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ScienceDirect
Estuarine, Coastal and Shelf Science 75 (2007) 21–34
ESTUARINE COASTAL AND SHELF SCIENCE
www.elsevier.com/locate/ecss

Environmental drivers of phytoplankton distribution and composition in Tagus Estuary, Portugal

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Received 15 October 2006
Available online 5 July 2007

Abstract

A 7-year (March 1999–November 2005) monitoring program was developed in the Tagus estuary to study phytoplankton dynamics and several key controlling factors, namely nutrient content, light availability, atmospheric and hydrodynamic conditions (temperature, wind, rainfall, river flow, and salinity). Water was collected at four sampling sites on a monthly basis. Phytoplankton biomass, analyzed as Chl *a*, was moderate to low, when compared to other mesotidal estuaries: interannual average Chl *a* values ranged from 1.4 in winter to 8.0 μg L^{-1} in summer. A consistent seasonal pattern was observed, with a unimodal peak extending from late spring to summer. The phytoplankton community, as determined by biomarker pigment concentration using HPLC and CHEMTAX, was dominated by diatoms (57%), and included cryptophytes (23%), dinoflagellates (6.8%), chlorophytes (5.4%), euglenophytes (4.9%), and prasinophytes (2.6%). The method was capable of detecting phytoplankton taxa generally underestimated or overlooked when using standard microscopic techniques. Diatoms were the main bloom-formers in the summer Chl *a* maximum. A stepwise regression analysis showed that air temperature, river flow and irradiance explained 47% of the observed Chl *a* variance, illustrating the importance of climatic factors as driving forces for seasonal and interannual variability of phytoplankton.

Keywords: phytoplankton; environmental drivers; chlorophyll *a*; chemotaxonomy; monitoring; Tagus estuary

1. Introduction

The relevance of long-term studies in ecosystem management programs is now widely recognized. In order to measure anthropogenic influence on a certain ecosystem, a good knowledge of the system natural variability is a necessary requisite. Estuaries are, by definition, transitional states, suffering more acutely the outcome of climate variability. However, variability and unsteadiness are intrinsic properties of estuarine ecosystems; their biological communities are well adapted to several temporal variability scales and to spatial gradients of key factors, such as salinity or temperature.

Phytoplankton, as the basis of the trophic chain, constitutes the biological community in which scientific attention is focused when a management plan is needed or an assessment of the ecosystem health is required (e.g. Monbet, 1992; Cloern, 1999; Sin et al., 1999). Estuarine phytoplankton is submitted to superimposed temporal scales derived from tidal regime and seasonal freshwater runoff, which greatly affect water column stability, residence time, light and nutrient availability. Vertical mixing of the water column is also largely influenced by less regular events such as wind-driven water mixing. The influence of these processes on phytoplankton biomass and productivity has been a recurrent subject in the recent literature, and is still the subject of debate.

Knowledge of the taxonomical composition of phytoplankton is essential to study the spatial and temporal community dynamics and to characterize it into functional groups. Furthermore, an index of ecosystem eutrophication is the shift

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doi:10.1016/j.ecss.2007.05.014

from a diatom dominating to a phytoflagellate phytoplankton community (Cloern, 1996). The identification and distribution of phytoplankton classes is increasingly done by the detection of photosynthetic pigments in the water column by high-performance liquid chromatography (HPLC) analytical techniques. HPLC pigment analysis does not have the taxonomic precision of microscopy, but is suitable for the analysis of the hundreds of samples required in ecological studies (Wright et al., 1996). Quantitative estimates of various phytoplankton classes from biomarker pigment concentrations has been attempted using several statistical approaches (Gieskes et al., 1988; Everitt et al., 1990; Letelier et al., 1993), but these generally suffer from a number of difficulties that limit their application (Mackey et al., 1996). CHEmical TAXonomy software (CHEMTAX) has been described by Mackey et al. (1996), using pigment/chlorophyll *a* ratios to characterize algal classes. There have been a vast number of recent studies on phytoplankton community structure using pigment analysis and CHEMTAX in ocean waters (e.g. Wright et al., 1996; Rodriguez et al., 2002) but more rarely in estuaries (Pinckney et al., 1998; Ansotegui et al., 2001). The distribution and composition of phytoplankton in the Tagus estuary using HPLC pigment analysis and CHEMTAX from May 2001 till November 2005 is reported in the present study.

The relevance of interannual studies is enhanced with the recent public concern on global climate change. Rainfall is a proxy of interannual climate variability, whereas river flow reflects precipitation integrated both spatially (over the catchment) and temporally. The Tagus river basin is the largest in the Iberian Peninsula, where the rainfall regime is characterized by high temporal variability (Trigo et al., 2004). The variability observed in a 75-year period in the last century was correlated with North Atlantic oscillation (NAO). The effect of climatic factors on the distribution of phytoplankton biomass can only be assessed over pluri-annual monitoring programs.

A 7-year (March 1999–November 2005) monitoring program was developed with the intention of establishing a database on hydrography, nutrient concentrations, suspended particulate matter, vertical light attenuation, chlorophyll *a* (Chl *a*) and photosynthetic pigments, so that future management plans could take into consideration seasonal fluctuation patterns and interannual variability. The objectives of the present study were to examine seasonal, interannual and spatial variations on phytoplankton biomass and class composition in the Tagus estuary, and to determine the influence of environmental parameters on phytoplankton communities, with a particular emphasis on climatic factors.

2. Methodology

2.1. Study area

The present study was carried out in the upper part of the Tagus estuary (Fig. 1). This estuary is one of the largest estuaries on the west coast of Europe, with a broad shallow bay

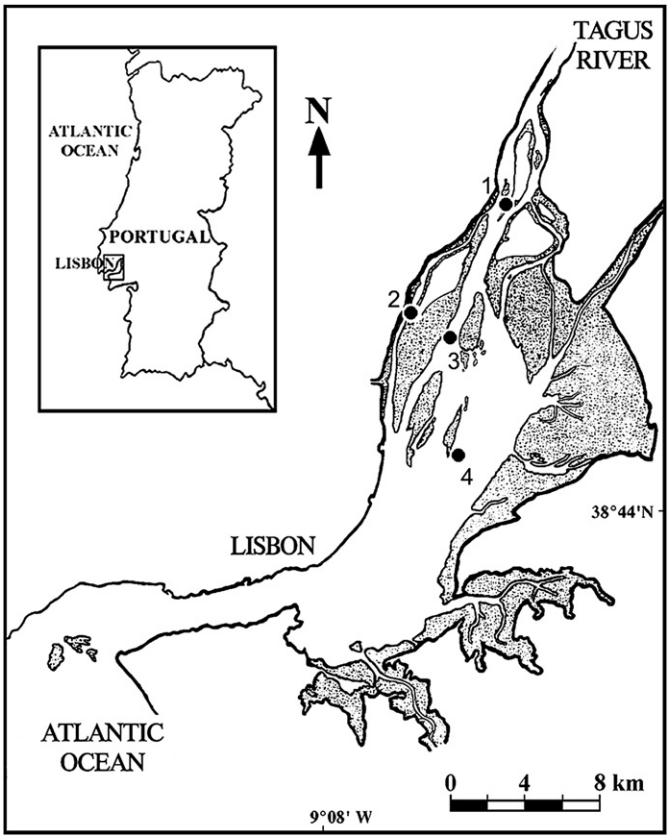

Fig. 1. Map of Portugal with enlarged portion of the Tagus estuary. Shaded areas represent intertidal zones and circles indicate the location of the sampling sites.

covering an area of about 320 km^2. It is located in the most populated area of Portugal. The Tagus river is the main source of freshwater to the estuary. The area affected by tides reaches 80 km landward of Lisbon. It is a mesotidal estuary with semi-diurnal tides. Table 1 summarizes some other relevant characteristics of the Tagus estuary. The sampling was carried out in the upper estuary, near the downstream limit. Four sampling sites were considered, hereafter designated sites 1, 2, 3 and 4. Sampling sites 1, 3 and 4 are located along a latitude gradient, whereas site 2 is situated in the Northern Channel where several industrial plants are established. The exact location of the study sites is shown in Fig. 1. Sites differed in relation to average water depth during high tide: site 1, 3 m; site 2, 2.5 m; site 3, 3.5 m; site 4, 5.5 m.

2.2. Sampling and field procedures

Water was collected at the surface of the four sites on a monthly basis from March 1999 to November 2005, making a total of 75 dates. Sampling was always conducted during high tide of neap tides to attenuate the influence of the spring–neap tidal cycle. Water temperature, salinity and pH were measured *in situ* with a thermometer, an ATAGO S/Mill-E refractometer and a HI9813 pH meter (Hanna Instruments), respectively. Incident irradiation (I_o) and light intensity at depth z (I_z) were determined with a LIcor-192SA

Table 1
Main physical, socio-economic and ecological characteristics of the Tagus estuary

Physical	
Total area	320 km^2
Total area of water basin	80629 km^2
Average depth	10 m
Average tidal range[a]	2.4 m
Mean estuary volume	19×10^8 m^3
Mean tidal volume	7.5×10^8 m^3
Residence time of freshwater	8 days at $Q = 813$ m^3 s^{-1} 26 days at $Q = 145$ m^3 s^{-1}
Maximum current speed	2.0 m s^{-1}
Average annual river discharge[b]	343 m^3 s^{-1}
SPM average year discharge	4×10^5 t (Vale and Sündby, 1987)
Socio-economic	
Human population around the estuary	1.6×10^6 (AML)[c]
Agricultural land and build area	33% and 60% (AML)[c]
Total annual nitrogen loading	26000 t (Cabeçadas et al., 2000)
Annual ship traffic	250000 containers, 11×10^6 t
Local fisheries activities	316 boats (DGPA)[d]
Ecological	
Salt marsh vegetation area	21 km^2
Residents and migratory fishes	44 species (Costa, 1999)
Residents and migratory bird population	120000 birds
Tagus estuary natural reserve	Created in 1976 (14 660 ha) (Ramsar site)[e]

[a] Average value at seaward end (0.9 m neap tide and 4.1 m spring tide).
[b] Long-term (25 years) averages of river discharge.
[c] AML, Metropolitan Area of Lisbon (http://www.aml.pt).
[d] DGPA, General Direction of Fisheries and Aquiculture (a central service of the Portuguese Ministry of Agriculture, Rural Development and Fisheries).
[e] Convention on Wetlands (Ramsar, Iran, 1971).

underwater quantum sensor at the water surface and at 0.5 m intervals throughout the water column. From these set of data, the vertical light attenuation coefficient (K_{par}, m^{-1}) was estimated, applying the equation $I_z = I_o \exp(-K_{par} \times z)$. Euphotic depth ($Z_{euf}$) was taken as the depth of 1% surface irradiance calculated from the measured light attenuation coefficient, and estimated as $4.6/K_{par}$. In our previous work (Gameiro et al., 2004), we verified the absence of stratification in the water column at the same sampling sites. Therefore, we assumed that phytoplankton was homogeneously distributed in the euphotic zone and the mixing depth (Z_{mix}) was considered as bottom depth at the sampling sites. Z_{mix} was divided by Z_{euf} to obtain Z_{mix}:Z_{euf} ratios.

