River damming leads to decreased phytoplankton biomass and disappearance of cyanobacteria blooms

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ABSTRACT

The large Alqueva dam was built in the early 2000s in the Guadiana River (southern Portugal) and has highly controlled the freshwater flowing into the Guadiana estuary, leading to significant changes in the natural hydrological regime. To evaluate the impacts of water restriction and flow regularization on estuarine phytoplankton and their environmental variables, sampling campaigns were conducted in the Guadiana estuary throughout a 14-year period, covering different phases related to the Alqueva dam construction. Significant alterations in phytoplankton and their environmental drivers were observed. In the post-filling period, river flow became more constant throughout the year and its natural seasonal variability, with maxima in the winter and minima in the summer, was greatly reduced, leading to higher river flows in the summer and lower in the winter, in relation to the pre-filling phase. Nutrient and light availability and, hence, phytoplankton dynamics, were greatly affected. Phytoplankton abundance and biomass decreased in the post-filling phase related to a decrease in diatoms and cyanobacteria. Since cyanobacteria blooms in the Guadiana are frequently dominated by toxic species, this constitutes an improvement in water quality. However, the overall decrease in phytoplankton biomass and, specifically, the decline in diatom biomass, will have major consequences for the higher trophic levels that depend on planktonic food.

1. Introduction

Dam construction is essentially driven by economic needs and it represents a main path for the social and economical development of many countries but even the most carefully engineered dams can create unforeseen problems (Milliman, 1997); however, the impact on estuaries and adjacent coastal waters is not always considered in environmental impact studies when dams are proposed on rivers (Wolanski, 2007). Increased eutrophication, pollution, coastline erosion and saltwater intrusion upriver and decreased offshore fish catches are just a few problems usually associated with water and sediment trapping in dams (Domingues et al., 2012).

River flow plays a crucial role in the regulation of abiotic and biotic variables, such as nutrient and light availability (Barbosa and Chícharo, 2011; Domingues et al., 2012), primary and secondary production (Schemel et al., 2004), and, eventually, coastal fish landings (Erzini, 2005). A decrease in river flow is the most obvious

and immediate change caused by dam construction, bearing a significant and negative economic impact to the ecosystem (Morais et al., 2009 and references therein). For instance, prior to the construction of the Aswan High Dam, high nutrient concentrations in the Nile River produced a very large bloom of phytoplankton which supported a productive fishery in the Egyptian coastal waters (Nixon, 2003 and references therein). With the loss of over 90% of the freshwater flow, phytoplankton blooms disappeared and fisheries collapsed (Aleem, 1972). Later, fisheries were able to recover due to high loads of fertilizers and sewage discharges in Egypt (Oczkowski et al., 2009).

These alterations in the upper trophic levels of the food web originate at its base. Inorganic nutrient inputs, which are one of the most important abiotic variables regulating primary producers, may decrease notably due to water and sediment trapping behind dams. Conversely, anthropogenic activities promote an enhancement of nutrient loadings downriver, thus compensating nitrogen (N) and phosphorus (P) removal. However, no such compensation is observed for silica (Humborg et al., 1997), given that the chemical weathering of silicates on land is the main process that supplies Si to the water (Ittekkot et al., 2000). The relative availability of

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inorganic N, P and Si will thus change and affect phytoplankton, the main primary producers in most aquatic ecosystems. River impoundment may therefore lead to significant changes in phytoplankton biomass and species composition (Smayda, 1990), especially when N and P increase, but not Si (Officer and Ryther, 1980). One of the most important examples of ecological problems associated with dam construction was a reduction of silica discharges from the Danube River due to the Iron Gates Dam, which led to changes in phytoplankton composition, from a diatom-based community to non-siliceous forms (Humborg et al., 1997). Indeed, a break in the diatom-zooplankton-fish food web, based on high diatom biomass, which benefits fish and other organisms that rely on planktonic food during their life cycle (e.g., Chícharo et al., 2006a; Beaugrand and Kirby, 2010), can ultimately be expected after dam construction.

In addition to alterations in the nutritional environment, sediment trapping behind dams leads to a decrease in sediment input to estuarine and coastal areas. Given that light attenuation in coastal ecosystems is strongly dependent on suspended particulate matter concentration (Cloern, 1987), river damming and consequent sediment trapping will promote an increase in light availability for phytoplankton. In turbid estuaries, this may lead to a transition from a light-limited environment to a more nutrient-limited one (Barbosa et al., 2010).

The Alqueva dam, located in a Mediterranean climate region in southern Portugal, was the last large dam to be built in the Guadiana River. When its floodgates were close, in February 2002 (Morais et al., 2009), a detailed assessment of its impact on estuarine and coastal zones and remediation measures were still lacking (Wolanski et al., 2004). Nonetheless, the Alqueva dam construction encouraged extensive scientific research, and since the late 1990s, research has been conducted in various fields, aiming to evaluate the impacts of this huge multipurpose infrastructure on estuarine and coastal dynamics. Phytoplankton dynamics have been extensively studied in the last decade and a detailed knowledge of phytoplankton variability and underlying environmental drivers has been acquired. Previous studies were focused on cyanobacteria blooms (Rocha et al., 2002; Sobrino et al., 2004; Galvão et al., 2008), effects of nutrient and light availability on phytoplankton composition and growth (Domingues et al., 2011a,b,c), tidally-induced variability of phytoplankton and environmental stressors (Domingues et al., 2010), influence of climatic and anthropogenic stressors on phytoplankton variability (Barbosa et al., 2010) and the development of an ecohydrological model (Chícharo et al., 2006b).

However, the effects of the Alqueva dam construction and operation on phytoplankton and their environmental drivers have only been briefly analysed; previous studies were focused on the seasonal variability of phytoplankton during specific time periods (Domingues et al., 2005, 2007, 2012; Domingues and Galvão, 2007). No integration of more than 14 years of data has been done to assess the impacts of the Alqueva dam construction and to compare the before and after scenarios. This work aimed, therefore, to understand the impacts of the Alqueva dam, particularly the effects of different hydrological regimes, on phytoplankton and their environmental drivers in the Guadiana estuary. This information is fundamental for a successful management of estuaries and adjacent coastal ecosystems and, to our knowledge, this study is one of the few that evaluate the effects of dam impoundment on estuarine phytoplankton dynamics. Given the importance of phytoplankton as a primary producer in many estuarine ecosystems worldwide, our study can be used to predict impacts of river impoundment on higher trophic levels, serving also as a basis for future environmental impact studies when new dams are proposed on rivers.

2. Material and methods

2.1. Study site and sampling strategy

The Guadiana estuary is located in a Mediterranean climate region, forming the border between south-east Portugal and south-west Spain (Fig. 1). The estuary is approximately 70 km long and less than 800 m wide at its widest point (river mouth area), with a total area of 22 km^2. It is subjected to semidiurnal, mesotidal tides, and it is usually well mixed in its upper section and partially stratified in its middle and lower sections, depending on river flow and tidal stage. Except for the river mouth area, the anthropogenic pressure along the Guadiana estuary margins is low, given that agricultural and industrial activities are nearly inexistent. The Alqueva dam is located in the Guadiana River, approx. 140 km from the river's mouth.

