

Use of a hydrotechnical infrastructure (Alqueva Dam) to regulate planktonic assemblages in the Guadiana estuary: Basis for sustainable water and ecosystem services management

Luis Chícharo*, M. Alexandra Chícharo, Radhouane Ben-Hamadou

*Universidade do Algarve, Faculdade de Ciências do Mar e do Ambiente (FCMA), Centro de Ciências do Mar (CCMAR),
Campus de Gambelas, 8005-139 Faro, Portugal*

Received 15 October 2005; accepted 26 March 2006

Available online 21 July 2006

Abstract

The phytoplankton and zooplankton assemblages in the Guadiana estuary (south Portugal) were compared in two contrasting hydrologic years, characterized by high and low freshwater discharges. Changes in salinity, nutrients and turbidity, which were mainly due to the changes in freshwater input, had an important influence on the structure of the planktonic assemblages. Nevertheless, coastal upwelling can also influence the productivity of the lower estuarine area during short periods both in winter and in summer. In 2000, a short-term freshwater pulse during May (average $126.46 \text{ m}^3 \text{ s}^{-1}$) was markedly beneficial; phytoplanktonic diversity increased, and this caused an increase in zooplanktonic diversity. As phyto-zooplankton coupling in these systems is an important regulator of processes in the trophic web, changes in the dominant groups can have consequences on water quality, especially on the occurrence of toxic plankton blooms. Modelling simulations indicate that a day discharge of $50 \text{ m}^3 \text{ s}^{-1}$, every week during critical periods, such as summer/autumn, could be enough to avoid cyanobacteria blooms. Since freshwater “pulses” can be managed by operating the freshwater release from hydrotechnical structures, understanding the relationships between the periodicity and magnitude of inflow pulse events and the estuarine ecosystem structure and healthy functioning is a crucial step towards the development of management modelling tools, as proposed in this study.

© 2006 Elsevier Ltd. All rights reserved.

Keywords: phytoplankton; zooplankton inflow; estuarine environment; Guadiana; ecohydrology; Harmful Algal Bloom HAB; modelling; water quality

1. Introduction

Studies of plankton dynamics in estuarine and coastal areas often neglect the role of freshwater inflow and runoff (e.g. for temperate estuaries, see Bode et al., 2005; Marques et al., 2006). However, factors affecting the balance between fresh and salt water are crucial and, worldwide, the modification in freshwater volume discharged into an estuary is a major determinant of the structure and function of the estuarine ecosystem (Sklar and Browder, 1998). Modifications in the freshwater discharge affect the estuarine biological and non-

biological factors, both in the short and long term. Major biological effects include: (a) modifications in productivity of the various trophic levels (Binet et al., 1995); (b) shifts in phytoplankton species composition (Alpine and Cloern, 1992; Cloern and Dufford, 2005); (c) alterations in the distribution areas of zooplanktonic species (Kingsford and Suthers, 1994); (d) invasions by alien species and effect on endemisms (Bunn and Arthington, 2002); and (e) reduction of fish stock mainly caused by physical obstructions to migration, decrease in availability of food items (Doornbos, 1982; Chícharo et al., 2002) and the destruction of spawning and nursery areas (Costa, 1988; Drake et al., 2002). Modifications to freshwater discharge are also responsible for non-biological effects such as: (a) changes in the downstream sediment transport to estuaries and coastal areas (Gonzalez et al., 2001; Dias et al., 2004);

* Corresponding author.

E-mail address: lichichar@ualg.pt (L. Chícharo).

and (b) changes in the input of nutrients, mainly affecting the concentration of dissolved silicate and the N:P:Si ratio (Rocha et al., 2002).

In the Guadiana estuary, under the influence of the Mediterranean climate, natural river inflows typically vary markedly, within and between years, as a result of seasonal and annual fluctuations in rainfall. In addition to this natural variation in freshwater discharge into the estuary, extensive urban and agricultural development in the Guadiana River basin, mainly since the 1950s, has progressively reduced the amount of freshwater, thereby, contributing to a decrease in the quantity (Dias et al., 2004) and quality of the water that reaches the estuary and the coastal zone (Chícharo et al., 2001; Rocha et al., 2002).

However, it is the beginning of the operation of the Alqueva Dam, in February 2002, that by doubling the volume of water already stored in the Guadiana River dams to approximately 8,000 hm³, will be responsible for most changes in the ecosystems downstream. The decrease in freshwater discharge and nutrient loading affects the primary productivity of the estuary and coastal zone (Sklar and Browder, 1998) and, hence, influences the trophic structure and ecosystem sustainability in these areas. The first effects are expected to occur in the planktonic assemblages. Decreases in the availability of silica relative to N and P may result in a shift in the phytoplanktonic community from a dominance of diatoms to other phytoplanktonic forms as cyanobacteria (Rocha et al., 2002). Moreover, anthropogenic inputs in the Guadiana basin, mainly of agricultural fertilizers, can cause phosphorus and nitrogen to be reintroduced into the system, thereby, leading to eutrophication.

Frequently, planktonic processes in estuaries are analysed within a trophic group, e.g. phytoplankton, zooplankton or ichthyoplankton (e.g. Ramos et al., 2006; Marques et al., 2006), and not in an integrative way. A holistic approach should also include the major factors responsible for changes in the ecosystem, such as hydrotechnical infrastructures. The present work aimed to: (1) characterize the planktonic assemblages in the Guadiana estuary under two different freshwater regimes, in order to establish a point of reference; (2) evaluate the contribution of major environmental variables related with the freshwater inflow on the estuarine planktonic assemblages; and (3) use the results as the basis for modelling the use of the hydrotechnical infrastructure to manage the planktonic response to modification in the freshwater inflow.

2. Materials and methods

2.1. Study area

The Guadiana River estuary is on the southeastern border between Portugal and Spain (Fig. 1). Its catchment basin is the fourth largest in the Iberian Peninsula, ~67,500 km². The climate, typically Mediterranean, is classified as semi-arid, being arid in July and August, and temperate-humid from November to January (Morales, 1993). Accordingly, the Guadiana area shows clear seasonal and inter-annual variations, characterized by severe droughts and heavy floods. Historical data of the freshwater flow, measured at the

hydrometric station of Pulo do Lobo (37°48'N, 7°38'W) a few kilometres above the last point of tidal influence (Mértola) and from the uppermost sampling station (Alcoutim), record values between 6.07 and 2491.70 m³ s⁻¹. The average flow of the wettest year (1963/1964) was 436.4 m³ s⁻¹, and of the driest year (1980/1981) was 7.99 m³ s⁻¹.

The Guadiana basin is under increasing pressure for exploitation of water resources, especially since the 1960s, with the construction of dams and reservoirs that affect the river and its tributaries. With the Alqueva Dam, operating since 2002, the total volume of water retained in the Guadiana River and not reaching the estuary and coastal area is estimated to be 13,000 hm³ year⁻¹ (Dias et al., 2004). The resulting regulation of the freshwater discharge affects the frequency and style of the floods in the river basin. The duration of the flood events has diminished and the water level downstream usually rises abruptly, within the space of one because dam floodgates are opened when water in the storages reaches critical levels.

As a consequence, the Guadiana estuary shifts between being freshwater dominated during winter and flood periods, and being a salt-wedge estuary during neap tides in spring and summer months (Rocha et al., 2002). The Guadiana estuary is also influenced by upwelling events.

In this study, the Guadiana estuary was divided into three sub-areas: upper, middle and lower estuary, according to the studies by Olausson and Cato (1980), Gonzalez (1995) and Chícharo et al. (2001). The upper area is mainly the freshwater section, still with a tidal influence, but with salinity close to zero (tidal freshwater < 0.5). The middle section is the salinity-mixing zone (0.5–25), while in the lower area, salinity is usually very close to that of seawater (>25).

2.2. Environmental parameters

Freshwater inflow data measured at the Pulo do Lobo hydrometric station were obtained from the Instituto Nacional da Água (<http://snirh.inag.pt/>). The data covered a hydrologic year, starting in October and ending in September of the following year. Wind direction data were obtained from the INAG meteorological station in Castro Marim, in the lower estuarine area.

Water temperature in the oceanic area for the sampling days was inferred from satellite images (size 760 × 1100 and resolution of 8 bits/pixel) obtained by NOAA 17 (www.npm.ac.uk/rsdas/data).

Six sampling stations – two in each estuary sub-area (Fig. 1) – were sampled during ebb tides, with a periodicity of two/three months, from December 1999 to November 2001.

At each station, water column temperature and salinity profiles were analysed with a YSI 6600 CTD. Water samples for analysis of concentrations of seston, nutrients (ammonia, nitrate, orthophosphate and silicate) and chlorophyll *a* were collected at the surface and near the bottom with a Van Dorn bottle, and kept in the dark and cooled until sample processing.

Seston and organic matter were determined by filtering water samples through pre-ignited and pre-weighed 0.7-µm pore filters (Whatman GF/F). After sample filtration, filters were washed with distilled water, dried at 60 °C and

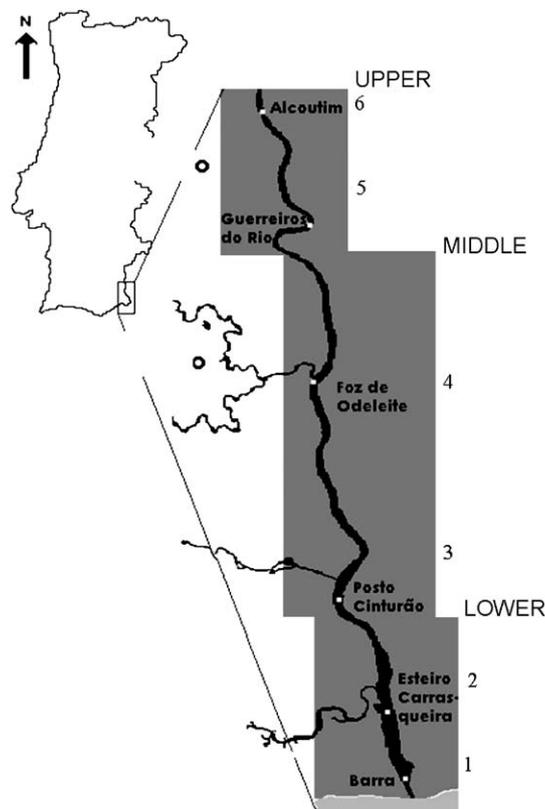


Fig. 1. Division of the Guadiana estuary into upper, middle and lower estuary areas.

reweighed after cooling for seston determination. To determine organic matter, dried filters were placed in an oven for 4 h at 450 °C to burn all the organic matter retained in them, and the filters were reweighed after cooling.

For nutrient analyses, water samples were filtered through 0.45- μ m pore cellulose filters (MSI) and preserved frozen until spectrophotometric analyses (Kirkwood, 1996), which were conducted according to the protocols of Grasshoff et al. (1983).