Aliquots of samples of water were used for determination of suspended particulate matter (SPM), dissolved inorganic nutrients (ammonium: NH_4^+, nitrate: NO_3^-, nitrite: NO_2^-, soluble orthophosphates: PO_4, and silicates: SiO_2), chlorophyll *a* (Chl *a*) and other photopigment (carotenoids and chlorophylls) concentrations.

Mean daily river discharge (Q) of the Tagus river and the total daily rainfall (Rf) at the sampling area was obtained from the Water National Institute in a public database (http://www.inag.pt). Daily data on air temperature (T), irradiance (I), wind direction and speed (Ws) were obtained from the Meteorological Institute.

2.3. Laboratory procedures

For determination of suspended particulate matter (SPM) concentration, triplicate water samples were filtered through pre-weighed GF/C Whatman filters, which were subsequently dried at 80 °C for about 24 h and reweighed.

Triplicate samples for inorganic nutrient concentrations were collected and filtered through GF/C Whatman filters and immediately frozen for later colorimetric analysis with a Tecator FIAstar™ 5000 Analyser. Nitrate was determined according to Grasshoff (1976), nitrite according to Bendschneider and Robison (1952), phosphates and silicates according to Murphy and Riley (1962) and Fanning and Pilson (1973) respectively. Ammonium concentrations were determined using manual colorimetric methods in filtered samples according to Koroleff (1969/1970). Analysis for dissolved inorganic nitrogen (DIN) ($NO_3^- + NO_2^- + NH_4^+$), were performed during the whole sampling period, whereas PO_4 and SiO_2 were determined only from May 2001 onwards.

Triplicate samples for Chl *a* analyses were filtered onto Whatman GF/F glass fiber filters and extracted in 90% acetone at 4 °C in darkness for 24 h (Lorenzen, 1967). Chl *a* was quantified with a Shimadzu UV-1603 spectrophotometer before and after acidification to correct for pheopigments.

2.4. HPLC analysis

Water samples (1–2 L) were filtered onto Whatman GF/F filters (nominal pore size 0.7 μm and 4.7 cm diameter). The filters were frozen and kept at −80 °C until extraction. Photosynthetic pigments were extracted at −20 °C for 30 min with 95% cold buffered methanol (2% ammonium acetate), using a ramrod for filter grinding and further sonication during 1 min at low temperature at the beginning of the extraction period. The samples were centrifuged at 2500 rpm for 5 min, at 4 °C. Extracts were filtered (Millipore membrane filters, 0.2 μm) and immediately injected into the HPLC.

The filtered extracts were injected into a Shimadzu HPLC comprised of a solvent delivery module (LC-10ADVP) with system controller (SCL-10AVP), a photodiode array (SPD-M10AVP) and a fluorescence detector (RF-10 AXL). Chromatography separation was carried out using a C18 column for reverse phase chromatography (Supelcosil, 0.46 × 25 cm, 5 μm particles). The solvent gradient followed Kraay et al. (1992) adapted by Brotas and Plante-Cuny (1996) with a flow rate of 0.6 mL min^{-1}, an injection volume of 100 μL, and a duration of 35 min. Pigments were identified by comparing retention times and absorption spectra with pure crystalline standards, including chlorophyll *b*, Chl *a* and β-carotene from Sigma and chlorophyll c_3, chlorophyllide *a*, chlorophyll c_2, peridinin, fucoxanthin, neoxanthin, prasinoxanthin, violaxanthin, diadinoxanthin, antheraxanthin, alloxanthin, diatoxanthin, lutein and zeaxanthin standards from DHI, and

concentrations calculated from peak areas in the photodiode array detector.

The HPLC method used allowed the discrimination of 17 pigments, corresponding to all the peaks detected in the phytoplankton samples, except for chlorophylls c_1 and c_2 that co-eluted as a single peak (Fig. 2, peak 3). Peak identification, average retention times and spectral absorbance maxima are shown in Table 2. The linear regression (Model II) performed on Chl *a* determined by spectrophotometry and by HPLC gave a significant positive correlation ($r = 0.83$; $n = 203$), with a slope close to 1. Hence, the relative contribution of each algal class was estimated in relation to Chl *a* determined by HPLC.

In order to obtain pigment ratios typical of species abundant in the Tagus estuary, cells were isolated from phytoplankton samples collected in the study area and cultivated in the laboratory. Cultures of the diatom *Detonula pumila* were grown on a 12 h light:12 h dark cycle at 19 °C in f/2 medium (Guillard and Ryther, 1962) with a light intensity of 80 µmol photons m^{-2} s^{-1}. The cultures of the dinoflagellate *Scrippsiella* sp. were maintained in GSe medium (a variation of G medium with the addition of 1×10^{-8} mol L^{-1} selenium; Doblin et al., 1999) at the same temperature, photoperiod and irradiance levels described above.

2.5. CHEMTAX and class abundance

The abundance of phytoplankton classes contributing to total Chl *a* were estimated from the concentrations of biomarker pigments using CHEMical TAXonomy software (Mackey et al., 1996; Wright et al., 1996). CHEMTAX is a matrix-factorization program for the calculation of algal class abundances from concentrations of algal chemosystematic-marker photopigments, chlorophylls and carotenoids (Mackey et al., 1996, 1998; Wright et al., 1996). This program, running under MATLAB™, uses a factor analysis and a steepest descent algorithm to find the best fit to the data based on an initial pigment ratio matrix for the classes to be determined. Our initial pigment ratio matrix (Table 3) was based on pigment ratios determined for *Detonula pumila* and two *Scrippsiella* sp. collected at the Tagus estuary and from pigment ratios published in the literature for estuarine species (Schlüter et al., 2000). The complexity of the estimated community structure depends upon the number of phytoplankton groups defined *a priori* by the researcher. We took a conservative approach in defining the initial ratio matrix, restricting our resolution to Bacillariophyceae (diatoms), Chlorophyceae, Cyanobacteria, Cryptophyceae, Prasinophyceae, Dinophyceaea and Euglenophyceae. Except for Prasinophyceae, detected by the presence of the diagnostic pigment prasinoxanthin, other algal classes were chosen as they were previously detected microscopically in the Tagus estuary (Gameiro et al., 2004).

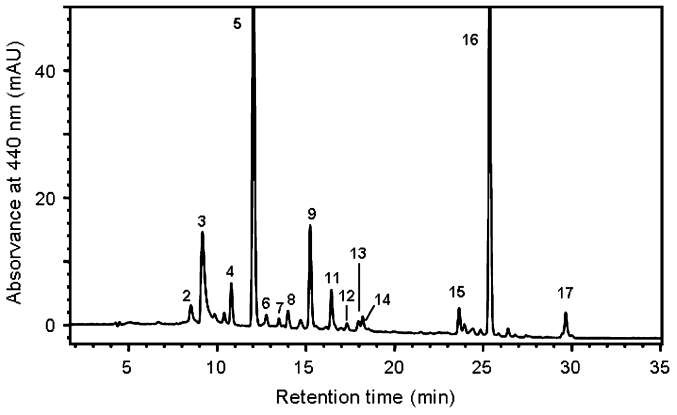

Fig. 2. Selected HPLC chromatogram ($\lambda = 440$ nm) showing the pigment pattern of phytoplankton extracts from a sample collected at site 1 on July 2005. Peak identification as shown in Table 2.

2.6. Statistical analysis

The values used in all statistical analysis for climatic factors, irradiation, air temperature and river discharge, were obtained as the daily mean of the 8 days before sampling date, whereas the rainfall data were the sum of the 8 days before sampling date.

Basic statistics and linear regression analysis were calculated using the Statistica 6.0® software package. Spearman's rank correlation coefficients were used to investigate statistical correlations between the various parameters. The data used in the multiple regression analysis based on stepwise method were logarithmically transformed in order to approximate the distribution to normality and reduce the proportion of variances.

3. Results

Climatic factors registered during the period of the survey (March 1999 to November 2005) are shown in Fig. 3. Daily air temperature values are plotted with the average monthly temperature for 55 years (1939–1995) (Fig. 3A), showing the seasonal interannual variability, with the maximum value (33.5 °C) attained in summer 2003 and the minimum value (6.4 °C) in winter 2005. Fig. 3B shows that the monthly mean of wind speed attains higher values in summer (2.9–4.6 m s^{-1}), with the dominant wind generally from N and NW (shown in open circles) with the exception of the 2000 and 2005. The discharge of the Tagus river (*Q*) shows a pronounced dry/wet season signal. The annual discharge varied between a critical very dry year (52.8 m^3 s^{-1}) in 2005 to a wet year during 2001 (735.5 m^3 s^{-1}). The average monthly discharges fluctuated from 5.6 m^3 s^{-1} (October 2005) to 2914 m^3 s^{-1} (January 2001, Fig. 3C). The mean annual river discharge from the Tagus river in the past 25 years was 343 m^3 s^{-1} (Table 1).

