Nutrients, light and phytoplankton succession in a temperate estuary (the Guadiana, south-western Iberia)

Rita B. Domingues*, Ana Barbosa, Helena Galvão

CIMA – Centre for Marine and Environmental Research, FCMA, Universidade do Algarve, Campus de Gambelas, 8005-139 Faro, Portugal

Received 26 August 2004; accepted 9 February 2005
Available online 2 April 2005

Abstract

Seasonal changes in freshwater flow, leading to alteration of the nutritional environment and hence affecting phytoplankton composition, will probably be enhanced in the Guadiana estuary (SW Iberia) by the recently built Alqueva dam. The main goal of this study is to assess the relationship between dissolved inorganic macronutrient concentrations and ratios, light availability and phytoplankton succession in the upper estuary of the Guadiana River prior to the completion of the dam.

From April to October 2001 three locations along the upper estuary were sampled fortnightly. Several physical and chemical parameters were analysed and phytoplankton composition, abundance and biomass were determined through inverted and epifluorescence microscopy. Phytoplankton showed a uni-modal cycle with a biomass maximum during spring. A relationship between phytoplankton succession and nutrient ratios seemed to exist. In early spring, N:P was high, Si was abundant and a diatom bloom occurred. This bloom collapsed and an increase in green algae abundance was observed later in spring, with low Si and high N:P. In the summer, N:P and Si were low, and a cyanobacteria bloom developed. This bloom included the potentially toxic Microcystis. Light was probably limiting throughout the sampling period, particularly to non-motile cells. Enhancement of cyanobacteria blooms can be expected, and as the river water is used by local human populations, continued monitoring of the Guadiana estuary will be necessary to evaluate the effects of the Alqueva dam construction on phytoplankton dynamics.

© 2005 Elsevier Ltd. All rights reserved.

Keywords: phytoplankton succession; nutrient concentrations; nutrient ratios; light; Guadiana estuary; Alqueva dam; south-western Iberia

1. Introduction

One of the main goals of phytoplankton ecology has been to understand the factors that regulate phytoplankton production (Pennock and Sharp, 1994) and their seasonal succession in aquatic ecosystems (Gallegos and Jordan, 1997). Several factors have been linked to limitation of phytoplankton production, but nutrient availability has frequently outweighed all others (Roelke et al., 1999 and references therein).

Dam construction promotes a decrease in nutrient loadings to rivers and coastal areas, due to the removal of nutrients in reservoir sediments (Humborg et al., 1997), whereas other anthropogenic activities are usually responsible for an enhancement of nutrient loading to these areas. Anthropogenic discharges typically have a high nitrogen:phosphorus (N:P) content because of the preferential removal of P in sewage treatment (Flynn, 2002), while the N:P of fertilizers added to agricultural lands is low; thus, nutrient removal can be over-compensated by anthropogenic N and P. However, no such compensation has been observed for silica (Si) (Humborg et al., 1997), as chemical weathering of silicates on land is the main process that supplies
dissolved and particulate silicate to rivers (Ittekkot et al., 2000) and Si recycling is a slow process dependent upon temperature and biogeochemical equilibriums (Wetzel, 1993). Changes in nutrient supply are often accompanied by alterations in nutrient ratios (Yin et al., 2001) and as the relative availability of nutrients plays a major role in structuring phytoplankton communities (Tilman, 1977), alterations in nutrient ratios can change phytoplankton biomass and species composition (Smayda, 1990), especially if there is an increase in N and P availability, but not Si (Officer and Ryther, 1980). Furthermore, competition between phytoplankton species under conditions of variable nutrient supply ratios may influence the development of nuisance algal blooms regardless of eutrophication (Flynn, 2002).

It is broadly accepted that in marine systems N is the limiting nutrient, whereas P limits freshwater systems. There is, however, evidence of seasonal and temporal variations of the limiting nutrient in estuarine systems (D’Elia et al., 1986; Fisher et al., 1992). Fisher et al. (1999) refer to several studies showing P limitation in winter and spring, and N limitation during summer; Si may also be limiting to diatom populations especially in the spring period.

Light limitation by turbidity has been frequently referred to as another factor controlling phytoplankton growth, during the whole year (Cloern, 1987; Irigoien and Castel, 1997) or seasonally (Pennock and Sharp, 1994; Fisher et al., 1999; Kocum et al., 2002a,b), and much of the temporal variability of phytoplankton biomass in estuaries can be related to variations in light availability (Cloern, 1987). Differences in algal production and biomass in equally nutrient-rich estuaries can be related to differences in light conditions (Cloern, 1999). In addition, different estuarine areas can be limited by different factors (Fisher et al., 1999; Mallin et al., 1999); maximum turbidity zones, for instance, are most likely light limited, whereas further down or up-river, other factors may be limiting.