Chlorophyll *a* concentration was determined by filtering water samples through 0.7- μ m pore filters (Whatman GF/F). The filtration pressure did not exceed 100 mm Hg, and the filters were kept frozen (–20 °C) until spectrofluorimetric analysis using acetone as the extraction solvent (Welschmeyer, 1994).

2.3. Planktonic community

Planktonic samples were collected simultaneously with the measurements of environmental parameters. Phytoplankton samples were preserved in acid lugol (6.25% v/v, final concentration) and processed according to the Utermöhl method (Hassle, 1978). Samples were amplified 400 \times in a Zeiss IM35 inverted microscope and cells were counted according to Venrick (1978).

Mesozooplankton (Holo and merozooplankton) samples were collected with a conical net (0.37 \times 1.60 m, 0.3 mm mesh), towed horizontally at 1 m depth for approximately 10 min, at a constant speed of two knots. The volume filtered in the tows

was determined from the number of rotations of the flow meter placed in the mouth of the net. Samples were preserved in 4% buffered formaldehyde solution and processed for taxonomic identification and counting with a binocular microscope.

2.4. Data analysis

The diversity of the planktonic assemblage was calculated with the Average Taxonomic Distinctness Index developed by Clarke and Warwick (2001), which reflects the expected taxonomic distance between any two individuals chosen at random from a sample, provided those two individuals are not of the same species. This index removes the effect of dominant species and reflects more purely the taxonomic hierarchy, while these are discrete distances in a standard Linnaean classification. According to this index, the greatest taxonomic distance (ω) between two species is considered to be 100 and equal step lengths between each successive taxonomic level are assumed. Thus, three taxonomic levels are considered: species, genus and order or class, respectively, valued as: $\omega = 33.3$ (species of the same genus), $\omega = 66.6$ (same order or class but different genera) and $\omega = 100$ (species from different classes or orders).

Seasonal and spatial patterns in phytoplankton and zooplankton assemblages were analysed by non-metric multidimensional scaling (MDS) based on triangular matrices of Bray–Curtis measures of similarity of log-transformed data.

The resultant groups of stations were further analysed by non-parametric ANOSIM to determine whether they were significantly different.

When appropriate, *R*-statistic values for pair-wise comparisons provided by ANOSIM were used to determine the dissimilarity between groups. Values close to 1 indicate a very different composition, while values near 0 show little difference. *R*-statistic values were also used to test the null hypothesis that within a group of samplings there have been no changes in community structure. All these statistical analyses used the PRIMER 5 Software ‘Plymouth Routines in Multivariate Ecological Research’.

Horizontal water column profiles showing the surface distribution of temperature and salinity were made with *Surfer 8.01* (Golden Software, Inc.), using kriging as the gridding method. Other graphs were elaborated with the program Microsoft Excel 2002 (Microsoft®).

Existence of significant relationships between the parameters analysed was tested with Spearman’s correlation. To avoid assuming a significant correlation due to random processes, the Bonferroni’s inequalities were used in the analysis. The value of $t_{0.05}$ was corrected to $t_{0.05/n'}$ (where n' is the number of pairs of correlations in the correlation matrix). Only after applying this correction did we verify whether a correlation was significant using Spearman’s test. The latter statistical analyses were made with the software package STATISTICA V.5 (StatSoft®).

2.5. Hydro and phytotechnology model

A generic model to illustrate the major dynamic properties of phytoplankton succession under bottom-up and top-down control was developed using MATLAB 6.5 Software. The model consists of three nutrient compartments (Nitrogen “N”, Phosphate “P” and Silica “Si”), two phytoplankton compartments (Diatoms “D” and Cyanobacteria “CB”) and a grazer compartment “H”. Nutrient inputs are conditioned by flow discharge. Both phytoplankton groups assimilate N and P, Silica is taken up only by diatoms. Nutrient assimilation

is conditioned by light limitation “LL” modelled as a sinusoidal function. Herbivore grazing concerns both phytoplankton groups, but preferentially upon diatoms. The nitrogen compartment is regenerated by herbivore releases. All biological state variables are affected by mortality, which eliminates the relative biomass from the system. Biological parameters were derived mainly from field experiments conducted at the Guadiana estuary. The structure of the model (state variables and the process linking them) is schematically illustrated in Fig. 2 and mathematically explained in Appendix 1.

3. Results

3.1. Environmental data

During the hydrologic year of 2000 (October 1999 to September 2000) an abnormal situation was registered, with low inflow values during winter months, with a minimum in March ($8.68 \text{ m}^3 \text{ s}^{-1}$) and a maximum during spring, in May ($128.46 \text{ m}^3 \text{ s}^{-1}$). Guadiana River, monthly during the hydrologic year of 2001 (October 2000 to September 2001) averaged $242.35 \text{ m}^3 \text{ s}^{-1}$, with its maximum in winter ($721 \text{ m}^3 \text{ s}^{-1}$ in February) and lower values throughout spring and summer (as low as $6.6 \text{ m}^3 \text{ s}^{-1}$ in September 2001) (Fig. 3A). The 2001 values were above average ($158 \text{ m}^3 \text{ s}^{-1}$ for the period 1948–1990), whereas the 2000 values were below average. The low inflow situation was interrupted in early May 2000, with the occurrence of a peak of freshwater discharge (Fig. 3B).

The minimum water temperatures occurred in the upper estuary: $12.4 \text{ }^\circ\text{C}$ in January 2001, and $13.8 \text{ }^\circ\text{C}$ in December 1999. Maximum values occurred in all estuary in September in both 2000 ($23.1 \text{ }^\circ\text{C}$) and 2001 ($23.7 \text{ }^\circ\text{C}$).

Highest salinity values were registered in the lower estuarine area, in July 2000 (37.98), and in September 2001 (35). As expected, the lowest salinity values were measured in the upper estuary: in the spring of 2001 (0.18 in May) and in the winter of 2000 (0.0 in February). In 2000, the low inflow year, salinity values were higher and more homogeneous through the estuary, without seasonal variation. In 2001, the

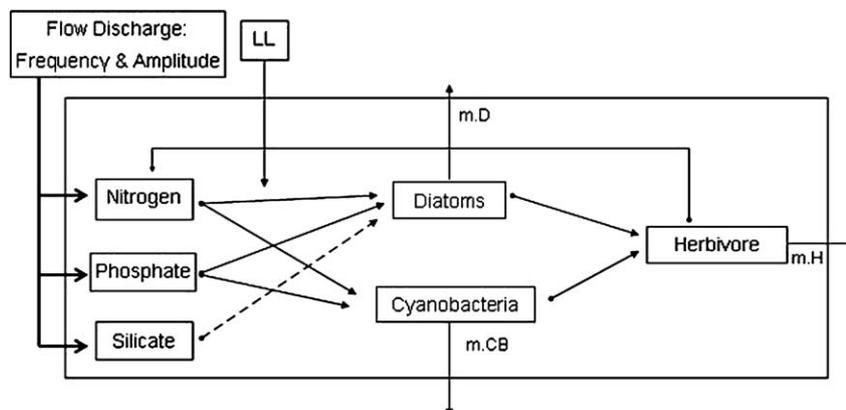


Fig. 2. Model structure with state variables and processes linking them. Mathematical explanation in Appendix 1. (Nitrogen “N”, Phosphate “P” and Silica “Si”, Diatoms “D” and Cyanobacteria “CB”, grazer compartment “H”, light limitation “LL”).

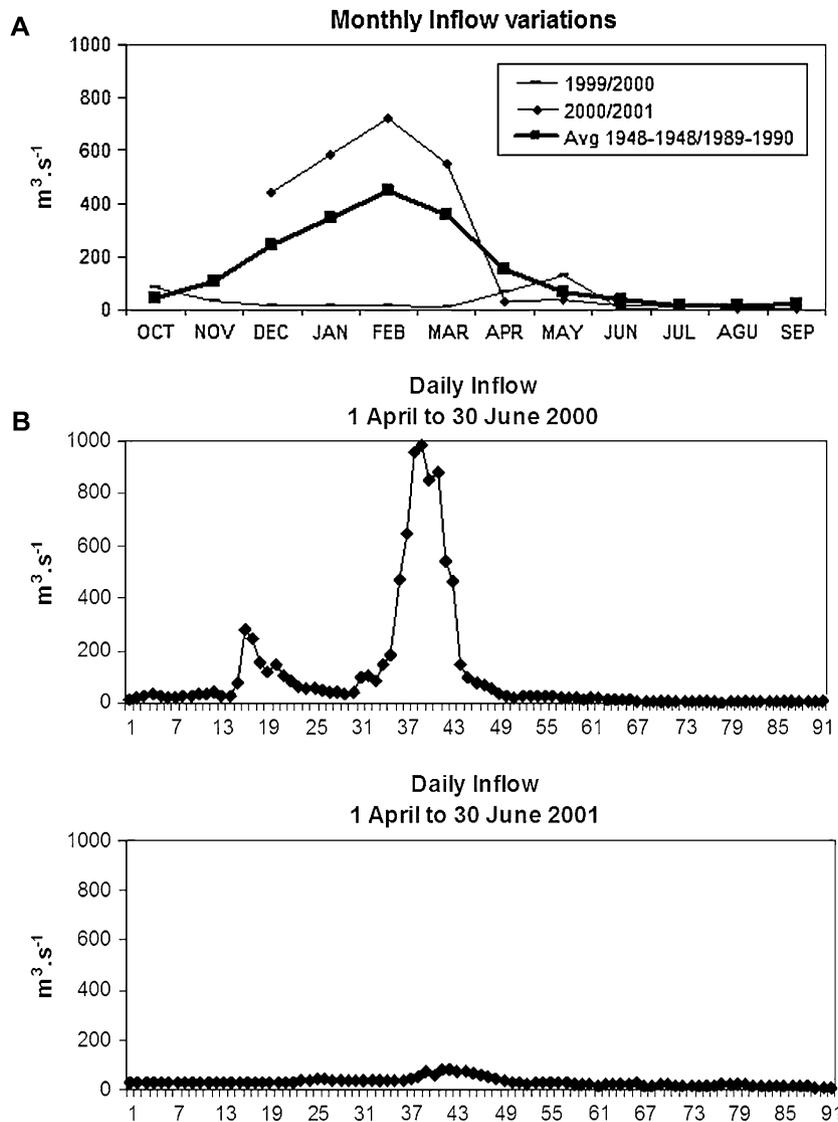


Fig. 3. (A) Average monthly inflow variations in two contrasting hydrologic years in the Guadiana estuary: 1999/2000 and 2001/2002. Freshwater outflow was measured at the hydrometric stations Pulo do Lobo and Rocha da Galé a few kilometres above the last point of tidal influence (Mértola) and from the last station (Alcutim). (B) Average daily inflow variations during Spring/Summer, 1999/2000 and 2001/2002. Data obtained through the INAG web site (<http://snirh.inag.pt/>).

high-inflow year, the estuary was characterized by a sharp decrease in salinity from the sea to the upper estuary, and from summer to winter (Fig. 4).