The seasonal range and average water temperature, pH and salinity are shown in Table 4. Water temperature varied seasonally from 12.8 °C in winter to 22.9 °C in summer. The water pH values varied between 5.6 and 9.0. A marked salinity gradient was observed in spatial and seasonal scales. Considering the whole period, the mean salinity ranged from 15 at site 1 to 24 at site 4. During summer the average salinity was 20 and 28 at sites 1 and 4 respectively, and in winter

Table 2
Peak identification, retention time, spectral absorbance maxima and maximum concentrations of phytoplankton pigments detected in Tagus estuary samples from May 2001 to November 2005. The number in parentheses is the site where the maximum pigment concentration was found

Peak no.	Pigment	Abbreviation	Retention time (min)	Maxima in eluant (nm)	Max. concentrations of pigments found at Tagus estuary (site) (μg L^{-1})
1	Chlorophyll c_3	Chl c_3	7.58	454, 586	0.72 (4)
2	Chlorophyllide *a*	Chlide *a*	7.95	431, 665	3.10 (2)
3	Chlorophyll $c_1 + c_2$	Chl $c_1 + c_2$	8.69	445, 581, 630	2.95 (1)
4	Peridinin	Per	10.48	475	1.99 (1)
5	Fucoxanthin	Fuc	11.80	448, 465	11.77 (1)
6	Neoxanthin	Neo	12.71	414, 438, 466	0.25 (3)
7	Prasinoxanthin	Pra	13.51	454	0.72 (3)
8	Violoxanthin	Vio	14.23	417, 441,471	1.40 (2)
9	Diadinoxanthin	Diad	15.45	424, 448, 477	0.22 (1)
10	Antheraxanthin	Anth	16.53	424, 444, 476	0.11 (3)
11	Alloxanthin	Allo	16.84	429, 454, 483	0.99 (4)
12	Diatoxanthin	Diat	17.77	430, 454, 482	0.51 (3)
13	Lutein	Lut	18.24	425, 447, 475	0.26 (1)
14	Zeaxanthin	Zea	18.71	430, 454, 481	0.35 (1)
15	Chlorophyll *b*	Chl *b*	22.97	457, 596, 646	1.20 (3)
16	Chlorophyll *a*	Chl *a*	25.00	430, 617, 663	23.28 (1)
17	β-Carotene	β-Car	28.02	430, 454, 481	0.27 (1)

11 for site 1 and 21 for site 4. Salinity lowest values attained in 2001, a very wet year, with a significant inverse correlation with river discharge ($r = -0.75$; $p < 0.001$; $n = 35$).

Daily average atmospheric surface solar radiation (I) varied between 25 and 700 μmol m^{-2} s^{-1} (PAR). The light attenuation coefficient (K_{par}) ranged from 0.4 to 8.0 (Table 4). Site 2 recorded the highest values and site 4 the lowest, and the overall mean was typically near 1.8 m^{-1}. The light attenuation coefficient was highly correlated with SPM ($r = 0.62$; $n = 222$; Table 5), but not with Chl *a*, suggesting that the turbidity was due to suspended particles rather than phytoplankton cells. The range obtained for SPM was 3.9 to 113.3 mg L^{-1} (Table 4).

The ratio between mixing depth (Z_{mix}) and euphotic depth (Z_{euf}), ranged from 0.4 at site 4 to 4.1 at site 2 (Fig. 4). The average Z_{mix}:Z_{euf} ratios were similar for all seasons (1.3). Values of Z_{mix}:Z_{euf} ratio lower than 1 indicated that the entire water column was located within the euphotic zone; this occurred only in 22% of sampling occasions. According to Cole and Cloern (1984), a ratio of 5 is the upper limit for net primary productivity and bloom initiation. In our all data set this value was never reached. Z_{mix}:Z_{euf} ratios were significantly correlated ($r = 0.66$, $p < 0.001$) with K_{par} values (Table 5).

The monthly value for each nutrient analyzed in the four sites, as well as their average is presented in Fig. 5A–C. DIN and SiO_2 concentrations displayed the typical seasonal pattern of temperate estuaries, where the source of nitrogen and silicate is mostly riverine (Fig. 5A,C). Significant positive correlations were obtained between river discharge and DIN and silicates (Table 5). DIN concentrations decreased from

Table 3
Initial pigment ratio input matrix and output values calculated by CHEMTAX. *Pigment ratios from our cultures

Algal groups	Pigments: Chl *a* ratio								
	Peridinin	Fucoxanthin	Alloxanthin	Lutein	Zeaxanthin	Neoxanthin	Violoxanthin	Prasinoxanthin	Chlorophyll *b*
Initial values									
Dinoflagellates*	0.639	–	–	–	–	–	–	–	–
Cryptophytes	–	–	0.392	–	–	–	–	–	–
Chlorophytes	–	–	–	0.260	0.099	0.043	0.011	–	0.145
Cyanobacteria	–	–	–	–	1.620	–	–	–	–
Diatoms*	–	0.755	–	–	–	–	–	–	–
Prasinophytes	–	–	–	0.032	0.157	0.082	–	0.497	0.568
Euglenophytes	–	–	–	–	0.104	0.072	0.012	–	0.211
Best-fit values									
Dinoflagellates*	0.639	–	–	–	–	–	–	–	–
Cryptophytes	–	–	0.339	–	–	–	–	–	–
Chlorophytes	–	–	–	0.196	0.099	0.043	0.011	–	0.188
Cyanobacteria	–	–	–	–	1.620	–	–	–	–
Diatoms*	–	0.560	–	–	–	–	–	–	–
Prasinophytes	–	–	–	0.032	0.157	0.082	–	0.497	0.568
Euglenophytes	–	–	–	–	0.104	0.072	0.012	–	0.211

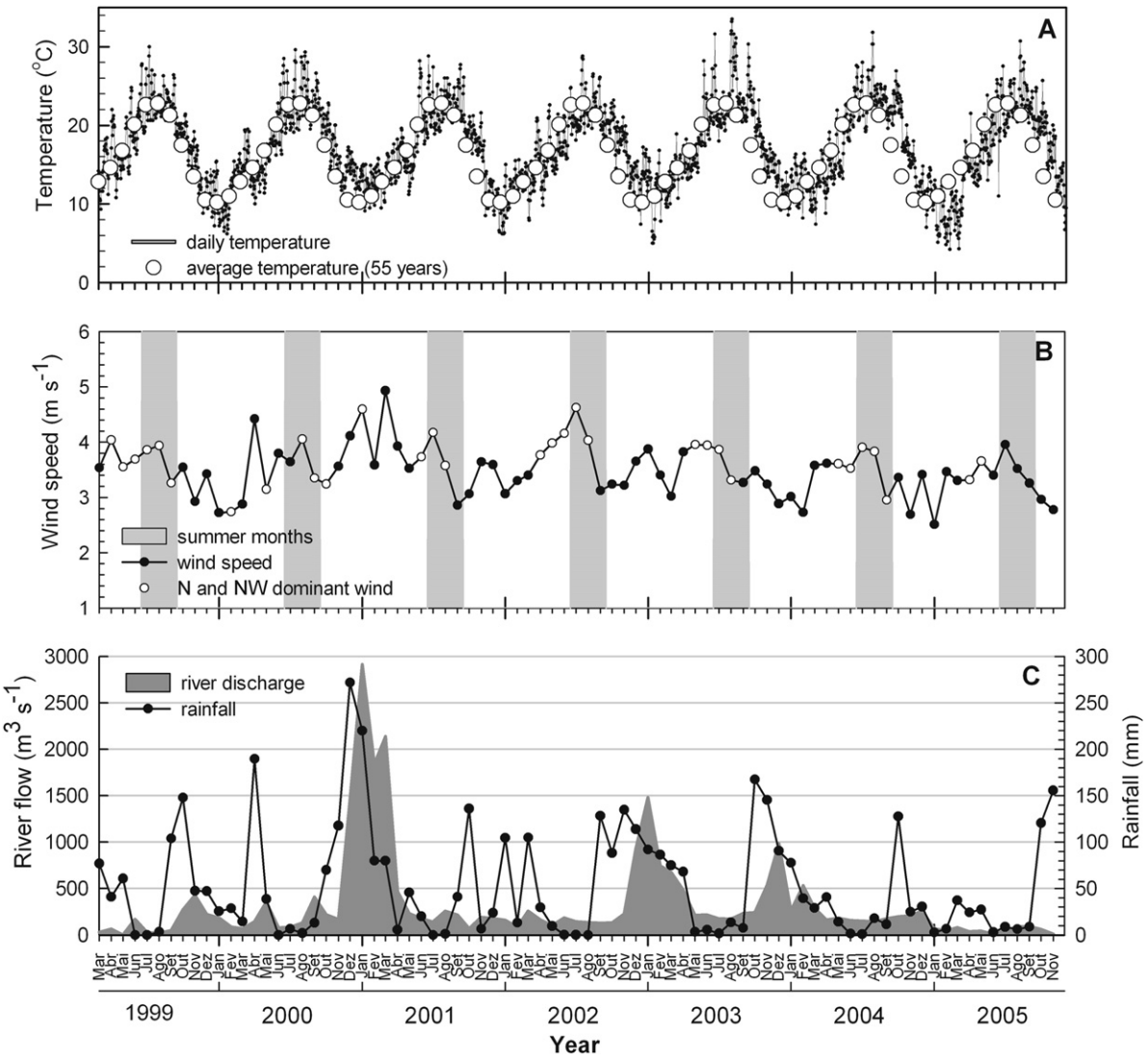

Fig. 3. (A) Time series of daily air temperature (°C) and monthly average air temperature of 55 years dataset (1934–1994) for the Tagus estuary region from March 1999 to November 2005. (B) Monthly average wind speed (m s^{-1}) (●) and North (N) and Norwest (NW) wind direction when they are dominant on each date (○). (C) Mean monthly river discharge (m^3 s^{-1}) and total monthly rainfall data (mm).

site 1 (mean concentration of 75.5 μM) to site 4 (36.1 μM; Fig. 5A). Nitrate was the dominant form of DIN in the estuary with a mean value of 41 μM. Ammonium concentrations were, on average, less than 12 μM, and nitrite concentration was just 3.5% of the total DIN, with average concentrations of 2 μM. At all sites, during the summer months, DIN and SiO_2 concentrations dropped to very low values (Fig. 5A,C), the minima being reached at site 4, with 0.18 μM and 4.6 μM, respectively. The mean SiO_2 concentration was higher in site 1 (63 μM). Phosphate concentration did not show a consistent seasonal pattern; however, an interannual trend seems to exist, with higher values in 2004 and 2005 (Fig. 5B).

