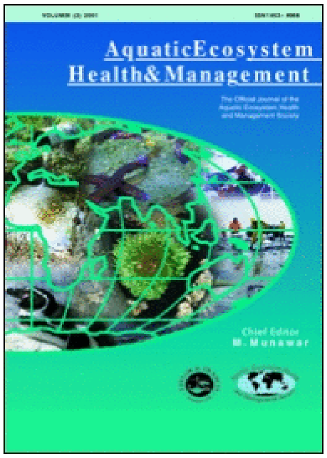

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Status of the Guadiana Estuary (south Portugal) during 1996-1998: An ecohydrological approach

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Abstract

The aims of this study were to monitor basic hydrological and ecological characteristics of the Guadiana Estuary (1996–1998) before the construction of the Alqueva dam. This work was carried out to determine how environmental factors affect seasonal and tidal variations of plankton populations in the estuary. The available information on the subcatchment of the estuary (e.g., urban, agricultural and forested areas) was integrated into a geographic information system-based software program. Mean monthly river flow varied markedly on a seasonal and yearly basis. River flow near Mértola (ca 50 km upstream from the mouth) reached 3400×10^6 m^3 in winter and decreased to 42×10^6 m^3 in summer. With respect to nutrients, nitrogen to phosphorus ratios indicated some limitation by phosphorus, except at the end of summer, when nitrogen limitation appeared. During this period, cyanobacterial blooms usually occurred in the upper/middle estuary. Estuarine Turbidity Maximum may significantly influence the retention of zooplankton in the estuary. The fish larval life cycle, especially sensitive to environmental alterations, showed high ratios of ribonucleic to desoxyribonucleic acids indicating good physiological condition. It was concluded that an ecohydrological approach, allowing integration of different elements from the cellular to the habitat level into a geographic information system, can contribute to a better understanding of the processes that influence the aquatic biota of the estuary. The approach will be a useful assessment tool for monitoring the estuary following dam completion.

Key words: plankton, GIS, RNA/DNA ratios

1. Introduction

Extensive development of the Guadiana Estuary basin over the last century has resulted in significant alteration of river flow regimes and in anthropogenic nutrient enrichment. The construction of the Alqueva dam (end of 2000) is imminent and it will pose problems for maintenance of water quantity and quality in the Guadiana Estuary. It will be necessary to develop an ecological evaluation of this important Iberian estuary and an assessment of anticipated impacts of environmental change.

Ecohydrology is defined as the science of relating hydrological processes to the biological dynamics of ecosystems over varied spatial and temporal scales (Zalewski et al., 1997). Ecohydrology is considered a useful discipline for aiding in sustainable management of freshwater resources (Zalewski et al., 1998). Evaluation of how and to what extent biological processes might modify the flow of water, nutrients, sediments and pollutants in aquatic ecosystems and the surrounding landscape is a logical further step in the development of the sciences of hydrology and ecology (Zalewski, 1995; Schiemer et al., 1995). Integration of

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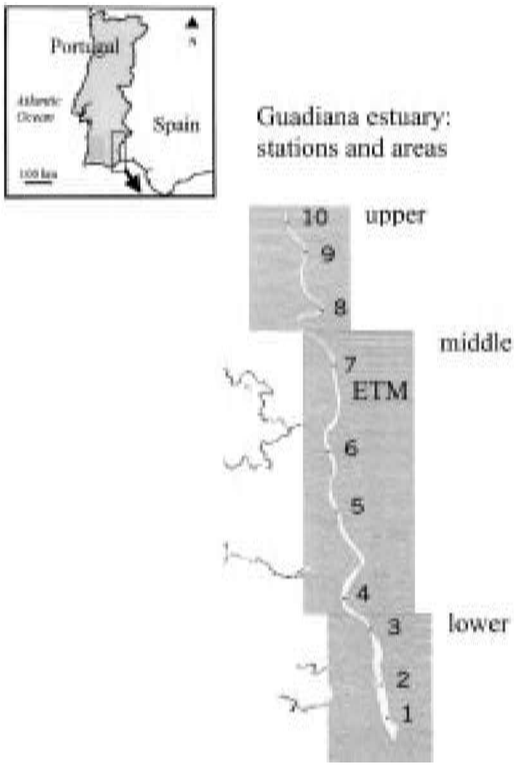

Figure 1 Sampling stations and subareas (upper, middle and lower) defined to the Guadiana estuary.

the dynamics of the three components, catchment, water and biota, determines the management target: the maintenance of homeostatic equilibrium as measured by indices of biodiversity, water quality and quantity (Zalewski et al., 1997).

Condition indices have been widely used to assess the health of individuals under certain circumstances (Barron and Adelman, 1984; Chícharo et al., 1994). It is very important to know whether an aquatic organism captured in the field is in some kind of stress, and therefore may be more susceptible to negative influences in the environment, for example, low prey availability or pollution (Chícharo et al., 1994). Determination of the physiological condition by measurement of the RNA/DNA ratio has been used on a wide range of aquatic organisms (Anger and Hirche, 1990; Chicharo et al, 1998). However, it appears to give a more consistent index for fish larvae (Bullow, 1970; Buckley, 1984; Robinson and Ware, 1988; Suthers, 1992; Clemmesen, 1994; Chícharo, 1997, 1998; Chícharo et al., 1998). Larvae in good condition tend to have a higher RNA/DNA ratio (e.g., Robinson and Ware, 1988) and larvae with an RNA/DNA ratio below 1 (the 'minimum ratio', sensu Clemmesen, 1994) are considered to be in very poor condition, with survival threatened. The use of this index is based on the assumption that the amount of DNA, the primary carrier of genetic information, is stable under changing environmental situations, while the amount of RNA is directly involved in protein synthesis and by inference, with nutritional condition, and therefore more susceptible to negative influences of the environment (Buckley, 1984; Bergeron, 1997)

Until recently, river systems have often have been regulated by engineering works, without consideration of ecosystem processes. As there is little information about the biological dynamics of the Guadiana Estuary, the results of this study will provide an important tool to evaluate ecosystem response to changes in river flow and increases in anthropogenic stressors.

The general aim of this study was to monitor basic hydrological and ecological characteristics of the Guadiana Estuary before construction of the Alqueva dam. The specific goals of the study were: i) to study the form of estuary subcatchment use and integrate that information in a geographic information system (GIS); ii) to study spatial and seasonal variations of plankton and environmental parameters in the estuary; iii) to investigate small-scale temporal distribution patterns of plankton over 12 to 24 h sampling; and iv) to analyse the biochemical (nucleic acids) indices (RNA/DNA ratios) of fish larvae in the estuary.

2. Methods

2.1 Study area

The estuary of the Guadiana River is located in the Mediterranean area on the border between Portugal and Spain (Fig. 1). Its catchment basin is the fourth largest in the Iberian Peninsula (ca. 67 500 km^2) and is larger than the area of Portugal (11 475 km^2). Mean monthly river flow volumes vary markedly on a seasonal and yearly basis, although the Alqueva dam will artificially regulate them after 2000. Water inflow near Mértola (ca 50 km upstream from the lower estuary) can reach 200 to 600 m^3 s^{-1} in winter, and decrease from 0.1 to 20 m^3 s^{-1} in summer. The year-to-year variability of inflow is also high, totalling as little as 20×10^7 m^3 in a dry year (1980), and as much as 140×10^7 m^3 in a wet year (1962) (Fig. 2). The mean yearly rainfall in the Guadiana Estuary catchment area is 400 to 600 mm, but the summer months are usually very dry and hot. The average temperature in

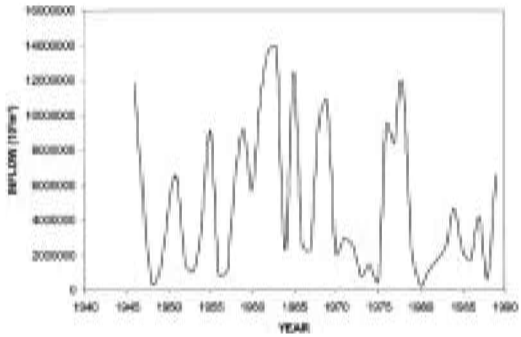

Figure 2 Variations in Guadiana Estuary inflow from 1970 to 1990.

the cool months (December–January) is less than 18 °C, and in the warm months greater than 22° C.