Seston and organic matter values were highest in the upper estuary (seston 690 mg L^{-1} , December 2000), and lowest in the low estuary (seston 25 mg L^{-1} , July 2000) (Fig. 5). The highest silicate concentrations were obtained from water samples collected in the upper estuary, during winter ($283.17 \mu\text{M}$ in March 2001) although there was a sharp increase from 2000 to 2001. The lowest silicate concentration ($0.23 \mu\text{M}$) occurred in the low estuary, in July 2001.

Nitrate concentration varied from $134.9 \mu\text{M}$, in March 2001 at the uppermost station, to $0 \mu\text{M}$ during summer months at the lower estuary stations, in both the years analysed.

Phosphate concentrations varied markedly between years, with higher values during 2000. The maximum concentration measured during the entire sampling ($48.0 \mu\text{M}$) occurred in the low estuary area, in May 2000. The minimum

phosphate concentration also occurred in this area, in July 2001 ($0.02 \mu\text{M}$). Similarly, ammonia concentration was higher during 2000; the highest concentration was measured in the middle estuarine area ($62.43 \mu\text{M}$), and the lowest ($0.5 \mu\text{M}$) in the lower estuary (Fig. 5).

The correlation between salinity and nutrient concentrations indicates an inverse relationship with 95% significance between salinity and nitrates ($r = -0.383$) and between salinity and silicates ($r = -0.303$). Nutrient concentrations were also correlated with values of the freshwater discharge; significant (95%) and positive relations were observed for inflow with silicate ($r = 0.315$) and nitrate ($r = 0.498$) concentrations (Table 1).

Higher values of chlorophyll *a* were usually measured in the middle–upper estuary areas, with the highest being in October in both 2000 ($9.75 \mu\text{g L}^{-1}$) and 2001 ($10.89 \mu\text{g L}^{-1}$). Lower values were measured in the low estuary, under the predominantly marine influence, with the minimum being

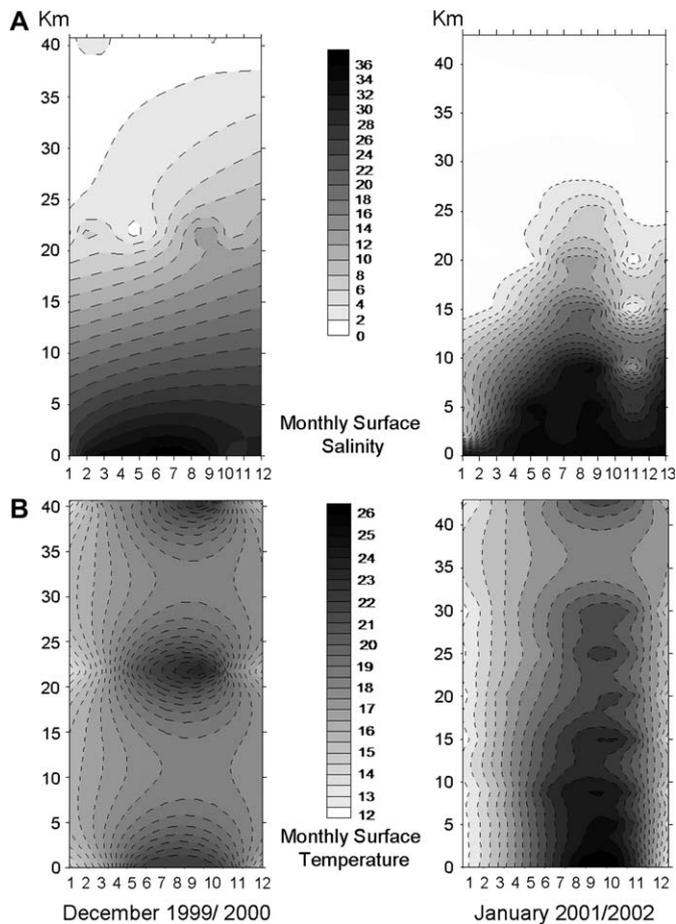


Fig. 4. (A) Longitudinal gradient of isohalines; (B) longitudinal gradient of isothermics in the Guadiana estuary.

$0.01 \mu\text{g L}^{-1}$ in March 2001 (Fig. 5). However, high chlorophyll concentrations were measured in the low estuary in December 1999 ($10.56 \mu\text{g L}^{-1}$) and July 2001 ($9.98 \mu\text{g L}^{-1}$). On both these occasions, sea surface temperature (SST) data obtained from satellite images indicate a decrease in water temperature in the coastal area adjacent to the Guadiana estuary. These periods were coincident with the occurrence of the westward winds (Fig. 6).

3.2. Planktonic assemblages

3.2.1. Phytoplankton

Typically, phytoplankton abundance was higher during spring and summer months, and was dominated in the upper/middle estuary (freshwater – oligohaline) by chlorophytes (mainly *Dictyosphaerium reniforme*, *Crucigenia tetrapedia*, *Scenedesmus acutus*), unidentified Cryptophyceae, diatoms (*Cyclotella* sp., *Melosira* sp., *Leptocylindrus minimus*) and cyanobacteria (mainly *Microcystis* spp.). In the lower marine meso-polyhaline estuary, the most abundant groups were: dinoflagellates (*Gymnodinium* spp.), plastidic nanoflagellates and diatoms (mainly *Thalassionema nitzschioides*, *Chaetoceros* spp., *Leptocylindrus danicus*, *Guinardia delicatula*, *Skellertonema costatum* and *Navicula* spp.) (Fig. 7). However,

despite the above-described general trend, there was an important shift in the phytoplankton assemblages in the estuary between the two hydrologic years analysed: phytoplankton abundance was very low in 2000 in comparison with 2001. Moreover, MDS plots of density of the phytoplanktonic community in 2000 and 2001 (Fig. 8A) show a modification in the community structure. The temporal and spatial shifts in the distribution of the phytoplanktonic assemblages within the Guadiana estuary were stressed by the ANOSIM run between the two hydrologic years, where global R is equal to 0.402 with a significance level of 0.1%. As shown above for silicate, the environmental factors linked to this change are dependent on inflow regime (Fig. 8B).

3.2.2. Zooplankton

In the upper and middle areas of the estuary, the zooplankton was dominated by copepods (mainly *Acartia clausii* and *Calanipeda aquaedulcis*) and cladocerans (mainly *Bosmina longirostris*). In the lower area, besides holozooplankton such as Copepoda (*A. clausii* and *Euterpina acutifrons*) and Cladocera (*Evadne* spp. and *Podon* spp.), several groups of merozooplankton were present namely, decapod larvae (e.g. *Paleomon* sp. zoea), fish eggs and larvae (mainly *Engraulis encrasicolus*) and mysids (especially *Mesopodopsis slabberi*). Major zooplankton taxa did not appear in consistent numbers during periods of high river inflow (winter), although meroplankton was especially present with highest densities during the summer that followed a winter with high inflow, i.e. the hydrologic year of 2001. Holozooplankton groups were more abundant during spring (copepods: 599×10^4 individuals 100 m^{-3} in April 2000 in the middle estuary) and summer (e.g. cladocera: 25×10^4 individuals 100 m^{-3} in June 2000 in the upper estuary) (Fig. 7). Merozooplankton taxa were especially abundant during summer, e.g. crustacean larvae 8124 individuals 100 m^{-3} (September 2000) and fish eggs 1.9×10^4 individuals 100 m^{-3} (June 2001) (Fig. 7).

Also the zooplankton assemblages, as also observed with the phytoplankton, had shifted between the two hydrologic years. During the dry year of 2000, very low abundances of merozooplankton taxa occurred. However, holozooplankton abundance was high for the same period. Thus, there was a change in both abundance and dominance of zooplanktonic groups – meroplankton and holozooplankton – from 2000 to 2001, i.e. from a dry to a wet year. This change in community structure was shown in MDS plots of abundance of the zooplanktonic community in different areas and months in the Guadiana estuary and by the ANOSIM run for the 2000 and 2001 hydrologic years (Global R , 0.245; significance level 0.1%) and by changes in diversity (Fig. 9).

Spearman's correlation test, calculated for both years, showed a significant correlation between copepod density and ammonia concentration ($r = 0.531$, <0.05) (Table 1).

3.3. Model simulations

Simulations with different settings (outflow amplitude from the dam at different frequencies of dropping) show a large

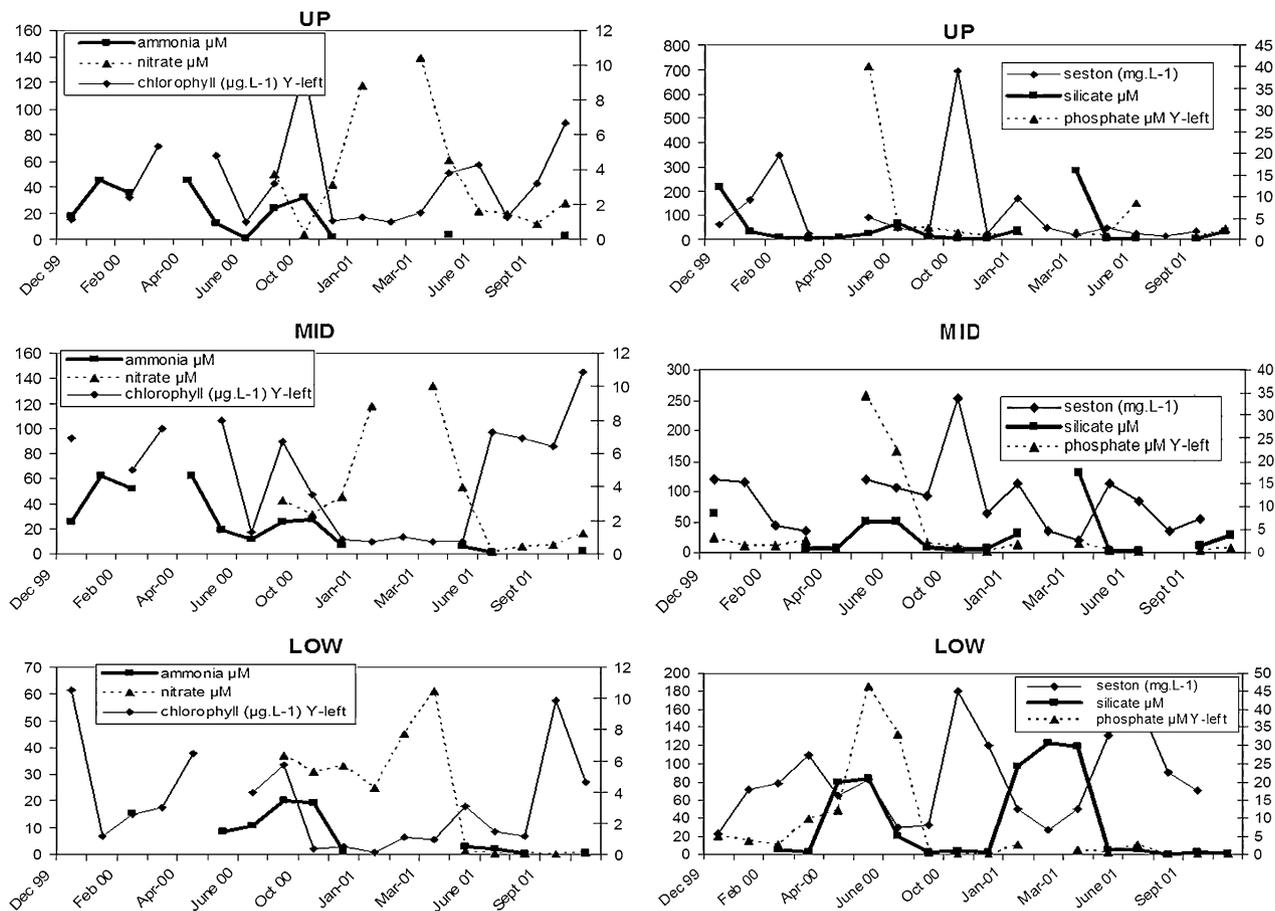


Fig. 5. Average surface and bottom water parameters in 2000 (hydrologic year 1999/2000) and in 2001 (hydrologic year 2000/2001).