Previous studies on phytoplankton dynamics in the Guadiana have mainly been focused on the relationship between nutrient availability and cyanobacteria blooms (Rocha et al., 2002) and bloom toxicity (Cabeçadas and Brogueira, 1981; Caetano et al., 2001; Sobrino et al., 2004). These studies have reported the occurrence of toxic cyanobacteria blooms during summer, especially in years of reduced freshwater flow (Cabeçadas and Brogueira, 1981), and related the summer dominance of cyanobacteria to silica depletion (Rocha et al., 2002). The Guadiana estuary faces today serious water quality and quantity problems (Chicharo et al., 2001) and due to the recent construction of the Alqueva dam, more than 81% of the freshwater flow will be artificially regulated from 2002 onwards (Morales, 1997). Alteration of the nutritional regime is expected, especially declining silica concentrations, which may produce transitions from diatom-based communities to non-diatom assemblages (Kilham, 1971). Dominance of flagellates or cyanobacteria is adverse in aquatic ecosystems, given that these groups represent undesirable food sources for higher trophic levels, whereas a diatom-based food web contributes directly to the development of large fish and shellfish populations (Officer and Ryther, 1980; Ryther and Officer, 1981). In addition, many cyanobacteria species produce one or more of a range of potent toxins; if water containing high concentrations of toxic cyanobacteria or their toxins is ingested, it presents a risk to human health (Bartram et al., 1999).

In order to assess the effects of the dam, it is necessary to understand the ecosystem status before dam construction. Since estuarine phytoplankton displays strong inter-annual variability (Cloern, 1996; Li and Smayda, 1998; Sin et al., 1999; Jassby et al., 2002), it is important to develop the current knowledge of the system before dam closing. The main goal of this study was to assess the relationship between dissolved inorganic nutrient concentrations and ratios, and phytoplankton succession in the Guadiana River upper estuary, prior to the construction of the Alqueva dam. Based on a previous study (Rocha et al., 2002), it is hypothesized that the occurrence of a summer cyanobacteria bloom may occur under N and Si deficiency.

2. Material and methods

2.1. Study site

The Guadiana, a mesotidal estuarine system, arises in Spain, at Campo de Montiel, in the province of Ciudad Real, and drains to the sea between Vila Real de Santo António and Ayamonte (Fig. 1). Its 70 km estuary is located in a temperate climate area, with moderate, humid winters and hot, dry summers. The river’s total length is 810 km, of which 550 km is in Spanish territory, 150 km in Portugal and 110 km serves as a border between the two countries. Guadiana has the fourth largest drainage basin of the Iberian Peninsula, 67,840 km² in area (Rocha and Ferreira, 1980), of which 75% is artificially regulated by more than 40 dams (Morales, 1997). From early 2002, more than 81% of the freshwater flow will be controlled, due to the recently built Alqueva dam, 140 km from the river mouth, which provides irrigation to the largest agricultural area in Portugal. Variations in rainfall and water retention in dams account for sharp differences in freshwater inputs to the estuarine zone (Rocha et al., 2002). In the last two decades the mean monthly freshwater flow at Pulo do Lobo (70 km upstream) has varied abruptly from 200–600 m³ s⁻¹ in the winter to 0.1–20 m³ s⁻¹ in the summer (INAG — Portuguese National Water Institute public
In addition, the estuary receives freshwater inputs from some tributaries (especially Odeleite stream), whilst other inputs include sewage, mainly near the mouth, due to the densely populated cities of Vila Real de Santo António and Ayamonte. Nevertheless, the urban pressure along the upper estuary is small (Chicharo et al., 2001), industry is nearly non-existent, the soil is made of schist, therefore inadequate for agricultural use, and the scarce agricultural lands that exist along Guadiana estuary margins are used only for subsistence farming.

2.2. Sampling strategy

Sampling stations were established along a longitudinal transect covering the upper estuary, into the freshwater zone. Stations sampled were Mértola, Alcoutim and Foz de Odeleite, respectively, 60, 35 and 20 km from the river mouth. Mean depth of the sampling stations is 6.5 m (Bettencourt et al., 2003). From April to October 2001, at fortnight intervals, water samples were collected during neap tides, immediately after flood tide, beginning at the landward end (Mértola) and hence accompanying the ebb flow. Since there is neither thermal nor haline vertical stratification in the upper estuary, independent of season and tidal cycle (Rocha et al., 2002; Domingues, unpublished data), samples were collected near the surface (approx. 0.5 m depth) and it was assumed that the entire water column was well mixed.

2.3. Environmental variables

Subsurface water temperature and dissolved oxygen were determined in situ using a WTW dissolved oxygen temperature meter Oxi 197 connected to a WTW TA 197 sensor, and an Atago S/Mill refractometer was used for salinity measurements. Concentration of suspended particulate matter (SPM) was determined gravimetrically using Whatman GF/F filters (pore diameter = 0.7 μm).

