Prasinophyceae, representing 2% of total abundance, developed mainly at the estuary mouth (St. 1) (Table 1) and its principal species was *Phaeocystis pouchetii*. Euglenophyceae accounted only for 1% of total abundance, being mostly due to the development of *Eutreptiella marina* in the lower estuary. The contribution of Cyanobacteria and Dinophyceae and Ebriidea was below 1%. The most abundant dinoflagellates were nanoplanctonic *Amphidinium* species (Sts. 1 and 6) and the most common Cyanobacteria was *Merismopedia tenuissima* (St. 12).

### 3.2.2. Diversity

A total of 236 phytoplankton species were identified in Tagus estuary during the study period. However, species richness (S) was moderate and no clear trend was apparent along the estuary (Fig 5). The minimum value (25 species) was detected at Sts. 3, 18 and 19 in the euhaline-mesohaline zone, and the maximum (37 species) was detected at St. 10 in the oligohaline-freshwater zone (Fig. 5), indicating the importance of freshwater species in the estuarine community. Shannon diversity (H’) presented high values, most of them (84%) over 3.0, ranging from 1.7 (St. 11) to 4.6 (Sts. 6 and 17) (Fig. 5). Evenness (J’) was also high, and 79% of values surpassed 0.6 (Fig. 5). Changes
in $J'$ closely mirrored changes in $H'$, varying from a minimum of 0.4 at St. 11 to a maximum of 0.9 at St. 17. The occurrence of a simultaneous reduction of $H'$ and $J'$ at St. 11, where phytoplankton maximum was attained, reflects the strong dominance of *Chaetoceros socialis* and *Melosira moniliformis* (Fig. 4b,c).

3.3 Statistical Analysis

The application of PCA analysis to environmental data reveals that the first three PCs had eigenvalues greater than one (Table 2), indicating that all of them were significant. These components accounted for 89% of total variance, and represent a very good description of the environmental structure.

Variables $T$, $NO_3$, $PO_4$, $Si(OH)_4$, TN, and TP present highest positive loads to PC1 while $S$ loads negatively (Table 3). This component, accounting for 59.7% of total variance (Table 2) is interpreted as the gradual mixing of nutrient-enriched fresh water with nutrient-poor saline water along the estuary (Fig. 6a,b). Position of most of stations along this axis gives support to this interpretation, St 14 (the most riverine) and St. 1 (at the estuary mouth) being more distant from each other.

PC2 accounted for 19% of total variance and variables $NH_4$ and DO are, respectively, the main positive and negative contributors to this axis (Table 3). This can be interpreted as a representation of “poor water quality” in specific areas as opposed to the well oxygenated main body of the estuary. The isolation of St. 15, and to a minor extent St. 4 and St. 16, connected with higher values of $NH_4$ and lower values of DO, supports this interpretation (Fig. 6a,b). The remaining environmental variable, SPM, contributes mostly to PC3 which still explains 10% of variation (Table 3). This component is mainly associated with higher turbidity at Sts. 4 and 5, both located nearby Trancão river discharge.
The MDS ordination of stations generated by phytoplankton abundance is illustrated in Figure 7. Stress value associated with this 2-dimensional plot is 0.11, revealing that this representation of stations is still sound. However, the 3-dimensional MDS (not shown) presents a lower stress (0.07) indicating that samples are likely to fit more easily into three dimensions. The successive position of the stations reveals algal communities differences along the estuary. St 1 and St 14 are the most distinct sites as they differ both in abundance and species composition (respectively marine and freshwater taxa).