variation in biological responses despite the realistic values considered. Model sensitivity tests (Fig. 10) showed that for an equal mean flow during the simulation period ($5 \text{ m}^3 \text{ s}^{-1}$), but corresponding to different pairs of flow amplitude and frequencies, the frequency of the discharge events seems to be more critical to the dynamics of the different biological compartments than is amplitude. Keeping a low, but continuous inflow within the estuary provides a stationary state of the different biological compartments. With an increase in flow amplitude but separated in time the biological system presents an unstable dynamic. For high frequencies, cyanobacteria could surpass the diatoms until new nutrient enrichment (particularly silicate) is provided for the estuary system. Model sensitivity tests (Fig. 11) showed that for a low flow such as the ecological flow for the Guadiana River ($1\text{--}2 \text{ m}^3 \text{ s}^{-1}$), cyanobacteria blooms will occur every day during the simulation period (60 days).

The third set of simulations (Fig. 12) confirms this statement, although high-amplitude flushes ($50 \text{ m}^3 \text{ s}^{-1}$ freshwater pulse every week) can, when repeated, exclude the cyanobacterial community from the system.

4. Discussion

This study has showed how phytoplankton and zooplankton assemblages are affected by natural modifications in

freshwater discharge. Such observations have been used to estimate the effect of the reduction of freshwater discharge in the Guadiana River, and the consequences, to the estuarine and coastal planktonic assemblages, of the construction of the Alqueva Dam across the river. Management of the freshwater discharge by this hydrotechnical infrastructure is crucial to the sustainability of planktonic assemblages and the dependent food-web structure, particularly under the forecast changes in hydrologic regimes associated with climatic changes.

Since planktonic assemblages are the first trophic level in aquatic ecosystems, they are highly susceptible to changes in abiotic conditions (bottom-up control). The contrasting abiotic conditions observed during the years analysed in this study can be attributed to the differences in the volume of freshwater discharged. Such differences can be related to global phenomena, such as the North Atlantic Oscillation (NAO) (Dias et al., 2004). In the year 2000, a positive phase of the NAO (2.80) occurred, whereas in 2001 a negative phase was recorded (-1.89). A positive phase of NAO reflects below-normal pressure in the northern North Atlantic and above-normal pressure over the central North Atlantic, leading to strong westerly winds associated with a warm and moist air mass across northern Europe during winter and increased precipitation. The negative phase reflects the converse in circulation and air temperature, resulting in less precipitation in southern Europe.

Table 1
Correlations between analysed parameters: SALIN Salinity, TEMP temperature, CHL α chlorophyll α , MO organic matter, PHOSP Phosphates, AMMO Ammonia, NITRA Nitrates, DIATO Diatoms, CHLOR Chlorophyceae, CYANO Cyanobacteria, CRUS Crustacean larvae, LAR_P Fish Larvae, EGG_P fish eggs, CLAD Cladoceran, COPE Copepod, MYSID Mysidacea. Marked correlations are significant at $p < 0.05$, corrected by the Bonferroni's method

	SALIN	TEMP	CHL α	SESTO	MO	SILICA	PHOSP	AMMO	NITRA	INEFLOW	DIATO	CHLOR	CYANO	CRUS	LAR_P	EGG_P	CLAD	COPE	MYSID
SALIN	1.000	-0.061	0.039	0.089	-0.126	-0.201	0.102	-0.341	-0.383*	-0.191	-0.374*	-0.256	-0.299*	0.332	0.129	0.178	0.544*	0.269	-0.130
TEMP		1.000	0.023	-0.175	0.202	-0.421*	0.067	-0.474*	-0.116	-0.257	0.039	0.360	0.306	0.191	0.061	0.071	0.057	0.106	-0.054
CHL α			1.000	-0.107	0.190	-0.276	0.119	0.235	-0.461*	-0.424*	-0.063	0.108	-0.163	-0.097	-0.184	-0.044	0.022	0.146	0.053
SESTON				1.000	0.483*	-0.272	-0.008	0.269	-0.099	-0.124	-0.374*	-0.119	-0.182	0.111	0.170	0.154	-0.182	-0.163	0.165
MO					1.000	-0.222	0.119	0.294	-0.276	0.045	-0.117	0.210	-0.023	-0.079	0.003	0.034	-0.054	-0.195	-0.084
SILICA						1.000	-0.003	0.643*	0.315*	0.334*	0.016	0.043	-0.023	-0.229	-0.149	-0.098	-0.134	-0.031	-0.125
PHOSP							1.000	-0.042	0.133	-0.108	-0.174	-0.123	-0.231	-0.164	-0.093	0.387	0.053	0.164	
AMMO								1.000	0.216	-0.145	-0.251	-0.104	-0.160	-0.230	-0.187	-0.186	0.531*	0.277	
NITRA									1.000	0.498*	0.242	-0.078	0.045	0.056	0.013	-0.394	-0.283	0.039	
INEFLOW										1.000	0.096	0.038	-0.015	-0.182	-0.112	-0.088	-0.187	-0.114	
DIAT											1.000	-0.009	0.020	0.048	0.062	-0.107	-0.189	-0.171	
CHLOR												1.000	0.604*	-0.093	-0.060	-0.079	-0.158	-0.097	
CYANO													1.000	-0.112	-0.077	-0.132	-0.078	-0.098	
CRUS														1.000	0.336*	0.030	-0.066	0.375*	
LAR_P															1.000	0.563	0.007	-0.085	
EGG_P																1.000	0.143	-0.070	
CLADO																	1.000	-0.107	
COPEP																		1.000	
MYSID																			1.000

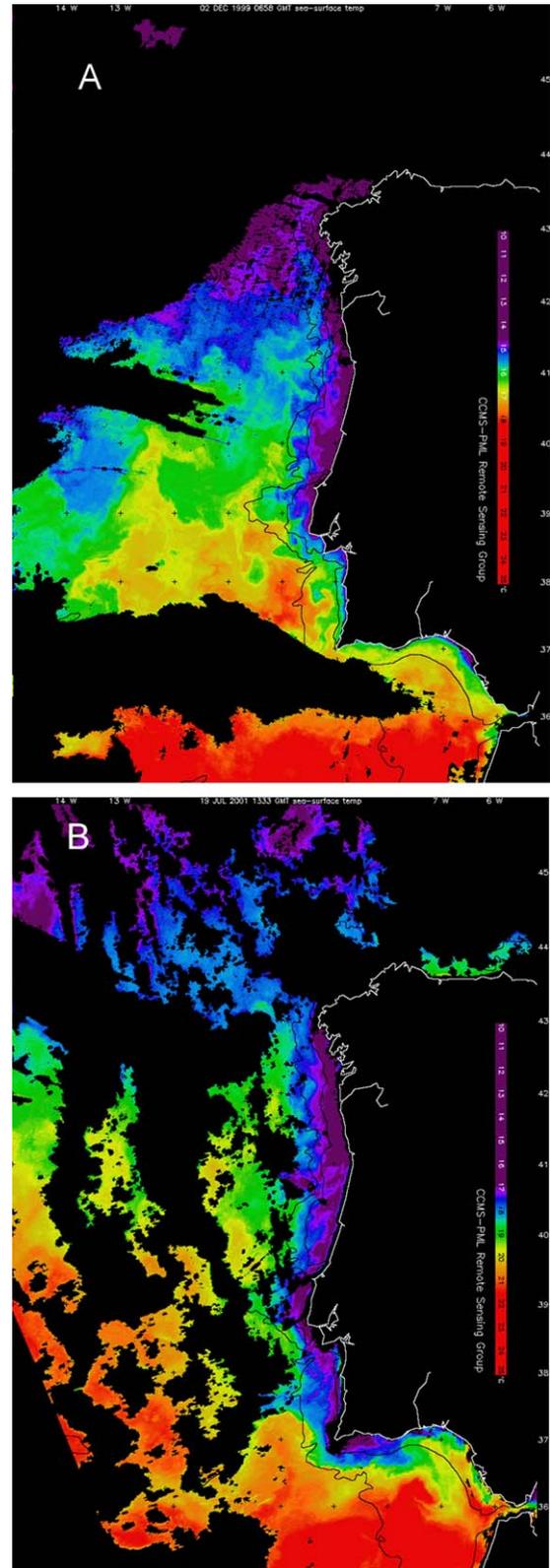


Fig. 6. Distribution of satellite-derived sea surface temperatures ($^{\circ}\text{C}$) off NW Iberia obtained on 2 December 1999 (A) and 19 July 2001 (B), i.e. during the set up of westward winds. Black color correspond to cloud cover.

