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Environmental Forcing of Phytoplankton in a Mediterranean Estuary (Guadiana Estuary, South-western Iberia): A Decadal Study of Anthropogenic and Climatic Influences

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Abstract Phytoplankton seasonal and interannual variability in the Guadiana upper estuary was analyzed during 1996–2005, a period that encompassed a climatic controlled reduction in river flow that was superimposed on the construction of a dam. Phytoplankton seasonal patterns revealed an alternation between a persistent light limitation and episodic nutrient limitation. Phytoplankton succession, with early spring diatom blooms and summer–early fall cyanobacterial blooms, was apparently driven by changes in nutrients, water temperature, and turbulence, clearly demonstrating the role of river flow and climate variability. Light intensity in the mixed layer was a prevalent driver of phytoplankton interannual variability, and the increased turbidity caused by the Alqueva dam construction was linked to pronounced decreases in chlorophyll *a* concentration, particularly at the start and end of the phytoplankton growing period. Decreases in annual maximum and average abundances of diatoms, green algae, and cyanobacteria were also detected. Furthermore, chlorophyll *a* decreases after dam filling and a decrease in turbidity may point to a shift from light limitation towards a more nutrient-limited mode in the near future.

Keywords Phytoplankton · Estuary · Light · Nutrients · Seasonal variation · Interannual variation · Dam

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Introduction

Phytoplankton are the dominant primary producers in most aquatic ecosystems. They have significant impacts on water quality and are key players in global biogeochemical processes (Cloern and Dufford 2005). Phytoplankton are also highly sensitive to environmental perturbations and thus they are used in the assessment of ecological conditions (Hays et al. 2005; Paerl et al. 2006). The distribution patterns of phytoplankton reflect the interplay between their growth rates, commonly regulated by light, inorganic nutrients, and turbulence, and their loss rates, controlled by grazing, viral lyses, programmed cell death, sinking, and advection (Bidle and Falkowski 2004; Cloern and Dufford 2005). Thus, both phytoplankton growth and loss rates can potentially be driven by natural environmental changes, such as climate change occurring at global and local scales, and by human-induced alterations. In estuaries, both types of environmental changes are relevant (Harley et al. 2006; Lotze et al. 2006).

Climatic change is considered to be an important driver of phytoplankton variability, namely seasonality, through its influence on water column stratification and surface radiation. In estuaries, climatic forcing is additionally linked to freshwater inflow that can further influence other environmental properties relevant to phytoplankton (e.g., turbidity, nutrient concentrations, water residence times, see Iriarte and Purdie 2004). Short-term episodic climatic perturbations, such as heat-waves or floods, can also lead to abrupt phytoplankton changes (Cloern et al. 2005; Wetz and Paerl 2008). Recent studies have shown that long-term climate variability may induce alterations in the magnitude and position of phytoplankton blooms (McQuatters-Gollop et al. 2007; Borkman et al. 2009), and changes in their composition and phenology (Leterme et al. 2005; Wiltshire et al. 2008), that may be propagated to higher trophic levels (Edwards and Richardson 2004; Nixon et al. 2009).

In estuaries, climatic forcing is tightly linked to phytoplankton interannual variability, but phytoplankton responses to climate change can be attributed to multiple mechanisms: (1) direct changes in nutrient concentrations (Rudek et al. 1991; Mallin et al. 1993; Harding 1994), light (Mallin et al. 1999; Howarth et al. 2000; Iriarte and Purdie 2004), or water residence time (Sin et al. 1999; Paerl et al. 2006; Valdes-Weaver et al. 2006), associated to river flow variability; (2) indirect changes in top predators associated to river flow variability (Reaugh et al. 2007); (3) direct and indirect effects of temperature alterations on phytoplankton (Thompson et al. 2008; Villate et al. 2008) and their grazers (Li and Smayda 1998; Oviatt 2004); and (4) changes in the advection of phytoplankton (Glé et al. 2007), or grazers (Cloern et al. 2007) from adjacent oceanic water masses. Therefore, the degree to which climate change will influence phytoplankton dynamics in estuaries will not only depend on their geomorphological and hydrological characteristics, but also on phytoplankton composition and taxa-specific attributes and controls (Paerl et al. 2006; Smetacek and Cloern 2008).

In addition to changes in climate forcing, coastal ecosystems have also been subjected to concomitant anthropogenically induced alterations (Lotze et al. 2006). Indeed, the general increase in population densities led to increased nutrient loads and shifts towards a lower relative availability of silicate. These changes, sometimes aggravated by artificial hydraulic retention by dams (Humborg et al. 1997, 2008), led to enhanced phytoplankton productivity and biomass, increased occurrence of noxious algal blooms, and a relative decrease in diatoms in favor of flagellated taxa (Cadée 1992; Cloern 2001; Danielsson et al. 2008). Other anthropogenic perturbations that affect phytoplankton variability include dredging (Facca et al. 2002), hydraulic manipulations (Wetsteyn and Kromkamp 1994; Filipsson et al. 2005; Petersen et al. 2008), species introductions (Alpine and Cloern 1992; Caraco et al. 1997; Kideys et al. 2008), inputs of toxic contaminants (Yoshiyama and Sharp 2006), and overfishing (Cadée and Hegeman 2002).

Overall, multiple natural and anthropogenic environmental changes, operating on different phytoplankton properties, are superimposed and interact in every ecosystem, making the identification of main drivers extremely difficult (Livingston 2007; Kideys et al. 2008). Information on long-term trends in phytoplankton is crucial to separate anthropogenic influences from natural variability and to identify the driving forces underlying phytoplankton variability, thus enabling a higher predictive ability and a more rationale management of aquatic ecosystems (Paerl 2006; Smetacek and Cloern 2008; Borkman et al. 2009).

The present study focuses on phytoplankton variability in the Guadiana estuary, south-western Iberia (see Fig. 1), located in a very vulnerable area to climate change (IPCC 2007). Phytoplankton in the Guadiana estuary has been extensively studied due to an anticipated increase in cyanobacterial blooms caused by the construction of the Alqueva dam (Rocha et al. 2002). However, most studies have evaluated phytoplankton variability during only 1 to 2-year periods and specifically addressed relationships between phytoplankton and inorganic nutrients (Rocha et al. 2002; Sobrino et al. 2004; Domingues et al. 2005; Chícharo et al. 2006; Domingues et al. 2007; Domingues and Galvão 2007; Galvão et al. 2008; Morais et al. 2009). The aims of this study were: (1) to describe phytoplankton seasonal and interannual variability in the Guadiana upper estuary over a 10-year period (1996–2005), a period that encompassed the construction of the Alqueva dam superimposed on a high interannual river flow variability and (2) to understand the driving forces underlying phytoplankton variability, and discern the relative importance of climatic and anthropogenic influences.

Materials and Methods

Study Location

The Guadiana River is located in the south-western Iberian Peninsula, Portugal (Fig. 1). It has a drainage basin of 67,840 km^2 and is subjected to a Mediterranean climate with humid moderate winters and hot dry summers. The Guadiana estuary is a mesotidal system (tidal amplitude, 1.3–3.5 m), with a length of 70 km, a maximum width of ca. 550 m, and an area of 22 km^2. It has an average depth of 6.5 m, and an average water residence time of 12 days (Domingues and Galvão 2007; Vasconcelos et al. 2007). The maximum upstream extent of saltwater intrusion is usually located close to Alcoutim (ca. 38 km from the river mouth), whereas the tidal influence usually extends to Mértola (ca. 70 km from the river mouth; see Fig. 1). The lower estuary ranges from partially stratified to well-mixed, whereas the upper estuary is well-mixed under all conditions (Rocha et al. 2002; Cravo et al. 2006; Oliveira et al. 2006; Morais et al. 2009).

The freshwater inputs to the estuarine zone exhibit pronounced intra- and interannual variability depending on global climate patterns, such as the North Atlantic Oscillation (NAO) and rainfall, and water retention in dams (Trigo et al. 2004). The freshwater flow has been mainly constrained by numerous dams which regulated ca. 75% of the freshwater input into the estuary. The recent construc-

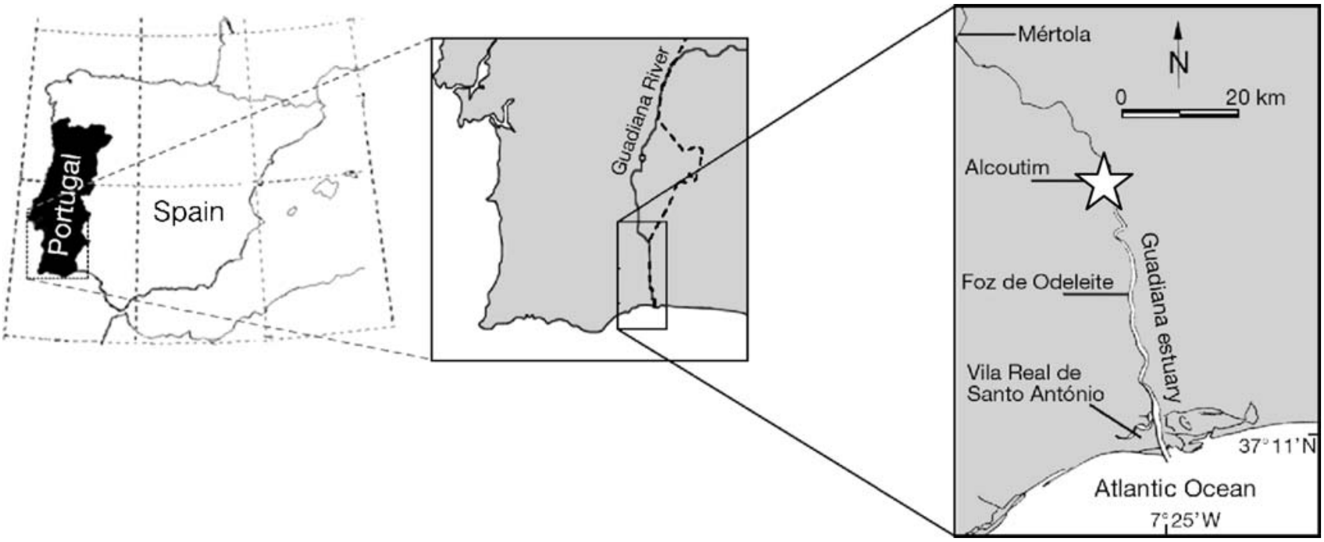

Fig. 1 Location of the Guadiana estuary and sampling station (Alcoutim, see *star*)