Spatial and temporal distribution of phytoplanktonic biomass, measured as Chl *a*, is illustrated in Fig. 6. There is a clear general pattern, repeated throughout the 7 years, with a unimodal increase of Chl *a* in late spring and summer months, and very low values during winter. Landward site 1 presents higher biomass values, with a maximum of 32 μg L^{-1} in summer 1999. The nature of the spring/summer bloom changes from year to year, either biomass changes in accordance at all sites, or short-lived events occur in a single site. For example, the 1999 spring bloom was an event occurring only at site 4.

Table 5 displays the matrix obtained with Spearman's rank mean correlation coefficients between all studied parameters, showing the seasonality and relationship between climatic, hydrographic and biological factors. A stepwise multiple regression was performed on Chl *a* against several potentially important variables, considering the whole study period and the assemblage of results from all sites ($n = 272$). The mean values of air temperature, river flow and irradiance explained 47% of the observed Chl *a* variance, according to the expression:

$$\ln(\mathrm{Chl}a + 1) = -4.729 + 1.430 \times \ln(\mathrm{AirTemp\_8}) - 0.131 \times \ln(\mathrm{RiverFlow\_8}) + 0.376 \times \ln(\mathrm{Irradiance\_8}),$$

Table 4
Descriptive statistics for the different physical parameters, nutrients and chlorophyll concentrations studied from March 1999 to November 2005, considering all sites

	n	Mean	Median	SD	Range
River flow ($m^3\ s^{-1}$)[a]					
Spring	103	256.5	194.9	395.8	3.7–2421.0
Summer	79	139.7	122.6	80.2	16.8–349.4
Autumn	49	190.0	178.3	119.7	12.3–380.3
Winter	41	942.4	162.9	1207.9	34.6–3340.1
All data	272	314.0	163.0	592.7	3.7–3340.1
Water temperature (°C)					
Spring	102	17.7	17.6	2.4	13.0–24.0
Summer	82	22.9	23.0	1.4	20.0–26.0
Autumn	57	17.4	18.0	3.2	12.0–24.0
Winter	44	12.8	13.0	2.1	8.0–18.0
All data	285	18.4	19.0	4.1	8.0–26.0
Salinity					
Spring	103	18.8	18.0	8.5	1.0–37.0
Summer	80	24.8	26.4	7.5	6.0–36.0
Autumn	57	19.4	21.0	7.7	5.0–32.0
Winter	45	16.4	16.0	9.4	2.0–32.0
All data	285	20.2	8.7	21.0	1.0–37.0
pH					
Spring	91	8.1	8.2	0.3	7.1–9.0
Summer	69	7.9	8.1	0.6	5.6–8.5
Autumn	48	7.9	8.0	0.3	6.6–8.4
Winter	34	8.1	8.0	0.3	7.5–8.6
All data	242	8.0	0.4	8.1	5.6–9.0
K_{par} (m^{-1})					
Spring	68	1.7	1.3	1.3	0.6–8.0
Summer	63	1.9	1.5	1.2	0.4–7.7
Autumn	56	1.8	1.4	1.2	0.6–6.5
Winter	33	2.1	1.8	1.4	0.6–6.3
All data	220	1.8	1.3	1.4	0.4–8.0
SPM (mg L^{-1})					
Spring	103	26.2	22.0	17.6	3.9–94.9
Summer	83	35.7	32.9	15.1	13.7–82.5
Autumn	57	26.2	22.1	16.9	8.3–113.3
Winter	45	30.2	22.7	20.6	8.4–102.6
All data	288	29.6	17.7	24.4	3.9–113.3
DIN ($\mu mol\ L^{-1}$)					
Spring	102	64.8	58.6	32.1	0.2–146.3
Summer	83	27.2	26.4	19.2	0. 8–123.6
Autumn	57	67.0	60.3	35.5	7.7–182.4
Winter	45	84.2	78.3	32.9	32. 5–142.3
All data	287	57.4	36.0	54.6	0.8–182.4
PO_4 ($\mu mol\ L^{-1}$)[b]					
Spring	73	3.5	3.2	1.4	2.0–8.4
Summer	61	3.9	3.5	1.6	1.8–9.2
Autumn	47	4.5	3.6	3.0	1.9–19.1
Winter	28	3.4	3.5	1.1	1.4–6.0
All data	209	3.8	1.9	3.5	1.4–19.1
SiO_2 ($\mu mol\ L^{-1}$)[b]					
Spring	78	47.8	42.9	32.3	3.7–135.4
Summer	62	32.1	28.8	22.2	4.6–110.6
Autumn	47	73.0	56.6	49.9	15.3–258.3
Winter	30	62.3	48.0	44.8	14.3–177.5
All data	217	50.8	39.3	44.1	3.7–258.4
Chl a ($\mu g\ L^{-1}$)					
Spring	103	4.5	3.1	4.1	0.5–24.3
Summer	83	8.0	6.8	4.8	1.7–32.3
Autumn	57	2.4	1.7	1.7	0.2–7.0
Winter	45	1.4	0.9	1.7	0.3–8.4
All data	288	4.6	4.4	3.3	0.2–32.3

[a] Derived as a mean of the 8 days before the sampling date.
[b] From May 2001 to November 2005.

where AirTemp_8, RiverFlow_8 and Irradiance_8 are the air temperature, river flow and irradiance daily averages of the 8 days prior to sampling.

This result indicates that climatic factors are the principal driving factors for biomass fluctuation. The predicted Chl *a* values derived from this model are plotted against observed ones in Fig. 7, where it can be seen that observed high biomass values, occurring mainly in summer, are underestimated by this regression.

CHEMTAX analysis identified diatoms as the dominant algal group at all studied sites and seasons (Fig. 8), contributing on average with 57% of total Chl *a*. However, occasionally, especially in situations of low phytoplankton biomass, dinoflagellates or cryptophytes were the most abundant class of microalgae. Cryptophytes were identified as the second dominant algal group, contributing on average with 23% to total Chl *a*. Dinoflagellates, chlorophytes, euglenophytes, and prasinophytes contributed on average with 6.8, 5.4, 4.9 and 2.6% to total phytoplankton biomass. Contribution of cyanobacteria to total Chl *a* was negligible (<0.1%).

Regarding spatial variability, contribution of diatoms to the phytoplankton community registered higher values in sites 1 and 2 (62%) compared to sites 3 and 4 (53%). The relative distribution of cryptophytes followed the salinity gradient, as this group increased from site 1 (19.9%) to site 4 (26.9%). Euglenophytes were more abundant in sites 3 and 4 (6%), than the other two (3.8%). Chlorophytes were significantly more abundant in riverine site 1 (8.7%), with intermediate contributions in sites 2 and 3 (5.6 and 5%, respectively), and significantly lower importance in site 4 (2.5%). Dinoflagellates showed a higher contribution at site 4 (9.4%) compared to an average contribution of 6% at sites 1, 2, and 3. Prasinophytes were more abundant in sites 3 and 4 (3.7%) than in sites 1 and 2 (1.5%).

Interannual variability of phytoplankton taxonomic classes is not outstanding; however, the increase of euglenophytes in summer 2005 is clear. Moreover, a trend seems to exist regarding the relative contribution of diatoms, higher in the period 2002/2003 (63%) than in the subsequent period (54%), counterweighed by the opposite pattern found for cryptophytes with increasing contributions from 2001 (13%) to 2005 (29%).

Fig. 9 plots the contribution of each class, throughout the four seasons, considering the average values for the four sites. Diatoms constituted the dominant group, reaching the maximum contribution to the total Chl *a* pool (65.4%) in summer months. Cryptophytes were the second most abundant group, reaching the maximum contribution during summer–autumn. The chlorophyll *a* value attributed to dinoflagellates was higher in summer. Euglenophytes and prasinophytes were more abundant during summer and autumn, being almost

Table 5
Spearman's rank-mean correlation coefficients of irradiance (I), temperature (T), wind speed (Ws), Tagus river discharge (Q), rainfall (Rf), salinity (S), suspended particular matter (SPM), light attenuation coefficient (K_{par}), mixing depth:euphotic depth ratio (Z_{mix}:Z_{euf}), dissolved inorganic nitrogen (DIN = $NH_4^+ + NO_3^- + NO_2^-$), soluble orthophosphates (PO_4), silicates (SiO_2) and chlorophyll a (Chl a) from March 1999 to November 2005. Coefficients represent data including all four sites ($n = 209–288$ depending upon the variable; $^*p < 0.05$, $^{**}p < 0.01$ and $^{***}p < 0.001$)

	I	T	Ws	Q	Rf	S	SPM	K_{par}	Z_{mix}:Z_{euf}	DIN	PO_4	SiO_2	Chl a
I	1	–	–	–	–	–	–	–	–	–	–	–	–
T	0.587***	1	–	–	–	–	–	–	–	–	–	–	–
Ws	0.327***	–	1	–	–	–	–	–	–	–	–	–	–
Q	−0.294***	−0.289***	0.135*	1	–	–	–	–	–	–	–	–	–
Rf	−0.551***	−0.418***	–	0.229**	1	–	–	–	–	–	–	–	–
S	0.201**	0.279***	−0.261***	−0.514***	−0.325***	1	–	–	–	–	–	–	–
SPM	–	0.124*	–	–	−0.154**	0.345***	1	–	–	–	–	–	–
K_{par}	–	–	0.196**	–	–	−0.196**	0.620***	1	–	–	–	–	–
Z_{mix}:Z_{euf}	0.655***	–	–	–	–	–	0.554***	0.655***	1	–	–	–	–
DIN	−0.401***	−0.534***	–	0.401***	0.399***	−0.673***	−0.192**	0.228**	–	1		–	–
PO_4	−0.215**	–	−0.425***	−0.291***	–	0.281***	–	−0.171**	–	–	1		–
SiO_2	−0.401***	−0.290***	–	0.330***	0.571***	−0.538***	−0.319***	–	−0.155*	0.610***	0.254**	1	–
Chl a	0.385***	0.515***	–	−0.246***	−0.324***	0.188**	0.198**	–	–	−0.404***	–	−0.351***	1

absent from the phytoplankton community during spring and winter. Chlorophytes reached their maximum in spring.