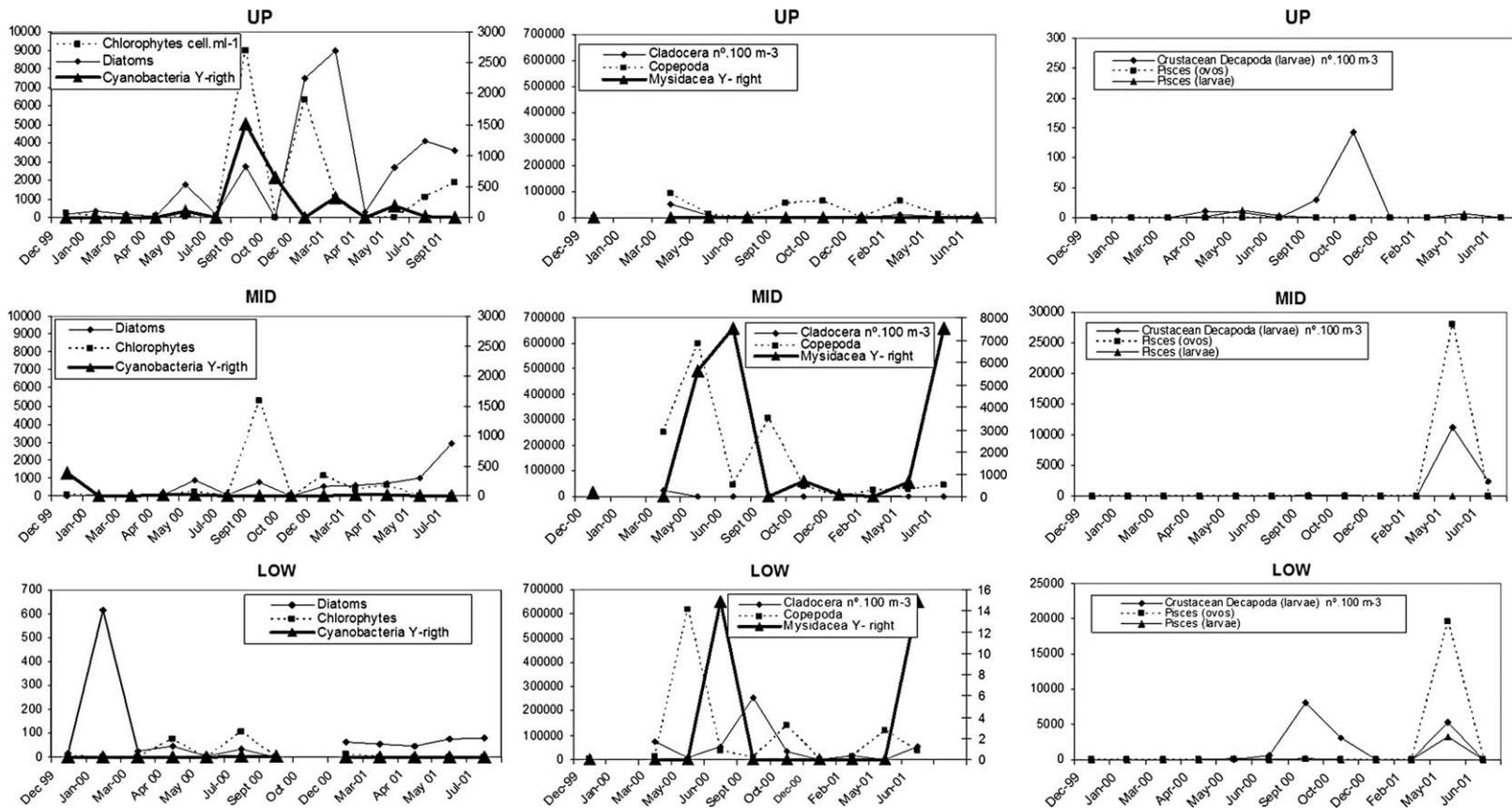


Fig. 7. Phytoplanktonic densities of major groups and zooplanktonic densities of major groups in 2000 (hydrologic year 1999/2000) and in 2001 (hydrologic year 2000/2001).

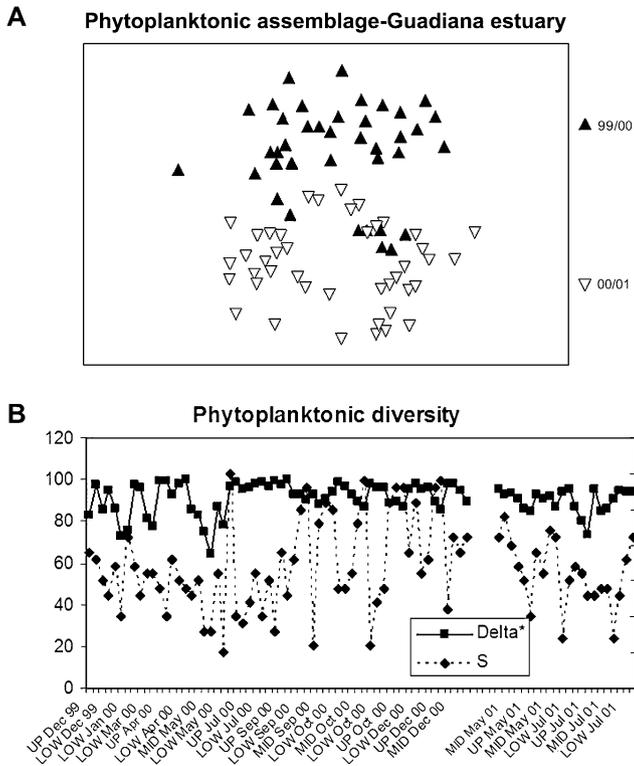


Fig. 8. (A) MDS plots of density of phytoplanktonic community of Guadiana estuary during 1999/2000 and 2000/2001 in different areas and months; plots based on triangular matrices of Bray–Curtis similarities using $\log(1+x)$ transformed species abundance data. These techniques were used with PRIMER Software. (B) Average Taxonomic Distinctness Index (Delta*) *sensu* Clarke and Warwick (2001) and Specific richness (S) of phytoplankton along sampling months and areas of the Guadiana estuary.

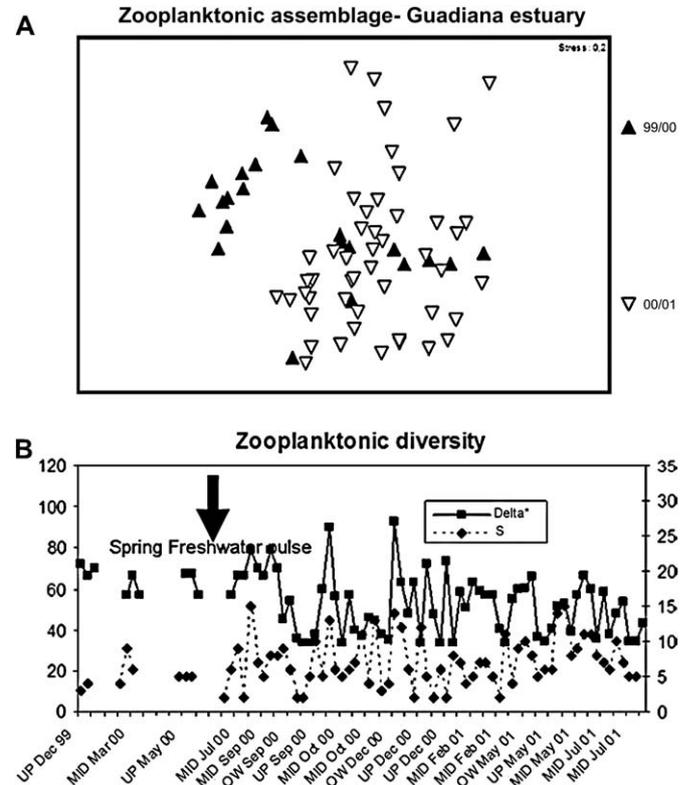


Fig. 9. (A) MDS plots of density of zooplanktonic community of Guadiana estuary during 1999/2000 and 2000/2001 in different areas and months; plots based on triangular matrices of Bray–Curtis similarities using $\log(1+x)$ transformed species abundance data. (B) Average Taxonomic Distinctness Index (Delta*) *sensu* Clarke and Warwick (2001) and Specific richness (S) of phytoplankton along sampling months and areas of the Guadiana estuary.

During the low inflow registered during 1999/2000, the dry year, values of salinity and temperature in the estuary were higher than in the high-inflow year, 2000/2001. Accordingly, nutrient concentrations also varied between sampling years: the regenerated nutrients ammonia and phosphates occurred in higher concentrations in the water during the low inflow year, while concentrations of the “new” nutrients nitrate and silicate were higher in the high-inflow year. Such observations agreed with several studies that indicated a dependence of nitrates and silicates on freshwater input (Kimmerer and Schubel, 1994; Binet et al., 1995; Sklar and Browder, 1998; Rocha et al., 2002; Wolanski et al., 2004), whereas regenerated nutrients, such as ammonia and phosphate, are mostly dependent on biological activities (Ikeda et al., 1982; Dolan, 1997).

The effects of freshwater inputs on nutrient concentrations were responsible for changes in the productivity of the estuary, particularly in the lower estuary. Productivity, estimated by chlorophyll *a* concentration, decreased with the reduction of freshwater discharge in the low flow year. According to Wolanski et al. (2004), during low flow periods, an important reduction of nutrient export from river to coastal areas occurs due to an increase in residence time of the estuary. However, in our study, such a decrease was attenuated as a result of

upwelling events in the adjacent coastal area. Such “compensatory” effect of upwelling events on estuarine productivity during reduced river discharges was also highlighted by Taylor (1992) and Pérez et al. (2000). According to the observations by Chicharo et al. (2003) and Ribeiro et al. (2005) on the western coast of Portugal, this productivity is related to the low mixing of the river outwelling layer, which counterbalances the highly mixed coastal waters; this conserves static stability at the level needed for phytoplankton growth, especially during winter, when water turbulence usually inhibits primary production.

Plankton productivity at the estuary varied with freshwater discharge and reflected associated modifications in planktonic assemblages. Environmental conditions during the low inflow year, limited the occurrence of the winter/spring bloom that has been typically described for this system (Rocha et al., 2002; Domingues et al., 2005). Basically, there can be two major reasons for this change: modification of the bottom-up or the top-down processes. Nutrient limitation, despite the decrease in freshwater discharge, seems an unlikely explanation, since nutrients regenerate cumulatively with the shallow depth of the estuary (maximum 10 m), and increased retention times would typically ensure, as is common in estuaries around the world, nutrient concentrations sufficient to sustain phytoplankton