The tidal regime of the estuary is mesotidal, with average amplitude of 2 m. The estuary is classified as a partially stratified estuary, with a highly variable freshwater inflow according to Michel (1980). During low inflows, estuary salinity becomes vertically homogenous with mesohaline conditions depending on position in the tidal cycle, and during high inflows, the estuary is stratified.

For this study, the Guadiana Estuary was divided into three sub-areas: upper, middle and lower (Fig. 1). This classification is consistent with the sedimentology of the area adopted by Gonzalez (1995) and it is also the method commonly used to subdivide estuaries (Olausson and Cato, 1980). The upper area is mainly the freshwater section, where the effect of tides still reaches, but the salinity is usually close to zero. In this area, riparian vegetation is very well developed with *Populus alba*, *Fraxinus angustifolia*, *Salix atrocinarea*, *Tamarix* sp., *Nerium oleander* and, sporadically, *Alnus glutinosa* (Pinto Gomes, pers. comm. University of Évora, Department of Ecology). The Portuguese border, with a lower slope, contains a larger area of riparian vegetation than the Spanish border, which has a higher slope. The middle area of the estuary is the salinity transition zone, and here the Portuguese margin is surrounded by intensively farmed meadows (i.e., very intense agricultural land use). In areas with high slope, there are *Populus* and introduced *Eucalyptus*. In this area, we observed a decrease in density of riparian vegetation (with only *Tamarix* sp. persisting) and the beginning of stands of *Spartina* and *Salicornia*. This middle estuarine area contains mainly brackish water. In the lower or marine part of the estuary, the salinity is very close to that of seawater. The vegetation of this area of saltmarshes is dominated by *Spartina*, *Salicornia*, *Zoostera* and *Arthrocnemum*.

2.2 Environmental parameters

Ten stations were sampled seasonally (every two months) during intermediate tides from October 1996 to March 1998 (Fig. 1). Additional sampling took place over 12 h near the Estuarine Turbidity Maximum (ETM), between stations 6 and 7, and over 24 h at the mouth of the estuary during the spring tide of 27 May 1997.

River inflow was measured (INAG public access data) at the hydrometric station Pulo do Lobo (lat. 37° 48' N, long. 7° 38' W), located a few km above the last point of tidal influence (Mértola) and from the last station (10). Water temperature and salinity were measured with a conductivity-temperature-depth (CTD) device. Turbidity was determined by Secchi disk depth and by collection of water samples, the volumes of which were measured and then filtered through pre-weighed cellulose acetate filters. The filters were washed with distilled water and dried in a warm hot plate at 60° C for 24 h. Dried filters were then weighed to determine the amount of suspended particulate matter (mg l^{-1}). Nutrient analyses were carried out with an autoanalyser, according to the methods described in Strickland and Parsons (1972) and Grasshoff et al. (1983). Phytoplankton density index was measured via chlorophyll a, using fluorometric analysis of ethanol/acetone extracted samples (Welschemeyer, 1994).

The phytoplankton samples were collected from 1 m below surface and from 1 m above the bottom with a Van Dorn bottle. Lugol-fixed phytoplankton cells were counted and identified using a Zeiss IM35 inverted microscope. Horizontal, sub-superficial tows for mesozooplankton collection were made at a constant speed of 2 knots at 1 m depth with a conical net (0.37 m×1.60 m, 0.5 mm mesh-size). The net was equipped with a fluxometer to measure the flow. Samples were preserved in 4% buffered formaldehyde solution for taxonomic counts using a binocular microscope.

2.3 Fish larvae: Nucleic acids assays

Fish larvae were collected monthly from the Guadiana River between May and September 1997. Some fish larvae from zooplankton tows were sorted in a black glass tray, immediately frozen in liquid N at –197° C and later stored at -80° C. The larvae were thawed and measured (to the nearest 0.1 mm) under a dissecting microscope equipped with an ocular micrometer. A highly sensitive fluorometric method for nucleic acid (RNA, DNA) quantification in individual organisms was employed in a subsample of 206 fish larvae. The analytical procedure, described by Esteves et al. (in press), involves purification of tissue homogenates and subsequent fluorescence-photometric measurements using ethidium bromide (EB), a fluorochrome dye specific for nucleic acids.

2.4 GIS software program

The GIS system used to analyse the characteristics of the subcatchment areas (e.g., 1:25 000 charts, 1998 aerial photographs, land use, and 1995 Corine land cover biotopes) was ArcView GIS Version 3.1. This allowed integration of available information on the area (urban areas, agriculture areas, forestry and other natural areas, wetlands and water bodies).

2.5 Data analysis

Total abundance of phytoplankton was expressed in cells ml^{-1}, and for zooplankton as organisms per 100 m^3. Contours of abundance were produced by interpolation using kriging. The trophic system index (TSI) was calculated based on the Secchi disk depth (PDS) in m, according to Carlson (1977):

$$TSI=10(6-\log 2PDS).$$

Tests for significant relationships between some environmental parameters were made using Spearman's correlation. To avoid assuming a significant correlation due to random processes, the Bonferroni inequalities (Snedecor and Cochran, 1989) 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 if a correlation was significant using Spearman's test.

3. Results

3.1 Land use

We classified forestry and other natural areas with vegetation, wetlands and water bodies as natural units, and urban areas and agriculture areas as anthropogenic units. The information on land use in the subcatchment basin of the Guadiana Estuary indicated a high proportion of natural units (Fig. 3). The lower estuary is where important urban areas, such as Vila Real de Santo António in the Portuguese margin and Ayamonte in the Spanish margin, are located. However, in general, the Portuguese border area, for the entire extent of the estuary is more urbanised than the Spanish border area. Nevertheless, along both estuary margins, areas occupied by vegetation (forestry and similar areas) constituted significant proportions of the total area.