Sampling campaigns were conducted in the Guadiana estuary, from 1996 to 2009. Sampling frequency was fortnightly during 2001–2005 and 2007–2008, monthly during 1996–1999 and 2009, and occasional in 2000 and 2006. All seasons were sampled during the four periods, except in the excavation years, during which phytoplankton was sampled only in the summer; to avoid misleading results, these data were not included in the analysis. Campaigns were conducted in Alcoutim, a small village located approximately 40 km from the river mouth, due to its representativeness of the upper, freshwater estuarine zone and accessibility by land. Mean depth of the sampling station is 9.4 m and sampling was usually conducted during neap tides, immediately after high tide.

2.2. Physical-chemical variables

Data on daily river flow at Pulo do Lobo hydrometric station (85 km from river mouth), monthly accumulated rainfall at Alcoutim and hourly solar radiation, used to derive daily solar irradiance, at São Brás de Alportel (50 km eastwards from Alcoutim), were obtained from the Portuguese National Water Institute public database (http://snirh.pt). Daily solar irradiance (W m^{-2}) was used to estimate daily photosynthetically active radiation (PAR) at the surface (I_0, converted to μmol photons m^{-2} s^{-1} according to Morel and Smith, 1974), considering that PAR constitutes 45% of the total radiation reaching the water surface and a 4% reflection at the surface (Baker and Frouin, 1987). Mean PAR intensity in the mixed layer (I_m, μmol photons m^{-2} s^{-1}) for each sampling date was subsequently calculated according to Equation (1):

$$I_m = I_0\left(1 - e^{(-K_e Z_m)}\right)(K_e Z_m)^{-1} \qquad (1)$$

where Z_m (m) represents the mean depth of the mixed layer (9.4 m in Alcoutim) and K_e (m^{-1}) is the light attenuation coefficient (Riley, 1957). K_e was estimated either as 1.44/Z_{SD} (from 1996 to 2006), where Z_{SD} is the depth of disappearance of the Secchi disk and 1.44 an empirical constant used for turbid waters (euphotic zone < 5 m: Holmes, 1970), or using vertical profiles of PAR intensity versus depth (from 2007 onwards) obtained with a LI-COR radiometer, according to Equation (2):

$$I_Z = I_0 e^{-K_d Z} \qquad (2)$$

where I_z represents the light intensity at depth Z (m) and I_0 is the light intensity at the surface.

Vertical profiles of water temperature and salinity were measured using appropriate probes and showed that the water column at the sampling station is well mixed; therefore, only sub-superficial (approx. 0.5 m) water samples were taken. Samples for
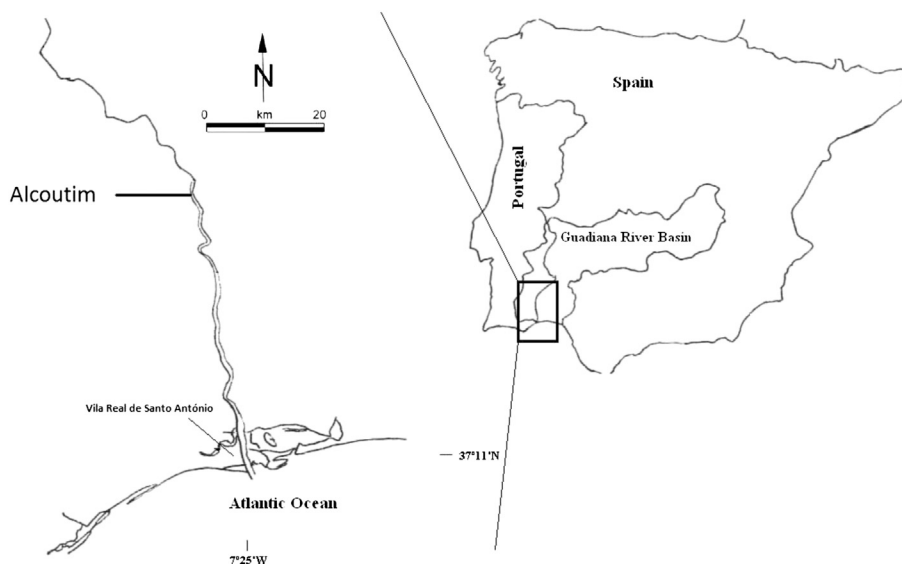
Fig. 1. Location of the Guadiana estuary and sampling station.

dissolved inorganic macronutrient analysis were filtered through 0.2 μm cellulose acetate filters. Soluble reactive phosphorus (SRP) and dissolved silica (DSi) were immediately analysed using the colourimetric methods described by Grasshoff et al. (1983) on a spectrophotometer Hitachi U-2000. Samples for nitrate (NO_3^-) determination were frozen (-20 °C) until analysis on a Skalar autoanalyser. Suspended particulate matter was determined gravimetrically using pre-combusted 0.7 μm glass fibre filters.

2.3. Phytoplankton variables

Phytoplankton identification and quantification were carried out using inverted microscopy for microphytoplankton (>20 μm) and epifluorescence microscopy for nano- ($2-20$ μm) and pico-phytoplankton (<2 μm), following the methods of Utermöhl (1958) and Hass (1982), respectively. Samples for inverted microscopy were preserved in acid Lugol's solution (final concentration approx. 0.003%), settled in sedimentation chambers and observed with a Zeiss Axiovert S100 inverted microscope at $400\times$ magnification. Samples for epifluorescence microscopy were preserved with glutardialdehyde (final concentration 1%) and filtered onto black polycarbonate 0.45 μm membranes, which were mounted on a glass slide with non-fluorescent Cargille Type A immersion oil. Slides were observed at $787.5\times$ magnification on a Leica DM LB epifluorescence microscope. For both methods, a minimum of 50 random visual fields, 400 cells in total and 50 cells of the most common taxon were counted, assuming a counting precision of $\pm10\%$ (Venrick, 1978).

Chlorophyll *a* (Chl*a*) concentration, used as a proxy for phytoplankton biomass, was determined according to the spectrophotometric method described in Parsons et al. (1984). Water samples were filtered onto 0.7 μm glass fibre filters and Chl*a* was extracted using acetone 90%.

2.4. Data analysis

All discrete data collected during 1996–2009 were aggregated into four different phases related to the Alqueva dam construction. The first period, 1996–1998 and 2001, corresponds to the pre-filling situation, when the Alqueva dam was being built, with no significant water retention. The years 1999–2000, although belonging to the pre-filling period, correspond to an intense dam construction

phase, the land excavation period, so these years were analysed separately. The Alqueva floodgates were closed in February 2002 and the dam attained its maximum level by the end of 2003; these years (2002–2003) correspond to the dam filling phase. The fourth period, 2004–2009, represents the post-filling situation, with the dam operating at its full capacity.

Data normality and variance homogeneity, assumptions of parametric statistical analysis, were tested with Shapiro and Bartlett tests, respectively. Differences in variables between periods were tested, considering a significance level of 0.05 and using a one-way analysis of variance or a Kruskal–Wallis on ranks test, depending on data normality. Relationships between variables were assessed using a Spearman rank correlation. All data analyses were carried out using the programming language R. The statistical software Statistica was used for plotting the data as box-whiskers plots.

