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Scientific Editors

Aquatic Ecology of the Mondego River Basin
Global Importance of Local Experience

Coimbra • Imprensa da Universidade

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Coordenação editorial
Imprensa da Universidade de Coimbra

Concepção gráfica
António Barros

Infografia
António Resende
Estimulus [design] • Coimbra

Execução gráfica
GRAFIASA

Ilustração da capa
P. P. Cunha e J. Dinis

ISBN
972-8704-04-6

ISBN Digital
978-989-26-0336-0

DOI
http://dx.doi.org/10.14195/978-989-26-0336-0

Depósito legal
175038/02

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IMAR – INSTITUTO DO MAR
IPIMAR – INSTITUTO DE INVESTIGAÇÃO DAS PESCAS E DO MAR

AQUATIC ECOLOGY OF THE MONDEGO RIVER BASIN.
GLOBAL IMPORTANCE OF LOCAL EXPERIENCE

1. INTRODUCTION AND DESCRIPTION OF THE AREA

2. COMMUNITY STRUCTURE AND DIVERSITY

FRESHWATERS

ESTUARY

3. NUTRIENT CYCLE AND PLANT DYNAMICS

FRESHWATERS

ESTUARY

4. ECOLOGY OF FAUNAL POPULATIONS

FRESHWATERS

ESTUARY

5. DEALING WITH ENVIRONMENTAL PROBLEMS

6. THE MONDEGO RIVER AS A CASE STUDY FOR INNOVATIVE TOOLS IN RESEARCH AND DEVELOPMENT

João Carlos Marques [1]
Manuel Augusto Graça [1]
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INTRODUCING THE MONDEGO RIVER BASIN

General Introduction

The source of the Mondego river is located at the "Estrela" Mountain and extends along 227 Km, draining a hydrological basin of approximately 6670 Km^2, the largest entirely comprised in Portuguese territory (Lourenço 1986) (Figure 1).

The main tributaries of the Mondego are the rivers Dão, Ançã and Foja, in the right margin, and the rivers Alva, Ceira, Cernache, Ega, Arunca, and Pranto, in the left margin (Lourenço 1986). The Pranto river converges with the Mondego already in the estuarine area. The Mondego valley between Aguieira and Coimbra is considerably deep, but the river spreads below this town to form a vast alluvial plain, the Lower Mondego Region, which consists of 15 000 hectares of good agricultural land.

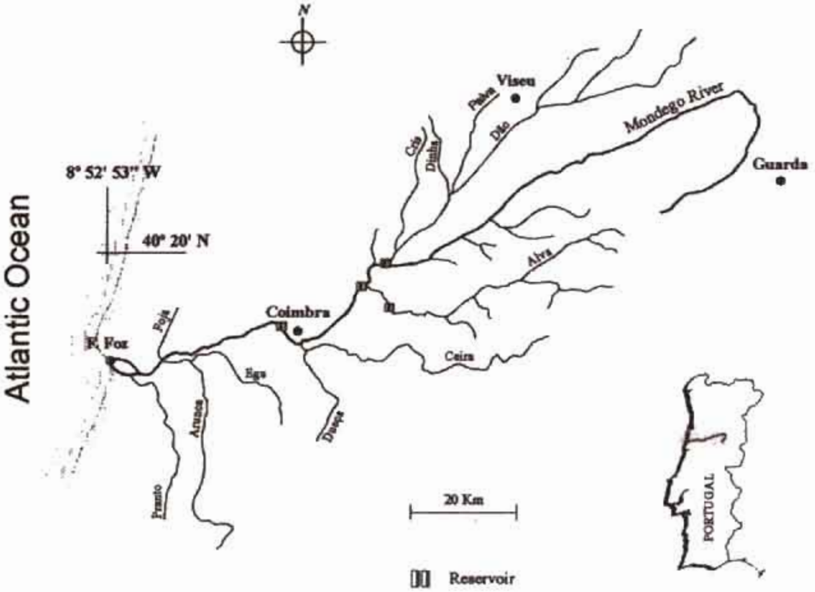

Figure 1. The Mondego river hydrological basin.

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The Mondego river basin plays an important role regarding the activities and day-by-day life of more than half a million people. Nevertheless, in the early sixties, the basin was still under-utilised. From 1962, the "Direcção Geral de Recursos e Aproveitamentos Hidráulicos", prepared a hydro-agricultural plan aiming to improve the use of the basin water resources and alluvial plain by means of the river flow control. The plan had also the objectives of, providing water to populations, industries, and agriculture, and produce electric power.