3.2 Spatial and seasonal variation of environmental parameters

The monthly inflow of the Guadiana River during the analysed period, 1996 to 1998, was high in winter, reaching 3400×10^6 m^3, and low in spring/summer, declining to 42×10^6 m^3 (Fig. 4). Salinity was lower in the upper estuary. Temperature was higher in summer and in the upper estuary (Table 1). Chlorophyll a showed a high value in the upper estuary (spring and autumn) (Fig. 4). The turbidity measure by the Secchi disk depth was lower in the middle estuary (average 58 cm) (Table 1). Phosphate and ammonia concentrations were higher in summer (Fig. 5), and the other analysed nutrients, nitrate and silicates, were higher in winter. The spatial distributions indicated sources of N in the lower estuary and sources of silicates and phosphates in the upper estuary (Table 1). Phytoplankton abundance exhibited maxim during summer, and was dominated in the upper/middle estuary by chlorophytes (mainly *Pediastrum* spp.), by diatoms (*Melosira granulata, Melosira* spp.) and cyanobacteria (mainly *Chroocococcus* spp. and *Microcystis* spp.) (Fig. 6), and in the lower estuary by dinoflagellates, plastidic nanoflagellates and diatoms (mainly *Chaetoceros* spp., *Leptocylindrus* spp., *Skeletonema costatum* and *Nitzschia* spp.). Zooplankton population in the estuary was dominated by cladocerans (mainly *Bosmina longirostris* in the upper estuary, and *Evadne* spp. and *Podon* spp. in the lower) and copepods (mainly *Acartia clausii*) (Fig. 7). Decapod larvae appear especially in the spring/summer in the middle/lower estuary. Major zooplankton taxa did not appear in consistent numbers in high river inflow periods (winter), although mysids (*Mesopodopsis slabberi*) were present throughout the year, with highest densities in autumn.

The analysed parameters showed high and significant relationships to salinity. A direct relationship was found between salinity and ammonia, and an inverse one with phosphate, nitrate and chlorophyll (Fig. 8). A temporal fluctuation of nutrient limitations occurred. In spring and early summer, some limitation of P was found

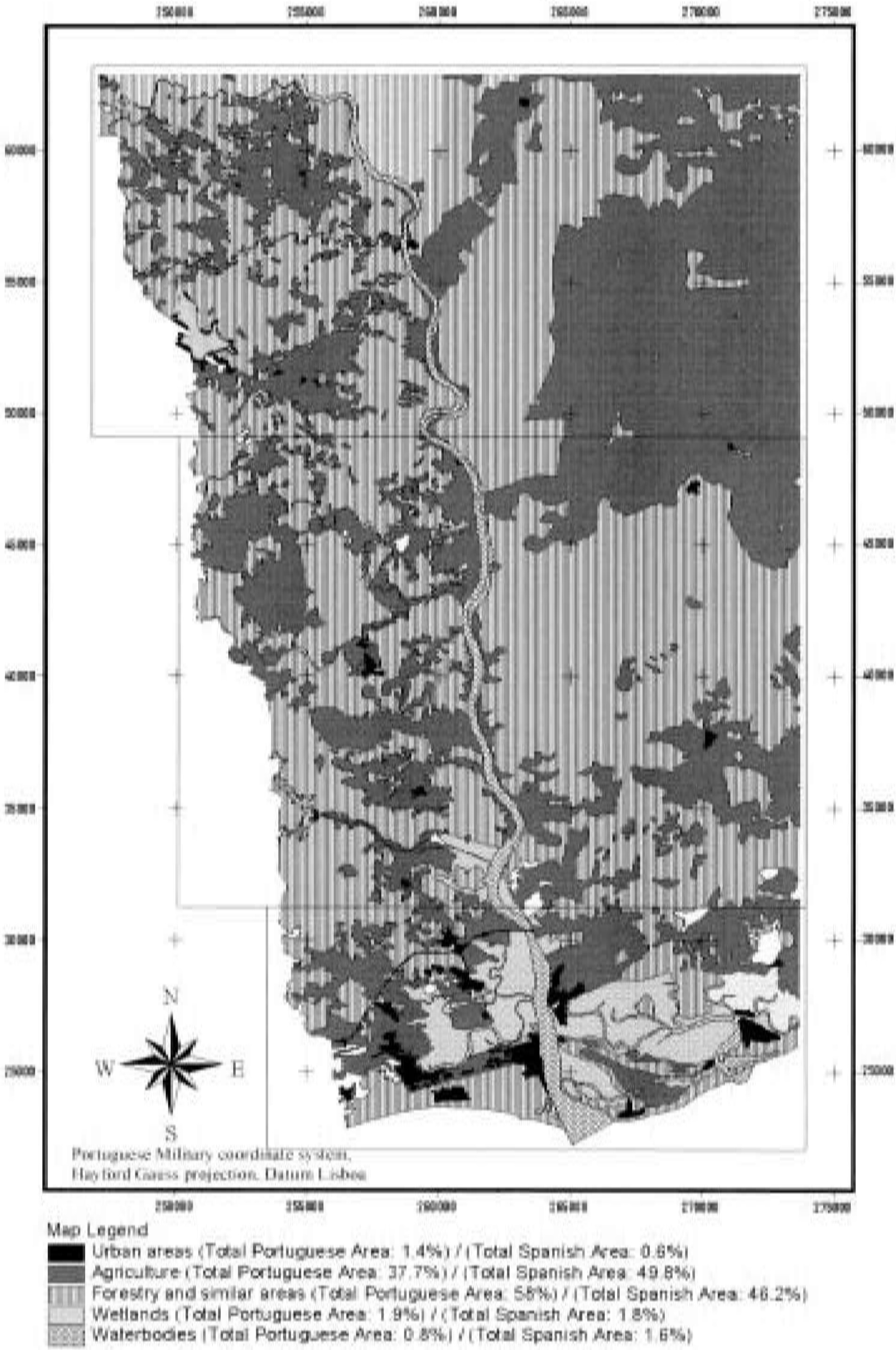

Figure 3 Form and intensity of subcatchment use (Land cover Corine) information integrated in the GIS system.