According to cluster analysis (not shown) stations located in lower/middle estuary (St. 1-4 and 15-19) constitute a group (A) characterized by the presence of marine and estuarine species (Thalassionema nitzschioides, Guinardia delicatula, Asterionellopsis glacialis, Nitzschia longissima). Sts. 5-10 and 12, mostly located in the middle estuary, formed another distinct group (B) with a mixed dominance of marine, estuarine and some freshwater species (Melosira moniliformis, Chaetoceros subtilis, Thalassionema nitzschioides, Chaetoceros socialis, Stephanodiscus hantzschii). Group C including Sts 11, 13 and 14 individualize the upper estuary due to higher abundance and prevalence of freshwater phytoplankton species (Stephanodiscus hantzschii, Aulacoseira distans, Cyclotella meneghiniana, Scenedesmus opoliensis). Consequently, Groups A and C present the highest dissimilarity (81%, SIMPER analysis) while the average dissimilarity between groups B-C and A-B attained respectively 65% and 62%.

BIOENV analysis reveals that environmental parameters have a strong correlation with phytoplankton community along Tagus estuary (Table 4). The combination of variables that best explained the phytoplankton pattern in the study period were T, S, Si(OH)₄ and TP (r =0.803) although correlation with S alone was nearly as large (0.796). Salinity is a dominant factor and is the single variable that is retained in all of the best 10 sets of
variables. SPM is still included in the best 10 results, though the respective group of variables have relatively lower correlation with phytoplankton abundance ($r = 0.776$).

The application of GLM analysis revealed the following relationship between phytoplankton abundance and environmental variables:

$$\log(\text{Phyto abundance}) = 6.98815 - 0.07298\log(S) + 0.42153\log(\text{PO}_4) - 0.02145\log(\text{Si(OH)}_4)$$

This relationship, which includes $S$, $\text{Si(OH)}_4$ and $\text{PO}_4$, has a high explanatory power (63%) for phytoplankton abundance.

### 4. Discussion

Both environmental and phytoplankton variables showed an upper-lower gradient along Tagus estuary, despite nutrient point sources other than Tagus river being important inputs to specific areas of the system.

The identified phytoplankton assemblages including, respectively, freshwater, estuarine and freshwater, and estuarine and marine species, were dominated by Bacillariophyceae. The dominance of this group has been already reported by other authors, either along the estuary (Oliveira, 2000; 2003) or in the upper (Gameiro et al., 2004) and lower estuary (Cabeçadas, 1999).

The total number of species (236) identified in this study was lower than that referred by Moita and Vilarinho (1999). However, these authors also included phytoplankton data from winter period, when Tagus river discharge is higher, and many freshwater species enter the estuary. The majority of these allochthonous species do not remain as components of the phytoplankton community all year round. In fact, in March 2001, after a very rainy winter period, a considerable number of freshwater species (115) were identified all along the estuary (Oliveira, 2003). By contrast, in July 2001 (data included in this work) only 28 of these freshwater species survived at the upper estuary. On the
other hand, the screening model developed by Ferreira et al. (2005), using data from 1929 to 1998, predicted that in Tagus estuary, from a total number of 342 species, 120 were combined riverine and oceanic species, and only 222 were real estuarine. This number compares well with the one found in our study.

The strong correlations revealed by BIOENV analysis support the view that, in the study period, the phytoplankton in Tagus estuary, were more likely controlled by abiotic factors than by biotic mechanisms, such as grazing by zooplankton. Despite data on zooplankton not being available for all the study period, Monteiro (2003) reported the occurrence in July 2001 of two zooplankton maxima, respectively, one at the oligohaline-freshwater zone coinciding with the phytoplankton maximum, and the other at the estuary mouth, with zooplankton abundance being low in the other estuarine zones.