As a whole, this plan was only partially accomplished, but we shall not utter here any opinion concerning the plan it self or its execution. Floods control was achieved through river margin regularisation and by the construction of several dams ("Aguieira", "Raiva" and "Açude de Coimbra", in the Mondego, and "Fronhas", in the Alva river). Flood control, had doubtless a considerable environmental impact, namely in the Mondego hydrological regimen (Dias and Rebelo 1984).

The Lower Mondego river valley, at the present, consists essentially of agricultural land where the main crop is rice (60% of the valley). Other significant crops are corn and beans (18% of the area). Non-cultivated areas, such as swamps, are usually located in the perimeter of the valley and exhibit a flourishing fauna and flora. Drainage channels, which are widespread across the whole valley, also constitute a biological reservoir (Anastácio et al. 1995).

The drainage of this Lower Mondego Region contributes with an important load of nutrients and several chemical pollutants into the Mondego estuary, located in the western coast of Portugal. Besides, the estuary it self constitutes an important system to support human activities, correspond to a considerable concentration of people and goods. Actually, the estuary is the location of a mercantile harbour, "Figueira da Foz", which has considerable regional importance, namely regarding the export of wood pulp for paper production. Urban waste-waters are still let out into the Mondego without treatment, and besides the harbour facilities, the estuary supports industrial activities, salt-works, and aquaculture farms. Additionally, the city of "Figueira da Foz" constitutes an important centre for tourism activities, which implies a seasonal increase of the human pressure on the system.

Brief description of the freshwater systems

Algae and macrophytes are the producers in rivers and streams. At the University of Coimbra there has been a strong tradition in phycological studies. However, most of the studies dealt with cell biology and taxonomy of individuals sampled from streams, lakes, ponds, fountains and terrestrial systems. The ecological studies are therefore scarce. The construction of reservoirs for irrigation and hydro electrical proposes created new environments for plankton taxa. The crescent use of fertilisers leaded to eutrophic conditions in some of those reservoirs, which resulted in blooms of cyanobacteria producing toxins and therefore affecting human populations.

The vegetation of the low Mondego River has been studied from the perspective of plant invasion into channels (Almeida et *al.* 1991, Morais et *al.* 1994). A recent study on the aquatic and riparian species of the artificial Mondego River channel indicated that nearly 10% of the 212 recorded taxa are invading species (Aguiar et *al.* 1999, 2001). The dominant taxa of the Mondego river channel are all exotic: *Eryngium pandanifolium* (piteirão), *Tradescantia fluminensis, Paspalum paspalodes* (graminhão) and *Acacia dealbata* (mimosa), among others. *Eryngium pandanifolium*, a South American species was reported for the first time in 1947 near Figueira da Foz (Garcia 1947). The expansion of this specie seems to be related with channel modification (Aguiar *et al.* 2001).

The number of Hidrophyte *taxa* is low. However, the more abundant taxa are the exotic *Azolla filiculoides* e *Myriophyllum aquaticum* (pinheirinha-de-água). The riparian vegetation is dominated by willows, ash, alder and poplar.

Besides primary production, organic matter entering the streams and rivers is an important energy source for aquatic systems. Deciduos and some evergreen trees dominated the original forest in the Mondego basin. Due the canopy cover, many small streams are always heterotrophic: most of their energy consist in organic matter rather than benthic algae. In small streams, invertebrates feeding in fine and coarse particulate organic matter are the most abundant.

As a result of the temperature decrease in autumn, many deciduous trees lose their leaves, leading to an input of organic matter to small streams. The result of this autumnal energy input coupled with a decrease in summer precipitation is a seasonal abundance of many aquatic insects. Many *taxa* have an aquatic stage in autumn-winter (high food in the aquatic system) and an aerial stage in summer (low water level).

One of the high economic activities in the Mondego river basin is the wood extraction for pulp production. The main tree used for such purposes is *Eucalytus globulus*, a species introduced from Australia. Due to the energy linkage between riparian vegetation and the aquatic systems, forest replacement is likely to affect aquatic communities. Studies in the Mondego river basin have shown that leaves of *Eucalyptus* are protected against microbial degradation and therefore they are not readily taken by invertebrates as food source. The plantations with eucalyptus are therefore likely to affect the structure and functioning of aquatic systems.