Light penetration in the water column was determined using a Secchi disc ($D_S$, m) and light extinction coefficient ($k_e$, m$^{-1}$) was calculated as recommended by Holmes (1970), where $k_e = 1.4/D_S$, with 1.4 an empirical constant used for highly turbid waters (euphotic depth < 5 m). Euphotic zone depth ($Z_{eu}$, m) was calculated as $4.61/k_e$, assuming that irradiance at the bottom was 1% of surface irradiance (Cloern, 1987). Total daily radiation (W m$^{-2}$) from São Brás de Alportel meteorological station, located approximately 50 km from Alcoutim (obtained from the Portuguese National Water Institute public database), was used to estimate the photosynthetically active radiation (PAR) at the surface ($I_0$), considering that PAR constitutes 45% of the total radiation reaching the water surface and a 4% reflection at the surface (Baker and Froiun, 1987). $I_0$ values were divided by the length of the light
period (10.6–14.5 h) and subsequently converted using 4.587 μEinstein s⁻¹ W⁻¹ (Morel and Smith, 1974). Mean light intensity in the mixed layer \( I_m = \mu \text{Einstein m}^{-2} \text{s}^{-1} \), which corresponds to the whole water column depth (6.5 m), was calculated according to the following equation (Jumars, 1993):

\[
I_m = I_0 \left( 1 - e^{-k_e Z_m} \right) (k_e Z_m)^{-1}
\]

where \( I_0 \) is the light intensity at the surface, \( k_e \) the light extinction coefficient (m⁻¹) and \( Z_m \) the depth of the mixed layer (m). The ratio mixing depth:euphotic depth \( (Z_m/Ze_u) \) was calculated as proposed by Cloern (1987).

Data on freshwater flow in Pulo do Lobo (70 km from the river mouth) and daily precipitation in Alcoutim were obtained from the Portuguese National Water Institute public database.

2.4. Nutrient concentration

Dissolved inorganic macronutrients (nitrate NO₃⁻, nitrite NO₂⁻, ammonium NH₄⁺, silicate SiO₄²⁻, orthophosphate PO₄³⁻) concentrations were determined in triplicate samples according to the spectrophotometric methods described by Grasshoff et al. (1983), using a spectrophotometer Hitachi U-2000, after being filtered through cellulose acetate filters (Whatman, pore diameter = 0.45 μm), treated with mercuric chloride (Merck) to a final concentration of 20 mg L⁻¹ (Kirkwood, 1992) and chilled (4 °C) until analysis.

2.5. Nutrient limitation

The potential nutrient limitation effect over phytoplankton growth will be discussed with respect to half-saturation constants described in the literature (Tilman et al., 1982; Dortch and Whitledge, 1992; Sarthou et al., 2005) and the optimal molar ratios between N, P and Si, i.e., N:P:Si = 16:1:16 (Redfield et al., 1963; Brzezinski, 1985) for marine phytoplankton growth.

2.6. Phytoplankton enumeration

Phytoplankton community composition was studied at Alcoutim through epifluorescence and inverted microscopy, following the methods of Haas (1982) and Utermohl (1958), respectively. Samples for enumeration of pico- and nanophytoplankton and cyanobacteria were preserved with glutaraldehyde (Merck, glutaraldehyde solution 25% for electron microscopy, final concentration 2% v/v), stained with proflavine (Fluka, 250 mg L⁻¹, final concentration 1% v/v) and filtered (<100 mmHg) onto black polycarbonate membrane filters (Whatman, pore diameter = 0.45 μm). Preparations were made with glass slides and non-fluorescent immersion oil (Cargille type A), within 24 h of sampling and then frozen (−20 °C) in dark conditions, to minimize loss of autofluorescence. Enumeration was made at 1250× magnification using the epifluorescence microscope Leica DM LB. Samples for enumeration of microphytoplankton were preserved with acid Lugol’s solution, settled in sedimentation chambers and observed at 400× magnification with the inverted microscope Zeiss Axiosvert S100 with phase contrast. In both techniques, phytoplankton cells were identified, whenever possible, to genus level, and 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 the cells were randomly distributed, the counting precision was ±10% (Venrick, 1978). Mean cell volume (MCV, μm³ cell⁻¹) was calculated using appropriate geometric configurations (Hillebrand et al., 1999), after measuring 20 cells of each taxon with a calibrated ocular micrometer. Biovolume was converted to biomass using appropriate allometric models. Cell carbon content (Cc, pg C cell⁻¹) was estimated with the equation \( Cc = aV^b \), where \( a \) and \( b \) are constants. For inverted microscopy the constants used were those described by Rocha and Duncan (1985), where \( a = 0.120 \) and \( b = 1.051 \). Verity et al. (1992) constants, \( a = 0.436 \) and \( b = 0.863 \), were used for epifluorescence microscopy. Phytoplankton composition is presented in terms of four taxonomic classes: diatoms (Bacillariophyceae), green algae (Chlorophyceae), cyanobacteria (Cyanophyceae) and others (comprises euglenophytes, dinoflagellates and other flagellates such as cryptophytes).