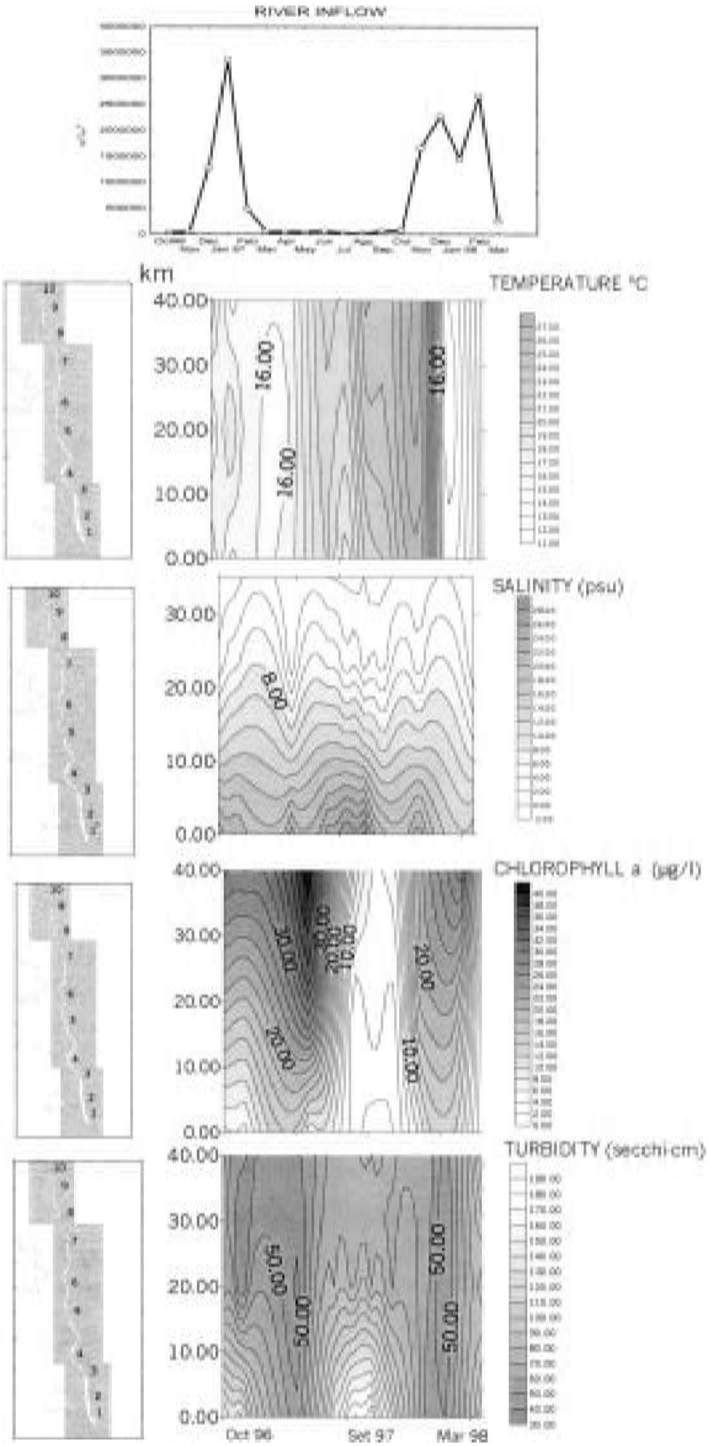

Figure 4 Interactions between hydrology and physic and chemical parameters in Guadiana Estuary.

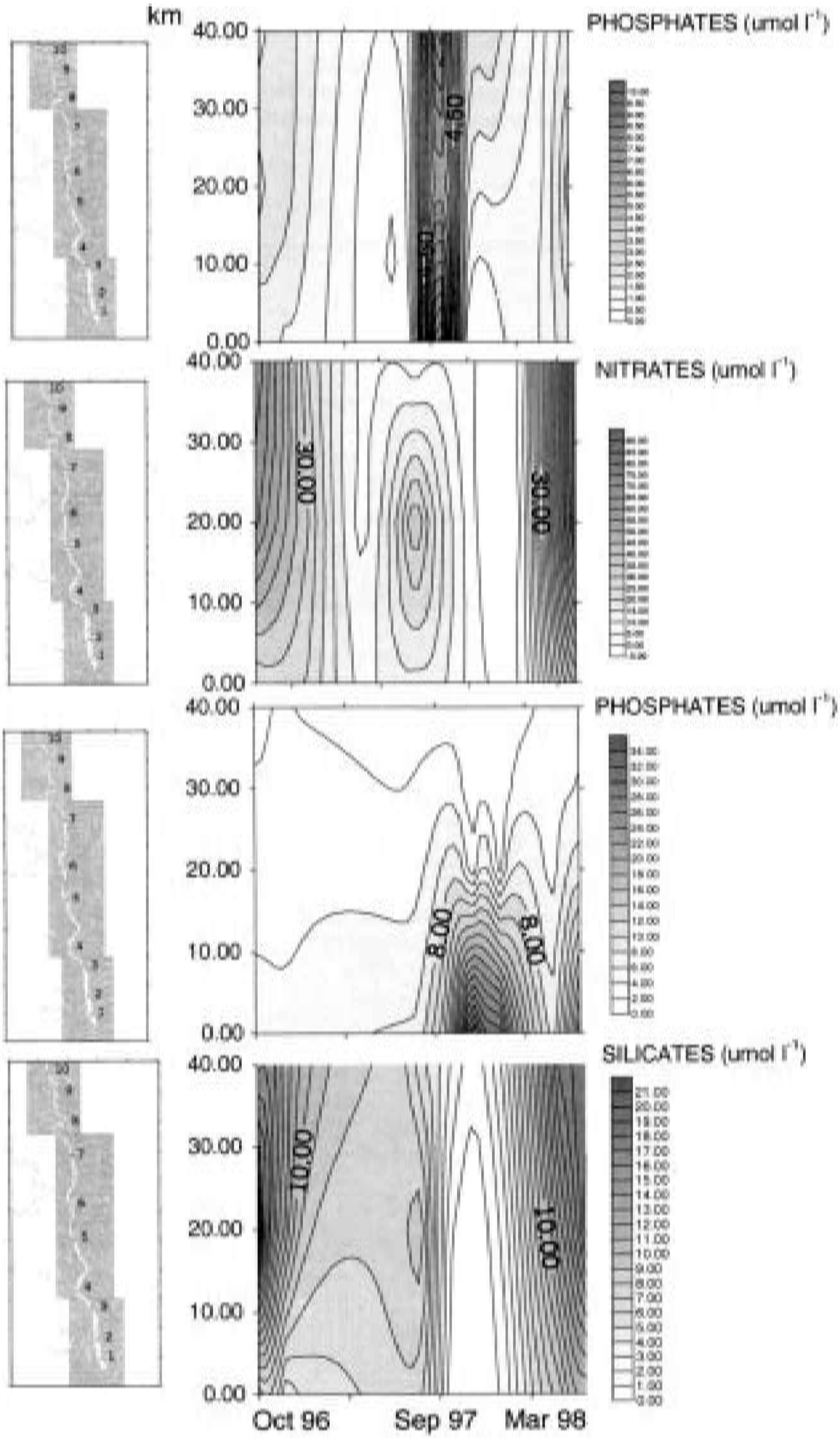

Figure 5 Interactions between hydrology and nutrients in the Guadiana Estuary.