New water treatment plants were constructed in recent years to decrease organic pollution. On the other hand, there has been a general decrease in the agricultural area in the Mondego river basin. At the same time, the amount of fertilisers has increased and the number of industries increased as well. Those changes affect water quality. Water quality of streams and rivers draining the Mondego basin range from very clean to heavily polluted, as assessed by the use of chemical physical and biological data. Because aquatic communities directly reflect changes in the chemical and physical environment, the use of biological methods for biomonitoring proposes is urgent. Moreover, such methodology will be compulsory by the new EC regulations.

Macroinvertebrates are food for fish and vertebrates. They also impact economic activities or may be resources for human populations. This is the case of the crayfish *Procambarus clarkii*. In the lower Mondego river valley, the introduction of this species had a significant effect on economic activities. For that reason a large effort was made to know the ecology of *Procambarus clarkii*.

Another very important component of aquatic communities are fish communities. Due the biogeographic conditions, cyprinids are very important in the rivers of the Iberian Peninsula. Damming destroys the longitudinal continuity of rivers and create lentic (reservoirs) environments were new species can be established. The Mondego river basin is therefore in a transitional phase and new studies are needed to assess the impact of such modifications. Some effects are already noticed. The creations of reservoirs hamper lampreys of completing their migratory movements. Such species is highly appreciated in the area as food for human populations.

Rice fields in the lower Mondego river also created habitats for new fish species. That is the case of the mosquitofish *(Gambusia affinis)* which can attain large numbers and high production. Marshlands or abandoned rice fields are also ideal habitats for amphibians.

Finally, the Mondego river basin is the home of one mammal with a high protection status in the European Union area: the otter *Lutra lutra*. This species is widely distributed in the Mondego river basin and their survival depends on the preservation of habitat, especially bank river vegetation.

Brief description of the estuary hydrographical and ecological conditions

The Mondego estuary is located in a warm temperate region with a basic continental temperate climate. It consists of two arms, north and south (Figure 2) separated by an island. The two arms become separated in the estuarine upstream area, at about 7 Km from the sea, and join again near the mouth. These two arms of the estuary differ in their hydrographic characteristics. The north arm is deeper (5 to 10 m during high tide, tidal range about 2 to 3 m), while the south arm (2 to 4 m deep, during high tide) is almost silted up in the upstream areas, which causes the freshwater of the river to flow essentially by the north arm. The water circulation in the south arm is mostly due to tides and to the relatively small fresh water input of a tributary, the Pranto River, which is artificially controlled by a sluice, located at 3 km from the confluence with the south arm of the estuary. In addition, due to differences in depth, the penetration of the tide is faster in the north arm, causing daily changes in salinity to be much stronger, whereas daily temperature changes are higher in the south arm (Marques 1989, Marques *et al.* 1993a, 1993b, Flindt *et al.* 1997).

Eutrophication is increasing in most estuaries all over the world, probably as a result of excessive nutrient release into coastal waters, and the Mondego estuary is no exception. Seasonal intertidal macroalgae blooms (mainly of *Enteromorpha* spp.) have been reported in the south arm of the estuary for several years (Marques et al. 1993a, 1993b, Pardal 1998, Pardal et al. 2000, Lillebø et al. 1999, Martins et al. 2001). As a pattern, although there is a clear inter-annual variation as a function of hydrological conditions, *Enteromorpha* spp. biomass increases from early winter (February/March) up to July, when an algae crash usually occurs. A second but much less important algae biomass peak may normally be observed in September followed by a decrease up to the winter.

The *Zostera noltii* beds, which represent the richest habitat with regard to productivity and biodiversity, are being drastically reduced in the south arm of the