2.7. Statistical analysis

A non-parametric one-way analysis of variance (Kruskal–Wallis) was used to test differences in environmental variables, nutrient concentrations and nutrient ratios between sampling stations. Spearman rank correlations were used to evaluate relations between variables.

3. Results

3.1. Environmental variables

From February to October 2001, total daily precipitation at Alcoutim ranged between 0.0 and 48.5 mm (Fig. 2A) and was mostly concentrated in February, September and October. Freshwater flow at Pulo do Lobo, located 70 km from the river mouth, varied between 1.88 and 11.695.05 m³ s⁻¹, in August and February, respectively (Fig. 2B). High freshwater flow periods followed high precipitation events (\( r = 0.331, p < 0.001 \)).
Subsurface water temperature ranged from 17.7 to 28.0 °C with lower values during spring and autumn, and maximum values in the summer. Salinity varied only at the seaward end, Foz de Odeleite, with maximum values (7) in the summer; salinity at Mértola and Alcoutim was generally below detection level. Dissolved oxygen concentration ranged between 3.8 and 11.8 mg L\(^{-1}\), with higher values (> 8 mg L\(^{-1}\)) during spring and lower values from early summer onwards, at all stations (Table 1). Although temperature and dissolved oxygen concentration were not significantly different between stations (\(p > 0.05\)), there was a slight increase in temperature and decrease in oxygen concentration from downriver to upriver stations.

Suspended particulate matter (SPM) ranged from 9 to 135 mg L\(^{-1}\) (Table 1) and excluding an extreme value associated to an autumn precipitation event at Mértola, was significantly higher at Foz de Odeleite (\(p < 0.001\)). Secchi depth values varied from 0.2 to 1.1 m, were significantly lower at Foz de Odeleite and higher in Mértola (\(p < 0.001\)) and were negative and significantly correlated with SPM in these stations (\(r = -0.805\) and \(r = -0.780\), respectively, \(p < 0.001\)). Consequently, euphotic zone depth was lower at Foz de Odeleite (1.2 ± 0.6 m) and higher at Mértola (2.1 ± 0.6 m). \(Z_{\text{m}}:Z_{\text{eu}}\) ratio was significantly lower (\(p < 0.001\)) in Mértola, increasing downriver.

Mean light intensity in the mixing layer (\(I_m\)), which corresponds to the whole water column, varied between 12 and 110 μEinstein m\(^{-2}\) s\(^{-1}\) and was higher in Mértola, decreasing downriver (Table 1, Fig. 3). In Mértola and Odeleite, SPM was negative and strongly correlated with \(I_m\) (\(r = -0.756\) and \(r = -0.807\), respectively, \(p < 0.001\)), while in Alcoutim the correlation was not so strong (\(p < 0.05\)).

### 3.2. Nutrient concentrations and ratios

Analysis of variance showed that neither nutrient concentrations (DIN, PO\(_4\)\(^3-\), SiO\(_4\)\(^2-\)) nor nutrient ratios (N:P, Si:N, Si:P) were statistically different between the three sampling stations (\(p > 0.05\)). Table 2 shows minimum and maximum values of nutrient concentrations and ratios for different seasons at Alcoutim.

Dissolved inorganic nitrogen (DIN = NO\(_3\) + NO\(_2\) + NH\(_4\)) concentrations during the sampling period ranged between 6 and 100 μM (Fig. 4); the highest values were in the spring, diminishing through the summer. DIN concentration was dominated by NO\(_3\); this ion reached a minimum value of 1.5 μM in July and a maximum of 58.0 μM in April. Relative maximums in DIN in Mértola and Alcoutim (45.5 and 99.7 μM) are due to high NH\(_4\) (respectively 40.5 and 43.7 μM) and not due to an increase in NO\(_3\). Aside from these peaks, ammonium concentration was significantly higher (\(p < 0.05\)) at the landward end (9.4 ± 9.7 μM) than at the seaward end (3.2 ± 2.3 μM). Nitrite concentration was usually less than 1.0 μM throughout the sampling period.