4. Discussion

Marine ecosystems are sensitive to a variety of physical factors, where hydrodynamics (including stratification), solar energy input and temperature often prevail (Wirtz and Wiltshire, 2005). In estuarine ecosystems, apart from these factors, rainfall is a major external forcing factor influencing the income of nutrients from freshwater origin, the runoff of fertilizers and organic detritus from surrounding agricultural and urban margins, and light penetration through the transport of particulate matter and sediment resuspension. Lehman (1992), among others, stresses the influence of precipitation patterns and stream flow on the density and species composition of estuarine phytoplankton. The study period covered contrasting years in terms of climatic regimes: from very dry years (1999 and 2005) to wet years, 2001, with extreme rainfall over a short period.

The seasonal and interannual variability of phytoplankton biomass in the Tagus estuary was partly explained by external physical factors. In fact, modeling Chl a as a function of air temperature, irradiance and river flow predicted 47% of long-term temporal evolution, estimating a biomass increase in late spring forming a plateau from June to August, and lower values in autumn and winter, in accordance with the observed results. The occurrence of blooms, however, was not predicted by the model. In fact, the short scale temporal variability is strongly dependent on tidal variations, with daily and fortnightly scales, which, by influencing light and nutrient availability play a major role on bloom generation, in a complex and yet unpredictable way. Biomass values attained cannot be considered elevated when compared to the bibliographic references cited by Monbet (1992) or Underwood and Kromkamp (1999). Considering blooms as episodes where Chl a exceeded 10 μg L^{-1} (Sin et al., 1999), we registered blooms on 20 sampling dates, 6 in late spring and the rest in the summer months. Within these dates, the occurrence of blooms was mostly reported in riverine site 1 (80%), and less in site 4 (in only 15% of the dates). Consequently, the Chl a simulation provided by the stepwise regression analysis had a best fit in site 4 and the least in site 1.

During this study, nutrient levels were seldom below half-saturation constants reported for estuarine phytoplankton (Fisher et al., 1988). DIN values were below 2 μM only on four sampling occasions (in June and July 1999), SiO_2 concentrations below 5 μM were registered only twice, and for PO_4 the lower limit of 0.5 was never attained. However, we can discuss the Redfield N:P molar ratios as indicators of potential N or P limitation, in terms of temporal and spatial scales: this ratio was <16, expressing a potential nitrogen deficiency in the majority of the summer months in all sites; at 30% occasions at site 1 and 80% at site 4, evidencing spatial heterogeneity conditions (Fig. 10). The divergence of the Si:N ratio from 1 displays a seasonal pattern. During winter, Si:N <1 was obtained in more than 72% of sampling dates. This was due to the increase of N, rather than the decrease of available Si.

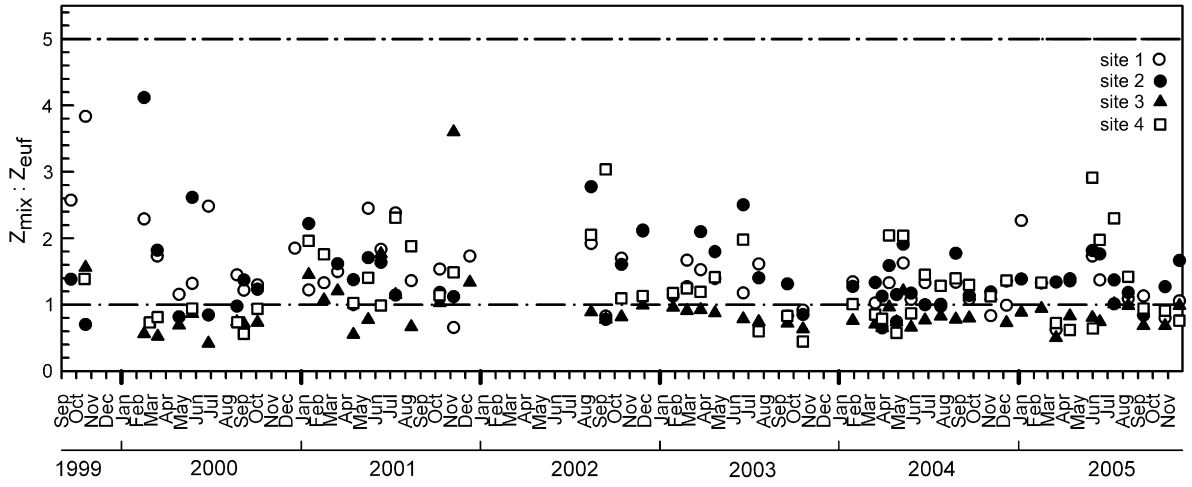

Fig. 4. Critical mixing ratio (Z_{mix}:Z_{euf}) for four sampling sites in the Tagus estuary.

SiO_2 and DIN showed decreasing concentrations down the estuary, as their origin is mainly from the Tagus river, shown by their positive correlation with river discharge and the negative correlation with salinity (Table 5). Unlike NO_3^- and NO_2^-, ammonium was not correlated to river flow, probably because it derives from sediment resuspension. Ammonium concentrations were higher in the shallower sites 1 and 2. Phosphate concentrations show a negative relation with river discharge and a positive correlation with salinity, excluding a freshwater origin (Table 5). In conclusion, nitrogen availability is a possible limiting factor for phytoplankton development after bloom initiation during the summer period, in particular at site 4.

In shallow and turbid estuaries, phytoplankton cells live in a turbulent medium partitioned into an upper euphotic zone (Z_{euf}) that sustains photosynthesis, and a lower aphotic zone that does not (Alpine and Cloern, 1988). The Z_{mix} (mixing depth):Z_{euf} ratio determines the time spent by cells in the light and the possibility of phytoplankton growth. The ‘‘critical mixing depth’’ approach (Sverdrup, 1953) assumes that the

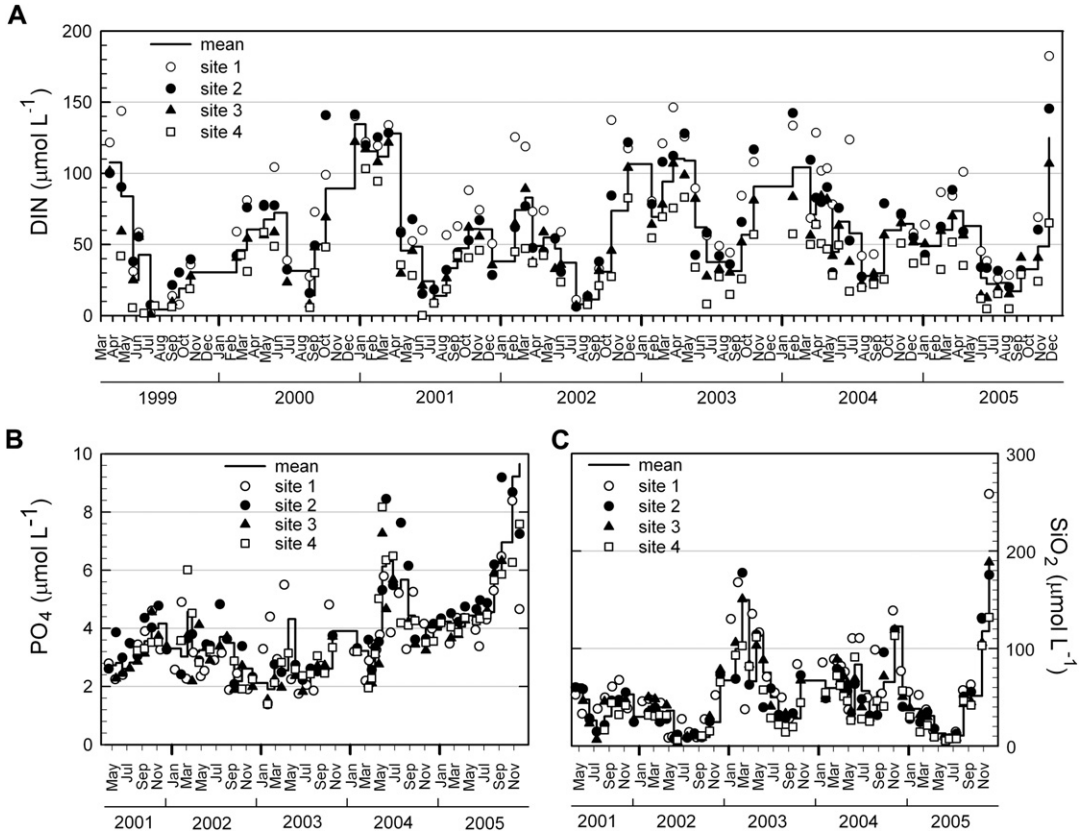

Fig. 5. (A) Dissolved inorganic nitrogen concentrations (DIN, µmol L^{-1}) for sites 1, 2, 3 and 4 from March 1999 to November 2005. (B) Soluble orthophosphate concentration (PO_4, µmol L^{-1}) from May 2001 to November 2005. (C) Silicate concentration (SiO_2, µmol L^{-1}) from May 2001 to November 2005. Line represents the mean concentration for all sampling sites on each date.