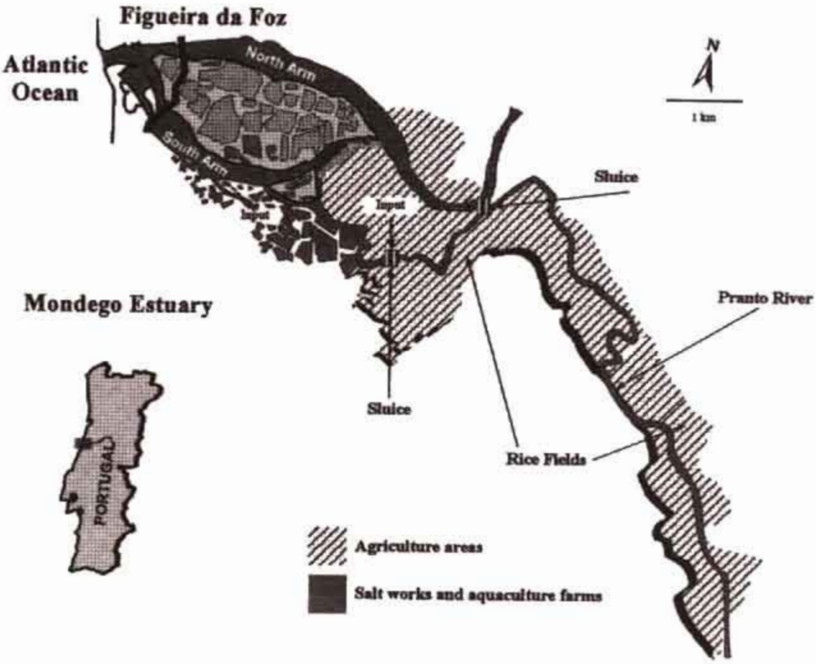

Figure 2. The Mondego estuary consists of two arms, north and south separated by an island. The two arms become separated in the estuarine upstream area, at about 7 Km from the sea, and join again near the mouth. The water circulation in the south arm is mostly due to tides and to the relatively small fresh water input of a tributary, the Pranto River, which is artificially controlled by a sluice, located at 3 km from the confluence with the south arm of the estuary.

Mondego estuary, presumably as a function of competition with *Enteromorpha*. (Raffaelli *et al.* 1991, Hodgkin and Hamilton 1993), resulting from the different strategies of macroalgae and macrophytes to uptake nutrients (Fairweather 1990 *in* Hardy *et al.*1993). Such shift in the benthic primary producers due to eutrophication may probably affect the structure and functioning of the communities, including the species composition (Marques et al. 1997, Pardal 1998), and through time such modifications may determine a selected new trophic structure.

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PRECIPITATION AND THE HYDROLOGY OF THE MONDEGO CATCHMENT: A SCALE – INVARIANT STUDY

Abstract

Precipitation in the Mondego drainage basin, in Portugal, is a highly variable input into the hydrological system of this region, strongly affecting water resources management. Precipitation is a highly non-linear hydrological process that exhibits wide variability over a broad range of time and space scales. The strongly irregular fluctuations of precipitation, which in certain cases lead to catastrophic events, have strong socio-economic impacts, related to the occurrence of floods and droughts, reservoir management policies, etc.

Many approaches to the study of precipitation fail to grasp the extreme variability of this process. The invariance of properties across scales and the multifractality of precipitation may offer an alternative approach to quantify this variability. Thus, to increase our understanding of the precipitation variability in the Mondego basin, this work explores the invariance of properties manifested across scales and the fractal and multifractal behaviour observed in the temporal structure of precipitation, using daily precipitation data from two locations in this catchment: Coimbra and Penhas Douradas. The data cover a period of 54 years. The study is based on spectral analysis, box-counting analysis, and investigation into the scaling of probability distributions and statistical moments of the precipitation intensity. Results show the presence of scale-invariant and multifractal properties in the temporal structure of precipitation.

Introduction

Precipitation exhibits a high non-linear variability over a wide range of time and space scales. This variability involves a large dynamic range, which in certain cases leads to catastrophic events. Precipitation phenomena range from cells (associated with

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cumulus convection), to synoptic areas (frontal systems). Precipitation cells have an area of the order of 1-10 km^2 and lifetimes of several minutes. Synoptic precipitation fields can cover areas of 10^4 km^2 and have a lifetime of one to several days.

Precipitation is the driving agent of many other processes. Its temporal and spatial variability are important issues in many studies and areas of research (e.g. hydrology, hydraulics, agronomy, soil pollution, water resources). However, information on the amount and distribution of precipitation in space and time is often restricted precisely because of this strong temporal and spatial variation, which many approaches to the study of precipitation fail to grasp.

The purpose of this work is to contribute to a better understanding of precipitation in the river Mondego drainage basin, in the Centre of Portugal (Figure 1). The strongly irregular fluctuations of river discharge, in both the Mondego River and its main first-order tributary streams, have led to different actions being taken with respect to the watercourses, aiming at diminishing the effect of hydrological extremes. In the past, until the end of the seventies, frequent flooding of the lower-lying lands near the estuary of the river (downstream of Coimbra, see Figure 1) had strong socio-economical impacts. These floods occurred nearly every year, with calamitous consequences. In the same region, fresh water was also often scarce due to the uneven distribution of precipitation during the year, and intrusion of saline water in the river and in the groundwater.