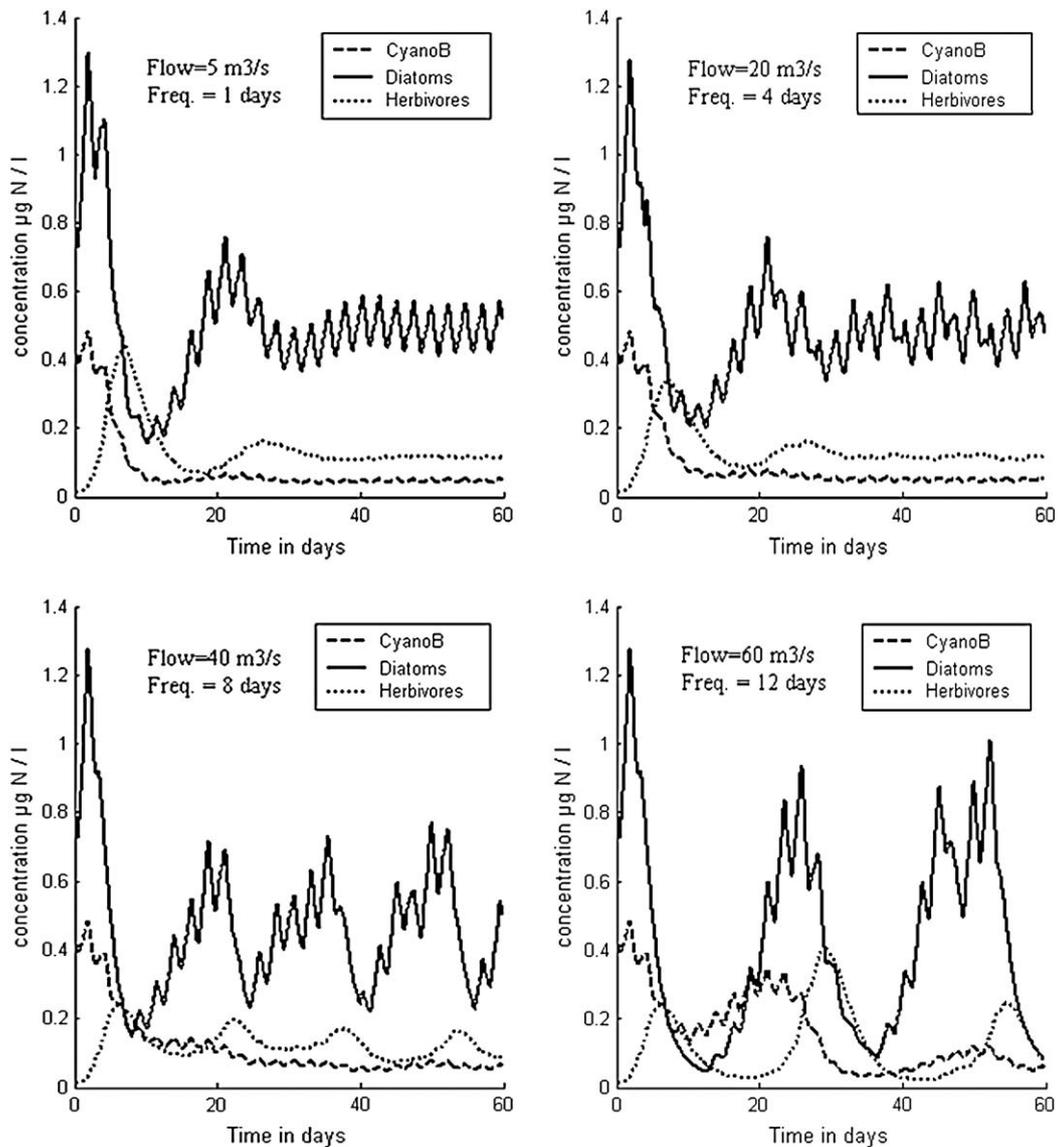


Fig. 10. Model sensitivity tests identified that for an equal mean flow during the simulation period ($5 \text{ m}^3 \text{ s}^{-1}$) but corresponding to different pairs of flow amplitude and frequencies.

growth. In fact, we noticed a sharp decrease in silicates from December 1999 to March 2000, which indicates an important consumption by diatoms. Accordingly, in December 1999, we recorded the highest value of chlorophyll *a* ($>10 \mu\text{g L}^{-1}$), a value considered arbitrarily by Iriarte and Purdie (2004) as an indicator of bloom events. Both the consumption of silicates and the peak in chlorophyll concentration seem to indicate the occurrence of a diatom bloom, although this was not evident in our sampling. Therefore, top-down control on phytoplankton assemblages seems to represent a more consistent hypothesis to explain the “missing” phytoplankton bloom at the Guadiana estuary. During this period, grazing activity by very abundant zooplanktonic taxa, especially copepods and cladocerans, may have caused the reduced phytoplankton density. In fact, zooplankton grazing activity — especially copepods (Hunter, 1981; Houde, 1989; Jones et al., 1997; Esteves et al., 2000) — may be triggered by the particular environmental

conditions related to an increasing residence time, such as water stability and increased temperature (Oviatt et al., 2002); in fact, an increase in zooplankton grazing was confirmed by the higher concentrations of ammonia (cf. Priddle et al., 2003).

The abundance of merozooplanktonic groups, such as decapods or fish larvae, decrease in the lower estuary, and this represents a loss in the use of the estuary as a nursery area. In fact, according to Govoni and Chester (1990), Grimes and Finucane (1991) and Kingsford and Suthers (1994) during low inflow years the associated decrease in river discharge to the coastal areas reduces the turbidity plumes and hampers the orientation of adult fishes in their search for suitable spawning and nursery areas. However, a very significant increase in freshwater discharge may also negatively affect the distribution and abundance of these species. In fact, comparison of the merozooplanktonic abundances between May 2001 and May 2000 suggested that the discharge peak in May 2000 had caused

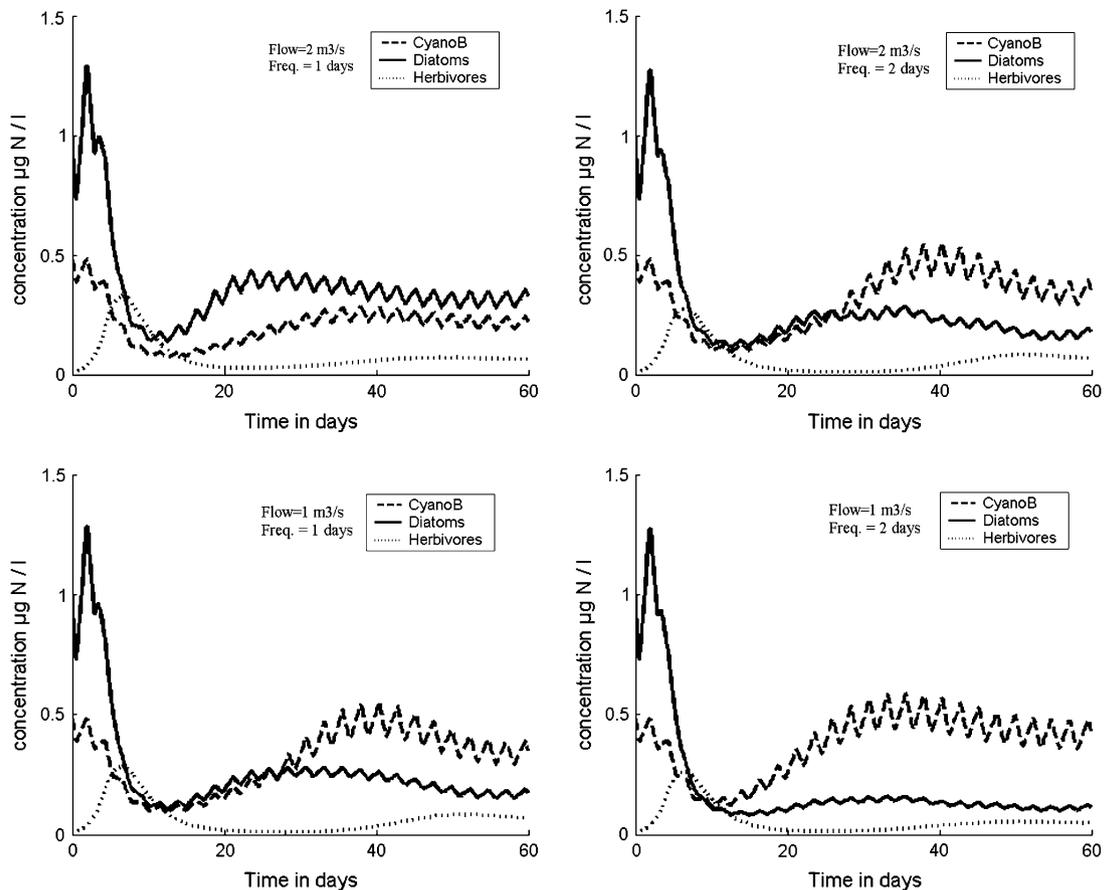


Fig. 11. Model sensitivity tests for previous established ecologic mean flow ($1-2 \text{ m}^3 \text{ s}^{-1}$).

the flushing of almost all merozooplanktonic species out of the estuary.

However, such a sudden increase in river discharge, if of short duration (a freshwater pulse), may affect positively the zooplankton abundance, especially copepods. Copepods are selective feeders (Reynolds, 1984) and benefit from an availability of a more diverse prey assemblage. Higher grazing pressure by zooplanktonic species promotes phytoplankton diversity and the suppression of competitive exclusion processes, owing to the changes in physicochemical conditions and to top-down control (Hutchinson, 1961; Reynolds, 1984); this avoids dominance of a particular group of algae and, therefore, reduces the risk of eutrophication in the estuarine area (Roelke, 2000; Roelke et al., 2003). Moreover, freshwater pulses can help to avoid the establishment of alien species that might otherwise benefit from the stable conditions during periods of low inflow (Bunn and Arthington, 2002).

Control of the volume, timing and duration of freshwater discharge into the Guadiana estuary allows regulation of the estuarine food-web. However, in practice no simple solutions exist, and beneficial and prejudicial consequences can result from increasing or decreasing the volume of freshwater discharge. In natural systems, the regulation of processes in the estuaries is dependent on natural phenomena, such as droughts or rainy periods. However, in reality, estuaries

worldwide are under high anthropogenic pressure, and natural processes usually are not capable of self-sustainability and of maintaining healthy ecological status in these systems. Hydrotechnical infrastructures have a marked impact on estuaries by causing a significant decrease in the volume of freshwater entering the estuary, even if the “ecological flow” is maintained. Often, this ecological flow is considered as an “average” flow and the timing of water release is not considered as a crucial factor for the sustainability of the estuarine ecosystem.

Development of simple models will contribute to a better management of the estuaries under the influence of dams, allowing the sustainability and the ecological uses of the estuarine ecosystem. In the model we have developed for the Guadiana estuary, simulations have clearly demonstrated that limitation of freshwater inflow, in amplitude rather than in frequency, determines the structure of the phytoplankton community, which in turn constrains the structure and functioning of the planktonic food-web. As shown in model outputs, the freshwater pulses ($50 \text{ m}^3 \text{ s}^{-1}$ — every week) can disturb and enrich the estuary with nutrients, thereby, excluding the cyanobacterial community from the system.