3. Results

3.1. Environmental drivers

Rainfall in Alcoutim varied between 0 and 285 mm d^{-1} throughout the study period (1996–2009), with a mean value of 42 ± 51 mm d^{-1} (Fig. 2A, Table 1). No significant differences in rainfall were found between the four different phases related to the Alqueva dam construction. River flow was significantly different ($p < 0.0001$) between the four phases of the Alqueva dam construction. The highest river flows were recorded during the pre-filling period; the years 1996–1998 were exceptionally wet, with a mean river flow of 497 ± 1270 m^3 s^{-1} and a median of 24 m^3 s^{-1}. Yet, considering a broader pre-filling period (from 1990 to 1998 and 2001), mean river flow was 221 ± 875 with a median value of 12 m^3 s^{-1}. River flow during the excavation (1999–2000) and filling (2002–2003) periods was significantly lower than during the other periods, with mean and median values of 31 ± 99, 34 ± 74, 12 and 9 m^3 s^{-1}, respectively. In the post-filling phase, river flow was significantly higher than in previous phases, but lower than in the pre-filling situation, with mean and median values of 26 ± 36 m^3 s^{-1} and 16 m^3 s^{-1}, respectively (Fig. 2B, Table 1).

Variability patterns of river flow during summer (June–September) and winter (November–February) months were different than the general trend (Fig. 2C, Table 1). Overall, median
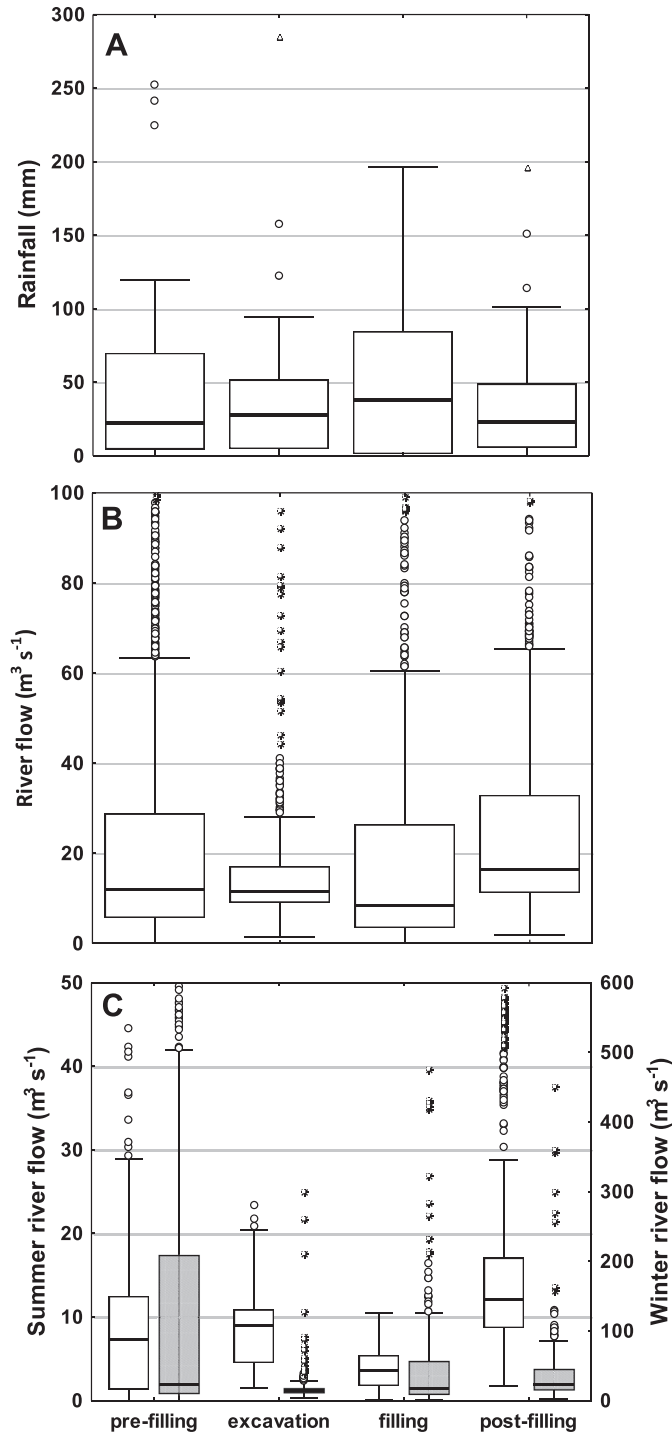
Fig. 2. (A) Monthly accumulated rainfall (mm) in Alcoutim, (B) daily mean river flow in Pulo do Lobo ($m^3 \, s^{-1}$) and (C) daily mean river flow ($m^3 \, s^{-1}$) during summer (transparent columns) and winter (patterned columns) months in Pulo de Lobo, during different phases of the Alqueva dam operation. Median value is represented by the horizontal line within the box, box edges represent 25th and 75th percentiles, the 5th and 90th percentiles are depicted by the error bars, circles represent outliers and triangles represent extreme values. Due to the scale of yy axes, outlier and extreme values are not all represented in the plots.

three previous phases (Table 1). Similarly, winter river flow was significantly higher ($p < 0.0001$) during the pre- ($548 \pm 1310 \, m^3 \, s^{-1}$, median $23 \, m^3 \, s^{-1}$) and post-filling (36 ± 58, median $23 \, m^3 \, s^{-1}$) phases relative to the excavation ($20 \pm 29 \, m^3 \, s^{-1}$, median $16 \, m^3 \, s^{-1}$) and filling ($54 \pm 101 \, m^3 \, s^{-1}$, median $18 \, m^3 \, s^{-1}$) periods. No significant differences in winter river flow were found between the pre-filling and post-filling phases.

Overall, accumulated monthly rainfall and mean monthly river flow were positively correlated throughout the whole study period

Table 1
Minimum, median, maximum, mean, standard deviation (SD) and number of values (n) for several abiotic and biotic variables during the four periods related to the Alqueva dam construction and operation (pre-filling: 1996–1998, 2001; excavation: 1999–2000; filling: 2002–2003; post-filling: 2004–2009; for rainfall and all river flow data pre-filling is 1990–1998 and 2001). Rainfall is monthly accumulated rainfall and all river flow data are mean daily values.