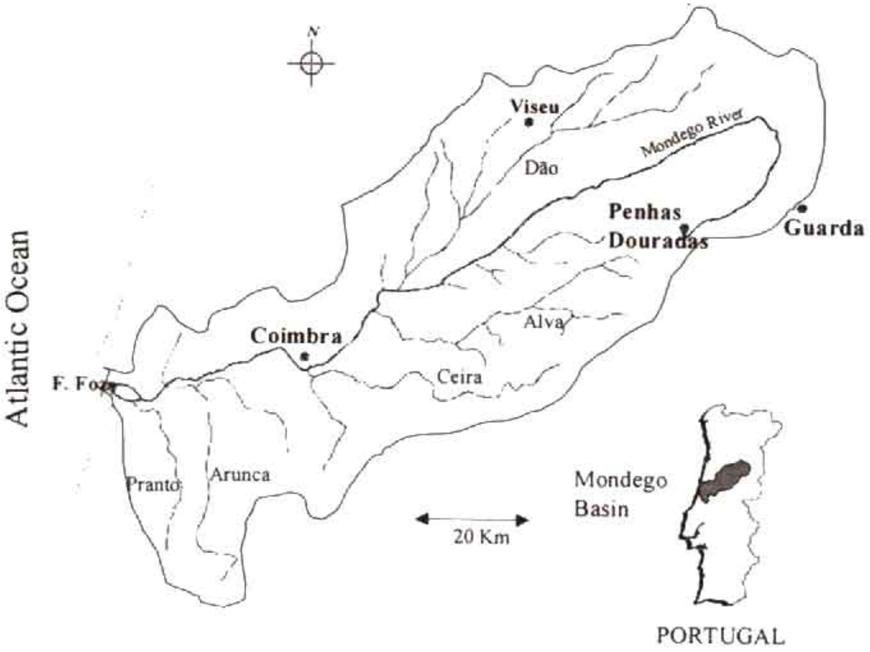

Figure 1. Map of Mainland Portugal localising the Mondego Basin, and an enlarged view of the catchment area locating the River Mondego and its main first-order tributaries, the main cities, and the two meteorological stations used in this study (Coimbra and Penhas Douradas).

These and other problems led to the development of a multipurpose hydro-agricultural project in the Mondego basin. The works carried out in the river and in the lower part of the drainage basin include flood protection works, and irrigation and drainage works. The management of its different components is very much dependent on the highly variable precipitation input into the hydrological system. Some difficulties arise from the lack of knowledge on the temporal and spatial distributions of precipitation in the catchment. For example, hydrological models usually have to conceptualise processes based on simple, often homogeneous, approximations of nature (e.g. precipitation is expressed as a mean over large areas, and as depths over periods of a day). Such generalised conceptualisations often lack sufficient temporal and spatial resolution to permit a detailed modelling of complex hydrological processes.

Recent events (December 2000/January 2001), with catastrophic consequences related to the occurrence of floods in the Lower Mondego region due to several ruptures of the protection dikes of the Mondego River, had a strong socio-economic impact and put pressure on decision makers to re-evaluate the entire project.

An important contribution towards increasing our understanding of the non-linear variability in precipitation is being given by scale-invariant studies of this process. The invariance of properties being maintained across scales can be mathematically investigated using fractal and multifractal theories. These theories offer an alternative to ('conventional') approaches that study one scale independent of the other. Instead, they investigate the presence of certain features of a dynamic system that are independent of scale. The present study uses these theories to investigate the scale-invariant temporal structure of precipitation in the Mondego drainage basin. The work uses daily precipitation data from two locations in the drainage basin, Coimbra and Penhas Douradas, over a period of 54 years.

General description of the Mondego River drainage basin and its hydrology

The Mondego River is the longest watercourse whose entire course lies within Portugal. Its source is in the Serra da Estrela, in Central Portugal, at an altitude of 1,547 m, and it flows into the Atlantic Ocean, near the city of Figueira da Foz, 234 km later (see Figure 1). The average slope of the Mondego riverbed is 0.637% (Lencastre and Franco 1984). The main first-order tributary streams entering the Mondego River are the Dão, on the right bank, and the Pranto, Arunca, Ceira and Alva, on the left bank.