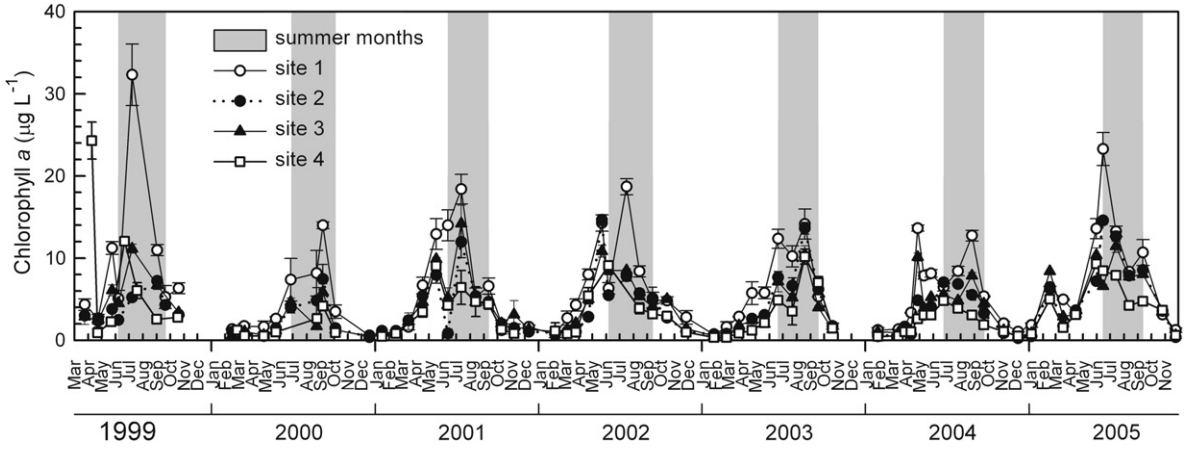

Fig. 6. Spatial and temporal variability of phytoplankton biomass as chlorophyll *a* concentration (μg L^{-1}) in surface water at the four study sites from March 1999 to November 2005. The grey area corresponds to summer months.

phytoplankton population is homogeneously distributed over depth and takes into account light-dependent growth rates as well as light absorption by the water column and has been used to explain the development of spring blooms. Talling (1971) analyzed the effect of mixing in relation to the euphotic depth and derived a maximum Z_{mix}:Z_{euf} ration of 5 in order to allow cell growth. Platt et al. (1991) pointed out that the Sverdrup critical depth criterion is a necessary but not sufficient condition for an initiation of phytoplankton blooms, it only indicates whether net growth is possible. Recently, Huisman et al. (1999) showed that there are two distinct and independent mechanisms for the development of phytoplankton blooms; one would be the classical critical depth theory of Sverdrup (1953) and the other, a critical turbulence mechanism, based on the rate of turbulent mixing, whereby a bloom could develop if turbulent mixing rates are less than a specific critical turbulence. This condition is irrespective of the depth of the water column and demonstrates that the development of blooms in the absence of water column stratification is possible. Such an approach makes sense in shallow estuarine environments, where tidally induced mixing prevents stratification of the water column and relatively small-scale fronts appear, forming and dispersing every tide (Mann and Lazier, 1991). In the Tagus estuary, throughout the year, the depth of the euphotic zone is lower than the mixing zone on most occasions (75%).

In what concerns light as a limiting resource, our results point to the existence of high turbidity levels throughout the year, concomitantly with the high SPM values. Suspended matter originates from freshwater runoff (and hence their maximum value in spring) and from sediment resuspension, which, according to Vale and Sündby (1987) is mostly tidal driven. Yet, to our knowledge, the effect of wind on water mixing and sediment resuspension was not studied in detail. Wind intensity can attain considerable values in all seasons, indirectly affecting the light environment. It should be noted that attenuation coefficient values in summer are elevated, suggesting that in periods where the increased water residence time allows the occurrence of continued cell growth, phytoplankton biomass is mostly controlled by light availability.

Light as the main controlling factor for phytoplankton growth was found by Cabeçadas (1999) for the Tagus estuary, and frequently in other estuarine systems (see Cloern, 1999). Despite the fact that K_{par} values of sites 1 and 2 were higher than sites 3 and 4, the average time spent by phytoplankton cells in the euphotic zone (given by the Z_{mix}:Z_{euf} ratio) is equivalent in all sites. Hence the spatial variability found in Chl *a* concentration and bloom occurrence should only by attributed to nutrient availability and differences in taxonomic composition. Site 1, where the highest biomass and bloom occurrence were found, presented high nutrient concentrations (all data average DIN value 76 μM, SiO_2 63 μM) and a freshwater-type community. On the opposite situation, site 4 displays the lowest nutrients values (all data average DIN value 36 μM, SiO_2 42 μM).

In this study, phytoplankton community analysis was based on photopigment detection. HPLC pigment analysis and CHEMTAX have been used previously as a valuable

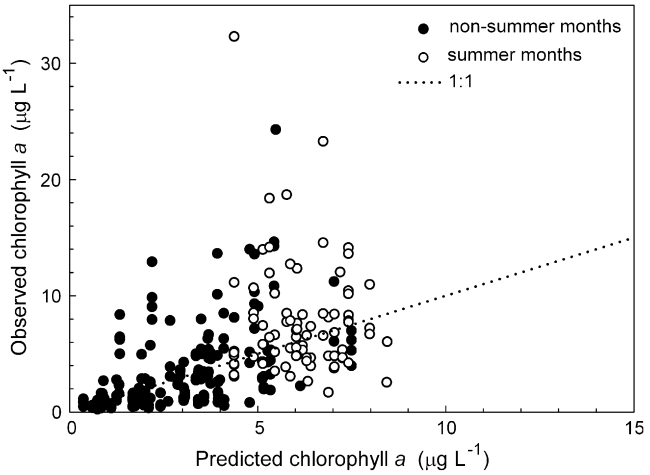

Fig. 7. Relation between observed chlorophyll *a* concentrations in the four sampling sites and those predicted using the multiple regression analysis equation. Non-summer months (●) and summer months (○). The dashed line is the theoretical 1:1 relationship.

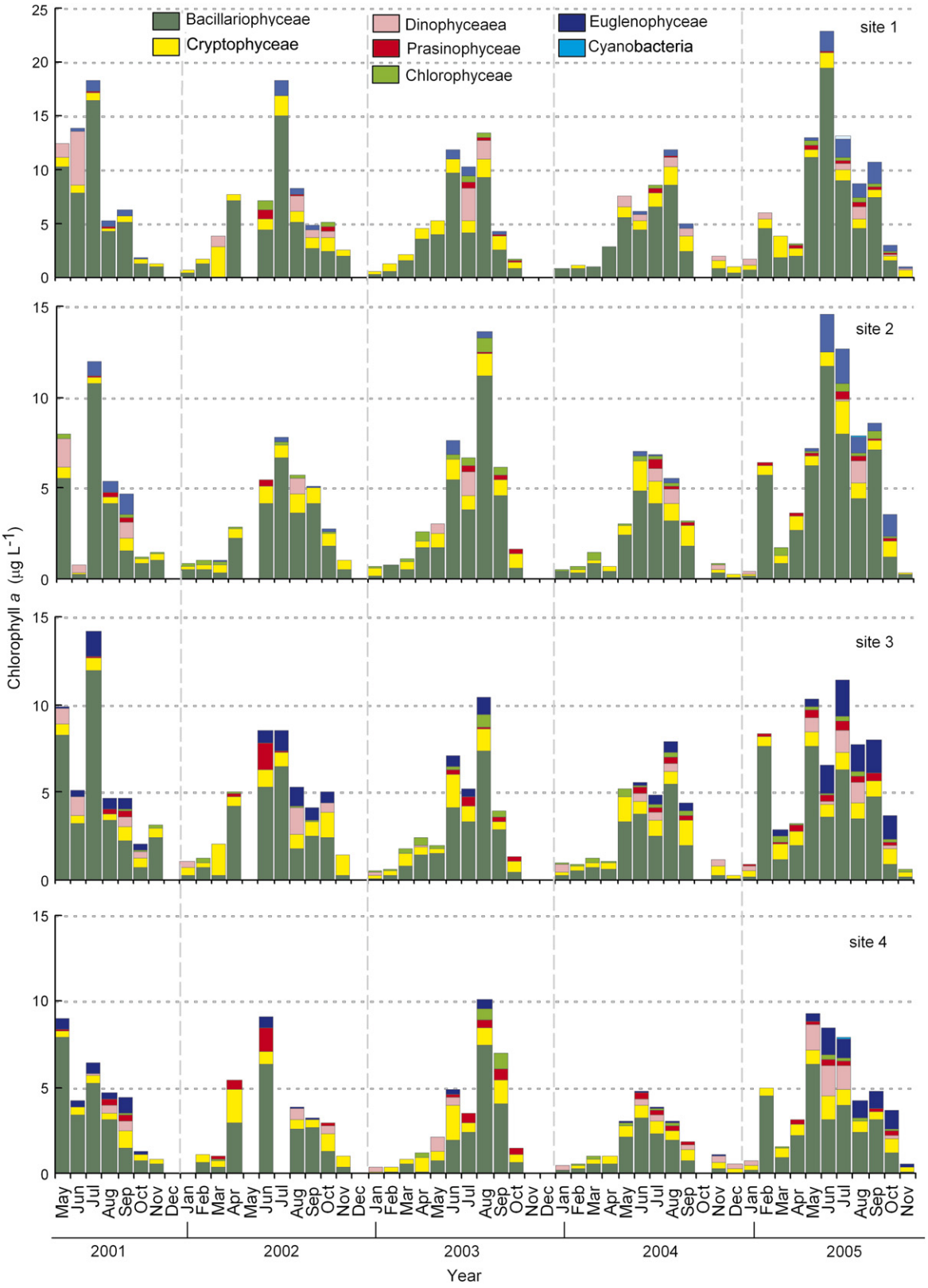

Fig. 8. Estimated contributions of the various phytoplankton groups to the total concentrations of chlorophyll *a* at four sites of the Tagus estuary from May 2001 to November 2005, as determined by interpretation of pigment HPLC data using CHEMTAX.