Dissolved reactive silicate (DSi, measured as SiO\(_4\)\(^2-\)) peaked at all stations in early spring, and showed an

### Table 1

Means (\(n = 15\)), standard deviations (s.d.), minimum and maximum values for the environmental variables measured or estimated at different sampling stations (Mértola – upriver; Odeleite – downriver)

<table>
<thead>
<tr>
<th></th>
<th>Mértola</th>
<th>Alcoutim</th>
<th>Foz de Odeleite</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean ± s.d.</td>
<td>Minimum</td>
<td>Maximum</td>
</tr>
<tr>
<td>Temperature (°C)</td>
<td>24.0 ± 3.4</td>
<td>18.5</td>
<td>28.0</td>
</tr>
<tr>
<td>Salinity</td>
<td>0.0 ± 0.0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Dissolved oxygen (mg L(^{-1}))</td>
<td>7.9 ± 2.1</td>
<td>3.8</td>
<td>10.9</td>
</tr>
<tr>
<td>SPM (mg L(^{-1}))</td>
<td>23.6 ± 26.4</td>
<td>9.0</td>
<td>112.0</td>
</tr>
<tr>
<td>Secchi depth (m)</td>
<td>0.8 ± 0.2</td>
<td>0.3</td>
<td>1.1</td>
</tr>
<tr>
<td>Extinction coefficient (k(_{sc}) m(^{-1}))</td>
<td>2.0 ± 0.9</td>
<td>1.3</td>
<td>4.7</td>
</tr>
<tr>
<td>Euphotic depth (Z(_{eu}) m)</td>
<td>2.5 ± 0.7</td>
<td>1.0</td>
<td>3.6</td>
</tr>
<tr>
<td>(I_m) (μEinstein m(^{-2}) s(^{-1}))</td>
<td>72.1 ± 27.9</td>
<td>14.6</td>
<td>110.6</td>
</tr>
<tr>
<td>(Z_{m}:Z_{eu})</td>
<td>2.8 ± 1.3</td>
<td>1.8</td>
<td>6.6</td>
</tr>
</tbody>
</table>
an abrupt fall in mid-spring (from 100 to less than 10 \(\mu M\) at Mértola); during summer, DSi concentration was always lower than 20 \(\mu M\), beginning to rise in late summer. Dissolved reactive phosphate (DRP, measured as PO\(_4^{3-}\)) varied between 0.6 and 7.3 \(\mu M\); the highest values were determined in early autumn.

Si:N and Si:P ratios were, in the beginning of the sampling period, above 1 and 16, respectively (Fig. 5). From mid-spring to late summer Si:N ratio remained below 1 and Si:P above 16. From early spring to early summer, N:P ratio was above 16, but it remained below this value through summer. Finally, Si:N ratio increased in early autumn.

### 3.3. Phytoplankton community

Total phytoplankton abundance at Alcoutim ranged between \(1.8 \times 10^7\) cells L\(^{-1}\) in May and \(1.5 \times 10^8\) cells L\(^{-1}\) in the summer; the community was dominated by cyanobacteria, except during diatom spring bloom. Biomass varied from 221 \(\mu g\) C L\(^{-1}\) in early summer to 990 \(\mu g\) C L\(^{-1}\) in the spring; diatoms were always the main component of phytoplankton community biomass (Fig. 6).

#### Table 2

<table>
<thead>
<tr>
<th></th>
<th>Early spring</th>
<th>Late spring and summer</th>
<th>Autumn</th>
</tr>
</thead>
<tbody>
<tr>
<td>DIN</td>
<td>Min 59.5</td>
<td>4.6</td>
<td>10.8</td>
</tr>
<tr>
<td></td>
<td>Max 64.5</td>
<td>99.7</td>
<td>20.8</td>
</tr>
<tr>
<td>PO(_4^{3-})</td>
<td>Min 1.3</td>
<td>1.9</td>
<td>2.4</td>
</tr>
<tr>
<td></td>
<td>Max 2.0</td>
<td>3.1</td>
<td>2.7</td>
</tr>
<tr>
<td>SiO(_4^{4-})</td>
<td>Min 36.8</td>
<td>1.3</td>
<td>16.0</td>
</tr>
<tr>
<td></td>
<td>Max 87.0</td>
<td>36.0</td>
<td>78.9</td>
</tr>
<tr>
<td>N:P</td>
<td>Min 32.2</td>
<td>2.0</td>
<td>4.0</td>
</tr>
<tr>
<td></td>
<td>Max 45.8</td>
<td>34.4</td>
<td>7.7</td>
</tr>
<tr>
<td>Si:N</td>
<td>Min 0.6</td>
<td>0.1</td>
<td>1.4</td>
</tr>
<tr>
<td></td>
<td>Max 1.3</td>
<td>5.8</td>
<td>4.6</td>
</tr>
<tr>
<td>Si:P</td>
<td>Min 28.3</td>
<td>0.7</td>
<td>6.7</td>
</tr>
<tr>
<td></td>
<td>Max 43.5</td>
<td>11.6</td>
<td>18.3</td>
</tr>
</tbody>
</table>

In the beginning of the sampling period, the phytoplankton community was dominated by several flagellates, especially cryptophytes; diatom and green algae abundance was lower, less than \(3 \times 10^6\) cells L\(^{-1}\). In mid-spring, diatom abundance increased to \(10^7\) cells L\(^{-1}\), coincident with a decrease in flagellates and cyanobacteria abundance. This diatom spring bloom was mainly composed of centric chain-forming genera, such as *Melosira*. In late spring, an increase in green algae abundance was observed, dominated by *Scenedesmus*, decreasing in early summer. Then, a remarkable increase in cyanobacteria abundance was registered, reaching values higher than \(1.4 \times 10^6\) cells L\(^{-1}\). The dominant genus was the potentially toxic *Microcystis*, as well as non-identified picocyanobacteria. Although non-dominant, filamentous forms
such as *Anabaena* and *Oscillatoria* also displayed maximum abundance during summer. After the spring bloom, diatom abundance decreased to values of $10^6 \text{cells L}^{-1}$ until late summer (Fig. 6A); the most frequent genera were *Cyclotella*, *Coscinodiscus* and *Navicula*.