		Min	Median	Max	Mean	SD	n
Rainfall (mm)	*Pre-filling*	0.0	21.4	252.5	39.3	52.2	120
	Excavation	0.0	27.9	284.8	46.8	65.0	24
	Filling	0.0	37.7	196.4	48.7	49.8	24
	Post-filling	0.0	22.8	196.5	34.5	37.7	72
River flow ($m^3 \, s^{-1}$)	*Pre-filling*	0.0	12.0	10,072.3	220.5	875.0	3320
	Excavation	1.5	11.6	985.0	30.8	99.2	703
	Filling	0.1	8.5	796.0	33.8	74.1	727
	Post-filling	1.8	16.4	898.0	26.0	36.2	2147
Summer river flow ($m^3 \, s^{-1}$)	*Pre-filling*	0.0	7.3	138.7	8.4	11.8	1098
	Excavation	1.5	9.0	23.3	8.7	4.7	244
	Filling	0.1	3.6	10.5	4.1	2.7	241
	Post-filling	1.8	12.1	51.9	16.1	11.7	731
Winter river flow ($m^3 \, s^{-1}$)	*Pre-filling*	0.0	22.6	10,072.3	547.9	1310.0	1066
	Excavation	3.4	15.3	300.2	20.2	28.7	274
	Filling	1.6	17.8	795.7	54.2	100.7	240
	Post-filling	3.0	22.5	897.2	36.2	57.3	647
SPM (mg L^{-1})	*Pre-filling*	3.2	25.5	604.0	42.7	74.0	74
	Excavation	12.0	59.7	143.0	62.8	35.6	38
	Filling	8.8	44.0	104.0	48.2	23.7	25
	Post-filling	6.8	30.0	89.6	32.0	15.1	91
I_m (µmol photons $m^{-2} \, s^{-1}$)	*Pre-filling*	1.2	37.1	91.9	36.3	18.5	77
	Excavation	5.2	16.1	57.1	18.4	10.3	31
	Filling	6.5	20.9	46.9	22.8	10.0	58
	Post-filling	6.2	26.0	85.6	30.0	17.4	50
NO_3^- (µM)	*Pre-filling*	0.0	48.9	250.3	58.8	58.4	69
	Excavation	1.5	61.0	247.6	67.0	58.3	25
	Filling	8.1	33.3	153.1	49.0	35.5	59
	Post-filling	1.7	24.2	209.7	32.9	29.6	93
PO_4^{3-} (µM)	*Pre-filling*	0.0	2.2	8.7	2.3	1.6	25
	Excavation				0.7		1
	Filling	1.7	3.5	5.2	3.4	0.8	34
	Post-filling	0.1	2.5	4.4	2.5	0.8	79
SiO_4^{4-} (µM)	*Pre-filling*	0.2	14.7	176.5	26.6	38.0	25
	Excavation	5.0	12.5	63.0	18.6	17.6	10
	Filling	48.8	117.9	250.7	127.5	56.8	34
	Post-filling	3.3	40.0	125.7	47.0	30.1	79
Chl*a* (µg L^{-1})	*Pre-filling*	0.7	11.8	216.1	17.9	28.2	61
	Excavation	0.2	2.2	16.9	4.6	4.8	40
	Filling	1.0	7.9	24.4	8.3	4.9	46
	Post-filling	0.0	6.4	34.2	7.0	5.3	62
Phytoplankton abundance ($\times 10^7$ cells L^{-1})	*Pre-filling*	0.5	6.9	733.0	42.2	152.0	23
	Excavation	1.1	2.0	225.0	46.7	89.2	6
	Filling	0.6	3.3	17.1	5.2	5.0	34
	Post-filling	0	0.5	9.8	1.2	1.9	78
Diatom abundance ($\times 10^5$ cells L^{-1})	*Pre-filling*	2.9	27.0	180.0	40.2	45.3	23
	Excavation	No data					
	Filling	0.0	3.7	52.1	8.6	12.0	34
	Post-filling	0.0	4.1	71.3	8.8	13.2	75
Green algae abundance ($\times 10^5$ cells L^{-1})	*Pre-filling*	2.9	21.0	113.5	31.6	26.0	23
	Excavation	No data					
	Filling	0.0	3.6	41.2	7.1	8.8	34
	Post-filling	0.0	2.5	72.2	5.7	10.1	64
Cyanobacteria abundance ($\times 10^7$ cells L^{-1})	*Pre-filling*	0.1	6.6	733.0	48.6	149.0	27
	Excavation	No data					
	Filling	0.0	3.3	17.0	5.0	5.1	34
	Post-filling	0.0	0.7	9.7	1.6	2.1	57

summer river flow in the post-filling period was significantly higher than in the previous phases, whilst winter river flow in the post-filling period was similar to the pre-filling phase. Summer river flow increased significantly ($p < 0.0001$) in the post-filling period ($16 \pm 12 \, m^3 \, s^{-1}$, median $12 \, m^3 \, s^{-1}$) in relation to the

($r_S = 0.533$, $p < 0.0001$, $n = 152$) and during each individual phase of the Alqueva dam construction and operation. The strongest correlations between river flow and rainfall were observed in the pre-filling ($r_S = 0.6033$, $p < 0.0001$, $n = 44$), excavation ($r_S = 0.6688$, $p = 0.0004$, $n = 24$) and filling ($r_S = 0.6249$, $p = 0.0011$, $n = 24$) periods; in the post-filling period a weaker but still significantly positive correlation was observed ($r_S = 0.3503$, $p = 0.0061$, $n = 60$).

Throughout the whole sampling period, water temperature varied between 9.5 and 28.9 °C with a mean value of 20.0 ± 5.4 °C, and presented lower values in the winter and higher during summer. Water temperature did not vary significantly between the different periods related to the Alqueva dam construction. Salinity values were lower than 0.5 PSU throughout the sampling period, except during the filling phase, when salinity reached 5 PSU, with a mean value of 1.9 ± 1.8 PSU for that period.

Suspended particulate matter (SPM) was significantly ($p < 0.0001$) lower during the pre- (43 ± 74 mg L^{-1}, median 26 mg L^{-1}) and post-filling periods (32 ± 15 mg L^{-1}, median 30 mg L^{-1}) and higher during the excavation (63 ± 36 mg L^{-1}, median 60 mg L^{-1}) and filling (48 ± 24 mg L^{-1}, median 44 mg L^{-1}) phases (Fig. 3A, Table 1). Consequently, mean light intensity in the mixed layer (I_m) was significantly ($p < 0.0001$) higher during the pre- (36 ± 19 μmol photons m^{-2} s^{-1}, median 37 μmol photons m^{-2} s^{-1}) and post-filling (30 ± 17 μmol photons m^{-2} s^{-1}, median 26 μmol photons m^{-2} s^{-1}) phases and lower during the excavation (18 ± 10 μmol photons m^{-2} s^{-1}, median 16 μmol photons m^{-2} s^{-1}) and filling (23 ± 10 μmol photons m^{-2} s^{-1}, median 21 μmol photons m^{-2} s^{-1}) periods (Fig. 3B, Table 1). However, considering I_m values for the spring-summer period (April–September) only, significant differences ($p < 0.001$) were found between the pre- and the post-filling periods, with lower I_m values during the latter (35 ± 13 μmol photons m^{-2} s^{-1}, median 34 μmol photons m^{-2} s^{-1}) in relation to the former (44 ± 13 μmol photons m^{-2} s^{-1}, median 44 μmol photons m^{-2} s^{-1}). Suspended particulate matter and mean light intensity in the mixed layer were significantly and inversely correlated ($r_S = -0.7674$, $n = 148$, $p < 0.0001$) throughout all periods related to the Alqueva dam construction.