tion of the Alqueva dam (building, 1999–2001; floodgates closure and filling, 2002–2003), an infrastructure located 150 km from the Guadiana River mouth, further increased freshwater retention up to 81% (Morais et al. 2009). Climate models predict a reduction in precipitation and river flow, as well as a substantial warming, and an increased frequency of extreme heat-waves and droughts during the years 2070–2100 for this region (Miranda et al. 2002; Cunha et al. 2002). The main activities in the Guadiana catchment area are agriculture and agro-industry, but the main anthropogenic pressures are associated to river damming; indeed, the Guadiana is currently considered one of the best preserved and most vulnerable estuaries of the Iberian Peninsula (Vasconcelos et al. 2007; for details see Morais 2008).

Sampling Strategy

Data reported in this study were compiled from two databases, associated with different monitoring and scientific programs: (1) our database, including data collected during 1996–2005, previously published (Rocha et al. 2002; Caetano et al. 2001; Sobrino et al. 2004; Domingues et al. 2005, 2007; Domingues and Galvão 2007) and (2) the public database from the Portuguese National Water Institute, INAG (http://snirh.pt/). Occasionally, data acquired before 1996 (INAG) and after 2005 (INAG; Domingues, unpublished data) were also utilized.

From these databases, we selected the station Alcoutim, located in the Guadiana upper estuary, because it was the estuarine location sampled more frequently. This station is located at ca. 38 km from the river mouth and has a mean depth of 9.4 m (Fig 1). During the period 1996–2005, samples were usually collected from the Alcoutim pier, from subsurface levels (approximately 0.5 m) using a sampling bottle. During 1997, some samples were collected from a small research vessel (Rocha et al. 2002). Since neither thermal nor haline vertical stratification was detected in the upper estuary, independent of season and tidal cycle (Rocha et al. 2002; Morais et al. 2009), it was assumed that the entire water column was well mixed, and that subsurface samples were representative of the whole water column. Most water samples were collected close to high tide during neap tides. However, sampling under different tidal conditions may have introduced a possible source of bias. The sampling effort was not evenly distributed throughout each year and it was strongly concentrated in the spring–summer productive period.

Sample Analyses

Physical–chemical Variables

Subsurface water temperature and salinity were determined in situ using a field CT meter (WTW dissolved oxygen, temperature meter Oxi 197 connected to a WTW TA 197 sensor; YSI-30 and WTW LF196) and an Atago S/Mill refractometer. Water samples were kept in cold and dark conditions (approximately 2 h) until analysis. Concentration of suspended particulate matter (SPM) was determined gravimetrically using pre-weighed glass fiber Whatman GF/F filters (pore diameter=0.7 μm). Light penetration in the water column was determined using a Secchi disk (D_S, m), and the light extinction coefficient (k_e, m^{-1}) was calculated as $1.4/D_S$, according to an empirical model for turbid waters (Holmes 1970). When Secchi depth values were not available (1999–2000), SPM was used as an independent variable to estimate k_e in a linear regression model

$(y = ax + b)$. This model, based on discrete data collected at two upper estuarine locations (Alcoutim and Mértola, see Fig. 1) during 1996–2008, explained 55.4% of the variability in k_e ($a = 0.0691 \pm 0.0058$; $b = 0.7778 \pm 0.1361$; n=117; p<0.000001). The euphotic zone depth (Z_{eu}, m) was calculated as $4.61/k_e$, assuming that irradiance at the bottom of Z_{eu} was 1% of the surface irradiance. Total daily radiation (W m^{-2}; see other data sources) was used to estimate photosynthetically active radiation (PAR) at the surface (I_o; Baker and Frouin 1987). I_o values were divided by the length of the light period (9.5–14.5 h) and subsequently converted using 4.587 µmol photons s^{-1} W^{-1} (Morel and Smith 1974). Mean light intensity in the mixed layer (I_m, µmol photons m^{-2} s^{-1}), which corresponds to the water column depth at Alcoutim (9.4 m), was calculated according to the following equation (Jumars 1993):

$$I_m = I_o\left(1 - e^{(-k_e.Z_m)}\right)(k_e.Z_m)^{-1}$$

where I_o represents light intensity at the surface, k_e is light extinction coefficient (m^{-1}), and Z_m is the depth of the mixed layer (m).

Dissolved inorganic macronutrient (nitrate NO_3^-, nitrite NO_2^-, ammonium NH_4^+, silicate SiO_4^{4-}, and orthophosphate PO_4^{3-}) concentrations were determined in triplicate according to spectrophotometric methods (Grasshoff et al. 1983), after being filtered through cellulose acetate filters (Whatman, nominal pore diameter=0.45 µm), and fixed with mercuric chloride (Merck) to a final concentration of 20 mg L^{-1} (Kirkwood 1992). Samples were kept chilled (4°C) until analysis (see Rocha et al. 2002 and Domingues et al. 2005 for details). Since NO_3^- was the dominant nitrogen compound for the dissolved inorganic nitrogen pool ($DIN = NO_3^- + NH_4^+ + NO_2^-$), representing on average 76±2% (n=122) of the DIN in the upper estuary, NO_3^- was used as a proxy for DIN at Alcoutim when NH_4^+ data were missing.

Phytoplankton

Chlorophyll *a* (Chl *a*), used as a proxy for phytoplankton biomass (but see Domingues et al. 2008), was determined in duplicate aliquots, after pigment extraction with acetone, following the spectrophotometric method (Parsons et al. 1984). Phytoplankton composition and abundance were analyzed using inverted (Utermöhl 1958) and epifluorescence microscopy (Haas 1982). Samples for enumeration of microplankton were preserved with Lugol's iodine solution, settled in sedimentation chambers and observed at ×400 magnification with a Zeiss Axiovert S100 inverted microscope with phase contrast. Samples for enumeration of nano- and picoplankton were preserved with glutaraldehyde (final concentration 2% *v*/*v*), stained with proflavine and filtered (<100 mm Hg) onto black polycarbonate filters (pore diameter 0.45 µm). Slide preparations were made within 24 h of sampling, using non-fluorescent immersion oil (Cargille type A), and then frozen (−20°C) in dark conditions. Slide observation was performed at ×1,250 magnification, with a Leica DM LB epifluorescence microscope. A minimum of 50 random visual fields, at least 400 cells in total and 50 cells of the most common genus were counted. Assuming that cells were randomly distributed, the counting precision was ±10% (Venrick 1978). The phytoplankton assemblage was divided into four groups: diatoms (Bacillariophyceae), green algae (Chlorophyceae), cyanobacteria, and plastidic flagellates.

Other Data Sources

The North Atlantic Oscillation winter index, based on the difference of normalized sea level pressure between Lisbon, Portugal, and Stykkisholmur/Reykjavick, Iceland, from December through March, was obtained from the Climate Analysis Section, National Center for Atmospheric Research at http://www.cgd.ucar.edu/~jhurrell/indices.html (Hurrell 1995). Monthly NAO indices, based on the difference of normalized sea level pressure between Ponta Delgada (Azores), Portugal, and Akureyri, Iceland, were obtained from the Polar Meteorology Group, Ohio State University at http://polarmet.mps.ohio-state.edu/NAO/ (Rogers 1984).

Guadiana River flow data, measured at Pulo do Lobo hydrometric station (station reference, 27L/01H; 37.803° N, 7.633° W), located ca. 85 km from the river mouth, was obtained from the INAG public database (http://snirh.pt/). Approximately 90% of the freshwater flow entering the estuary flows through this location. Daily precipitation data (rainfall) recorded at Alcoutim meteorological station (station reference, 27M/01UG, 37.465° N, 7.472° W), was obtained from the same public database. Meteorological and hydrological data were integrated over annual (January–December) and over hydrological or water years (1 October year (t-1) to 30 September of year t). For the period 2001–2005, total daily radiation (W m^{-2}) measured at São Brás de Alportel meteorological station (station reference, 31J/01C; 37.163° N, 7.896° W), located approximately 50 km west of Alcoutim, was also obtained from this database. For the period 1996–2000, total daily radiation (W m^{-2}) measured at the Faro meteorological station (ca. 25 km south of São Brás de Alportel), was acquired from the Portuguese Meteorological Institute.

Furthermore, data on chlorophyll *a* concentration (n=67), nitrate concentration (n=109), water temperature (n=100), and suspended particulate matter (n=110), measured at Alcoutim during 1996–2005, were also obtained from the INAG database (station reference, 29M/02). The incorporation of these data into this study allowed a more accurate analysis of temporal variability, particularly in the winter period. The variables referred (water temperature, NO_3^-, SPM, and Chl *a*) were analyzed using standard methods and for similar time periods (month/year), INAG's median values for these variables were not statistically different from our own median values (Mann–Whitney test, $p<0.05$).