In fact BIOENV analysis identified salinity, temperature, silicate and phosphorus as variables explaining the phytoplankton spatial pattern, although salinity alone has an overriding role. Results from the GLM analysis agreed quite well with BIOENV by selecting variables salinity, silicate and phosphate as explanatory variables of phytoplankton abundance in Tagus estuary in the study period. The inclusion of silicate and phosphorus emphasizes the nutrients requirements of the dominant algal group (Bacillariophyceae), and may be related to the apparent unbalanced proportions of nutrients in the estuary, revealed by shortage of Si in the lower/middle estuary and of P in the upper estuary (van der Zee & Chou, 2005; Justic et al. 2005). Nevertheless, as nutrient levels in the study period were higher than typical half saturation constants for natural phytoplankton populations, growth of most species was unlikely to be nutrient limited (Reynolds, 1999; Estrada et al., 2003).
Regarding the SPM variable, which varied irregularly along the estuary, concentrations were above 10mg/L which represents a threshold value above which primary productivity is usually inhibited (De Master et. al., 1993; Ragueneau et. al, 2002). However, the apparent reduced light conditions appear adequate for Bacillariophyceae growth. In fact, studies carried out by Cabeçadas (1999) on phytoplankton photosynthetic responses in Tagus estuary concluded that, in the lower estuary phytoplankton seemed partially adapted to high turbid conditions. It is known (Smayda and Reynolds, 2001) that species of this group are characterized by lower-light requirements and adaptation to fluctuations in light intensity induced by turbulent mixing.

Despite nutrient discharges from point sources in specific areas of Tagus estuary, no evidence of poor water quality relationships with changes in phytoplankton structure could be found. In fact, Cyanobacteria and Dinophyceae, which are dominant in high trophic state ecosystems, as well as Cryptophyceae whose predominance during the summer is known to be an indicator of eutrophication (Moncheva et al. 2001), were all minor contributors to the algal community. Indeed, they represented only 1-2% of the phytoplankton total abundance, suggesting that, at present, cultural eutrophication is not a problem in this estuary. In addition, the high values of Shannon-Wiener index and Evenness confirm that most phytoplankton communities are at equilibrium in Tagus estuary.

This work can be valuable for the outlining of water quality monitoring programs, as it allows to take into account a reduced number of key environmental parameters able to characterize the phytoplankton community structure in Tagus estuary.

Acknowledgements
This work was financially supported by EU, under project P.O.MARE “Caracterização Ecológica da zona Costeira” Contract no. 22-05-01-FDR-00015 and project REBECCA Contract no. SSPI-CT-2003-502158. We are grateful to M. Nogueira, A.P. Oliveira, C. Gonçalves, V. Franco and L. Palma for helping in sampling and for assistance in measurements. We would also like to thank the anonymous reviewers for constructive comments and suggestions.

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Table 1
Dominant algal groups (cells/mL) along Tagus estuary in the productive period.

<table>
<thead>
<tr>
<th>Algal group</th>
<th>Stations</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1  2  3  4  5  6  7  8  9  10 11 12 13 14 15 16 17 18 19</td>
</tr>
<tr>
<td>Bacillariophyceae</td>
<td>332 147 223 224 238 206 432 833 269 672 1562 612 400 934 165 218 116 71</td>
</tr>
<tr>
<td>Chlorophyceae</td>
<td>13  3  4  7 19 10 24 7 29 5 48 11 59 229 2 6 0.7 0.6 0.8</td>
</tr>
<tr>
<td>Cryptophyceae</td>
<td>15 10 8 110 0.4 97 5 3 0.8 28 0.2 8 0.4 0 11 25 1 0.6 8</td>
</tr>
<tr>
<td>Cyanobacteria</td>
<td>0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0</td>
</tr>
<tr>
<td>Dinophyceae</td>
<td>15 4 1 5 3 16 5 3 0.2 2 0 3 0 0 4 11 0.3 0.8 0.4</td>
</tr>
<tr>
<td>Ebriidea</td>
<td>0 0.1 0 4 0 4 2 0.1 0.8 1 1 0 0 0 0 0 0 0 0</td>
</tr>
<tr>
<td>Euglenophyceae</td>
<td>0.6 1 0.7 1 36 34 2 9 4 1 0.6 0 0.1 0 0 1 4 2 0.7</td>
</tr>
<tr>
<td>Prasinophyceae</td>
<td>107 4 0 8 0.8 8 1 2 1 0.5 0 0 0 2 14 0 0.6 0</td>
</tr>
</tbody>
</table>
Table 2