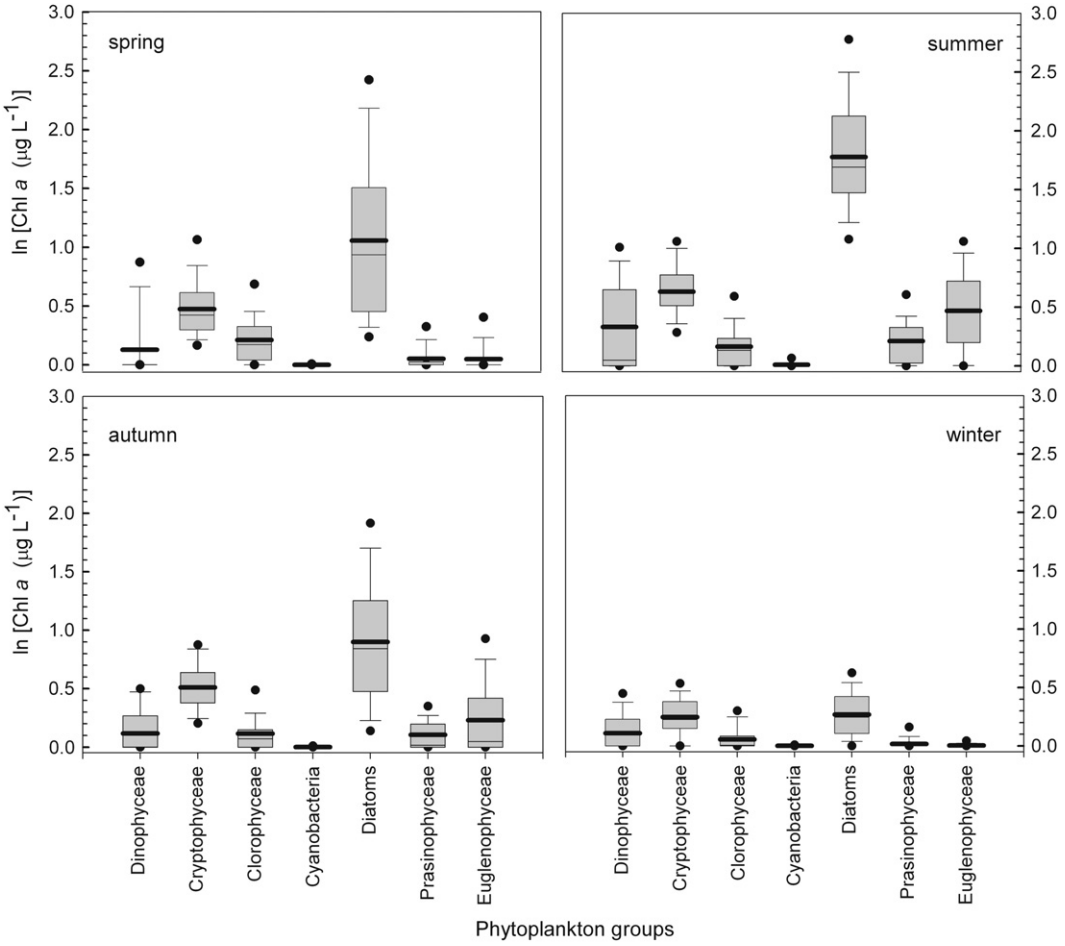

Fig. 9. Box–Whiskers plots of ln(chlorophyll *a* concentration) (µg L^{-1}) from initial samples distributed in the four different seasons and phytoplankton groups as allocated by CHEMTAX. Thick and thin horizontal lines within the box (comprising 25th and 75th percentiles) represent the mean and median, respectively. Error bars indicate 5th and 95th percentiles. Outliers are denoted by filled circles.

monitoring tool in estuaries for determining relationships between environmental variables and the absolute or relative biomasses of major phytoplankton classes as determined by shifts in pigment concentrations (Ansotegui et al., 2001; Paerl et al., 2003; Lewitus et al., 2005). The method has previously been shown to improve assessment of phytoplankton taxonomic composition in US estuaries (Lewitus et al., 2005), detecting phytoplankton taxa generally underestimated or overlooked by standard microscopic techniques (Schlüter et al., 2000; Wright and van den Enden, 2000; Ansotegui et al., 2001).

A crucial step to correctly estimate the contribution of different algal classes to total Chl *a* by CHEMTAX is the selection of the correct accessory pigment:Chl *a* ratios (Henriksen et al., 2002; Rodriguez et al., 2002). Therefore, pigment ratios to be used in CHEMTAX should come from the major phytoplankton species native to the area from which the samples were obtained (Mackey et al., 1996; Lewitus et al., 2005). In this study, we used pigment ratios from cultured species abundant in the Tagus estuary study sites, in the case of diatoms and dinoflagellates, as well as published ratios for other estuarine groups (Schlüter et al., 2000). Pigment ratios remained fairly constant and within the range reported in the literature after application of CHEMTAX (Table 3), indicating that the ratios used can be used to reconstruct the contribution of algal groups in our samples.

Diatoms were the dominant group of phytoplankton found in all sites and throughout the seasonal cycle and were responsible for the observed blooms (see Figs. 8 and 9). It has been shown previously that diatoms are the predominant phytoplankton group in estuaries (Lemaire et al., 2002; Gameiro et al., 2004). The three most frequent diatom species found by Gameiro et al. (2004) in the Tagus estuary were: *Detonula pumila*, *Skeletonema costatum* and *Thalassiosira minima*.

Dinoflagellates, euglenophytes and prasinophytes had a higher contribution to the phytoplankton community during summer, when vertical turbulence was low and water residence time higher. According to Huisman et al. (1999), a decrease of turbulent mixing processes allows the phytoplankton growth, a mechanism more important for buoyant species. This type of species aggregates more in the upper water column, having an advantage over sinking species. The upper three classes fit into the buoyant morphological category and their abundance increase during summer is consistent with

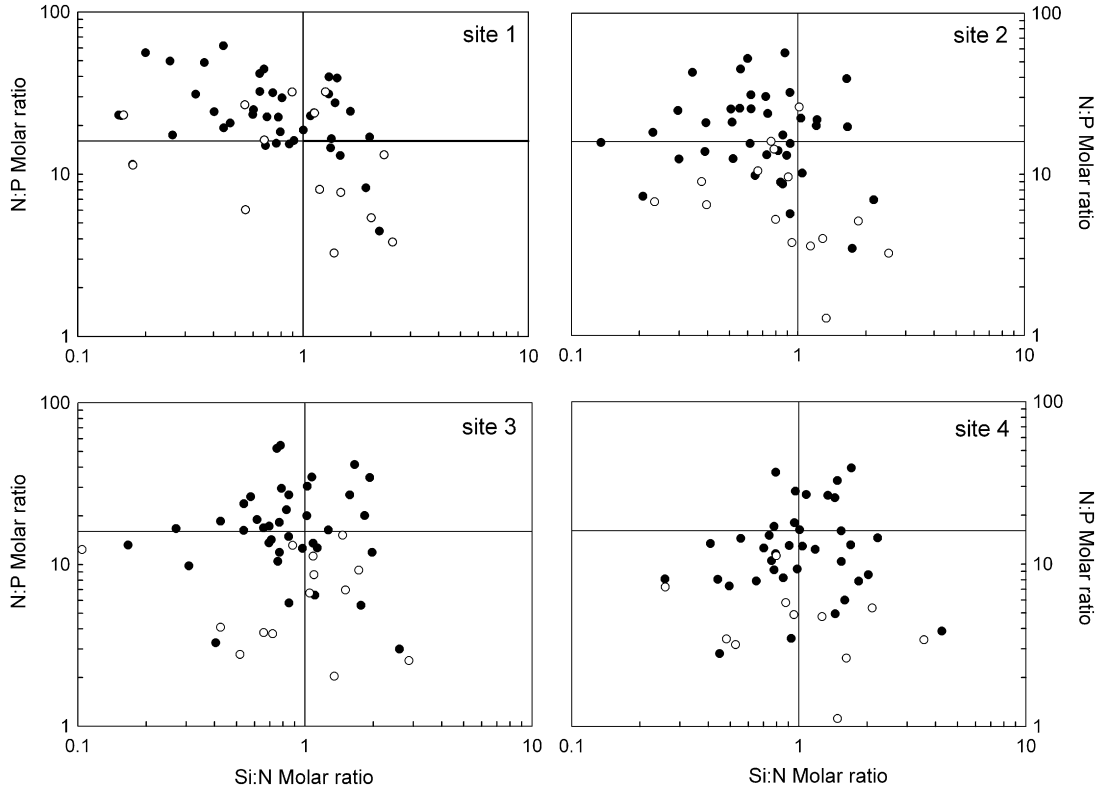

Fig. 10. N:P:Si molar ratios in the water column of the Tagus estuary from May 2001 to November 2005. Values of molar quotients between the concentrations of potentially limiting nutrients are delimited in logarithmic axes plots (log Si:N *vs* log N:P) for the four sampling sites; during non-summer months (●) and summer months (○). Horizontal (N:P = 16) and vertical (Si:N = 1) lines define four different areas within the plot, each one characterized by the potentially limiting nutrients (Redfield et al., 1963).

Huisman et al. (1999). Chlorophyceae had a higher contribution in the riverine site 1 and in spring and winter, clearly influenced by conditions of high freshwater input, low salinity and high DIN concentrations. Diatoms are more abundant in upper sites 1 and 2, while dinoflagellates and cryptophytes increase towards downwards sites 3 and 4. The river discharge was important in determining the temporal and spatial phytoplankton composition variability in the Tagus estuary, as found in Chesapeake Bay (Marshall and Alden, 1990).

The interannual comparison of average summer values gives higher Chl *a* concentrations for 1999 and 2005, the years when drought was more intense, and hence water residence time and water stability higher. Comparing the monthly concentrations of nutrients and Chl *a*, registered in this 7-year period with values of the same parameters obtained in 1980/1981, as well as the composition of phytoplankton community, we can state that no eutrophication trend is present. This is agreement with Cabeçadas et al. (2000) who state that coastal waters of southern Portugal are in relatively healthy condition, as a result partly of the hydrological and biological characteristics of the system and also as a consequence of removal of industrial complexes from this area, the installation of several waste treatment stations, and the creation of protected areas. de Jonge et al. (2002) consider flushing time, turbidity and nutrient input as the factors needed to predict which type of coastal ecosystem is sensitive to eutrophication; the results obtained for the Tagus estuary fit into the general classification scheme of lower eutrophication risk estuaries.

Acknowledgements

This work was funded by ValorSul SA and FCT by means of project INTAGUS (PDCT/MAR/58022/2004). C. Gameiro was funded by a PhD grant from FCT (POCI-2010/BD/13988/2003). We thank M.D. Mackey for a copy of the CHEMTAX software and to Dr Juan Zwolinski for help with the statistical analysis.

References

Alpine, A.E., Cloern, J.E., 1988. Phytoplankton growth rates in a light-limited environment, San Francisco Bay. Marine Ecology Progress Series 44, 167–173.

Ansotegui, A., Trigueros, J.M., Orive, E., 2001. The use of pigment signatures to assess phytoplankton assemblage structure in estuarine waters. Estuarine, Coastal and Shelf Science 52, 689–703.