Data Analyses

Basic statistics (mean; median; standard deviation; standard error, SE), and regression analyses were performed using Statistica 6.0® software package. Variables used in regression models were $\log_{10}$ transformed to confirm data normality and variance homogeneity. The strength of associations between variables, based on discrete data, was assessed using Spearman rank correlation coefficients (r). Climatological and hydrological variables used in regression and correlation analysis (e.g., river flow, rainfall, light intensity at the surface) were averaged over an 8-day period prior to sampling. All statistical analyses was considered at an $\alpha=0.05$ level.

In order to facilitate the analyses of temporal variability and to reduce the impact of unevenly distributed sampling, sampling during different tidal conditions, and extreme observations for each year, all discrete data of each variable were binned and averaged into monthly means. Subsequently, an annual mean was estimated using all 12 monthly means. Differences in annual mean values between particular periods were assessed using a Mann–Whitney rank sum test. To facilitate the comparison of phytoplankton seasonal patterns among years, the monthly deviations from the annual mean, normalized by the standard deviation of each annual mean were calculated. To analyze interannual variability and long-term trends, the time series was deseasonalized, i.e., the seasonal components were removed. First, for each variable, the average of each month was calculated (i.e. 1–12) for the whole study period (1996–2005) using monthly averages of each year. We then calculated the monthly anomaly as the difference between observed monthly mean values at a given year, and the monthly average for the time series. Subsequently, the monthly anomaly was divided by the month-appropriate standard deviation, generating the standard monthly anomaly. The 12 standard monthly anomalies of each year were averaged to give the normalized annual anomaly (Li and Harrison 2008). The strength of associations between time series of standardized monthly anomalies of different variables was assessed using Spearman rank correlations. Long-term linear trends for measured variables or their anomalies, based on annual mean values or monthly mean values, were evaluated using linear regression.

Results

Background Meteorological and Hydrological Conditions

During the period 1970–2007, the total annual rainfall measured at Alcoutim averaged 506 mm (range, 176–984 mm) and was negatively correlated with the North Atlantic Oscillation winter index. During this 38-year period, mean annual Guadiana River flow averaged 95 $m^3\ s^{-1}$ (range, 4–375 $m^3\ s^{-1}$; see Fig. 2), and was positively correlated to annual rainfall ($r=0.352$, $n=38$, $p<0.05$) and negatively correlated to the NAO index (Fig. 2). The study period (1996–2005) encompassed an exceptionally wet period, 1996–1998, and two very dry years, 1999 and 2005 (Fig. 2).

During 1996–2005, total monthly rainfall and average monthly river discharge (Table 1) exhibited a clear seasonality, with maximum values between October and March (Fig. 3a), and were positive and significantly correlated (Table 2). Major flood events with mean daily river discharge above 2,000 $m^3\ s^{-1}$, were observed during 1996, 1997, 1998, and 2001 (Fig. 3a). Both annual rainfall (-30.6 ± 13.3 mm $year^{-1}$, $R^2=0.40$, $p<0.05$) and mean annual river flow (-24.0 ± 6.9 $m^3\ s^{-1}\ year^{-1}$, $R^2=0.60$, $p<0.01$) exhibited significant linear declines throughout 1996–2005 (Table 1) that were strongly linked to declining trends observed during January (-18.3 ± 4.8 mm $year^{-1}$ and -145.0 ± 40.9 $m^3\ s^{-1}\ year^{-1}$, $n=10$, $p<0.01$). Time series of freshwater flow anomalies revealed a significant downward trend between 1996–2005 ($p<0.01$), particularly strong for the month of January, and during 1996–1999 (Fig. 4a). In addition to this climatic alteration during the construction and filling of the Alqueva dam, there was an

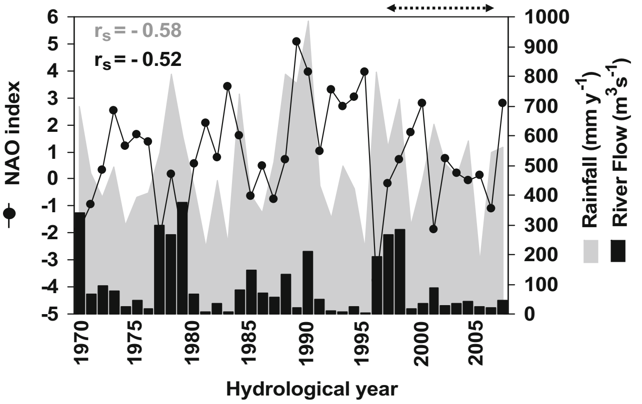

Fig. 2 Historical time series of North Atlantic Oscillation (NAO) winter index (December–March, *filled circle*), total annual rainfall ($mm^3\ year^{-1}$), measured at Alcoutim (*gray square*), and mean annual river flow ($m^3\ s^{-1}$), measured at Pulo do Lobo (*black square*), during the period 1970–2007. Spearman rank correlations between NAO and rainfall (*gray*) and NAO and river flow (*black*) are indicated ($n=38$; $p<0.001$). *Dashed horizontal line* indicates the study period

Table 1 Statistical information for key environmental variables and phytoplankton in the Guadiana upper estuary

	n	Range	Mean	Median	SD
RF (mm d^{-1})					
1996	366	0–60.2	1.96	0.71	2.88
1997	365	0–69.8	1.76	1.09	2.16
1998	365	0–40.0	1.03	0.49	1.22
1999	365	0–70.5	1.29	0.92	1.40
2000	366	0–138.3	1.76	0.93	2.67
2001	365	0–43.3	1.26	1.37	1.13
2002	365	0–43.3	1.54	1.39	1.35
2003	365	0–46.4	1.50	1.21	1.92
2004	366	0–33.5	0.81	0.52	0.84
2005	365	0–26.8	0.87	0.45	1.14
GRF (m^3 s^{-1})					
1996	366	3–2,894	213.9	19.2	443
1997	229	1–2,410	272.7	26.5	432.9
1998	365	4–2,853	170.7	39.4	328.5
1999	365	2–367	19.3	13.5	22.7
2000	338	3–423	26.9	13.8	36.0
2001	275	1–3,255	92.0	13.3	236.3
2002	365	2–656	23.4	7.9	26.6
2003	365	0–796	44.3	30.4	42.8
2004	366	4–897	37.4	34.2	25.5
2005	365	3–158	18.6	15.2	10.5
TEMP (°C)					
1996	13	12.3–26.5	18.7	5.4	19.0
1997	21	12.0–28.6	20.0	5.5	20.9
1998	15	9.6–27.8	19.0	5.9	18.9
1999	25	9.5–26.4	18.3	6.2	19.0
2000	12	11.0–25.1	18.3	5.2	17.1
2001	27	11.6–27.2	19.2	5.8	19.7
2002	29	10.9–26.2	18.9	5.0	18.9
2003	29	9.7–28.9	19.1	6.3	19.3
2004	26	12.3–26.8	19.3	5.2	18.6
2005	19	9.5–27.1	18.4	6.5	19.3
SPM (mg L^{-1})					
1996	12	8.6–604.0	87.6	166.3	33.1
1997	21	3.2–192.0	48.6	61.3	20.0
1998	15	8.0–73.2	30.1	18.6	23.9
1999	23	12.0–143.0	51.6	26.0	48.3
2000	15	34.0–140.5	73.2	33.0	69.8
2001	26	8.4–113.0	39.9	28.6	29.3
2002	13	19.0–104.0	56.7	23.8	51.6
2003	12	8.8–70.4	36.5	17.3	34.0
2004	23	7.75–51.0	28.8	8.8	27.2
2005	19	6.75–50.0	26.5	14.9	28.4
k_e (m^{-1})					
1996	14	1.4–42.5	6.7	11.5	3.0
1997	21	1.5–14.0	4.0	4.3	2.0
1998	15	1.2–5.8	2.9	1.3	2.4
1999	16	1.6–10.7	5.2	2.6	5.1
2000	15	3.1–10.5	5.8	2.3	5.6
2001	27	0.8–8.6	3.3	2.2	2.4
2002	30	2.1–8.0	4.5	1.2	4.4
2003	28	1.4–5.6	3.4	1.0	3.3
2004	27	1.3–5.6	3.1	0.7	3.2
2005	19	1.1–4.7	3.1	0.7	3.2
I_m (μmol photons m^{-2} s^{-1})					
1996	14	1.2–63.3	27.7	28.7	17.0
1997	20	3.5–70.7	37.4	46.4	20.7
1998	15	8.5–91.9	35.4	32.4	16.4
1999	23	9.5–57.1	20.8	16.1	11.1
2000	15	5.2–23.9	15.5	16.7	5.2
2001	24	7.6–66.1	33.7	36.6	18.4
2002	30	6.5–38.5	18.1	18.3	6.9
2003	28	8.1–46.9	25.2	24.7	10.8
2004	25	8.2–53.2	27.7	26.8	11.2
2005	19	13.8–49.6	30.9	26.8	11.2
NO_3^- (μM)					
1996	12	0.1–250.3	66.0	73.7	41.1
1997	19	0.0–216.4	73.6	74.3	48.9
1998	12	0.6–126.3	65.0	49.3	81.3
1999	12	12.6–247.6	71.7	69.0	50.0
2000	13	1.5–157.6	67.1	48.2	67.3
2001	27	2.5–141.9	69.6	50.7	70.8
2002	30	8.1–153.1	56.2	41.5	31.1
2003	29	10.3–127.4	62.6	34.8	69.2
2004	25	3.2–108.1	43.8	32.8	31.0
2005	18	1.7–209.7	30.4	56.9	17.3
Chl *a* (μg L^{-1})					
1996	2	31.8–31.9	–	–	–
1997	20	0.7–37.3	15.2	14.0	12.5
1998	15	1.6–35.6	6.3	3.7	5.4
1999	26	0.2–11.6	2.3	1.6	1.4
2000	14	1.1–16.9	8.5	9.6	4.9
2001	24	2.7–216.1	20.2	12.4	30.6
2002	29	1.0–15.3	7.4	6.9	2.8
2003	17	1.7–24.4	8.0	7.7	4.5
2004	17	0.9–23.0	4.3	3.0	4.3
2005	17	1.3–18.3	5.6	3.8	4.8