Nitrate concentration attained the highest values during the excavation period (67.0 ± 58.3 μM, median 61.0 μM) and the lowest in the post-filling phase (32.9 ± 29.6 μM, median 24.2 μM), although no significant differences were detected between periods (Fig. 4A, Table 1). The same pattern was observed for the spring-summer period; nitrate concentration was higher in the pre-filling phase (37.4 ± 42.1 μM, median 17.1 μM) relative to the post-filling period (26.0 ± 17.1 μM, median 24.1 μM). Soluble reactive phosphorus (SRP) concentration was significantly higher during the filling period (3.4 ± 0.8 μM, median 3.5 μM) relative to the pre- and post-filling phases (Fig. 4B, Table 1). Dissolved silica (DSi) concentration showed the same pattern as SRP with the highest values during the dam filling period (127.5 ± 56.8 μM, median 117.9 μM). Despite this, during the post-filling period, DSi concentrations were significantly higher (47.0 ± 30.1 μM, median 40 μM) than before dam filling (26.6 ± 38.0 μM, median 14.7 μM) (Fig. 4C, Table 1).

3.2. Phytoplankton

The lowest chlorophyll *a* concentrations were measured during the excavation period (4.6 ± 4.8 μg L^{-1}, median 2.2 μg L^{-1}) and the highest Chl*a* were detected in the pre-filling phase (17.9 ± 28.2 μg L^{-1}, median 11.8 μg L^{-1}) (Fig. 5, Table 1). Considering the productive period only (spring and summer months, April–September), Chl*a* was significantly lower ($p < 0.0001$) in the post-filling phase (7.3 ± 5.2 μg L^{-1}, median 6.2 μg L^{-1}) in relation to the
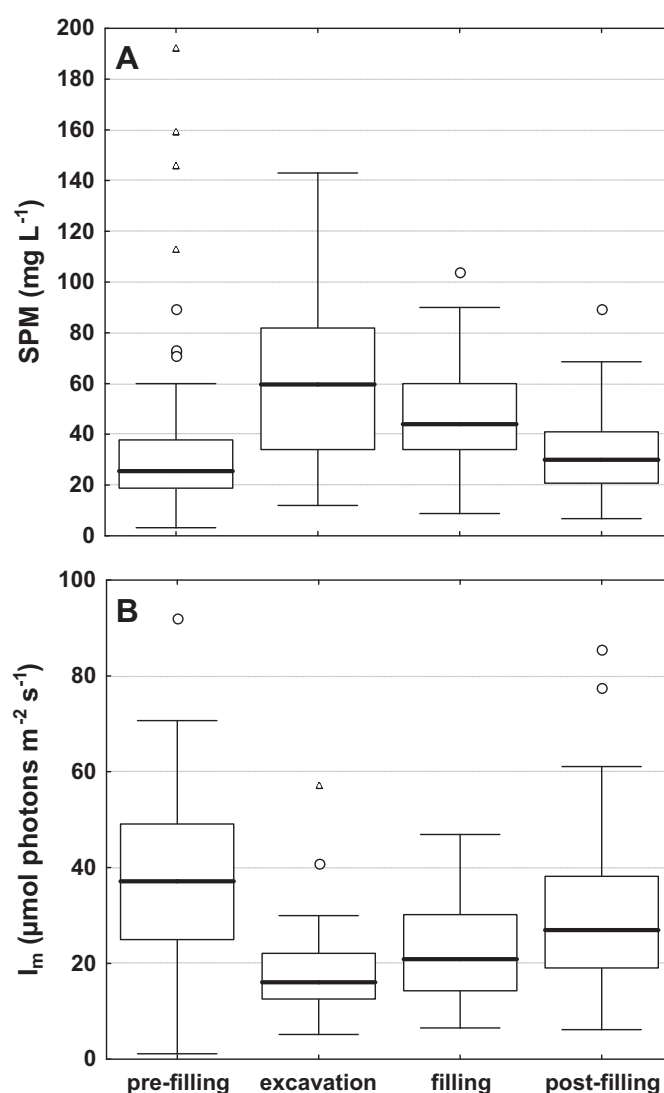

Fig. 3. (A) Suspended particulate matter concentration (SPM, mg L^{-1}) and (B) mean photosynthetically active radiation in the mixed layer (I_m, μmol photons m^{-2} s^{-1}) in Alcoutim during different phases of the Alqueva dam operation. Median value is represented by the horizontal line within the box, box edges represent 25th and 75th percentiles, the 5th and 90th percentiles are depicted by the error bars, circles represent outliers and triangles represent extreme values.

pre-filling (26.1 ± 40.0 μg L^{-1}, median 19.2 μg L^{-1}). Chl*a* was negatively correlated with river flow ($r_S = -0.2304$, $n = 113$, $p < 0.05$) and positively correlated with light availability ($r_S = 0.2599$, $n = 126$, $p < 0.01$).