Bendschneider, K., Robison, N.J., 1952. A new spectrophotometric determination of nitrite in seawater. Journal of Marine Research 11, 87–96.

Brotas, V., Plante-Cuny, M.R., 1996. Identification et quantification des pigments chlorophylliens et caroténoïdes des sédiments marins: un protocole d'analyse par HPLC. Oceanologica Acta 19, 623–634.

Cabeçadas, G., Brogueira, M.J., Cabeçadas, L., 2000. Southern Portugal: the Tagus and Sado estuaries. In: Sheppard, C.R.C. (Ed.), Seas at the Millennium: an Environmental Evaluation. Elsevier Science Ltd, Oxford, UK, pp. 151–165.

Cabeçadas, L., 1999. Phytoplankton production in Tagus estuary (Portugal). Oceanologica Acta 22, 205–213.

Cloern, J.E., 1996. Phytoplankton bloom dynamics in coastal ecosystems: a review with some general lessons from sustained investigation of San Francisco Bay, California. Reviews of Geophysics 34, 127–168.

Cloern, J.E., 1999. The relative importance of light and nutrient limitation of phytoplankton growth: a simple index of coastal ecosystem sensitivity to nutrient enrichment. Aquatic Ecology 33, 3–16.

Cole, B.E., Cloern, J.E., 1984. Significance of biomass and light availability to phytoplankton productivity in San Francisco Bay. Marine Ecology Progress Series 17, 15–24.

Costa, M.J., 1999. O Estuário do Tejo. Editora Cotovia Lda, Lisboa, 197 pp.

Doblin, M., Blackburn, S., Hallegraeff, G., 1999. Comparative study of selenium requirements of three phytoplankton species: *Gymnodinium catenatum*, *Alexandrium minutum* (Dinophyta) and *Chaetocerus* cf. *tenuissimus* (Bacillariophyta). Journal of Plankton Research 21, 1153–1169.

Everitt, D.A., Wright, S.W., Volkman, J.K., Thomas, D.P., Lindstrom, E., 1990. Phytoplankton community compositions in the western equatorial Pacific determined from chlorophyll and carotenoid pigment distribution. Deep Sea Research 37, 975–997.

Fanning, K.A., Pilson, M.E.Q., 1973. On the spectrophotometric determination of dissolved silica in natural waters. Analytical Chemistry 45, 136–141.

Fisher Jr., T.R., Harding, L.W., Stanley, D.W., Ward, L.G., 1988. Phytoplankton, nutrients, and turbidity in the Chesapeake, Delaware, and Hudson estuaries. Estuarine, Coastal and Shelf Science 27, 61–93.

Gameiro, C., Cartaxana, P., Cabrita, T., Brotas, V., 2004. Variability in chlorophyll and phytoplankton composition in an estuarine system. Hydrobiologia 525, 113–124.

Gieskes, W.W.C., Kraay, G.W., Nontji, A., Setiapermana, D., Sutomo, D., 1988. Monsoonal alternation of a mixed and a layered structure in the phytoplankton of the euphotic zone of the Banda Sea (Indonesia): a mathematical analysis of algal pigment fingerprints. Netherlands Journal of Sea Research 22, 123–137.

Grasshoff, K., 1976. Methods of Seawater Analysis. Verlag Chimie, New York, 520 pp.

Guillard, R.R.L., Ryther, J.H., 1962. Studies of marine planktonic diatoms. I. *Cyclotella nana* Hustedt and *Detonula confervacea* Cleve. Canadian Journal of Microbiology 8, 229–239.

Henriksen, P., Riemann, B., Kaas, H., Sørensen, H.M., Sørensen, H.L., 2002. Effects of nutrient-limitation and irradiance on marine phytoplankton pigments. Journal of Plankton Research 24, 835–858.

Huisman, J., van Oostveen, P., Weissing, F.J., 1999. Critical depth and critical turbulence: two different mechanisms for development of phytoplankton blooms. Limnology and Oceanography 44, 1781–1787.

de Jonge, V.N., Elliot, M., Orive, E., 2002. Causes, historical development, effects and future challenges of a common environmental problem: eutrophication. Hydrobiologia 475/476, 1–19.

Koroleff, F., 1969/1970. Direct determination of ammonia in natural waters as indophenol blue. International Council for Exploration of the Sea (ICES) Comm. Meet. Pap. 1969/C: 9, Interlab. Report 3, pp. 19–22.

Kraay, G.W., Zapata, M., Veldhuis, M.J.W., 1992. Separation of chlorophylls c_1, c_2, and c_3 of marine phytoplankton by reversed-phase-C18-high-performance liquid chromatography. Journal of Phycology 28, 708–712.

Lehman, P.W., 1992. Environmental factors associated with long-term changes in chlorophyll concentration in the Sacramento-San Joaquin delta and Suisun bay, California. Estuaries 15, 335–348.

Lemaire, E., Abril, G., De Wit, R., Etcheber, H., 2002. Distribution of phytoplankton pigments in nine European estuaries and implications for an estuarine typology. Biogeochemistry 59, 5–23.

Letelier, R.M., Bidigare, R.R., Hebel, D.V., Ondrusek, M., Winn, C.D., Carl, D.M., 1993. Temporal variability of phytoplankton community structure based on pigment analysis. Limnology and Oceanography 38, 1420–1437.

Lewitus, A.J., White, D.L., Tymowski, R.G., Geesey, M.E., Hymel, S.N., Noble, P.A., 2005. Adapting the CHEMTAX method for assessing phytoplankton taxonomic composition in Southeastern U.S. estuaries. Estuaries 28, 160–172.

Lorenzen, C.J., 1967. Determination of chlorophyll and phaeopigments: spectrophotometric equations. Limnology and Oceanography 12, 343–346.

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. Marine Ecology Progress Series 144, 165–283.

Mackey, D.J., Higgins, H.W., Mackey, M.D., Holdsworth, D., 1998. Algal class abundances in the western equatorial Pacific: estimation from HPLC measurements of chloroplast pigments using CHEMTAX. Deep Sea Research I 45, 1441–1468.

Mann, K.H., Lazier, J.R.N., 1991. Dynamics of Marine Ecosystems. Blackwell Scientific Publications, Boston, MA, 466 pp.

Marshall, H.G., Alden, R.W., 1990. A comparison of phytoplankton assemblages and environmental relationships in three estuarine rivers of the lower Chesapeake bay. Estuaries 13, 287–300.

Monbet, Y., 1992. Control of phytoplankton biomass in estuaries: a comparative analysis of microtidal and macrotidal estuaries. Estuaries 15, 563–571.

Murphy, J., Riley, J.P., 1962. A modified single solution method for the determination of phosphate in natural waters. Analytica Chimica Acta 27, 31–36.

Paerl, H.W., Valdes, L.M., Pinckney, J.L., Piehler, M.F., Dyble, J., Moisander, P.H., 2003. Phytoplankton photopigments as indicators of estuarine and coastal eutrophitcation. BioScience 53, 953–964.

Platt, T., Bird, D.F., Sathyendranath, S., 1991. Critical depth and marine primary production. Proceedings: Biological Sciences 246, 205–217.

Pinckney, J.L., Paerl, H.W., Harrington, M.B., Howe, K.E., 1998. Annual cycles of phytoplankton community-structure and bloom dynamics in the Neuse River Estuary, North Carolina. Marine Biology 131, 371–381.

Redfield, A.C., Ketchum, B.H., Richards, F.A., 1963. The influence of organisms in the composition of seawater. In: Hill, M.N. (Ed.), The Sea, vol. II. Wiley, New York, pp. 26–77.

Rodriguez, F., Varela, M., Zapata, M., 2002. Phytoplankton assemblages in the Gerlacje and Bransfield Straits (Antarctic Peninsula) determined by light microscopy and CHEMTAX analysis of HPLC pigment data. Deep Sea Research II 49, 723–747.

Schlüter, L., Møhlenberg, F., Havskum, H., Larsen, S., 2000. The use of phytoplankton pigments for identifying and quantifying phytoplankton groups in coastal areas: testing the influence of light and nutrients on pigment/chlorophyll a ratios. Marine Ecology Progress Series 192, 49–63.

Sin, Y., Wetzel, R.L., Anderson, I.C., 1999. Spatial and temporal characteristics of nutrient and phytoplankton dynamics in the York River estuary, Virginia: Analysis of long-term data. Estuaries 22, 260–275.

Sverdrup, H.W., 1953. On conditions for the vernal blooming of phytoplankton. Journal du Conseil pour l'Exploration de la Mer 29, 130–135.

Talling, J.E., 1971. The underwater light climate as a controlling factor in the production ecology of freshwater phytoplankton. Mitteilungen Internationale Vereiningung fuer Theoretische und Angewandte Limnologie 19, 214–243.

Trigo, R.M., Pozo-Vázquez, D., Osborn, T.J., Castro-Díez, Y., Gámiz-Fortis, S., Esteban-Parra, M.J., 2004. North Atlantic oscillation influence on precipitation, river flow and water resources in the Iberian Peninsula. International Journal of Climatology 24, 925–944.

Underwood, G.J.C., Kromkamp, J., 1999. Primary production by phytoplankton and microphytobenthos in estuaries. Advances in Ecological Research 29, 93–153.

Vale, C., Sündby, B., 1987. Suspended sediment fluctuations in the Tagus estuary on semi-diurnal and fortnightly time scales. Estuarine, Coastal and Shelf Science 25, 495–508.

Wirtz, K.W., Wiltshire, K., 2005. Long-term shifts in marine ecosystem functioning detected by inverse modeling of the Helgoland Roads time-series. Journal of Marine Systems 56, 262–282.

Wright, S.W., van den Enden, R.L., 2000. Phytoplankton community structure and stocks in the East Antarctic marginal ice zone (BROKE survey, January–March 1996) determined by CHEMTAX analysis of HPLC pigment signatures. Deep Sea Research II 47, 2363–2400.

Wright, S.W., Thomas, D.P., Marchant, H.J., Higgins, H.W., Mackey, M.D., Mackey, D.J., 1996. Analysis of phytoplankton of the Australian sector of the Southern Ocean: comparisons of microscopy and size frequency data with interpretations of pigment HPLC data using CHEMTAX matrix factorization program. Marine Ecology Progress Series 144, 285–298.