Sample number (*n*) and range refer to discrete data, and daily data in case of rainfall and riverflow; mean, median, and standard deviation (SD) were based on monthly mean values

Chl a subsurface chlorophyll *a* concentration, *GRF* Guadiana River flow, I_m mean light intensity in the mixed layer, k_e light extinction coefficient, NO_3^- subsurface concentration of nitrate, *RF* rainfall, *SPM* subsurface suspended particulate matter, *TEMP* subsurface water temperature

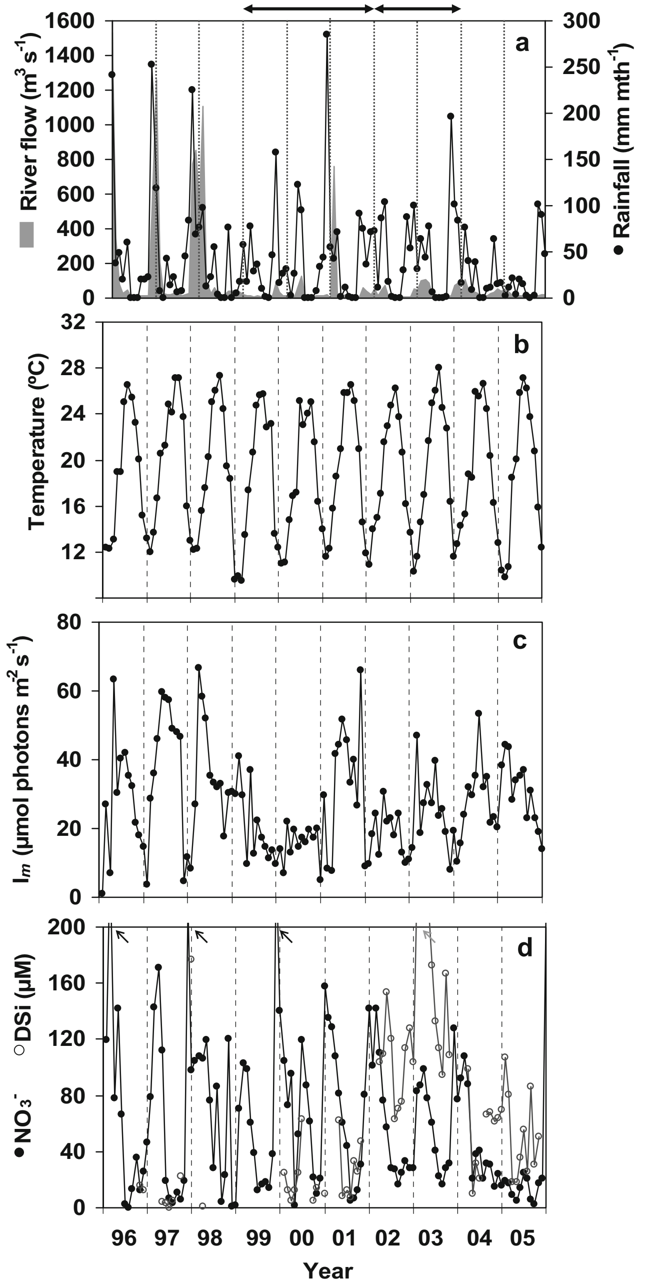

Fig. 3 Time series of mean monthly values of distinct physical and chemical variables at the Guadiana upper estuary (Alcoutim) during the study period (1996–2005). **a** total monthly rainfall (mm month^{-1}), and mean monthly Guadiana River flow (m^3 s^{-1}), measured at Pulo do Lobo; **b** water temperature (°C); **c** light intensity in the mixed layer, I_m (μmol photons m^{-2} s^{-1}); **d** concentration of NO_3^-, and silicate, DSi (μM); values above 200 μM (see *arrows*) were omitted for clarity. *Horizontal arrows* mark two phases of the Alqueva dam construction (pre-filling and filling)

increase in the ratio between annual rainfall and mean annual river flow, indicating increased water retention behind this dam. However, much higher water retention behind existing dams was apparent during 1992–1995, a period of sustained drought (see Fig. 2).

Table 2 Significant ($p<0.05$) associations (Spearman rank correlation coefficient, r_S) between environmental variables and phytoplankton at the Guadiana upper estuary (Alcoutim) considering all discrete data for the period 1996–2005, obtained from subsurface levels

Variables	r_S	*n*	*p* value
GRF and RF	+0.571	118	<0.000001
SPM and RF_8	+0.258	103	<0.01
k_e and SPM	+0.591	49	<0.00001
I_{m8} and RF_8	−0.336	212	<0.000001
I_m and SPM	−0.831	172	<0.000001
NO_3^- and GRF_8	+0.389	188	<0.00001
NO_3^- and RF_8	+0.313	196	<0.0001
DSi and RF_8	+0.259	87	<0.05
Chl *a* and RF_8	−0.273	170	<0.001
Chl *a* and GRF_8	−0.158	162	<0.05
Chl *a* and TEMP	+0.258	162	<0.001
Chl *a* and SPM	−0.199	126	<0.05
Chl *a* and I_{m8}	+0.354	157	<0.00001
Chl *a* and NO_3^-	−0.197	142	<0.05
Chl *a* and DSi	−0.305	78	<0.01
Chl *a* and SRP	−0.255	78	<0.05
Chl *a* and CB	+0.487	88	<0.00001
Chl *a* and GA	+0.284	82	<0.01
DI and GRF_8	+0.383	87	<0.01
DI and TEMP	−0.253	83	<0.05
DI and I_{m8}	+0.259	87	<0.05
DI and DSi	−0.564	81	<0.000001
DI and GA	+0.563	89	<0.000001
DI and CB	−0.242	88	<0.05
GA and GRF_8	+0.250	87	<0.05
GA and I_{m8}	+0.332	87	<0.01
CB and RF_8	−0.397	94	<0.0001
CB and GRF_8	−0.458	92	<0.00001
CB and TEMP	+0.608	89	<0.000001
CB and NO_3^-	−0.245	79	<0.05

Subscript "8" refers to variables averaged on the 8-day period prior to sampling; *n* refers to number of observations

CB abundance of cyanobacteria, *Chl a* chlorophyll *a* concentration, *DI* abundance of diatoms, *DSi* concentration of dissolved reactive silicate, *GA* abundance of green algae, *GRF* monthly mean Guadiana River flow, I_m mean light intensity in the mixed layer, k_e light extinction coefficient, NO_3^- concentration of nitrate, *RF* total monthly rainfall, *SPM* suspended particulate matter, *SRP* concentration of soluble reactive phosphate, *TEMP* water temperature

Physical Conditions

During the period 1996–2005, the subsurface water temperature at Alcoutim (Table 1) fluctuated between 9.5°C (February 1999 and 2005) and 28.9°C (August 2003) and showed the lowest values in winter and highest in summer (Fig. 3b). At the interannual scale, the period 1999–2000

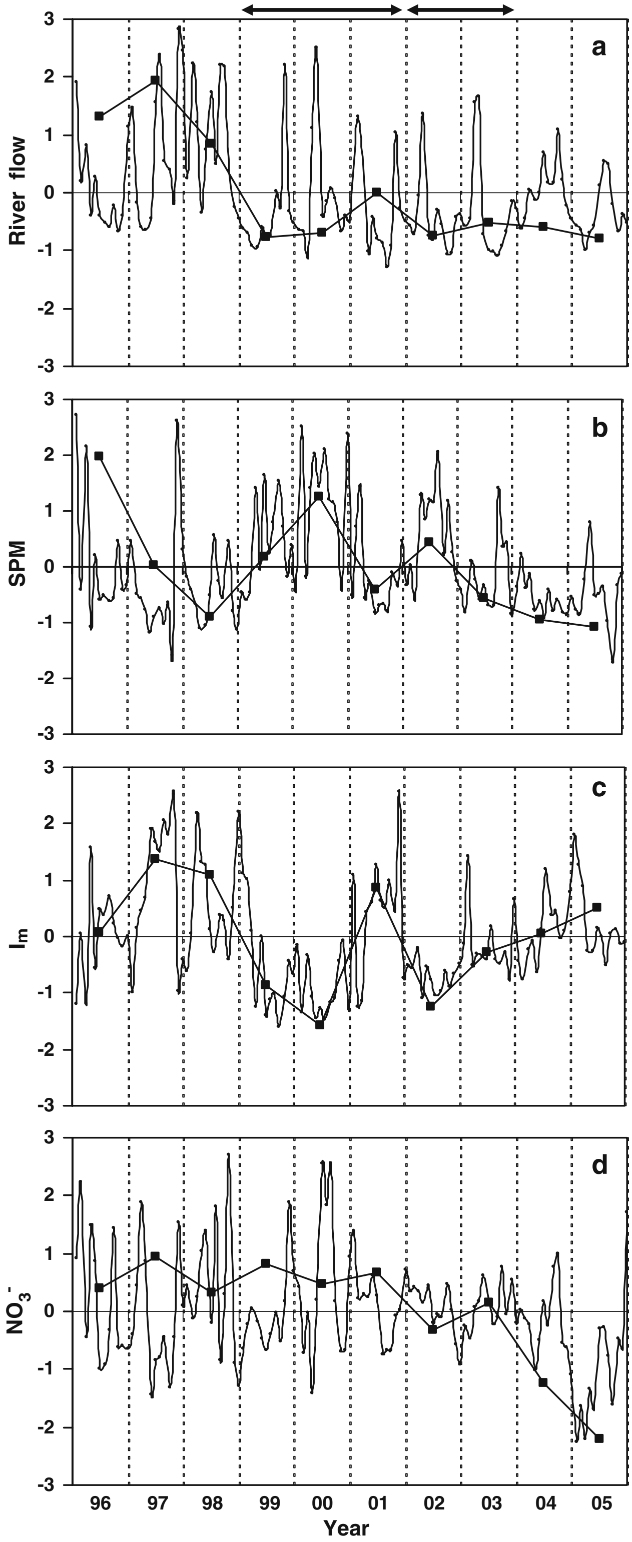

Fig. 4 Time series of deseasonalized standardized annual (*filled square*) and monthly (*filled circle*) anomalies of distinct physical and chemical variables at the Guadiana upper estuary (Alcoutim) during the study period (1996–2005). **a** Guadiana River flow; **b** suspended particulate matter, *SPM*; **c** mean light intensity in the mixed layer, I_m; and **d** concentration of NO_3^-. *Horizontal arrows* mark two phases of the Alqueva dam construction (pre-filling and filling)