In estuaries with hydrotechnical infrastructures to understand and model the relation between the infrastructure and the ecosystem, represents a step forward in the integrative

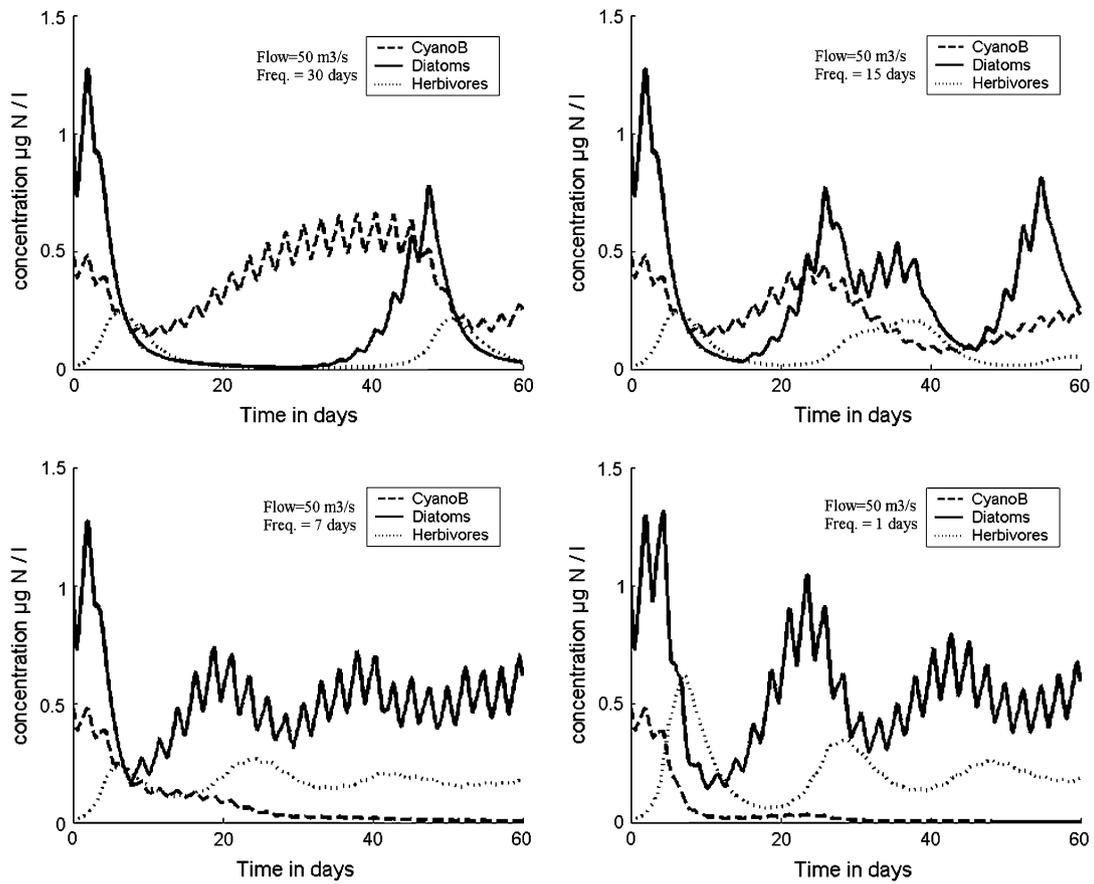


Fig. 12. Model sensitivity tests for medium flow rates of $50 \text{ m}^3 \text{ s}^{-1}$ but corresponding to different frequencies of discharges: every 30 days, every 15 days, every week, every day.

management of estuaries and may reduce drastically the negative effects of dam construction.

5. Conclusions

In an integrated ecohydrological management program, planktonic communities may play an important role as indicators of environmental changes and as active contributors to the robustness of the systems. The planktonic assemblages in the Guadiana estuary were highly dependent on the freshwater discharge from the river. Planktonic productivity decreases in dry years, but oceanographic processes — such as upwelling — can compensate for this limitation and sustain estuarine productivity. Reduced freshwater discharge in dry years also causes an increase in the residence time and may promote eutrophication. Natural short-term increase in freshwater discharge — freshwater pulse — has been shown to counterbalance this risk, by promoting the development of zooplankton, particularly copepod species, and thereby, exerting a top-down control on the algae. High peaks of freshwater discharge may flush out the larval phases of species using the estuarine area as a nursery ground. However, in some circumstances, such flushing may be used to avoid colonization by alien species and eutrophication in the estuary. Positive and negative effects on the estuarine ecosystem may result from

high or low freshwater discharges. Such lessons learned from natural systems should be considered for the management of dammed rivers. Regulation of river discharge needs to consider not only the quantity of water, but also the timing of the release, as demonstrated in the model developed for the Guadiana estuary. In this respect, hydrotechnical infrastructures, where present, may play an important role in allowing the sustainability of such already affected estuarine ecosystems.

Acknowledgements

This research was funded by the projects: “Study of environmental conditions in the Guadiana estuary and adjacent coastal area” — LNEC (subcontractor CCMAR), “Guadiana fisheries resources valorization” ODIANA — FEDER and “Effect of river flow changes on the ichthyofauna communities in Douro, Tejo and Guadiana estuaries and in its adjacent coastal areas. Ecological and socio-economical predictions” — FCT/P/MAR/15263/1999. R. Ben-Hamadou was supported by a fellowship from the FCT (SFRH/BPD/20649/2004). Thanks are due to Ana Barbosa for stimulating discussion about the data and to Ana Amaral, Isabel Gouveia, Rita Constantino, Pedro Morais, Silvia Condinho, Luís Cristóvão and André Iglésias for the participation or collaboration in field and

laboratory work. An acknowledgement is also due to the Remote Sensing Group at Plymouth Marine Laboratory, UK, for the SST images and browsing access to their satellite database.

Appendix I

Model equations and coefficient list corresponding to the conceptual diagram (Fig. 2) and estimating the flows between the state variables total nitrogen (N), phosphorus (P), silicate (Si), diatoms (D), cyanobacteria (CB) and herbivore (H) concentrations. All coefficients and abbreviations are explained in the table below.

A.1. Nutrient dynamics

Nutrient–phytoplankton interactions are described here by Michaelis–Menten kinetics; differential equations modelling flow exchange are:

$$\dot{N} = -\frac{\rho_{m1}ND}{K_{1.1} + N} - \frac{\rho_{m2}NCB}{K_{2.1} + N} + f(N) + e_H$$

$$\dot{P} = -\frac{\rho_{m1}PD}{K_{1.2} + P} - \frac{\rho_{m2}PCB}{K_{2.2} + P} + f(P)$$

$$\dot{Si} = -\frac{\rho_{m1}SiD}{K_{1.3} + P} + f(Si)$$

With $f(\cdot)$ is the nutrient enrichment function consequent to the flow discharge forcing according to the input frequency and with the amplitude A :

$$f(N/P/Si) = (e_N/e_P/e_{Si})A$$

P and Si concentrations are converted to nitrogen equivalent using:

$$\dot{P}^* = R_{np}P$$

$$\dot{Si}^* = R_{nsi}Si$$

A.2. Phytoplankton dynamics

Phytoplankton growth (=nutrient uptake) is equivalent to the net primary production in the model. Phytoplankton growth can be limited by light and nutrient availability according to von Liebig's law of the minimum. Since phytoplankton as a state variable is represented in the model in nitrogen units, phosphorus and silicate uptakes are recalculated into nitrogen with a fixed Redfield ratio.

Diatom and cyanobacterial biomass dynamics are given by the following logistic differential equations:

$$\dot{D} = K_{c1}D - b_1D^2 - m_D D - HI_m \frac{D}{K_D + D}$$

$$\dot{CB} = K_{c2}CB - b_2CB^2 - m_{CB}CB - HI_m \frac{CB}{K_{CB} + CB}$$

The growth rate K_c for diatoms and cyanobacteria considers possible limitations firstly by light and secondly either by nitrate or phosphorus for cyanobacteria and moreover by silicate for diatoms following von Liebig's law of the minimum:

$$K_{c1} = \rho_{m1} \lim 1(I) \min \left(\frac{N}{K_{1.1} + N}, \frac{P}{K_{1.2} + P}, \frac{Si}{K_{1.3} + Si} \right)$$

$$K_{c2} = \rho_{m2} \lim 2(I) \min \left(\frac{N}{K_{2.1} + N}, \frac{P}{K_{2.2} + P} \right)$$

The light intensity I is a sinusoidal time function as a day mean of light irradiance:

$$I(t) = 1/2 \sin \left(100 \left(\frac{2\pi(t-1)}{140} \right) + 50 \right)$$

Negative values of $I(t)$ were substituted by nil values, light limitations of the phytoplankton growth are given by:

$$\lim 1(I) = \frac{I(t)}{I_{1s}} \exp \left(\frac{1-I(t)}{I_{1s}} \right)$$

$$\lim 2(I) = \frac{I(t)}{I_{2s}} \exp \left(\frac{1-I(t)}{I_{2s}} \right)$$

A.3. Herbivore dynamics

Temporal changes in the herbivore biomass are due to grazing, mortality, intraspecific competition and excretion:

$$\dot{H} = aHI_m \left(\frac{D}{K_D + D} + \frac{CB}{K_{CB} + CB} \right) - H(m_H + c_i + e)$$

The numerical integration of all differential equations cited above was computed using the fourth-order Runge–Kutta method; coefficients, abbreviations and their values used in the model simulations are cited below.

Coefficient	Symbol	Value	Unit
Maximum uptake rate for diatoms	ρ_{m1}	0.8	j^{-1}
Maximum uptake rate for cyanobacteria	ρ_{m2}	0.5	j^{-1}
Diatom half saturation constant for nitrate absorption	$K_{1.1}$	0.4	$\mu\text{mol N l}^{-1}$
Diatom half saturation constant for phosphorus absorption	$K_{1.2}$	0.6	$\mu\text{mol P l}^{-1}$
Diatom half saturation constant for silicate absorption	$K_{1.3}$	0.2	$\mu\text{mol Si l}^{-1}$
Cyanobacterial half saturation constant for nitrate absorption	$K_{2.1}$	0.5	$\mu\text{mol N l}^{-1}$

(continued)

Coefficient	Symbol	Value	Unit
Cyanobacterial half saturation constant for phosphorus absorption	$K_{2.2}$	0.5	$\mu\text{mol P l}^{-1}$
Excretion rate of the herbivore	e	0.08	j^{-1}
Nitrogen pool per unit of discharge flow	e_N	420	$\mu\text{mol N m}^{-3}$
Phosphorus pool per unit of discharge flow	e_P	400	$\mu\text{mol P m}^{-3}$
Silica pool per unit of discharge flow	e_{Si}	180	$\mu\text{mol Si m}^{-3}$
Flow amplitude	A	Input	$\text{m}^3 \text{s}^{-1}$
N:P ratio conversion	R_{np}	16	undimensioned
N:Si ratio conversion	R_{nsi}	2	undimensioned
Diatom intraspecific competition rate	b_1	10/55	j^{-1}
Cyanobacterial intraspecific competition rate	b_2	1/3	j^{-1}
Diatom mortality rate	m_D	0.1	j^{-1}
Cyanobacterial mortality rate	m_{CB}	0.12	j^{-1}
Maximum ingestion rate of the herbivore	I_m	1	j^{-1}
Herbivore half saturation constant for diatom ingestion	K_D	6.2	$\mu\text{mol N l}^{-1}$
Herbivore half saturation constant for cyanobacterial ingestion	K_{CB}	4.5	$\mu\text{mol N l}^{-1}$
Light saturation intensity for diatoms	I_{1s}	90	$\mu\text{E m}^{-2} \text{s}^{-1}$
Light saturation intensity for Cyanobacteria	I_{2s}	90	$\mu\text{E m}^{-2} \text{s}^{-1}$
Assimilation efficiency of the herbivore	a	0.7	j^{-1}
Herbivore mortality rate	m_H	0.04	j^{-1}
Herbivore intraspecific competition rate	c_i	0.1	j^{-1}