Phytoplankton abundance in the freshwater tidal reaches of the Guadiana estuary showed an overall decreasing trend with the construction of the Alqueva dam. Total phytoplankton abundance was significantly lower in the post-filling, in relation to previous periods; mean phytoplankton abundance was maximum in the pre-filling period, with a mean of $4.22 \times 10^8 \pm 1.52 \times 10^9$ cells L^{-1} and a median of 6.93×10^7 cells L^{-1}, decreasing to a mean value of $1.24 \times 10^7 \pm 1.94 \times 10^7$ cells L^{-1} and a median of 5.26×10^6 cells L^{-1} during the post-filling phase. Phytoplankton abundance during the productive period (spring-summer) was also significantly lower ($p < 0.0001$) in the post-filling period, relative to the pre-filling, decreasing from $1.03 \times 10^8 \pm 1.14 \times 10^8$ cells L^{-1} and a median of 7.91×10^7 cells L^{-1} to $1.86 \times 10^7 \pm 2.27 \times 10^7$ cells L^{-1} and a median of 9.23×10^6 cells L^{-1}.
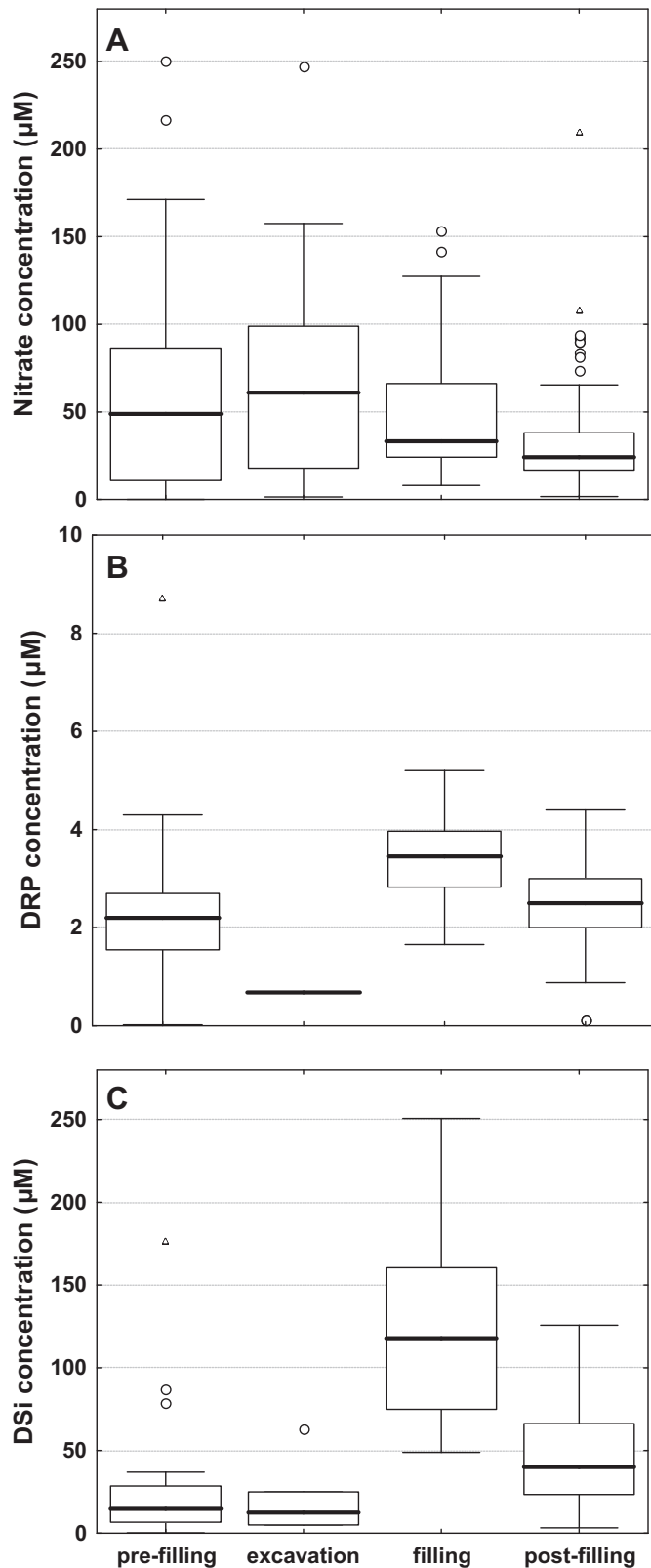
Fig. 4. (A) Nitrate, (B) dissolved reactive phosphorus (DRP) and (C) dissolved silica (DSi) concentration (μM) in Alcoutim during different phases of the Alqueva dam operation. Median value is represented by the horizontal line within the box, box edges represent 25th and 75th percentiles, the 5th and 90th percentiles are depicted by the error bars, circles represent outliers and triangles represent extreme values.
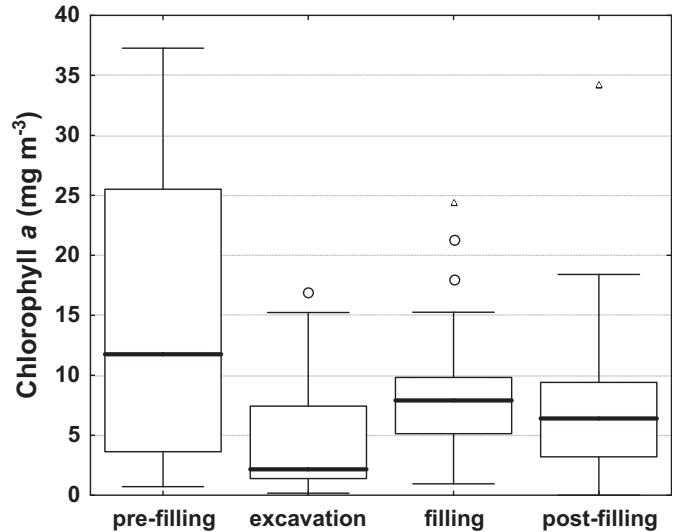

Fig. 5. Chlorophyll a concentration (mg m^{-3}) in Alcoutim during different phases of the Alqueva dam operation. Median value is represented by the horizontal line within the box, box edges represent 25th and 75th percentiles, the 5th and 90th percentiles are depicted by the error bars, circles represent outliers and triangles represent extreme values.

The abundance of the three most important phytoplankton groups in the freshwater zone of the Guadiana estuary, diatoms, green algae and cyanobacteria, showed the same decreasing pattern (see Fig. 6). Diatom abundance was significantly higher in the pre-filling period in relation to the filling and post-filling phases, decreasing from a mean value of $40.2 \times 10^5 \pm 45.3 \times 10^5$ cells L^{-1} and a median of 27.0×10^5 cells L^{-1} in the pre-filling period to a mean value of $8.8 \times 10^5 \pm 13.2 \times 10^5$ cells L^{-1} and a median of 4.1×10^5 cells L^{-1} in the post-filling phase (Fig. 6A, Table 1). Likewise, green algae abundance decreased from $31.6 \times 10^5 \pm 26.0 \times 10^5$ cells L^{-1} and a median of 21.0×10^5 cells L^{-1} in the pre-filling period to a mean value of $5.7 \times 10^5 \pm 10.1 \times 10^5$ cells L^{-1} and a median of 2.5×10^5 cells L^{-1} in the post-filling phase (Fig. 6B, Table 1). Cyanobacteria abundance was significantly lower in the post-filling period in relation to the previous phases of the Alqueva dam construction, decreasing from $48.6 \times 10^7 \pm 149 \times 10^7$ cells L^{-1} and a median of 6.6×10^7 cells L^{-1} in the pre-filling period to a mean value of $1.6 \times 10^7 \pm 2.1 \times 10^7$ cells L^{-1} and a median of 0.7×10^7 cells L^{-1} in the post-filling phase (Fig. 6C, Table 1). A strong negative correlation between cyanobacteria abundance and river flow was observed ($r_S = -0.4778$, $n = 69$, $p < 0.0001$) as well as a positive correlation between cyanobacteria abundance and chlorophyll a concentration ($r_S = 0.4583$, $n = 73$, $p < 0.0001$). Diatoms and green algae were both positively correlated with light availability ($r_S = 0.4029$, $n = 82$, $p < 0.001$ and $r_S = 0.3475$, $n = 78$, $p < 0.01$, respectively).