clearly displayed lower water temperatures (Table 1), but no significant trends were detected during 1996–2005. Standardized monthly anomalies in water temperature for this period were negatively rank correlated to NAO monthly anomalies (Table 3). Salinity at Alcoutim ranged between 0 and 5 with higher values during the summer period. However, values surpassing 3 were only observed during 1999 and during the Alqueva dam filling period (2002–2003), indicating a pronounced saltwater intrusion under minimum river discharge periods (data not shown).

Secchi depth (D_s) and suspended particulate matter at Alcoutim varied between 0.2 and 1.3 m, and 3.2 mg L^{-1} and 604.0 mg L^{-1}, respectively (Table 1). SPM was positively correlated to rainfall, but not to river flow (Table 2), and maximum values were usually associated with high precipitation events. Time series of standardized SPM anomalies (Fig. 4b) revealed marked interannual variability with an upward trend between 1997 and 2000 and a declining trend thereafter. High positive anomalies detected after 1997, under low river flow conditions were associated with the start of Alqueva dam construction (1999–2000) and the beginning of dam filling (2002). The light extinction coefficient at Alcoutim (k_e, 0.8–42.5 m^{-1}, Table 1) was correlated to SPM (Table 2), and long-term trends in k_e anomalies basically paralleled SPM anomalies (Table 3), suggesting that SPM was a prevalent driver of turbidity. Mean light intensity in the mixed layer (I_m) at Alcoutim ranged between 1.2 µmol photons m^{-2} s^{-1} (January 1996) and 91.9 µmol photons m^{-2} s^{-1} (March 1998), and was negatively correlated to both rainfall and SPM (Table 2). I_m usually depicted higher values during June–July, following trends in incident irradiance (Fig. 3c). At the interannual scale, deseasonalized I_m trends basically mirrored SPM trends (Table 3), depicting a downward trend

Table 3 Significant ($p<0.05$) associations (Spearman rank correlation coefficient, r_S) between deseasonalized standardized monthly anomalies (see Materials and Methods) of environmental variables and phytoplankton at the Guadiana upper estuary (Alcoutim) during 1996–2005

Variables	r_S	*n*	*p* value
TEMP and NAO	−0.204	117	<0.05
k_e and SPM	+0.910	120	<0.000001
I_m and SPM	−0.934	120	<0.00001
NO_3^- and I_m	−0.261	120	<0.01
Chl *a* and TEMP	+0.189	110	<0.05
Chl *a* and I_m (1996–2003)	+0.312	86	<0.01

Chl a chlorophyll *a* concentration, I_m mean light intensity in the mixed layer, k_e light extinction coefficient, *NAO* North Atlantic Oscillation, NO_3^- concentration of nitrate, *SPM* suspended particulate matter, *TEMP* water temperature, *n* refers to number of observations between 1997 and 2000 and an overall upward trend thereafter (Fig. 4c).

Inorganic Nutrients

The concentration of soluble reactive phosphate (SRP) at Alcoutim varied between 0.02 μM (March 1998) and 8.7 μM (May 1997). DRP was mostly higher than 1 μM, and exhibited a reduced intra- and interannual variability (data not shown) and was not significantly related to either river flow or rainfall (Table 2). The concentration of NO_3^- at Alcoutim (Table 1) ranged between 0.03 μM (September 1997) and 250.3 μM (February 1996), and was positively correlated to both river flow and rainfall, and negatively correlated to chlorophyll *a* concentration and cyanobacteria (Table 2). NO_3^- exhibited a pronounced and consistent seasonality with lowest values during summer and highest values in autumn–winter following major rainfall events (Fig. 3d). At the interannual scale, NO_3^- in February and April exhibited a significant linear decline throughout 1996–2005 (−12.8±3.5 μM NO_3^- year^{-1}, n=20, p<0.01), whereas other months showed no significant trends. The time series of NO_3^- standardized anomalies exhibited an overall significant declining trend (p<0.01) and was particularly significant (p<0.001) after 2001 (Fig. 4d). The concentration of dissolved silicate (DSi) at Alcoutim (Table 1) ranged between 0.21 μM (June 1997) and 250.7 μM (March 2003), and was positively correlated with rainfall, and negatively correlated to chlorophyll *a* concentration and diatoms (Table 2). DSi exhibited a distinct seasonality with higher values during winter and lower values between mid-spring and summer. DSi exhibited an obvious increase during the period of the Alqueva dam filling (2002–2003) that led to a significant increase in the Si:N and Si:P molar ratios (not shown), and a subsequent decline after its completion (Fig. 3d).

Phytoplankton

The concentration of chlorophyll *a* (Chl *a*) in the Guadiana upper estuary (Alcoutim) fluctuated between 0.2 μg L^{-1} (April 1999) and 216 μg L^{-1} (April 2001; Table 1). Chl *a* showed a clear seasonality with the highest values usually between spring and early autumn and the lowest values during winter. However, seasonal patterns varied remarkably among years, both in terms of amplitude and phasing (Fig. 5a). Most years revealed bi-modal annual cycles (1997–1999, 2002, and 2005), whereas other years exhibited patterns closer to uni-modal cycles (2000–2001, 2003–2004), and a Chl *a* maximum occurred in most months from January to October (Fig. 5b). The amplitude of each annual cycle also exhibited a large variability among years with particularly low values during 1999 and elevated values during 2001 (Fig. 5a).

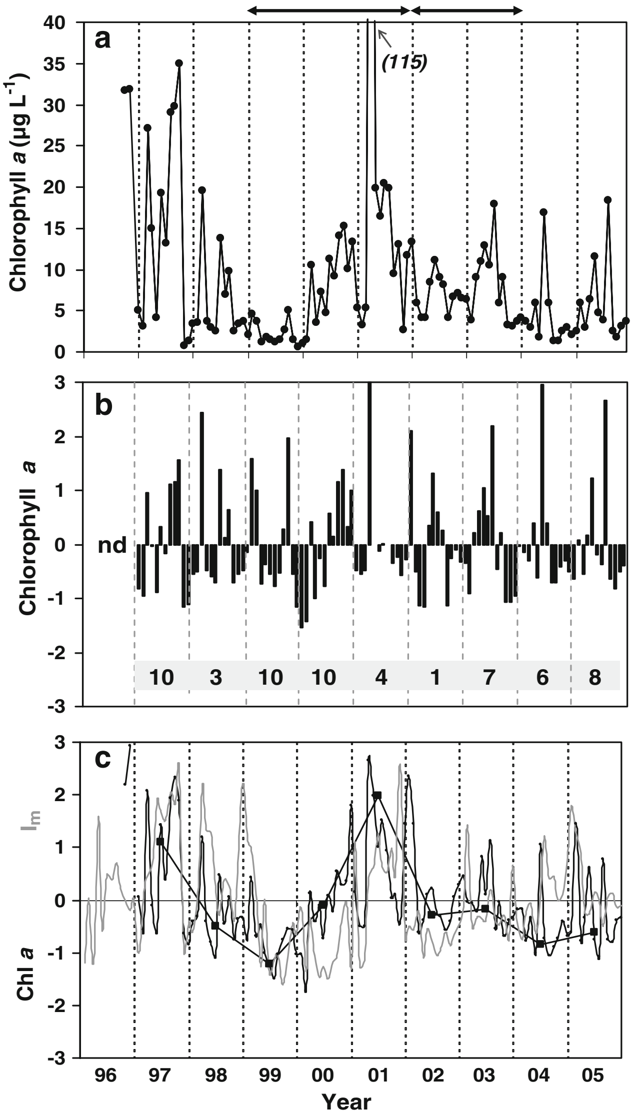

Fig. 5 Time series of chlorophyll *a* concentration (Chl *a*) at the Guadiana upper estuary (Alcoutim), during 1996–2005. **a** monthly means of Chl *a*; value for April 2001 (115 μg L^{-1}) was omitted for clarity; **b** standardized deviations of monthly means from annual means; values in *bold* represent the month number (i.e., 1–12) of average Chl *a* maximum for each year; *nd* no data available; **c** standardized deseasonalized annual (*filled square*) and monthly (*filled circle*) anomalies; anomalies of mean light intensity in the mixed layer (I_m) are shown in gray for comparison. *Horizontal arrows* mark two phases of the Alqueva dam construction (pre-filling and filling)

Considering all discrete data (1996–2005), Chl *a* was negatively correlated with river flow, rainfall, suspended particulate matter, nitrate, phosphate, and silicate. Conversely, Chl *a* was positively correlated to water temperature, mean light intensity in the mixed layer, green algae, and cyanobacterial abundance (Table 2). However, the analysis of each year separately yielded a reduced number of significant relationships between Chl *a* and environmental variables. Significant positive correlations with I_m were observed only in 1999 and 2002–2003, and those with temperature in 1997 and 2001; negative correlations with nitrate were detected only in 1997 and 2001 ($p<0.05$, not shown). At the interannual scale, Chl *a* in March, September, and October exhibited a significant linear decline throughout 1996–2005 (−2.6±0.5 μg Chl *a* L^{-1} year^{-1}, $n=28$, $R^2=0.522$, $p<0.00001$), whereas other months showed no significant trends. Time series of Chl *a* standardized anomalies revealed an overall declining trend during 1997–1999, an upward trend from mid-1999 to 2001, and a subsequent decline until 2005 (Fig. 5c). Deseasonalized Chl *a* trends were positively related to trends in temperature and remarkably paralleled trends in I_m (Fig. 5c), particularly after the exclusion of the post-dam filling period (2004–2005; Table 3).