The Mondego drainage basin has an area of approximately 6,645 km^2, being the second biggest river catchment area totally located in Portugal. Its shape is elongated, with its axis oriented approximately NE-SW, and is located approximately between 39°46' N and 40°48' N, and 7°14' W and 8°52' W. The highest altitude in the drainage basin is almost 2,000 m above sea level. The mean altitude is around 375 m.

The climate of the Mondego basin is Mediterranean and strongly influenced by the proximity of the Atlantic Ocean. The Atlantic influence increases the relative humidity of the air and affects temperatures and precipitation. In the summer, low precipitation and high temperature and insolation can occur due to the presence and influence of the Azores high-pressure system. In the winter, many days have precipitation and mild temperature, strongly influenced by the passage of frontal surfaces and depressions moving from West to East, coming from the Atlantic Ocean.

The average annual temperature in the basin is approximately 13 °C. The warmest part of the basin is near the coast (in the Lower Mondego region), where the average annual temperature is around 16 °C. The coldest regions are in the high lands of Estrela and Caramulo Mountains, where the average annual temperature is only 10 °C. Near the coast, the variation of temperature during the year is small because of the influence of the Atlantic Ocean. As the distance to the ocean increases, the temperature range increases. In the Mondego basin, the average temperature for the hottest months of July and August is 19 °C, and for the coldest months of December and January it is 6 °C.

The average annual insolation in the basin is around 2,400 h and the mean annual evapotranspiration is around 720 mm.

The mean annual precipitation in the basin is approximately 1,130 mm. The period from October to March is the humid semester, registering about 70% of the annual precipitation (for the two locations in the basin, see Figure 4(c)). The wettest month is December, with around 160 mm of precipitation. The driest months are July and August, with a monthly precipitation of around 15 mm. In the dry season, months often register zero-precipitation.

Topography has a striking influence on both temperature and precipitation (Figure 2). There is a clear increase in precipitation and decrease in temperature with altitude, as one goes from the lower lands towards the hills.

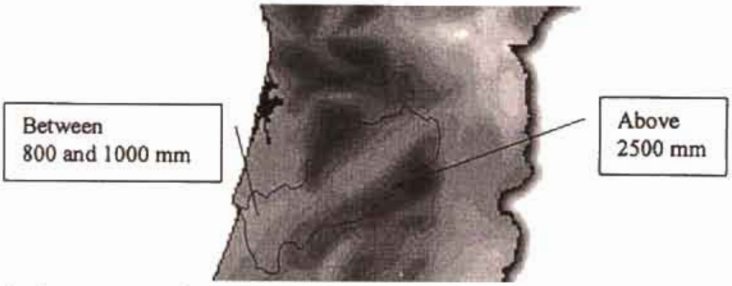

Figure 2. Spatial variation of mean annual precipitation in the Mondego River Basin. The highest values of precipitation (darker areas) are recorded in the mountainous regions of Estrela and Caramulo and the lowest (lighter areas) in the Lower Mondego Region, near the Atlantic coast. (Adapted from DGA 1989)

It is possible to define three hydro-morphologic regions in the Mondego basin, based on different morphologic and climatic characteristics, hydrologic regimes and land use:

- *Upper Mondego*, corresponding to the mountainous region, where the river runs through valleys formed in the ice age. This region includes, among others, parts of the drainage basins of the Dão and Alva tributaries, and the upper section of the Mondego River.

- *Middle Mondego*, which occupies the area lying between the outskirts of the Serra da Estrela mountains and the city of Coimbra. In this part of the Mondego catchment, the riverbed, which initially runs along a narrow valley, progressively broadens. This section of the river contains the outlets of the rivers Dão, Alva, and Ceira.
- *Lower Mondego* corresponds to the last section of the basin between Coimbra and the sea. In this area the river has a wide valley. The main tributaries of the Mondego River in this area are the rivers Arunca, Pranto and Foja.

The total resident population in the Mondego basin is around 680 thousand inhabitants (MA 2000). In this region the land is predominantly occupied by forest. In recent years the area occupied by forest has increased as the agricultural area diminished; forest accounted for 45% of ground cover in 1985 and 54% in 1990.