Considering the productive period for phytoplankton growth (spring-summer), the abundance of the three phytoplankton groups showed the same decreasing trend between the pre-filling and the post-filling phases. Diatom abundance decreased significantly ($p < 0.01$) from $40.7 \times 10^5 \pm 50.6 \times 10^5$ cells L^{-1} and a median of 17.5×10^5 cells L^{-1} to $9.6 \times 10^5 \pm 11.6 \times 10^5$ cells L^{-1} and a median of 5.5×10^5 cells L^{-1}. Green algae abundance also decreased significantly ($p < 0.0001$) from $31.8 \times 10^5 \pm 27.4 \times 10^5$ cells L^{-1} and a median of 20.0×10^5 cells L^{-1} in the pre-filling period to $7.3 \times 10^5 \pm 12.6 \times 10^5$ cells L^{-1} and a median of 3.0×10^5 cells L^{-1} in the post-filling phase. The same significant differences ($p < 0.001$) were observed for cyanobacteria abundance between the productive periods of the pre-filling and
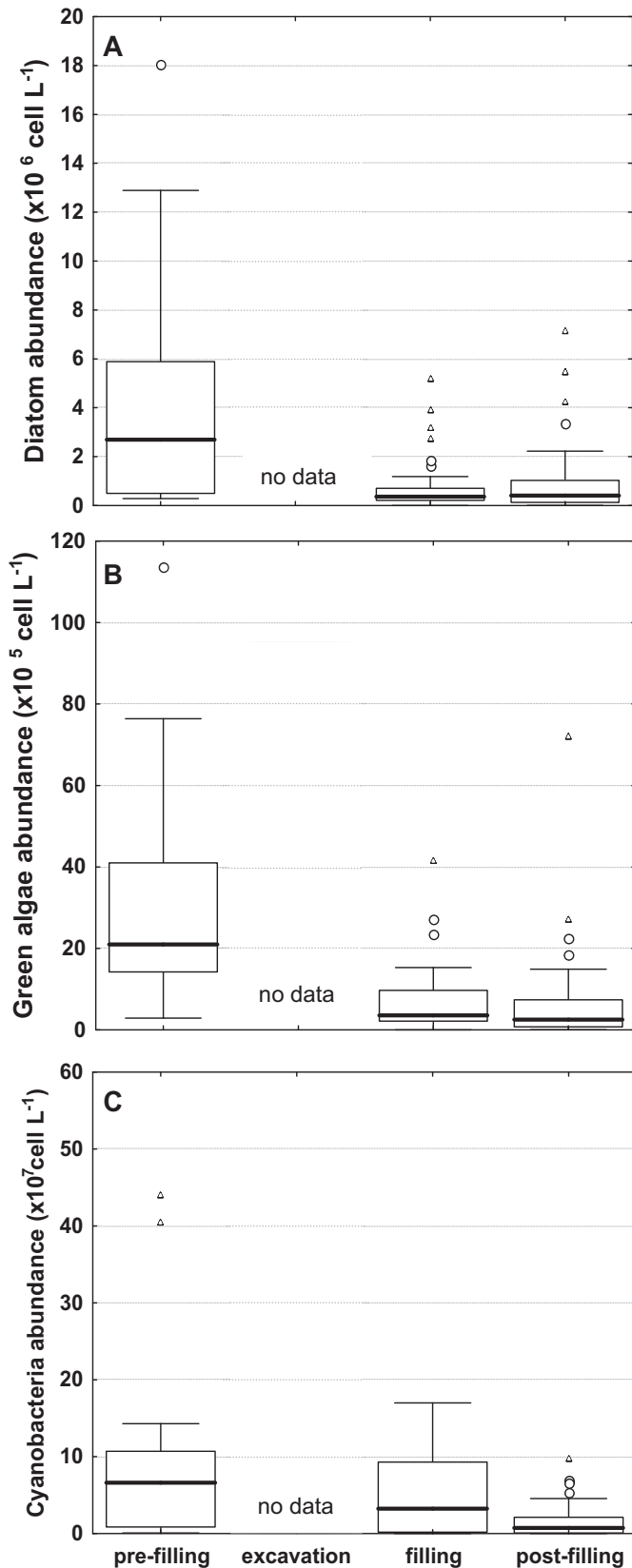
Fig. 6. Abundance of specific phytoplankton groups, (A) diatoms ($\times 10^6$ cells L^{-1}), (B) green algae ($\times 10^5$ cells L^{-1}) and (C) cyanobacteria ($\times 10^7$ cells L^{-1}), in Alcoutim during different phases of the Alqueva dam operation. Median value is represented by the horizontal line within the box, box edges represent 25th and 75th percentiles, the 5th and 90th percentiles are depicted by the error bars, circles represent outliers and triangles represent extreme values.

the post-filling phases, with a mean abundance value of $8.0 \times 10^7 \pm 9.8 \times 10^7$ cells L^{-1} and a median of 6.9×10^7 cells L^{-1} in the former and $2.0 \times 10^7 \pm 2.3 \times 10^7$ cells L^{-1} and a median of 1.2×10^7 cells L^{-1} in the latter.

4. Discussion

Freshwater flow has been described as one of the main variables regulating abiotic and biotic variability in estuaries (Iriarte and Purdie, 2004). Rainfall and dam management will induce significant intra- and inter-annual variability in river flow, which, in turn, may affect phytoplankton due to alterations in nutrient (González-Ortegón and Drake, 2011) and light availability (Jassby et al., 2002), water residence time (Maier et al., 2012), and changes in food web structure (Atwood et al., 2012). These environmental impacts of dams on estuaries and coastal zones are often neglected when dams are planned on rivers (Wolanski, 2007), but there is much evidence of the detrimental effects of dams on the ecosystems downriver (e.g., Aleem, 1972; Humborg et al., 1997; Dai et al., 2010; Liu et al., 2012).

In the Guadiana estuary, river flow and rainfall were significantly correlated, indicating that, despite intense impoundment, water flowing into the estuary is still largely controlled by rainfall. Nonetheless, the impact of dam operation on freshwater flow was obvious during summer months; river flow during summer increased significantly in the post-filling period. This was an unexpected, yet clear effect of river flow regularization by dam managers. The alteration of the flow regime resulted in the elimination of the natural high and low peaks in river flow, typical of Mediterranean climate regions, which in turn will have a significant effect on ecosystem dynamics. Indeed, river flow is more important than seasonality in explaining the interannual variability of several abiotic and biotic variables in the Guadiana estuary (Morais et al., 2009).

The observed variability in river flow contradicts previous predictions for the Guadiana estuary that anticipated a significant river flow reduction, especially during summer, as a result of increased water retention by the Alqueva dam (Rocha et al., 2002). This scenario was of concern as toxic cyanobacteria blooms were recurrent in the Guadiana estuary during summer, associated with low freshwater flows (Domingues et al., 2005). Indeed, cyanobacteria growth is usually enhanced by high water residence times, high water temperatures and low N:P ratios; under these conditions, cyanobacteria can grow abundantly and form extensive blooms and even scums at the water surface. In addition to the ecological consequences of these phytoplankton proliferations, such as shading, anoxia, alterations in carbon fluxes and effects on other algae, invertebrates and fish (Havens, 2008), cyanobacteria are able to produce potent toxins with irritant, neurotoxic and hepatotoxic effects on mammals (Vasconcelos, 2001). Cyanobacteria toxins have even been responsible for fatal human intoxications (e.g., Jochimsen et al., 1998), therefore representing a serious public health concern.

A major increase in the magnitude and frequency of cyanobacteria blooms was thus expected with the construction of the Alqueva dam and consequent freshwater flow reduction (Domingues et al., 2005). Indeed, although cyanobacteria abundance did not increase, cyanobacteria blooms during the filling phase occurred not only in the summer, but in the autumn and winter as well, associated with the lowest freshwater flows of the study period (Domingues et al., 2007). Low river flow periods, either artificially reduced by dam operation or associated with low rainfall, lead to decreased advection of cells and the occurrence of transient microstratification (Barbosa et al., 2010). This promotes a higher buoyancy capacity in cyanobacteria (Fernald et al., 2007)

and increased recruitment of resting stages from bottom sediments, resulting in higher cyanobacteria growth and biomass accumulation. However, river flow during summer actually increased in the post-filling phase, due to the elimination of natural flow peaks by dam operation. During this last period, cyanobacteria abundance decreased significantly from a mean value of approx. 49×10^7 cells L^{-1} during the pre-filling phase to approx. 1.6×10^7 cells L^{-1}, representing a reduction of 97% of cyanobacteria abundance in the post-filling phase.