With respect to key phytoplankton groups, the abundance of diatoms in the Guadiana upper estuary varied from values below detection levels (June 2003) to 18.0×10^6 cells L^{-1} (April 1997) and was negatively correlated to silicate, and temperature, and positively correlated to river flow and I_m (Table 2). However, when each year was considered separately, none of these relationships were statistically significant. Diatom maxima usually occurred during early spring (March–April), and during late summer to early fall secondary peaks were observed in 1997 and 2004 (Fig. 6a). Green algal abundance varied from values below detection levels observed during the period of strongest saline intrusion (August–September 2003), to 11.4×10^6 cells L^{-1} (May 1997) and was positively correlated to river flow and I_m (Table 2). However, when each year was considered individually, correlations with I_m were positive and significant only during 2002 and 2004, whereas correlations with river flow were positive in 2003 but negative in 1997 ($p<0.05$; not shown). Green algae showed a recurrent seasonal distribution with highest values during spring (April–June), usually following diatom blooms, and lowest values during winter (Fig. 6b). Cyanobacterial abundance in the Guadiana upper estuary (Alcoutim) ranged from below detection levels to a maximum of 7.3×10^9 cells L^{-1} (October 1996), and were positively correlated to water temperature, and negatively correlated with rain fall, river flow, and nitrate (Table 2). When each year was considered individually, correlations between cyanobacteria and water temperature and river flow were significant in all years except in 2005 (not shown). Cyanobacteria exhibited a consistent seasonal pattern with peaks during summer to early autumn (>20.5°C), following green algal blooms (Fig. 6c). However, during 1997–2000 (except 1999), cyanobacterial blooms were clearly more intense (2.2–7.3×10^9 cells L^{-1}) and delayed (September–October) than those observed during 2001–2005 (0.2–1.7×10^8 cells L^{-1}; Fig. 6c). At the interannual scale, since 2001, there was a notable decrease in the maximum and average abundances of diatoms, green algae, and cyanobacteria (Fig. 6). Furthermore, during the Alqueva dam filling period (2002–2003), diatoms peaked relatively later (May–August) and after green algae (Fig. 6a, b). The relative contribution of diatoms and green algae decreased and the relative contribution of cyanobacteria was clearly higher (Fig. 7).

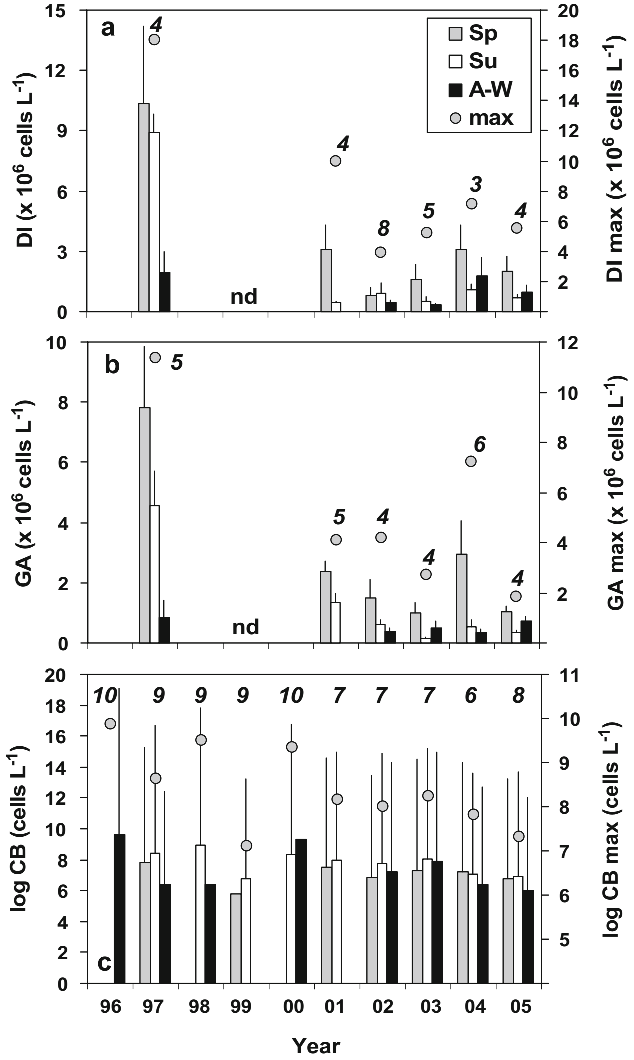

Fig. 6 Seasonally averaged abundances (*bars*) and maximum annual abundances (*circles*) of key phytoplankton groups in the Guadiana upper estuary (Alcoutim) during 1997–2005. **a** diatoms, *DI*; **b** green algae, *GA*; **c** cyanobacteria, *CB*. *Sp* Spring, *Su* Summer, *A-W* Autumn–Winter, *nd* no data available. *Vertical lines* indicate standard-errors and values in *italics* represent the month number (i.e., 1–12) of maximum abundance for each year. Note that *y*-axes have different ranges and cyanobacteria are represented on a log scale

Discussion

Regulation of Phytoplankton Resources

Phytoplankton seasonal and interannual variability is strongly linked to the availability of its major resources,

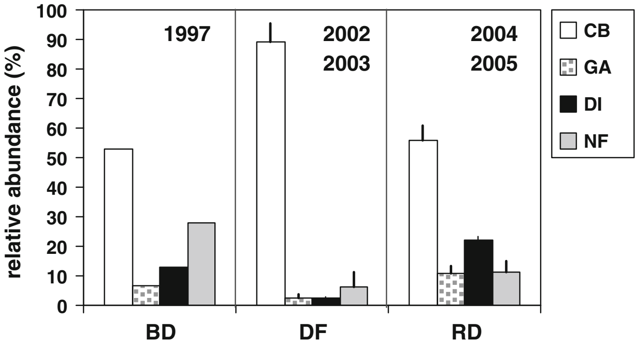

Fig. 7 Annually averaged contribution (%) of key phytoplankton groups (*CB* cyanobacteria, *GA* green algae, *DI* diatoms, *NF* nanoplanktonic flagellates) for total phytoplankton abundance at the Guadiana upper estuary during different periods. *BD* before Alqueva dam construction (1997, 2001); *DF* Alqueva dam filling period (2002–2003); *RD* regular dam functioning period (2004–2005)

dissolved inorganic nutrients, and light. Thus, knowledge of the drivers controlling resources is relevant to understand and predict phytoplankton dynamics. The mean light intensity in the mixed layer (I_m), which corresponds to the whole water column in the Guadiana upper estuary (9.4 m), is controlled by incident irradiance, typically under climate control, and water turbidity. In the Guadiana estuary, suspended particulate matter was a prevalent driver of turbidity and I_m, both on seasonal and interannual scales (Fig. 4), and was responsible for reduced annual mean euphotic zone depths (0.7–1.6 m, i.e., ca. 7–17% of the mixed layer depth), typical of turbid estuaries (Cloern 1987). SPM peak values were usually associated to winter rainfall events and river flow peaks, demonstrating the relevance of climate variability as a turbidity driver. Yet, the impact of the Alqueva dam construction clearly surpassed the influence of climate during 1999–2002. Indeed, the intense land excavation during 1999–2000, and the beginning of the dam filling period (2002) were associated to a major increase in SPM under low river discharge (Fig. 4). Conversely, the relative decrease in SPM during 2004–2005 after dam filling, could be attributed to some extent to its increased retention behind the dam, as described for other dam-regulated systems (Jassby et al. 2002; Humborg et al. 2006). Indeed, the annual mean SPM in the Guadiana upper estuary after the Alqueva dam filling was significantly lower than that observed during periods of similar annual mean river flow (<50 $m^3 s^{-1}$; Fig. 2), before the dam construction (1991–1995: 46.2±5.8 mg L^{-1}; 2004–2008: 31.4±2.6 mg L^{-1}; Mann–Whitney test, $p<0.05$). Thus, whereas seasonal variability in I_m was more regulated by climatic variability, interannual changes were more strongly related to human-induced hydrological alterations.