The Mondego River has a mean annual runoff of approximately 86 m^3/s (403 mm or 2.7×10^9 m^3), at the outlet. The mean runoff of the driest month is around 0.015×10^9 m^3 and of the wettest month is 0.530×10^9 m^3 (MA 2000). The hydrological discharges of the Mondego River and its main first-order tributary streams are extremely irregular. In Coimbra, approximately 40 km from the estuary, the river flow has reached discharges as high as 3,000 m^3/s, in strong contrast with discharges of less than 1 m^3/s, occurring several days a year (natural situation before the human intervention). This has frequently led to flooding, and water supply problems, mainly for agricultural purposes. Flooding is still a problem in the Mondego basin, although several dams have been constructed, and these are able to control the great majority of flood situations in roughly 80% of the basin area. For approximately 20 consecutive years, the regulation and protection scheme managed to control the river discharges against floods. Recent flooding events are leading to a re-evaluation of the project and of its management and maintenance.

The most important hydraulic works in the Mondego basin are the dams at Aguieira, Fronhas, Raiva and the barrage at Coimbra, the Serra da Estrela hydroelectric system, and the irrigation and drainage systems of the Lower Mondego region. The diversity of water uses (e.g. for supplying populations, agriculture, industry, energy production) is extremely important for the economy of the region. In fact, the Mondego basin is the one registering the most extensive use of water resources in Portugal, namely for hydroelectric power (annual production of 360 GWh) and agriculture. The Lower Mondego hydro-agricultural scheme, which includes large irrigation and drainage systems, enhances the economic development of the region, where there are 15,000 ha of good agricultural land.

For mainland Portugal and, in particular, for the Mondego river drainage basin, Figure 3 shows a drought situation recorded in 1945 (Pimenta 1998), classified according to Palmer (1965). The Palmer Drought Severity Index is based on the supply-and-demand concept of the water balance equation, and is calculated using precipitation and temperature data, and the available water content of the soil. The values of the Palmer Index that are indicated in Figure 3 are average values for the year of 1945. Pimenta (1998) and Pimenta and Lima (1999) studied the occurrence of droughts in mainland Portugal, using monthly precipitation and air temperature time series from 70 stations of the Portuguese climatological network, for the period 1941 to 1992. Their study shows that, during this period, there were droughts affecting most of the territory, including the Mondego catchment area. Because of the uneven distribution of precipitation during a year, with the dry season coinciding with the warmest months, the Palmer Index yearly average indicates the occurrence of extremely dry periods within a twelve-month period. These are often linked to important water shortage problems, already mentioned above.

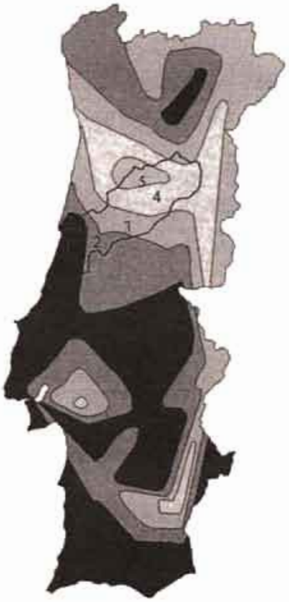

Figure 3. Impact of one of the most severe droughts (year of 1945) observed in Mainland Portugal and in the Mondego Basin, according to the Palmer classification, where: 1 – Extremely dry; 2 – Very dry; 3 – Moderately dry; 4 – Slightly dry; and 5 – Incipient dry. (Adapted from Pimenta and Lima 1999)

The precipitation data from Coimbra and Penhas Douradas

The precipitation data used in the scale-invariant analysis (see below) were recorded at two locations in the river Mondego drainage basin, in Portugal: Coimbra/ Geofísico and Penhas Douradas (Serra da Estrela). The co-ordinates of these stations are approximately 40°12' N and 8°25' W, for Coimbra, and 40°25' N and 7°33' W, for Penhas Douradas. The altitude of the measuring sites is, respectively, 141 m and 1,380 m above mean sea level. Figure 1 shows the location of the measuring stations in the Mondego basin.

The precipitation measuring devices are of the 20-14-G type (according to the classification by Sevruk and Klemm, 1989); they have horizontal openings of 200 cm^2 at 1.5 m height. The gauges were observed daily. The resolution of the measurements is 0.1 mm of precipitation. Trace precipitation of less than 0.1 mm is disregarded and such days are considered dry (zero-precipitation days).