Significant and negative correlations were found between river flow and cyanobacteria abundance throughout the entire study period, indicating that river flow is clearly a relevant regulator of phytoplankton community dynamics. However, changes in river flow will also induce changes in abiotic variables, which, in turn, will affect phytoplankton and estuarine dynamics. Therefore, most ecological problems associated with river damming are a direct consequence of changes in freshwater flow. These include: alterations in the geomorphology of the system, due to sediment trapping behind the dam; higher salinity intrusion upriver, due to lower freshwater flows, and a lower biological productivity, due to decreased nutrient inputs (Ibàñez and Prat, 2003). In addition, the decrease in nutrient inputs due to water and sediment retention behind dams may have serious consequences especially in non-eutrophic systems, such as the Guadiana estuary, where anthropogenic nutrient inputs are negligible.

The variability of nutrient inputs to estuaries and coastal zones after river impoundment is somewhat unpredictable and depends not only on changes in river flow, but also on human activities that may be enhanced due to damming. In the Guadiana estuary, nutrient concentrations, particularly dissolved silica (DSi), varied significantly throughout the study period and showed the highest increase during the dam filling phase. This increase was associated with the filling of the reservoir, which became the largest artificial lake in Europe (Chícharo et al., 2007); during its filling, a previously uncovered area of 250 km^2 was inundated, leading to soil displacement and hence particulate and dissolved silica inputs to the water. After dam filling, DSi concentrations remained at higher levels than in the pre-filling phase, as inputs of dissolved and particulate silica to the estuary are more regular due to a more constant river flow throughout the year. A decreasing trend in DSi inputs is typical after river impoundment and has been observed in many rivers, estuaries and adjacent coastal zones (Zhou et al., 2008), such as the Danube River and the Black Sea (Humborg et al., 1997), the Nile River and the eastern Mediterranean Sea (Turley, 1999), and the Yangtze River Estuary (Chai et al., 2009).

Nitrate showed the highest range of variability throughout the study period, but nitrate concentrations were not significantly different between the different phases. However, the mean, median and range of nitrate were lower in the post-filling phase in relation to the pre-filling period, indicating a lower variability and a decreasing tendency of nitrate concentrations in the Guadiana estuary. The Guadiana is not a highly human-impacted system; hence anthropogenic nutrient inputs to the estuary are not important. In contrast, the construction of the Three Gorges Dam in the Yangtze River led to an increase in population and fertilizer use in the river basin, which in turn promoted higher anthropogenic nutrient discharges and, consequently, an increase in dissolved nitrogen concentrations in the estuarine and coastal zones (Chai et al., 2009).

Although nutrient concentrations were mostly similar (N, DRP) or higher (DSi) than pre-dam values, phytoplankton abundance and biomass decreased significantly after dam filling in relation to the pre-filling phase. Phytoplankton biomass maxima in the Guadiana estuary were usually observed in the spring, corresponding to the diatom bloom and associated with high nitrate and light availability; given that nitrate inputs to the estuary depend on river flow, diatom maxima were higher in years of higher freshwater flows (Domingues et al., 2012). Phytoplankton abundance usually peaked in the summer due to cyanobacteria blooms, which were associated with low freshwater flows and high water temperature (Domingues et al., 2005). With the construction of the Alqueva dam, mean phytoplankton abundance decreased from approx. 42×10^7 cells L^{-1} in the pre-filling phase to approx. 1.2×10^7 cells L^{-1} after dam filling, mainly due to a decline in cyanobacteria abundance. Phytoplankton biomass, measured as chlorophyll a concentration, showed a similar behaviour, decreasing from a mean value of approx. 18 µg L^{-1} in the pre-filling phase to approx. 7 µg L^{-1} after dam filling; a significant decrease in diatom abundance was the main responsible for this large reduction in phytoplankton biomass in the Guadiana estuary. However, this leads us to question why phytoplankton did not recover after dam filling despite nutrient concentrations apparently recovering.

Estuarine phytoplankton growth is basically regulated by nutrient and light availability, which, in turn, depend on freshwater flow. Reduced river discharges usually boost phytoplankton growth, by allowing an increase in water residence time and a decrease in turbidity that leads to increased light availability (Butrón et al., 2009; Maier et al., 2009). The highest phytoplankton biomasses in estuaries are, thus, generally observed during dry years (e.g., Gameiro and Brotas, 2010). Moreover, nutrient addition experiments showed that phytoplankton growth in the Guadiana estuary after dam filling was clearly nutrient-limited, especially by nitrogen and during spring-summer months (Domingues et al., 2011a); nitrate concentrations in the productive period were indeed lower in the post-filling phase in relation to the pre-filling. Therefore, after dam filling, phytoplankton in the Guadiana estuary was subjected to higher freshwater flows during the typically productive period, and to limiting nutrient concentrations. In addition, light availability during the productive period was significantly lower in the post-filling phase in relation to the pre-filling; light enrichment experiments have shown than phytoplankton growth in the Guadiana is light-limited (Domingues et al., 2011b). These three factors — a higher river flow in the spring-summer period, and nutrient and light limitation — most likely prevented the development of intense phytoplankton blooms as those observed in the pre-dam period (e.g., Rocha et al., 2002).

The increase in river flow during summer has particularly affected cyanobacteria, both in terms of abundance and biodiversity. During the pre-filling period, several cyanobacteria genera, such as Oscillatoria, Microcystis and Anabaena were common in the Guadiana (Rocha et al., 2002); in recent years, only chroccoid picocyanobacteria have been identified (Domingues and Galvão, 2007; Domingues et al., 2012). In the freshwater tidal reaches of the Guadiana estuary, diatoms and green algae suffered a marked decline in their abundance, during and after dam filling. Diatoms are considered 'healthy' for the ecosystem; the benefits of a diatom-zooplankton-fish food web, based on high diatom biomasses, are evident not only in the Guadiana estuary, but in many other ecosystems (Beaugrand and Kirby, 2010). For instance, a diatom bloom in spring 2001 was clearly associated with high fish abundances in the Guadiana, specifically of species that depend on planktonic food during their larval stages (Chícharo et al., 2006a). Declines in diatom biomass can thus pose serious consequences for upper trophic levels. Although significant changes in phytoplankton community composition were not observed in the Guadiana estuary, in other coastal ecosystems, such as the East China Sea (Gong et al., 2006) and the Black Sea (Humborg et al., 1997), river impoundment led to a replacement of diatoms for flagellates as the dominant group. Shifts on phytoplankton community composition towards non-siliceous species are a concern for aquatic ecosystems, given that these organisms represent a low quality food for higher

trophic levels and many of them, such as cyanobacteria and dinoflagellates, can produce toxins, hence affecting other algae, invertebrates and fish populations.

In conclusion, river flow is clearly a critical regulator of abiotic and biotic variables in the Guadiana estuary. The human regulation of river flow and the disruption of natural flow regimes may, therefore, have negative impacts on the ecosystem. Indeed, many plant and animal species can only complete their life cycle if the natural contracting river flow regime is maintained (Bunn and Arthington, 2002). The elimination of flow peaks and the maintenance of more constant river flows (the so called "ecological flows") throughout the year can lead, as observed in the Guadiana estuary, to declines in phytoplankton biomass, therefore affecting all other trophic levels that depend on planktonic food throughout their life cycle. Ecological flows should thus be avoided for extended periods; instead, the establishment of a river flow that mimics natural river discharges could prevent negative impacts on the natural patterns of biological communities (Morais, 2008), including fish populations (Loneragan and Bunn, 1999).

Acknowledgements

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