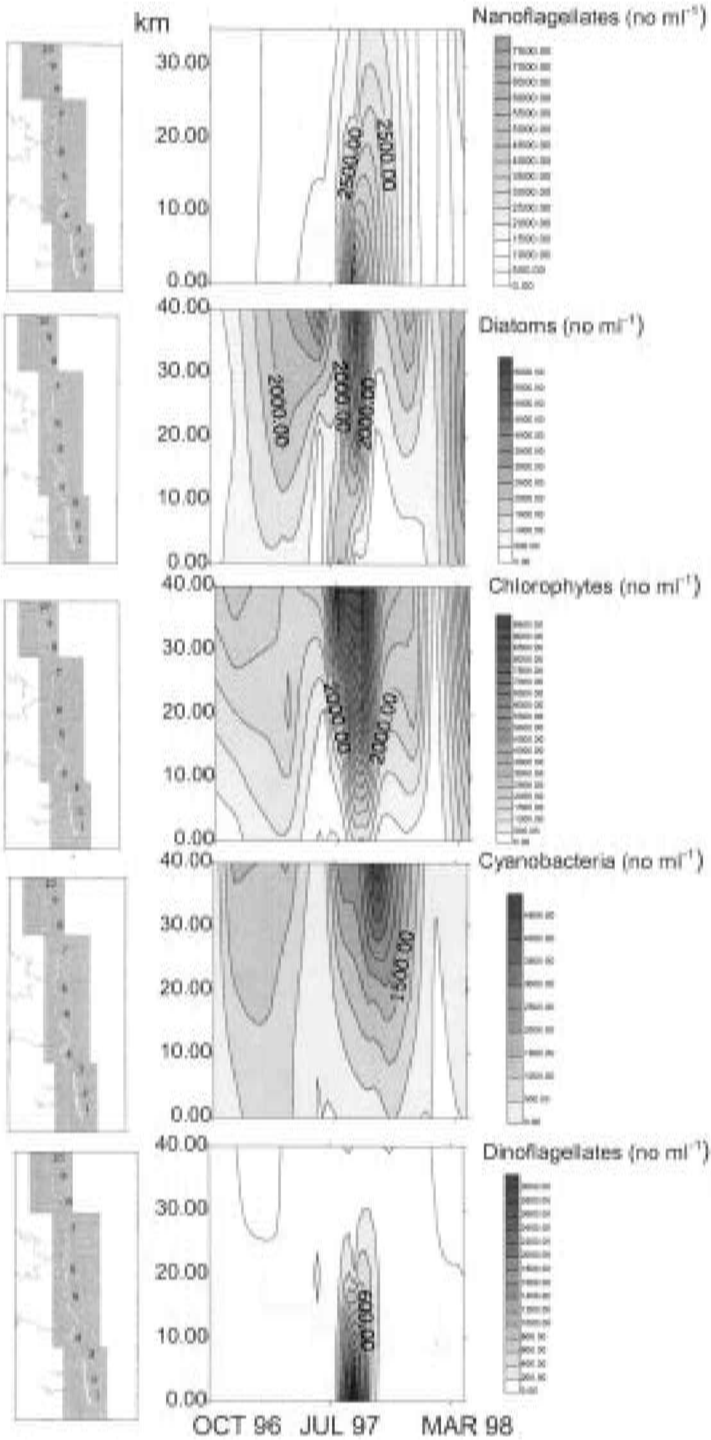

Figure 6 Interactions between hydrology and plankton densities in the Guadiana Estuary.

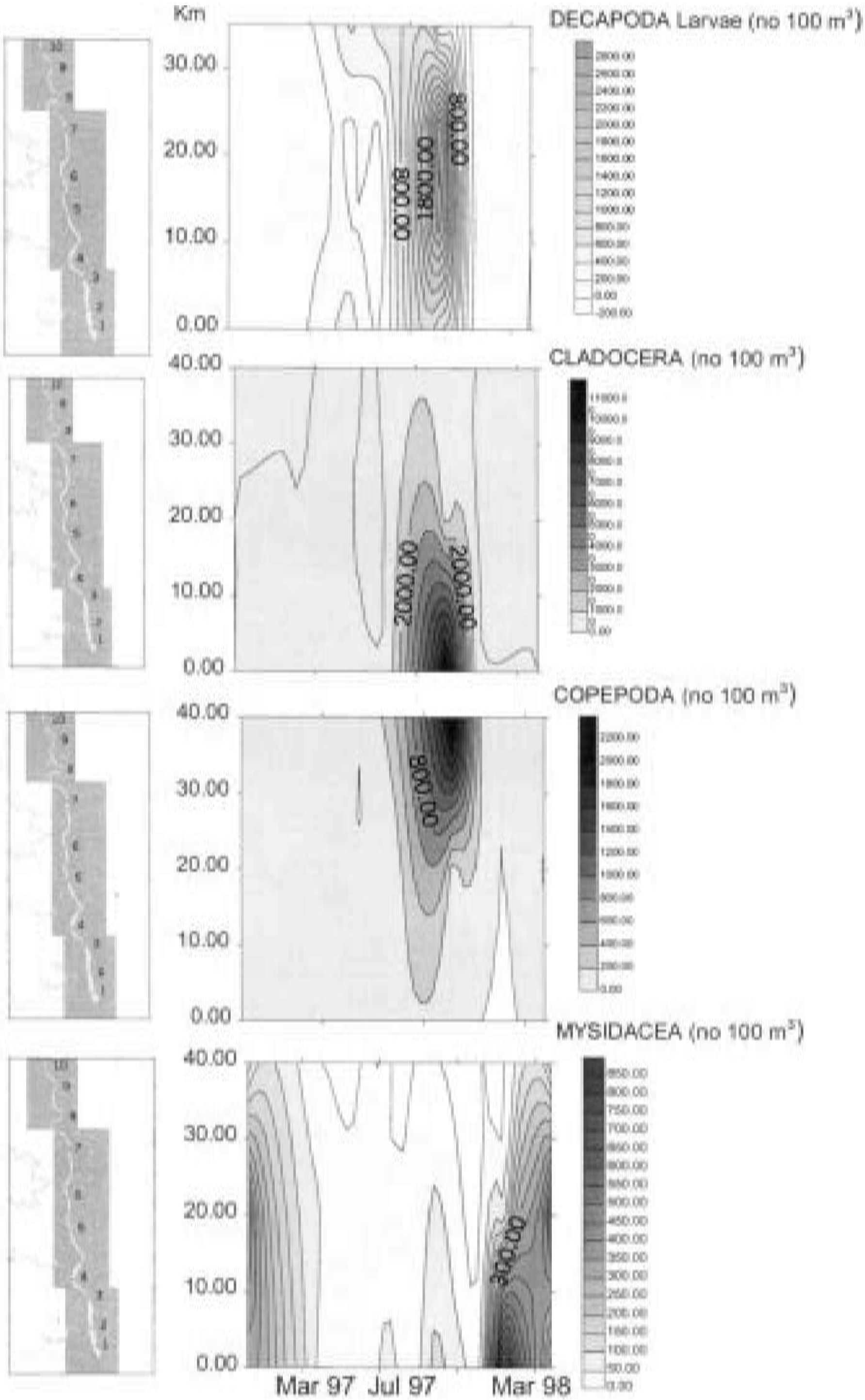

Figure 7 Interactions between hydrology and zooplankton densities in the Guadiana Estuary.

Table 1 Means and standard deviations of the parameters analysed in the different zones of the Guadiana Estuary. CHL a ($\mu g\ l^{-1}$), PDS-Secci disk depth (m), TSI-Trophic system index = 10 (6-log2PDS) (from Carlson, 1997).

	Zone		
Parameters	**Lower**	**Middle**	**Upper**
Temperature	19.72±4.76	23.3±2.8	24.2±2.75
Salinity	16.73±5.73	1.04±0.93	0.15±0.29
Secchi disk depth (m)	1.25±0.6	0.58±0.07	0.7 ±0.7
SIO_2 (µmol l^{-1})	2.24±4.78	4.13±5.57	6.23±6.66
PO_4^{3-} (µmol l^{-1})	0.86±0.66	2.32±2.04	3.72±3.6
NH_4^+ (µmol l^{-1})	21.98±12.42	2.87±2.81	1.82±1.29
NO_3^- (µmol l^{-1})	18.61±17.76	17.3±18.79	7.25±11.56
N/P	72.94±104.13	16.2±18.89	15.3±24.71
Chlor (µg l^{-1})	4.77±4.15	11.5±7.55	16.24±12.5
TSI	56.79±7.72	67.83±6.29	65.23±4.43
Seston (mg l^{-1})	59.48±18.4	25.5±8.83	17.6±7.347
Organic matter (mg l^{-1})	24.63±6.08	14.4±5.59	9.21±0.78

but in the end of summer and autumn N became the limiting nutrient (Fig 9). The trophic status of the estuary can be classified, according to chlorophyll a and the PDS index, as mesotrophic, except for the upper estuary which is eutrophic (Table 1).

3.3 Diel variations

Phytoplankton density was higher in the ETM area (Fig 10a), where cyanobacteria dominated, than at the lower estuary station, where nanoflagellates dominated (Fig 10b). However, no tidal or diel variations were exhibited by the phytoplankton.

Zooplankton, being transported into and out of the estuary at the entrance (lower estuary), were more abundant and more diverse than those entering the estuary up river (middle/upper estuary). Zooplankton transported into the estuary consisted mainly of coastal taxa such as *Podon* spp., *Evadne* spp., *Sagitta friderici* and *Oikopleura* spp. They were especially associated with the high tide and high salinity values. Copepods, fish larvae, Decapoda larvae and *Mesopodopsis slabberi* were also present. A diel effect was seen, with high values during the night (Fig. 11).