Seasonal variation of phytoplankton biomass showed a completely different pattern (Fig. 6B). Diatom dominated the community biomass throughout the spring–summer period. As described for the abundance, biomass peaked in mid-April, reaching 960 $\mu \text{g C L}^{-1}$ and then decreased to values of 130 $\mu \text{g C L}^{-1}$. This biomass maximum was coincident with a chlorophyll *a* concentration maximum (216.1 $\mu \text{g L}^{-1}$, data not shown). Cyanobacteria biomass never surpassed 9.5 $\mu \text{g C L}^{-1}$, which corresponded to the summer cyanobacteria bloom (1.4 $\times 10^8 \text{cells L}^{-1}$). Green algae biomass peaked during spring (280 $\mu \text{g C L}^{-1}$) and displayed minimum values in the end of summer (4 $\mu \text{g C L}^{-1}$). The remaining groups presented biomasses between 5.3 and 241 $\mu \text{g C L}^{-1}$.

Cyanobacteria abundance was positive and significantly correlated with water temperature ($r = 0.823$, $p < 0.001$) and negative and significantly correlated with both DIN ($r = -0.665$, $p < 0.05$) and N:P ($r = -0.765$, $p < 0.05$). All other correlations between phytoplankton groups and environmental variables were not statistically significant ($p > 0.05$).

4. Discussion

Variation in water temperature during the sampling period, with higher values in the summer and lower values in the spring and autumn, is typical of temperate regions. Salinity varied only at the seaward end, with maximum values in the summer, reflecting the effects of reduced freshwater flow and consequent seawater penetration upriver. Salinity fluctuations are indicative of the effects of the freshwater flow restriction from the Alqueva dam, completed in February 2002, which caused saltwater intrusion further upriver. Indeed, salinity values during summer 2002 and 2003 in Mértola and Alcoutim, previously freshwater locations, increased to 1 and 5, respectively, reaching 24 in Foz de Odeleite (C. Sobrino, personal communication). The negative and statistically significant correlations between SPM and $I_m$ indicate that light attenuation in the Guadiana upper estuary is mainly controlled by SPM. According to Cloern (1987), this is an important distinction between estuaries and the open ocean, where light attenuation is more strongly correlated with phytoplankton biomass.

Average light intensity in the mixed layer ranged between 12 and 110 $\mu \text{Einstein m}^{-2} \text{s}^{-1}$ and in Alcoutim was always lower than 83 $\mu \text{Einstein m}^{-2} \text{s}^{-1}$. These values are lower than saturating intensities referred for both phytoplankton monospecific cultures (ca. 200 $\mu \text{Einstein m}^{-2} \text{s}^{-1}$: Langdon, 1987) and estuarine phytoplankton communities (96–800 $\mu \text{Einstein m}^{-2} \text{s}^{-1}$: Fisher et al., 1982; Kocum et al., 2002b). Thus, light could have limited phytoplankton growth throughout the sampling period, particularly to non-motile phytoplankton cells. Indeed, from August onwards, $I_m$ values were even lower than the critical value of Riley (1957),...
40 langley d$^{-1}$ or 42 $\mu$Einstein PAR m$^{-2}$s$^{-1}$, and the ratio of the mixing depth vs. euphotic depth ($Z_m$/$Z_{eu}$) was higher than the critical mixing ratio value of 5 referred by Wofsy (1983) and Cloern (1987). This situation occurred permanently in Foz de Odeleite and discontinuously in Alcoutim, indicating that bloom development could be prevented. Nevertheless, other authors state that net primary production can still occur at ratios between 5 and 20 (Gobbelaar, 1990; Soetaert et al., 1994).