The concentrations of inorganic nutrients observed in the Guadiana upper estuary were comparable to those associated to slightly eutrophic estuaries (see Domingues and Galvão 2007), and reflected the relatively preserved/pristine nature of the Guadiana estuary (Vasconcelos et al. 2007). Phosphate and ammonium, usually classified as regenerated nutrients, were not related to rainfall or river flow, suggesting the relevance of biological sources and sedimentary fluxes (Sin et al. 1999). Nitrate, the predominant form of inorganic nitrogen in the upper estuary, was positively correlated with rainfall and river flow, and nitrate pulses were consistently linked to major rainfall and flood events (Fig. 3). The importance of river flow as a major nitrate source was previously described for the Guadiana estuary (Rocha et al. 2002; Chícharo et al. 2006; Domingues and Galvão 2007; Morais et al. 2009) and for other estuarine systems (Harding 1994; Sin et al. 1999; Borsuk et al. 2004). Silicate is commonly associated with river discharge (Turner et al. 2003), but it was not correlated with Guadiana River flow during 1996–2005. Indeed, a marked increase in silicate occurred during the period of Alqueva dam filling (2002–2003) under low river discharge (Fig. 3d). Hence, contrary to expectations that the Alqueva dam would lead to a decrease in estuarine silicate availability (Rocha et al. 2002; Humborg et al. 2006), at least during its filling phase, a 2-year silicate pulse occurred with a subsequent increase in Si:N and Si:P molar ratios, potentially favoring diatoms. This increase was probably related to weathering and dissolution of silicate minerals or non-diatom biogenic silica from newly inundated soils (Humborg et al. 2006; Domingues et al. 2007). Overall, seasonal and interannual nitrate variability in the Guadiana upper estuary during 1996–2005 was linked to river flow variability and clearly under climate control (Fig. 2; Trigo et al. 2004), whereas silicate was additionally controlled by the impact of dam filling. Nutrient concentrations showed no evidence of increased anthropogenic eutrophication (Harding 1994), or its mitigation (Borkman et al. 2009) in the Guadiana upper estuary.

Environmental Forcing of Phytoplankton Seasonal Variability

Chl *a* concentration in the Guadiana upper estuary (0.2 and 216.1 μg L^{-1}), with a grand-median of 7.4 μg L^{-1}, is within the intermediate range usually found in other estuaries (Cloern and Jassby 2008). Chl *a* was usually lower during winter and higher during spring–summer, but seasonal patterns varied remarkably among years, both in terms of amplitude and phasing, with Chl *a* maxima occurring between January and October (Fig. 5). Variable phytoplankton seasonal patterns within the same ecosystem are probably a result of the complexity of factors acting on phytoplankton in nearshore ecosystems (Cloern and Jassby 2008). Besides, phytoplankton in turbid nutrient-rich estuaries like the Guadiana estuary may be inherently more variable (Jassby et al. 2002).

Chl *a* seasonality reflects the interaction between phytoplankton growth and loss rates, and can therefore be associated to multiple mechanisms (Cloern 1996). In temperate ecosystems, light intensity is classically considered a primary trigger for the onset and end of the phytoplankton productive season (Iriarte and Purdie 2004). In the turbid Guadiana upper estuary, I_m was usually lower than the typical saturating light intensities for estuarine phytoplankton (96–800 μmol photons m^{-2} s^{-1}, Fisher et al. 1982; Kocum et al. 2002), and the ratio of mixing depth to euphotic depth (Z_{mix}:Z_{eu} mean 5.9; range, 1.7–100), was usually higher than the critical value of 5 (Wofsy 1983; Cloern 1987), suggesting light limitation. Moreover, the critical value of 42 μmol photons m^{-2} s^{-1} (Riley 1957), below which net phytoplankton growth may not occur, was exceeded only during restricted spring–summer periods, but it was never surpassed during 1999–2000 and 2002. Positive relationships between Chl *a* and I_m and the occurrence of the Chl *a* maxima at I_m values usually higher than 35 μmol photons m^{-2} s^{-1} provide further evidence for sustained light limitation, particularly for non-motile phytoplanktonic taxa. Moreover, photosynthesis/irradiance relationships in the Guadiana upper estuary showed light saturating intensities of ca. 500 μmol photons m^{-2} s^{-1} (Domingues, unpublished data), additionally supporting this hypothesis. Light limitation was probably aggravated between late fall and winter, and during 1999–2000 and 2002 (Fig. 3c), thus explaining low Chl *a* values during these years (Fig. 5a). Under these stringent light conditions, biomass increases may be explained by exogenous inputs of phytoplankton from upper riverine areas (Domingues and Galvão 2007; Domingues et al. 2007) or shallow areas, resuspension of microphytobenthos from shallow estuarine areas, reduced respiratory losses, and/or phytoplankton heterotrophy, either by uptake of dissolved organic compounds or phagotrophy (Grobbelaar 1990; Cole et al. 1992; Soetaert et al. 1994; Irigoien and Castel 1997).

Nutrient availability is also considered a key driver of phytoplankton seasonality and its reduction is classically considered an important driver of the collapse of algal blooms during late spring and summer in temperate ecosystems (Cebrian and Valiela 1999). Negative correlations between Chl *a* and inorganic nutrients suggest that phytoplankton represent an effective sink of nitrate and silicate in the Guadiana upper estuary, particularly during the spring–summer period. According to Dortch and Whitledge's (1992) criteria that uses comparisons of ambient nutrient concentrations with half-saturation constants and molar ratios, nutrient limitation in the Guadiana upper estuary only occurred sporadically in case of silica (DSi<2 μM; spring 1997–1998, and 2001) and nitrogen (DIN<1 μM; summer–fall 1997–1998), and *P* was possibly never limiting. Yet these inferences should be interpreted cautiously since non-saturating uptake kinetics (Lomas and Glibert 1999), and large inter- and intra-specific variability in half-saturation constants (Carpenter and Guillard 1971; Sarthou et al. 2005) may hinder the assessment of nutrient limitation. Indeed, in June 2001, silicate enrichment bioassays produced a significant increase in diatom net growth rate in the Guadiana upper estuary with an ambient DSi of 10 μM. Likewise, N limitation was also detected during summer 2005 and 2008, with ambient DIN concentrations ranging between 4 and 22 μM (Domingues, unpublished data). Thus, nutrient limitation could therefore explain, in part, Chl *a* decreases detected during the spring–summer period in the Guadiana upper estuary, particularly during 1997–1998, 2001, and 2005 (Figs. 3d and 5a; Rocha et al. 2002; Domingues et al. 2005). Subsequent recovery of phytoplankton biomass during summer to early autumn can be attributed to increased nutrient loading due to higher river flow, or increased in situ remineralization (Rocha et al. 2002), and its extension is constrained by light availability (Domingues et al. 2005).

During 1997–1998, 2001, and 2005, phytoplankton seasonal patterns in the Guadiana upper estuary indicated an alternation between sustained light limitation, aggravated from mid-autumn to late-winter, and episodic nutrient limitation, exacerbated during the spring–summer period. An alternation between nearly persistent light limitation and more rare nutrient limitation was also reported for other turbid, nutrient-rich estuaries (Pennock and Sharp 1994; Fisher et al. 1999; Mallin et al. 1999; Kocum et al. 2002), and may be responsible for bi-modal annual cycles (Fig. 5a, b). During 1999–2000 and 2002–2004, high concentrations of inorganic nutrients and a lower I_m suggest more sustained light limitation (Fig. 3c, d). Indeed, the occurrence of phytoplankton uni-modal annual cycles with summer blooms, detected in 2003–2004 is typical of light-limited mixed estuaries and of upper partially mixed estuaries (van Spaendonk et al. 1993; Lewitus et al. 1998; Mallin et al. 1999; Sin et al. 1999). The occurrence of bi-modal annual cycles (1999 and 2002), or a uni-modal cycle with the delayed Chl *a* maxima (2000), not commonly associated to light limitation, paralleled changes in I_m. Overall, light limitation is probably the most important factor controlling chlorophyll in the estuary, and should be considered for the improvement of currently available ecological models such as the model by Wolanski et al. (2006) and adequate data interpretation. For instance, differences in Chl *a* between 2000 and 2001 should not be attributed to changes in nutrient concentrations, as hypothesized by Chícharo et al. (2006), but instead to changes in light availability (Figs. 3c and 5a).

Our interpretation of phytoplankton seasonality is evidently constrained by limited information on phytoplankton life cycles and loss processes (e.g., predation, viral lyses, and advection). Monotonic and negative relationships between Chl *a* and Guadiana River flow were probably due to the combined effects of increased turbidity and phytoplankton advection down-estuary under high river flow regimes (Borsuk et al. 2004). These effects may prevent the development of phytoplankton blooms and/or delay phytoplankton seasonal development in upper estuarine regions (Harding 1994; Mallin et al. 1999; Sin et al. 1999; Lionard et al. 2008). As for zooplankton (Chícharo et al. 2006; Morais et al. 2009), major flood events or spring episodic freshwater pulses (May 2000, and April 2002; maximum 400–660 $m^3\ s^{-1}$) probably increased phytoplankton advective losses from the Guadiana upper estuary. Benthic and pelagic grazing may also effectively shape phytoplankton biomass and seasonality in estuaries (Malone et al. 1988; Reaugh et al. 2007; Thompson et al. 2008), and its impact was probably higher during summer due to the higher abundance of metazooplankton (Chícharo et al. 2006). Alterations in the factors regulating recruitment of phytoplankton resting stages from sediments to the water column (e.g., light, temperature, sediment resuspension, and bioturbation), may additionally exert a considerable impact on phytoplankton biomass, seasonality, and dominance patterns (Rengefors et al. 2004; Tan et al. 2008).

Regulation of Phytoplankton Succession

During 1997–2005, phytoplankton exhibited a clear annual succession with a diatom spring bloom (usually in March–April), followed by a mid-spring increase in the abundance of green algae (April–May), and a summer to early fall bloom of cyanobacteria (Fig. 6). This succession was previously reported for the Guadiana estuary (Rocha et al. 2002; Domingues et al. 2005; Domingues and Galvão 2007) and other estuarine systems (Andersson et al. 1994; Pinckney et al. 1998). The first groups, diatoms and green algae, dominate phytoplankton during periods of high freshwater discharge and low water residence time as a result of their faster nutrient uptake and growth rates (Paerl et al. 2006). Positive correlations between diatoms and I_m provide evidence for the role of light in the onset and end of their growing period, whereas negative correlations with DSi, and periods of DSi<2 μM and Si:N molar ratios <1 (Dortch and Whitledge 1992), probably indicate that silicate limitation was responsible, to some degree, for the collapse of diatom spring bloom (1997–1998, 2001). This hypothesis was previously suggested for the Guadiana (Rocha et al. 2002; Domingues et al. 2005) and other estuaries (Soetaert et al. 1994; Kocum et al. 2002).