Eigenvalues of the correlation matrix, proportion of variance explained by each PC and cumulative variation for the environmental PCA

<table>
<thead>
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<th>PC</th>
<th>Eigenvalues</th>
<th>% Variation</th>
<th>Cum. % Variation</th>
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</thead>
<tbody>
<tr>
<td>1</td>
<td>5.970</td>
<td>59.7</td>
<td>59.7</td>
</tr>
<tr>
<td>2</td>
<td>1.900</td>
<td>19.0</td>
<td>78.8</td>
</tr>
<tr>
<td>3</td>
<td>1.010</td>
<td>10.1</td>
<td>88.8</td>
</tr>
</tbody>
</table>
Table 3.

Variable loads of the environmental PCA

<table>
<thead>
<tr>
<th></th>
<th>PC1</th>
<th>PC2</th>
<th>PC3</th>
</tr>
</thead>
<tbody>
<tr>
<td>T</td>
<td>0.353</td>
<td>0.090</td>
<td>-0.036</td>
</tr>
<tr>
<td>S</td>
<td>-0.386</td>
<td>0.158</td>
<td>0.007</td>
</tr>
<tr>
<td>DO</td>
<td>0.164</td>
<td>-0.570</td>
<td>0.027</td>
</tr>
<tr>
<td>NO₃</td>
<td>0.396</td>
<td>-0.084</td>
<td>0.038</td>
</tr>
<tr>
<td>log (1+NH₄)</td>
<td>-0.091</td>
<td>0.652</td>
<td>0.256</td>
</tr>
<tr>
<td>PO₄</td>
<td>0.341</td>
<td>0.327</td>
<td>0.066</td>
</tr>
<tr>
<td>Si(OH)₄</td>
<td>0.368</td>
<td>-0.108</td>
<td>0.138</td>
</tr>
<tr>
<td>TN</td>
<td>0.359</td>
<td>0.185</td>
<td>0.091</td>
</tr>
<tr>
<td>TP</td>
<td>0.384</td>
<td>0.166</td>
<td>-0.037</td>
</tr>
<tr>
<td>SPM</td>
<td>0.077</td>
<td>0.172</td>
<td>-0.948</td>
</tr>
</tbody>
</table>
Table 4. Summary of the 10 best results (Spearman correlation, ρ) obtained from BIOENV analysis of combined environmental variables matching phytoplankton abundance

<table>
<thead>
<tr>
<th>Number of Variables</th>
<th>ρ</th>
<th>Variables</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>0.803</td>
<td>T, S, Si, TP</td>
</tr>
<tr>
<td>1</td>
<td>0.796</td>
<td>S</td>
</tr>
<tr>
<td>3</td>
<td>0.794</td>
<td>T, S, Si</td>
</tr>
<tr>
<td>5</td>
<td>0.789</td>
<td>T, S, NO, Si, TP,</td>
</tr>
<tr>
<td>4</td>
<td>0.784</td>
<td>T, S, NO, Si</td>
</tr>
<tr>
<td>3</td>
<td>0.781</td>
<td>S, NO, Si</td>
</tr>
<tr>
<td>2</td>
<td>0.779</td>
<td>S, Si</td>
</tr>
<tr>
<td>2</td>
<td>0.778</td>
<td>S, NO</td>
</tr>
<tr>
<td>5</td>
<td>0.776</td>
<td>T, S, Si, TP, SM</td>
</tr>
<tr>
<td>2</td>
<td>0.776</td>
<td>S, TP</td>
</tr>
</tbody>
</table>

Note: (S in bold as appeared in all combinations)
Fig. 1
Fig. 2
Fig. 3
Fig. 5
Fig. 6
Fig. 7