Phytoplankton biomass exhibited a uni-modal cycle with spring maxima. A diatom spring bloom was followed by increased abundance of green algae and a summer cyanobacteria bloom. This pattern has previously been observed in the Guadiana upper estuary (Rocha et al., 2002) and in other estuarine systems (Andersson et al., 1994). During the spring bloom, diatoms represented 52% of phytoplankton abundance and 97% of biomass. At the beginning of the sampling period (April 2001), the typical diatom spring bloom, widely described in the literature (Boney, 1989; Wetzel, 1993) and reported by Rocha et al. (2002) in the Guadiana estuary, had not yet occurred, since DSi concentrations were high, with Si:N > 1 and Si:P > 16. In the later work, DSi concentrations in early April, at Alcoutim, were inferior to 10 $\mu$M, therefore denoting a temporal delay on bloom initiation, which occurred in March in 1997 (Rocha et al., 2002) and in mid-April in 2001 (present study). An additional delay in diatom spring bloom inception probably occurred between the locations upriver and Foz de Odeleite, since DSi concentration fell abruptly only in May at the later location. Since spring blooms are classically associated with an increase in light intensity in the mixing layer, these differences in the timing of spring bloom may be related to inter-annual or spatial variations in spring irradiance (Iriarte and Purdie, 2004), given that nutrient concentrations are usually high and probably non-limiting throughout the winter (Galvão, unpublished data). In fact, Foz de Odeleite presented the lowest values of mean light intensity and the highest for SPM and is located in the estuarine turbidity maximum area (Bettemcourt et al., 2003). Furthermore, recent data show that phytoplankton biomass in the maximum turbidity zone is significantly lower ($p < 0.05$) than in the locations upriver (Domingues, unpublished data). However, variations in freshwater flow that can advect phytoplankton downriver (Sin et al., 1999), temperature and grazing pressure can also explain differences in spring bloom timing (Smayda, 1980).

The collapse of the diatom spring bloom occurred in early May in Alcoutim, and may be related to Si depletion due to a rapid consumption, and/or an increase in grazing impact. However, abundances of major zooplanktonic groups were relatively small during this period (Chicharo et al., 2001). According to Dortch and Whitledge (1992) (Si limitation if Si:N < 1 and DSi < 2 $\mu$M), Si was never limiting. However, application of criteria based on half-saturation constants should be done cautiously, because these constants have been obtained under equilibrium conditions, and are most likely to vary temporally, spatially, inter- and intra-specifically. For instance, Carpenter and Guillard (1971) refer that the same phytoplankton species have higher $K_S$ in coastal zones and lower in oceanic environments. In addition, both Tilman et al. (1982) and Sarthou et al. (2005) present a wide range of $K_S$ values for silica assimilation by freshwater and marine diatoms, between 0.12–19.5 and 0.2–22 $\mu$M, respectively, and DSi values at Alcoutim ranged between 1.9 and 15 $\mu$M from May to July.

Nutrient enrichment bioassays conducted in the Guadiana further support the hypothesis of silica limitation. Experimental addition of silica to Alcoutim water samples collected in June 2001 (initial concentration = 10 $\mu$M; concentration after Si addition = 140 $\mu$M) induced a significant increase in both silica consumption and diatom net growth rate relative to the control without silica addition (Domingues, unpublished data). By contrast, silica addition experiments undertaken in September 2001 (initial DSi = 29 $\mu$M) showed no significant effect in relation to the control. Overall, these data suggest that the diatom bloom collapse in May could be explained by silica limitation. Diatom spring bloom collapse in other temperate estuaries was also associated to silica limitation (Fisher et al., 1992; Kocum et al., 2002b). Additionally, other biomass losses, such as sinking, aggregation and cell autolysis, strongly dependent on the physiological state of the cells, may explain bloom collapse (Sarthou et al., 2005).

After the spring bloom, diatom abundance and biomass decreased and, simultaneously, an increase in green algae abundance was observed. Low Si and high N:P enhance green algae development whereas high N:P and Si:P ratios favour diatom growth (Roelke et al., 1999 and references therein). Moreover, transition from diatom to non-diatom species occurs between Si:P from 3 to 20 (Tilman et al., 1986) and Si:N from 0.3 to 1 (Sommer, 1996). In the Guadiana, Si:P and Si:N were, respectively, less than 16 and 1 during the spring–summer transition.

Green algae showed maximum contributions to phytoplankton abundance (12%) and biomass (38%) during May and June. According to Dortch and Whitledge (1992) (P limitation if N:P > 30 and PO$_4^{-}$ < 0.2 $\mu$M), P was never limiting to phytoplankton growth, even considering the $K_S$ values (0.03–1.89 $\mu$M) referred by Tilman et al. (1982). As such, according to Reynolds (1999) nutrient ratios should have been irrelevant to succession. However, a counter view, supported by extensive experimental evidence, is presented by
other authors (Bulgakov and Levich, 1999; Smith and Bennett, 1999), who state that variations in the ratios of growth-limiting nutrients can determine species relative biomass while absolute nutrient concentrations determine only the total biomass of the community. Considering the seasonal evolution of nutrient ratios and phytoplankton succession in the Guadiana, this view could also be acceptable. In fact, green algae were dominated by *Scenedesmus*, consistent with Rhee (1978), which indicated that the ideal N:P ratio for *Scenedesmus* growth is 30; indeed, N:P ratio was approximately 30 in Alcoutim during this period.