Salinity did not show great variability in the ETM station, but a peak of turbidities of 100 mg l^{-1} were representative of periods between 11:00 and 13:00 during the ebb tide. Zooplankton species characterising the ETM area were mainly *Mesopodopsis slabberi*, but copepods and fish larvae were also present (Fig. 11). Nevertheless, due to the early ending of sampling in the ETM area, the diel effect was more obvious for fish larvae than for copepods or *Mesopodopsis slabberi.*

3.3 Fish larvae condition (RNA/DNA Index)

The sampled larvae belonged mostly to the following taxonomic groups: Clupeidae (*Engraulis encrasiclous*, *Sardina pilchardus* and *Alosa* spp.), Blennidae (*Parablennius* spp.), Gobbidae (*Pomatochistus* spp.) and Atherinidae (*Atherina* spp.). Mean contents of nucleic acids were relatively high (Fig. 12). In fact, only low percentages of larvae in bad condition (RNA/DNA lower than 1, according to Clemmesen, 1994 and Chicharo, 1997) were found (Table 2).

4. Discussion

4.1 Land use

From the viewpoint of sustainable use of water resources, river ecosystem quality is dependent to a great

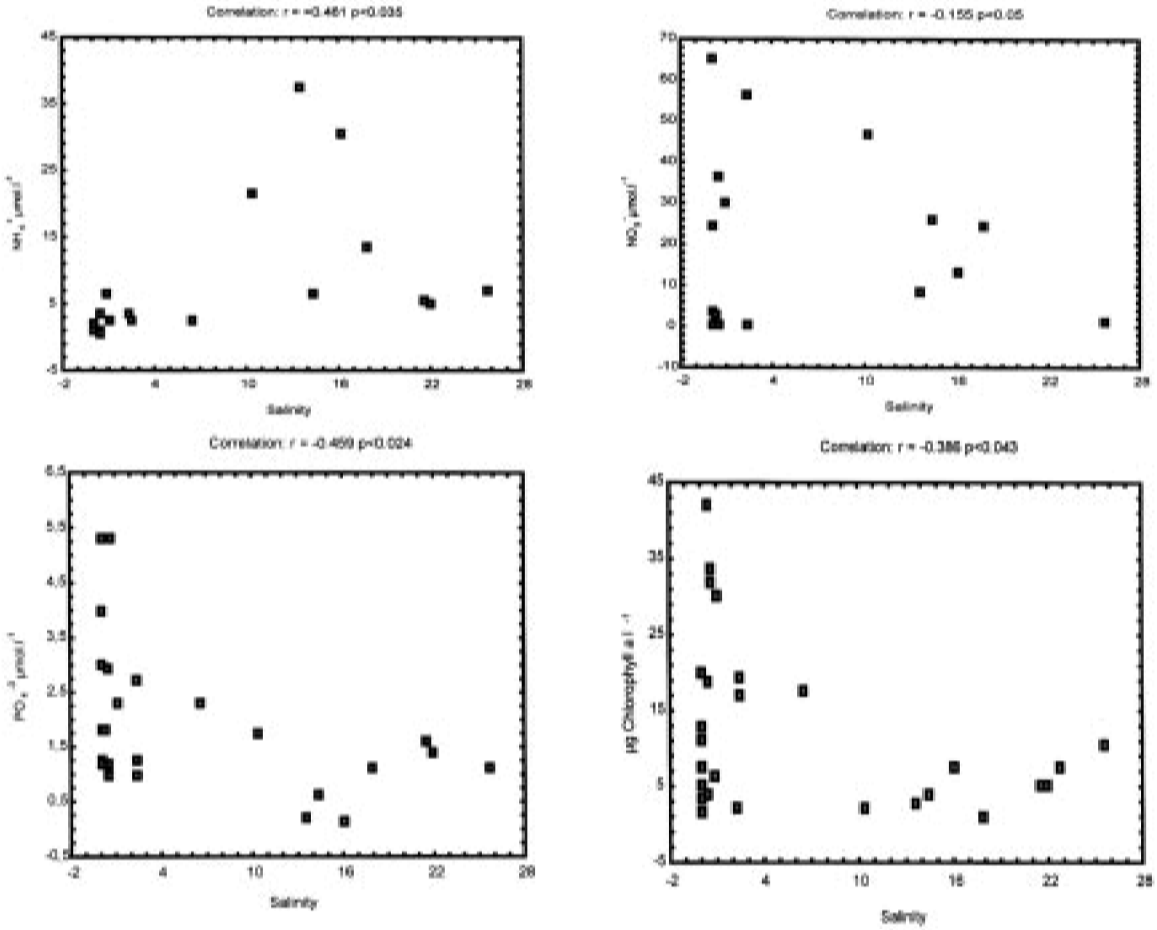

Figure 8 Relationships between salinity and nutrients (ammonia, nitrate and phosphate) and chlorophyll a.

Table 2 Number and percentages of fish larvae from each taxonomic group below 'minimum level' (RNA/DNA<1).

Fish larvae	n	Percentage
Clupeidae	39	0
Blennidae	63	11.1
Gobiidae	70	7.1
Atherinidae	34	2.9
Total	206	6.3

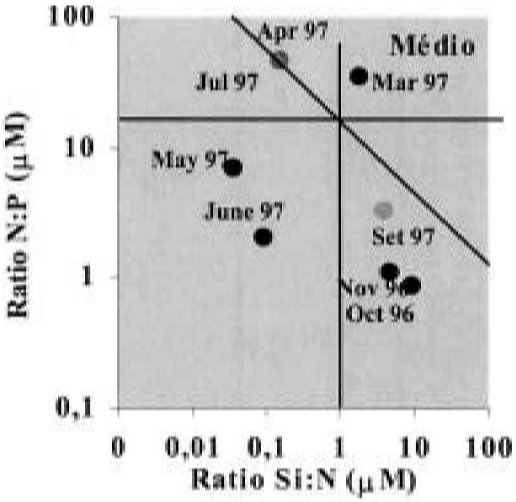

Figure 9 Temporal nutrient limitation in the Guadiana Estuary based on the N:P and Si:N ratios.

extent on catchment use, which directly influences water quality at any point in the river continuum (Zalewski et al., 1997). The importance of catchment to spatial and temporal variability in the Guadiana Estuary is reflected in the finding of high nutrient concentrations, especially ammonia, near urban areas (lower estuary stations 1-3) and during the summer. The GIS-based classification of information on land uses in the subcatchment basin of the estuary into natural and anthropogenic units showed that the subcatchment presently has low anthropogenic pressures. The lower estuary is more susceptible to negative influences owing to the concentration of important urban areas there. Nevertheless, both the Portuguese and Spanish margins of the estuary have a significant important percentages of occupation by natural habitats.

4.2 Spatial and seasonal variation of environmental parameters

With respect to nutrients, the N:P and Si:N ratios (according to the Redfield ratio, 106C:16N:1P) and considering that Si and N are assimilated in the same proportions (DeAngelis, 1992), the results indicate some limitation by P, except near the end of summer, when N limitation appeared. Estuarine waters are usually characterised by summer phosphate maximum, related to decomposition of detached leaves of salt-marshes (Knox, 1986) and this seemed the case in the Guadiana Estuary. Growth of cyanobacterial populations in summer/autumn in the middle/upper estuary is related to the normal succession in the algal community. Diatoms are the first step in the succession at the beginning of spring and lead to a decrease in silica availability. Chlorophytes then follow the diatoms and lead to a decrease of N availability. This factor controls the subsequent development of algal populations, namely the cyanobacteria, which are more competitive under conditions of low nutrient availability, owing to some species' ability to store P and fix N (Ejsmond-Karabin, 1983; Elwood et al., 1983; Zalewski and Wagner, 1998). In parallel with this process, the activity of aquatic zooplankton (e.g., Copepoda and Cladocera) is also more vigorous in that period in the upper/middle estuary, eliminating the smaller phytoplankton competing with cyanobacteria and allowing them to dominate successively (Holm et al., 1983).