The mid-spring increase in green algae may be attributed to their greater competitive capacity under reduced DSi and high N:P molar ratios (Roelke et al. 1999), while their decrease probably reflected the impact of increased nitrogen limitation during summer (see previous section). Cyanobacteria clearly dominated the Guadiana upper estuary during summer and early autumn as previously observed for the Guadiana (Oliveira 1991; Rocha et al. 2002; Domingues et al. 2005, 2007) and other estuaries (Pinckney et al. 1998; Adolf et al. 2006; Paerl et al. 2006; Fernald et al. 2007). Cyanobacteria were negatively correlated with river flow and positively correlated with water temperature in all years (except 2005), and annual peaks consistently occurred at temperatures above 20.5°C. Although these relationships may indirectly reflect their overall advantage under reduced nutrient concentrations, they could also indicate direct effects on cyanobacterial physiological processes. Indeed, cyanobacteria have a high temperature optimum compared with most diatoms and green algae, usually above 25°C, and exceptionally large Q_{10} values for growth (Breitbarth et al. 2007; Johnk et al. 2008). Besides being favored by higher temperature and reduced DIN concentrations and N:P molar ratios, cyanobacteria may also benefit from a high nutrient storage capacity, reduced sinking, and reduced grazing losses (Rocha et al. 2002). Furthermore, during summer, high water temperatures (up to 28.9°C) and reduced river discharge probably led to reductions in water viscosity and vertical turbulent mixing, eventually increasing the frequency of transient microstratification. This scenario promotes higher sinking losses of non-motile phytoplankton, such as diatoms and green algae, and higher buoyancy capacity in cyanobacteria (Fernald et al. 2007). Increased buoyancy regulation can constitute a substantial advantage. It may overcome light limitation, a persistent feature in the Guadiana upper estuary, while shading non-buoyant competitors, and evade benthic grazers (Mur et al. 1999; Fernald et al. 2007; Johnk et al. 2008), thus stimulating cyanobacterial growth and diminishing their losses. Overall, this phytoplankton successional pattern, observed most years (see next section for alterations during the Alqueva dam filling period), was apparently driven by changes in nutrients, water temperature, and turbulence, clearly providing evidence for the role of climatic variability.

Environmental Forcing of Phytoplankton Interannual Variability

The interannual variability of phytoplankton biomass in the Guadiana upper estuary, based on time series of Chl *a* deseasonalized standardized anomalies, exhibited three distinct phases: an overall declining trend during 1997–1999, an upward trend from mid-1999 to 2001, and a subsequent decline until 2005 (Fig. 5c). Chl *a* and water temperature trends during 1997–2005 were positively correlated, but since phytoplankton responses to temperature changes are usually not significant in light-limited assemblages (Underwood and Kromkamp 1999), water temperature was not considered a relevant driver of phytoplankton interannual variability. Conversely, positive and significant correlations between Chl *a* and I_m anomalies in 1997–2003 (Figs. 4c and 5c), during periods of either increasing or decreasing light intensities (Li and Harrison 2008), in combination with I_m values below saturating intensities, could be taken as evidence that light availability was a prevalent driver of phytoplankton interannual variability. Furthermore, the months that exhibited significant Chl *a* declines throughout 1997–2005 ($-2.6\pm0.5\,\mu g\ L^{-1}\ year^{-1}$) were located at the start (March), and end (September–October) of the phytoplankton growing season, under lower I_m, further supporting the importance of light. Light was also reported as a prevalent driver of phytoplankton interannual variability in other estuarine and coastal systems. In some cases, light variability was mostly attributed to climate changes (McQuatters-Gollop et al. 2007), whereas others reflected the impact of different anthropogenic activities (Cadée and Hegeman 2002; Facca et al. 2002), including increased SPM retention behind dams (Jassby et al. 2002; Jiao et al. 2007). Interannual variability of I_m in the Guadiana estuary was not driven by climatic variability, but it was mostly associated to the impact of the Alqueva dam construction, as previously referred. Thus, due to human-induced perturbations, a negative relationship between river flow and phytoplankton biomass, as would be expected for turbid light-limited estuaries (Mallin et al. 1999; Howarth et al. 2000; Jassby et al. 2002; Borsuk et al. 2004; Paerl et al. 2006), was not observed during this study (Figs. 4a and 5c).

Despite the putative role of light for most of the study period from 2003 onwards, the time series of Chl *a* and I_m anomalies seemed more uncoupled. Despite increasing light levels, Chl *a* continued to decrease, suggesting that nutrient concentrations that exhibited a marked decline, particularly in NO_3^-, may be more important than light (Figs. 4c, d and 5c). The trends of decreasing nutrient concentrations and increasing I_m that continued to the present (INAG; Domingues, unpublished data), probably reflected the influence of the Alqueva dam as observed for other dam-regulated ecosystems (Wetsteyn and Kromkamp 1994; Jassby et al. 2002; Jiao et al. 2007). Independent of biomass changes, these trends suggest that phytoplankton regulation may shift from light limitation to a more nutrient-limited mode in the near future, eventually indicating an increased susceptibility of the Guadiana estuary to anthropogenic eutrophication (Cloern 2001). During this study, interannual trends in chlorophyll *a* were used as proxies for changes in phytoplankton biomass. However, it is worth mentioning that alterations in carbon to chlorophyll *a* ratios caused by changes in phytoplankton composition or their physiological state, may eventually lead to Chl *a* changes without concurrent or parallel biomass changes. For instance, the chlorophyll *a* declining trend detected from 2003 onwards may partly reflect decreases in chlorophyll cell content caused by increased light availability, and/or decreased nutrient availability (Domingues et al. 2008 and references therein) during this period.

Phytoplankton composition and succession were altered during the Alqueva dam filling period (2002–2003) with relatively delayed diatom peaks (May–August) occurring after green algae (Fig. 6), decreased contributions of diatoms and green algae, and increased contributions of cyanobacteria (Fig. 7). Thus, as previously predicted for the Guadiana (Rocha et al. 2002) and observed in other coastal systems (Humborg et al. 1997; Gong et al. 2006), the Alqueva dam filling was associated to a relative increase in the proportion of non-siliceous phytoplankton, mostly cyanobacteria. However, this shift was transient (Fig. 7) and not permanent as recently assumed by Morais et al. (2009), and clearly not linked to increased silicate limitation (2002–2003: DSi>49 μM). Instead, the relative decrease in diatoms could probably be attributed to increased light limitation (Fig. 3c; Domingues et al. 2007), and extremely reduced summer mean river discharge (2002–2003: $4.8\pm1.8\ m^3\ s^{-1}$), thus benefiting cyanobacteria in comparison to other taxa. During 2004–2005, increased light availability and higher river discharge during summer probably promoted a reverse shift with a relative decrease in cyanobacteria and an increase in diatoms (Fig. 7). The significant increase in the summer mean Guadiana River flow detected after the Alqueva dam building (1980–2001: $9.7\pm6.4\ m^3\ s^{-1}$, $n=21$; 2004–2008: $20.3\pm10.5\ m^3\ s^{-1}$, $n=5$; Mann–Whitney test, $p<0.05$), combined with decreasing water residence time, and SPM (Fig. 4b), may favor fast-growing phytoplankton such as green algae and diatoms (Pinckney et al. 1998; Paerl et al. 2006), further supporting the suggestion of a future decrease in cyanobacteria. However, the forecasted climate alterations for the region that include a reduction in precipitation and an increased frequency of extreme heat-waves and droughts (Cunha et al. 2002; IPCC 2007) may eventually alter the predicted phytoplankton succession (Breitbarth et al. 2007; Johnk et al. 2008).

The analyses of mechanisms underlying phytoplankton interannual variability is of course highly dependent on station selection (Borsuk et al. 2004), sampling frequency, length of the time series (Jassby et al. 2002), and variables examined. As such, this 10-year analysis of phytoplankton variability in the Guadiana estuary represents a biased bottom-up perspective of an upper estuarine location, sampled during a period that encompassed a climatic controlled reduction in river flow superimposed on the construction of a dam. The role of overlooked biological controls, such as the proliferation of the introduced Asian clam *Corbicula fluminea* (Morais et al. 2009), and the impact of increased retention of suspended matter behind the Alqueva dam on the recruitment success of benthic algal life stages (Rengefors et al. 2004; Tan et al. 2008) should be further investigated.

Summary

During this 10-year study, phytoplankton seasonal patterns in the Guadiana upper estuary, a turbid Mediterranean ecosystem, exhibited an alternation between persistent light limitation and episodic nutrient limitation during the spring–summer period. Phytoplankton succession, with early spring diatom blooms, mid-spring green algal blooms, and summer to early fall cyanobacterial blooms was apparently driven by changes in nutrients, water temperature, and turbulence, clearly revealing the role river flow and climatic variability. Light availability was a prevalent driver of phytoplankton interannual variability, and increased turbidity caused by the Alqueva dam construction was linked to pronounced decreases in phytoplankton biomass, particularly at the start and end of the phytoplankton growing period. During dam filling, increased proportions of cyanobacteria and decreased proportions of diatoms were clearly unrelated to silicate variability. The uncoupling between light availability and chlorophyll *a* after dam filling in combination with reducing nutrient concentrations and decreasing turbidity, may point to a shift from a light limitation towards a more nutrient-limited mode in the near future.

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