References

- Alpine, A., Cloern, J., 1992. Trophic interactions and physical effects control phytoplankton biomass and production in an estuary. *Limnology and Oceanography* 37, 946–955.
- Binet, D., Reste, L.L., Diouf, P.S., 1995. The influence of runoff and fluvial outflow on the ecosystems and living resources of West African coastal waters. In: *Effects of Riverine Inputs on Coastal Ecosystems and Fisheries Resources*. FAO Fisheries Technical Papers, No. 349, pp. 89–118.
- Bode, A., Alvarez-Ossorio, T., Gonzalez, N., Lorenzo, J., Rodriguez, C., Varela, M., Varela, M.M., 2005. Seasonal variability of plankton blooms in the Ria de Ferrol (NW Spain): II. Plankton abundance, composition and biomass. *Estuarine, Coastal and Shelf Science* 63, 285–300.
- Bunn, S.E., Arthington, A.H., 2002. Basic principles and ecological consequences of altered flow regimes for aquatic biodiversity. *Environmental Management* 30 (No. 4), 492–507.
- Chicharo, M.A., Chicharo, L., Galvão, H., Barbosa, A., Marques, M.H., Andrade, J.P., Esteves, E., Miguel, C., Gouveia, C., 2001. Status of the Guadiana estuary (South Portugal) during 1996–1998: an ecohydrological approach. *Aquatic Ecosystem Health and Management* 4, 1–17.
- Chicharo, L., Chicharo, M.A., Esteves, E., Andrade, P., Morais, P., 2002. Effects of alterations in fresh water supply on the abundance and distribution of *Engraulis encrasicolus* in the Guadiana Estuary and adjacent coastal areas of south Portugal. *Journal of Ecohydrology and Hydrobiology* 1, 195–200.
- Chicharo, M.A., Esteves, E., Santos, A.M.P., dos Santos, A., Peliz, A., Re, P., 2003. Are sardine larvae caught off northern Portugal in winter starving? An approach examining nutritional conditions. *Marine Ecology Progress Series* 257, 303–309.
- Clarke, K.R., Warwick, R.M., 2001. *Changes in Marine Communities: An Approach to Statistical Analysis and Interpretation*, second ed. Primer-e, Plymouth, UK.
- Cloern, J.E., Dufford, R., 2005. Phytoplankton community ecology: principles applied in San Francisco Bay. *Marine Ecology Progress Series* 285, 11–28.
- Costa, M.J., 1988. The Tagus and Mira estuaries (Portugal) and their role as spawning and nursery areas. *Journal of Fish Biology* 33 (Suppl. A), 249–250.
- Dias, J.M.A., Gonzalez, R., Ferreira, Ó., 2004. Natural versus anthropic causes in variations of sand export from river basins: an example from the Guadiana River mouth (Southwestern Iberia). *Polish Geological Institute Special Papers* 11, 95–102.
- Doornbos, G., 1982. Changes in the fish fauna of the former Grevelingen estuary, before and after the closure in 1971. *Hydrobiological Bulletin* 16, 279–283.
- Dolan, J.R., 1997. Phosphorus and ammonia excretion by planktonic protists. *Marine Geology* 139, 109–122.
- Domingues, R.B., Barbosa, A., Galvão, H., 2005. Nutrient and phytoplankton succession in the Guadiana River upper estuary. *Estuarine, Coastal and Shelf Science* 64 (2–3), 249–260.
- Drake, P., Arias, A., Baldó, F., Cuesta, J.A., Rodríguez, A., Silva-García, A., Sobrino, I., García-González, D., Fernández-Delgado, C., 2002. Spatial and temporal variation of the nekton and hyperbenthos from a temperate European estuary with a regulated freshwater inflow. *Estuaries* 25 (3), 451–468.
- Esteves, E., Pina, T., Chicharo, M.A., Andrade, J.P., 2000. The distribution of estuarine fish larvae: nutritional condition and co-occurrence with predators and prey. *Acta Oecologica* 21 (3), 1–13.
- Grasshoff, K., Ehrhardt, M., Kremling, K., 1983. *Methods of Sea Water Analysis*. Verlag Chemie, Weinheim.
- Govoni, J.J., Chester, A.J., 1990. Diet composition of larval *Leiostomus xanthurus* in the Mississippi river plume. *Journal of Plankton Research* 12, 819–830.
- Gonzalez, J.A.M., 1995. *Sedimentologia del estuário del Rio Guadiana (SO Portugal)*. CEP. Biblioteca Universitária. Universidade de Huelva.
- Gonzalez, R., Dias, J.M.A., Ferreira, Ó., 2001. Study of a rapidly changing coastline using GIS: recent evolution of the Guadiana Estuary (Southern Portugal/Spain). *Journal of Coastal Research* 34 (S1), 516–527.
- Grimes, C.B., Finucane, J.H., 1991. Spatial distribution and abundance of larval and juvenile fish, chlorophyll and macrozooplankton around the Mississippi river discharge plume and the role of the plume in fish recruitment. *Marine Ecology Progress Series* 75, 109–119.
- Hasle, G.R., 1978. The inverted-microscope method. In: Sourmia, A. (Ed.), *Phytoplankton Manual*. UNESCO, Paris, pp. 88–96.
- Houde, E.D., 1989. Comparative growth, mortality and energetics of marine fish larvae: temperature and implied latitudinal effects. *Fishery Bulletin* U.S. 87, 471–495.
- Hunter, J.R., 1981. Feeding ecology and predation of marine fish larvae. In: Lasker, R. (Ed.), *Marine Fish Larvae: Morphology, Ecology and Relation to Fisheries*, second ed. Washington Sea Grant, pp. 34–40.
- Hutchinson, G.E., 1961. The paradox of plankton. *American Naturalist* 95, 137–147.
- Ikeda, T., Hing, E., Hutchinson, S.A., Boto, G.M., 1982. Ammonia and inorganic phosphate excretion by zooplankton from inshore waters of the Great Barrier Reef, Queensland I. Relationship between excretion rates and body size. *Australian Journal of Marine and Freshwater Research* 33, 55–70.
- Iriarte, A., Purdie, D.A., 2004. Factors controlling the timing of major spring bloom events in a UK south coast estuary. *Estuarine, Coastal and Shelf Science* 61, 679–690.
- Jones, D.A., Kumlu, M., Vay, L.Le., Fletcher, D.J., 1997. The digestive physiology of herbivorous, omnivorous and carnivorous crustacean larvae: a review. *Aquaculture* 155, 285–295.

- Kimmerer, W., Schubel, J.R., 1994. Managing freshwater flows into San Francisco Bay using a salinity standard: results of a workshop. In: Dyer, J., Orth, M. (Eds.), *Changes in Fluxes in Estuaries: Implications from Science to Management*. University of Plymouth, pp. 411–416. ECSA22/ERF Symposium.
- Kingsford, M.J., Suthers, I.M., 1994. Dynamic estuarine plumes and fronts: importance to small fish and plankton in coastal waters of NSW, Australia. *Continental Shelf Research* 14, 655–672.
- Kirkwood, D., 1996. *Nutrients: Practical Notes on their Determination in Sea Water*. ICES, Copenhagen, 25 pp.
- Marques, S., Azeiteiro, U., Marques, J.C., Neto, J.N., Pardal, M., 2006. Zooplankton and ichthyoplankton communities in a temperate estuary: spatial and temporal patterns. *Journal of Plankton Research* 28 (3), 297–312.
- Morales, J.A., 1993. *Sedimentología del estuário del Guadiana (SW Espana—Portugal)*. Ph.D. thesis, University of Sevilla, Spain.
- Olausson, E., Cato, I. (Eds.), 1980. *Chemistry and Biogeochemistry of Estuaries*. John Wiley, New York.
- Oviatt, C., Keller, A., Reed, L., 2002. Annual primary production in Narragansett Bay with no bay-wide winter–spring phytoplankton bloom. *Estuarine, Coastal and Shelf Science* 54, 1013–1026.
- Pérez, F.F., Álvarez-Salgado, X.A., Rosón, G., 2000. Stoichiometry of the net ecosystem metabolism in a coastal inlet affected by upwelling. The Ria de Arousa (NW Spain). *Marine Chemistry* 69, 217–236.
- Priddle, J., Whitehouse, M.J., Ward, P., Shreeve, R.S., Brierley, A.S., Atkinson, A., Watkins, J.L., Brandon, M.A., Cripps, G.C., 2003. Biogeochemistry of a Southern Ocean plankton ecosystem: using natural variability in community composition to study the role of metazooplankton in carbon and nitrogen cycles. *Journal of Geophysical Research* 108 (C4), 8082.
- Ramos, S., Cowen, R.K., Re, P., Bordalo, A., 2006. Temporal and spatial distributions of larval fish assemblages in the Lima estuary (Portugal). *Estuarine, Coastal and Shelf Science* 66, 303–314.
- Ribeiro, C., Peliz, A., Santos, M., 2005. A study of the response of chlorophyll-*a* biomass to a winter upwelling event off Western Iberia using SeaWiFS and in situ data. *Journal of Marine Systems* 53, 87–107.
- Reynolds, C.S., 1984. *The Ecology of Freshwater Phytoplankton*. Cambridge University Press, United Kingdom.
- Rocha, C., Galvão, H., Barbosa, A., 2002. Role of transient silicon limitation in the development of cyanobacteria blooms in the Guadiana estuary, south-western Iberia. *Marine Ecology Progress Series* 228, 35–45.
- Roelke, D.L., 2000. Copepod food quality threshold as a mechanism influencing phytoplankton succession and accumulation of biomass, and secondary productivity: a modelling study with management implications. *Ecological Modelling* 134, 245–274.
- Roelke, D.L., Augustine, S., Buyukates, Y., 2003. Directing the fall of Darwin's "grain in the balance": manipulation of hydraulic flushing as a potential control of phytoplankton population dynamics. *Texas Water Resources Institute* 245, 1–13.
- Sklar, F., Browder, J., 1998. Coastal environmental impacts brought about by alterations to freshwater flow in the Gulf of Mexico. *Environmental Management* 22, 547–562.
- Taylor, D.I., 1992. The influence of upwelling and short-term changes in concentration of nutrients in the water column on fluxes across the surface of a salt marsh. *Estuaries* 15, 68–74.
- Venrick, E.L., 1978. Water-bottles. In: Sournia, A. (Ed.), *Phytoplankton Manual*. UNESCO, Paris, pp. 33–40.
- Welschmeyer, N.A., 1994. Fluorometric analysis of chlorophyll *a* in the presence of chlorophyll *b* and pheopigments. *Limnology and Oceanography* 39, 1985–1992.
- Wolanski, E., Boorman, L.A., Chicharo, L., Langlois-Salious, E., Lara, R., Plater, A.J., Uncles, R.J., Zalewski, M., 2004. Ecohydrology as a new tool for sustainable management of estuaries and coastal waters. *Wetlands Ecology and Management* 12 (4), 235–276.