4.3 Diel Variations

Phytoplankton did not exhibit a diel pattern but showed higher densities by one order of magnitude in the ETM area. This may be related to some light attenuation in the light/dark regimen due to high turbidity in this area. According to Cloern (1999), in a low light environment, algal growth efficiency is enhanced by nutrient enrichment, which certainly seems to occur in the ETM area.

Cyanobacteria also dominated in the ETM area in agreement with the results from the seasonal study. Zooplankton characterising the ETM were dominated quantitatively by Decapoda larvae and by mysids, especially *Mesopodopsis slabberi*. This is an euryhaline mysid shrimp frequently very abundant in estuarine systems (Greenwood et al., 1989). This species occurs in more dense assemblages near the ETM than those found in the lower estuary.

Our results also show that the mysid *Mesopodopsis slabberi* ascended in the water column only after sunset and descended before dawn at the station near the mouth of the estuary, while at the ETM station no clear pattern of daily migration was observed for mysids or Decapoda larvae. This is probably related to the high attenuation of the light/dark regimen that is believed to be a cue to induce a circadian rhythm of activity (Chícharo et al., 1998). The appearance of peak turbidities of 100 mg l^{-1} were representative of periods between 11:00 and 13:00 during the ebb tide, typical of ETM phenomena as defined and described by Reed and Donovan (1994). However, low Secchi disk depths in the ETM station were continuously registered. It has

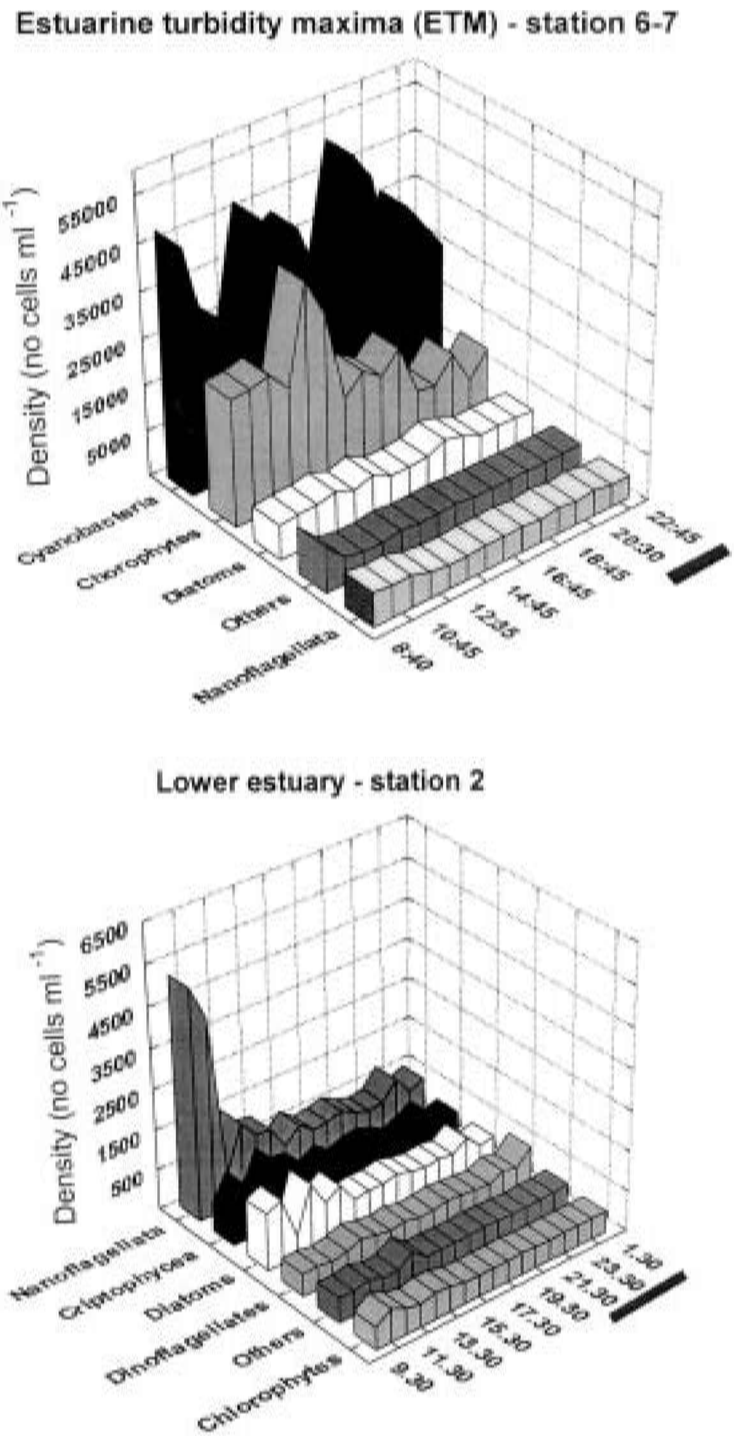

Figure 10 Phytoplankton diel variations in the a) Estuarine Turbidity Maxim (ETM) and b) at the lower estuary. Black box indicates the night period.