From late spring onwards cyanobacteria abundance showed a pronounced increase, attaining its maximum (1.4 × 10⁸ cells L⁻¹) during July. This bloom, composed mainly of non-identified picocyanobacteria and *Microcystis*, persisted throughout the summer. Maximum contributions of cyanobacteria reached 98 and 2% of total phytoplankton abundance and biomass during August. Green algae dominate over cyanobacteria in P competition, so they tend to have a competitive advantage with low Si:P and high N:P (Sommer, 1989). This happened while green algae bloomed, but in the summer N:P decreased to less than 5; according to Sommer (1999), the transition from green algae to cyanobacteria dominance lies around N:P ratios of 15, explaining the replacement of green algae by cyanobacteria. In these conditions, this group would have competitive advantage over green algae, thus developing and growing abundantly. This excess of P over N also favours nitrogen-fixing cyanobacteria (Sellner, 1997); indeed, nitrogen-fixing forms such as *Anabaena* were also more abundant during this period. Independent of nutrient ratios, the general decrease in both nutrient concentrations and light availability should promote higher contributions of smaller sized cells with a higher surface to volume ratio, such as picocyanobacteria and *Microcystis*. In fact, higher contributions of smaller cells during summer are reported for several estuaries (Cole et al., 1986; Sin et al., 2000). In addition to nutrients, the increase in temperature in summer, reaching values of 27 °C, could also explain cyanobacteria increase (Andersson et al., 1994); this probably happened in the Guadiana estuary, given that cyanobacteria abundance was positive and significantly correlated with water temperature. Furthermore, according to Tilman and Kiesling (1984) and Tilman et al. (1986) experiments, cyanobacteria are never dominant below 17 °C while at 24 °C this group is dominant at all N:P ratios inferior to 20. Also, it is well established that, in general, these organisms attain maximum growth rates at temperatures higher than for diatoms and green algae; this explains why in most temperate and boreal water bodies cyanobacteria bloom during summer (Mur et al., 1999). The fact that cyanobacteria suffer fewer cell losses than green algae by sedimentation (Sommer, 1989) and grazing (Sterner, 1989), may also have contributed to the dominance of these organisms during summer period. Engström-Ost (2002 and references therein) stated that, due to their early evolutionary history, cyanobacteria have developed an array of qualities that may favour them in competition with other organisms. These include, besides those already pointed, allelopathic compounds, grazing resistance, N and P storage capacity, ability to grow at low light, as well as tolerance of high pH or low CO₂ concentration and buoyancy. Actually, buoyancy control could represent an important competitive attribute during summer minimum mixed layer light intensities.

The increase in DSi concentration observed in late summer, probably due to the regeneration of diatom frustules and precipitation events that lead to terrestrial runoff and increased river flow, was already reported by Rocha et al. (2002) in the Guadiana estuary. However, contrary to the later study, diatom abundance did not increase during this event. The high metazooplankton abundance during this period (Chicharo et al., 2001), and the relatively low irradiance in the mixing layer, between 10 and 50 µEinstein m⁻²s⁻¹, could explain this result.

It is important to emphasize that phytoplankton community composition and its seasonal variability represent the interaction of species specific net growth rates, thus combining variability of specific growth and loss rates. Therefore, specific losses such as grazing, viral lysis and autolysis, could also explain phytoplankton succession and should be evaluated in future studies of the Guadiana estuary.

5. Conclusions

During 2001, phytoplankton succession in the Guadiana River upper estuary showed a clear transition from a diatom to non-diatom based assemblage. A relationship between phytoplankton succession and nutrient ratios seemed to exist. In early spring, N:P was high, Si was abundant and a diatom bloom occurred. This bloom collapsed and an increase in green algae abundance was observed later in spring, with low Si and high N:P. In the summer, N:P and Si were low, and a cyanobacteria bloom developed. In addition, light was probably limiting throughout the sampling period, particularly to non-motile phytoplankton cells.

But would the observed successional pattern be different if there was a high Si:N ratio, maintaining a low N:P, even though cyanobacteria are better competitors for N than diatoms? Recent data (2002–2003) from the Guadiana upper estuary show that even with high Si concentrations, high Si:N and low N:P, cyanobacteria dominate the phytoplankton community.
Cyanobacteria blooms seem to be a growing problem in the Guadiana, and are expected to be enhanced from 2002 onwards, due to severe water flow restriction by the Alqueva dam. Indeed, cyanobacteria blooms have recently occurred, in 2002—2003, not only during summer, but in the autumn and winter as well (Domingues, unpublished data).

Acknowledgements

The authors would like to thank Pedro Morais for numerous suggestions, friendship and for providing the maps, Susana Vidal for support during field work and all the staff at the Microbiology and Biological Oceanography labs for support during lab work. We would also like to thank the anonymous reviewers, whose comments greatly improved the original manuscript.

References