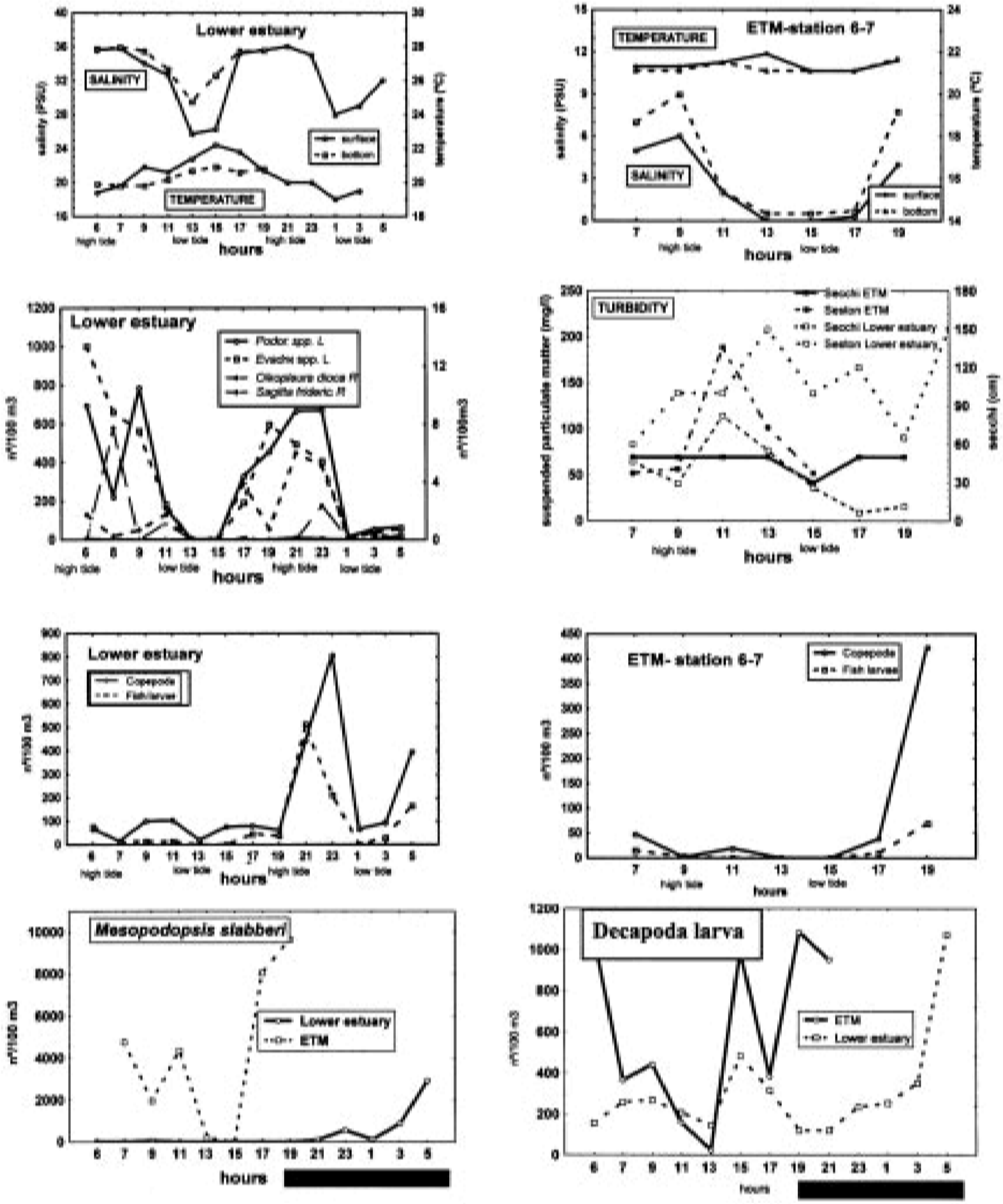

Figure 11 Zooplankton diel variations and turbidity in the Estuarine Turbidity Maxima (ETM) and the lower estuary. Black box shows night period. No data in the ETM after 19:00.

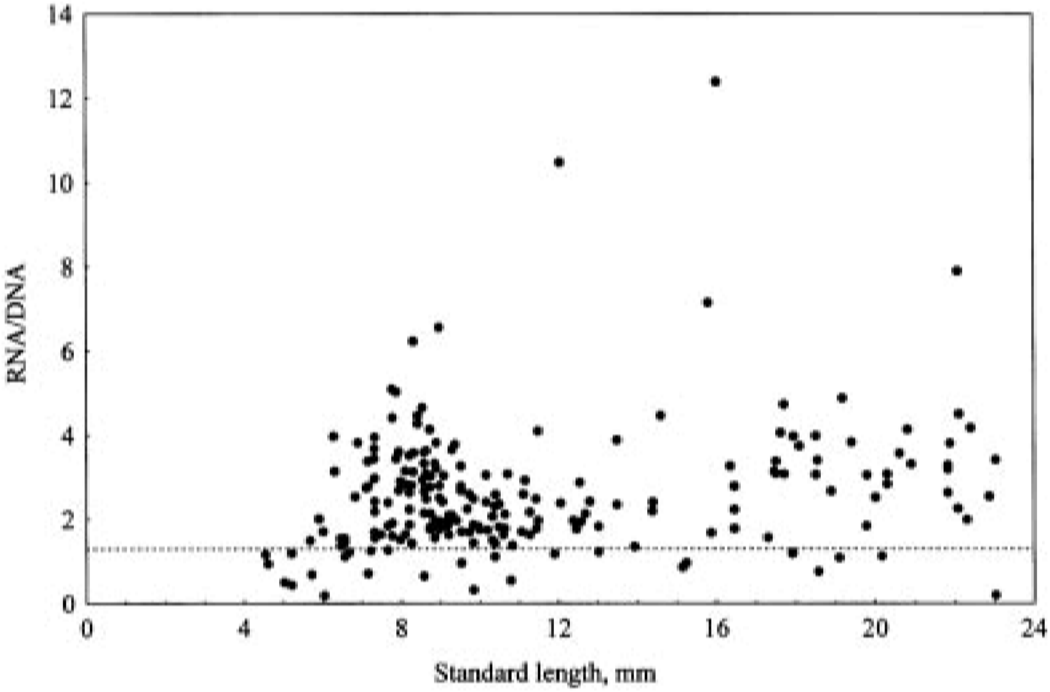

Figure 12 RNA/DNA ratios in fish larvae sampled in the Guadiana Estuary versus standard length.

been assumed that water column (i.e., planktonic) and near bottom (i.e., epibenthic) zooplankton associated with the ETM maintain reproductively viable populations by behavioural adaptations to ETM circulation, thus achieving high productivity owing to the concentrations of food particles within the turbid areas (Kingsford and Suthers, 1994). Moreover, the results of the seasonal study also showed some evidence of retention strategies by mysids, as they were usually more abundant during a high inflow period (autumn/winter). This is probably associated with the fact that under high inflow conditions, the estuary can be classified as a stratified estuary (Michel, 1980). This allows vertical migrations, and maintenance of organisms in seawater during ebb conditions.

Net seaward flow in estuaries often poses a retention problem for endemic zooplankton populations, although their continuous existence, albeit of limited spatial extent, provides evidence that they can successfully resist displacement forces. Particle trapping processes in estuaries at the estuarine turbidity maximum (ETM) may also significantly influence the retention of plankton (Simenstad et al., 1994).

4.4 Fish larvae condition (RNA/DNA Index)

Contaminants usually have subtle and long-term effects on biological systems, and especially affect the reproductive abilities of plants and animals as well the larval stages, which are more susceptible than adults (Chícharo, 1998). For this reason, the determination of the fish larval condition in an affected area has been developed as a diagnostic tool for water quality investigation (Kirk and Lewis, 1993). Studies on the ichthyoplankton of the Guadiana Estuary already indicated that this is an important nursery area for several commercial fish species (Chícharo and Teodósio, 1991). The present study showed that most larval specimens were in medium to good condition and the percentage of larvae with RNA/DNA ratios below the 'minimum ratio' to survival (Clemmesen, 1994; Chicharo, 1997) was very low, at 6.3%.

5. Conclusions

The integration of different tools of ecohydrology, from GIS to the cellular level, has contributed to a better understanding of the processes that influence the aquatic biota of the Guadiana Estuary. The classification of information about land use in the subcatchment basin of the estuary into natural and anthropogenic units showed that the subcatchment basin is under low anthropogenic pressure. With respect to nutrients, the N/P ratio indicated some limitation by P, except during summer, when some limitation by N was indicated. The trophic index showed that middle and lower estuary could be classified as mesotrophic. Water quality was usually good, which is reflected in the physiological condition of fish larvae. However, if a reduction of inflow and more intense uses of catchment were implemented, as is expected following construction of the Alqueva dam, then this will have consequences for the aquatic biota of the estuary. Attention should especially be given to the position of the ETM area, which will change. The estuary will probably become more vertically homogenous. These alterations will have consequences especially for the retention of zooplankton in the estuary, including fish larvae and crustacean decapod larvae. Moreover, the cyanobacterial blooms in the upper estuary, which already has eutrophication problems, may cause significant problems with respect to use of the estuary for fishing, mollusc harvesting and aquaculture, thereby affecting the economy of the region.

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