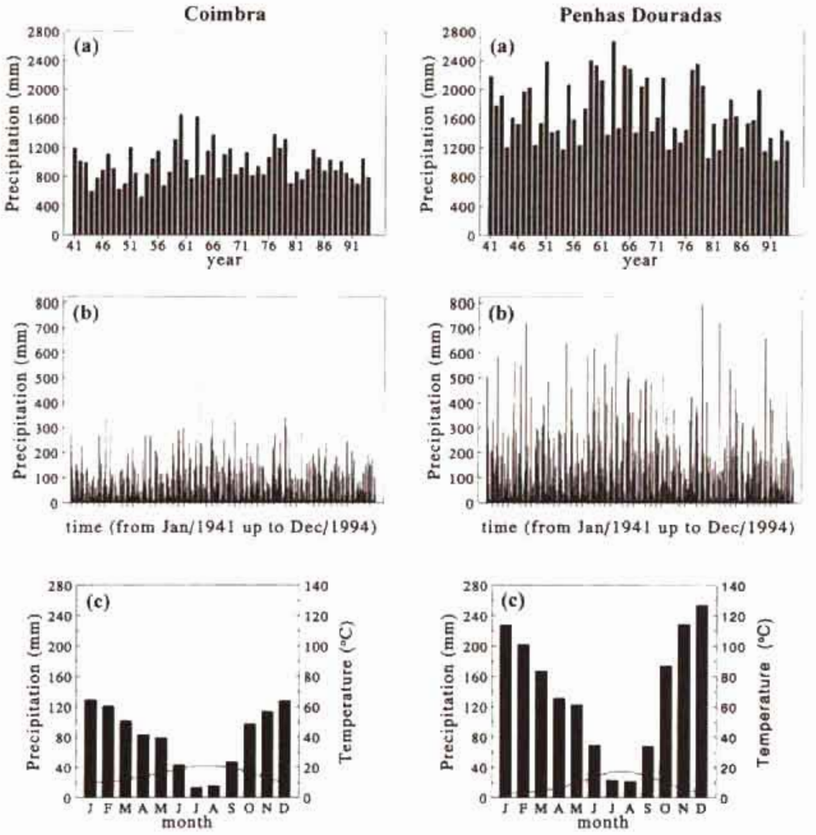

Figure 4. Precipitation in Coimbra and Penhas Douradas, in the Mondego drainage basin, for the years 1941-1994: (a) annual precipitation; (b) monthly precipitation; and (c) average monthly precipitation. Figure (c) also shows the average monthly temperature, for the years 1947-1982.

The precipitation recorded in Coimbra and Penhas Douradas, for the years 1941 to 1994, is shown in Figure 4 and in Table 1. Figure 4(a) shows the annual precipitation. Figure 4(b) shows the monthly precipitation and Figure 4(c) shows the monthly average precipitation. The precipitation recorded in Coimbra and Penhas Douradas illustrates the spatial variability of precipitation in the Mondego basin, where the lower-lying land registers less precipitation, and the higher ground gives higher precipitation figures. Both locations exhibit a marked seasonal distribution of precipitation during the year. In Coimbra, of the 648 months that constitute this sample, 16 months had less than 1 mm of precipitation (roughly 2.5%); among these, there are five months with less than 0.1 mm. In Penhas Douradas, in the same period, there were 24 months with less than 1 mm of precipitation (roughly 3.7% of the sample); among these, there are seven months with less than 0.1 mm of precipitation.

Figure 4(c) also shows the average monthly temperature in Coimbra and Penhas Douradas, recorded from 1947 to 1982. For this period, the mean annual temperature in Coimbra was 15.1 °C and in Penhas Douradas it was 8.9 °C. On average, the coldest month was January, with 9.6 °C in Coimbra and 2.8 °C in Penhas Douradas. In Coimbra, the warmest months were July and August with 20.9 °C, and in Penhas Douradas July was the warmest, with 17.1 °C.

Table 1. Summary of relevant precipitation data from Coimbra and Penhas Douradas, for the period 1941-1994.

Type of precipitation data (1941-1994)	**Measuring stations**	
	Coimbra	**Penhas Douradas**
Mean annual precipitation (coefficient of variation)	976.9 mm (0.24)	1692.2 mm (0.25)
Precipitation in wettest year (wettest year)	1651.4 mm (1960)	2669.3 mm (1963)
Precipitation in driest year (driest year)	524.2 mm (1953)	1023.9 mm (1992)
Average monthly precipitation (coefficient of variation)	81.4 mm (0.90)	141.0 mm (0.99)
Precipitation in wettest month (wettest month)	467.4 mm (November, 1963)	802.6 mm (December, 1978)
Precipitation in driest months (total number of months)	0 mm* (5 months)	0 mm* (7 months)
Average precipitation in wettest month (average wettest month)	129.5 mm (January)	253.8 mm (December)
Average precipitation in driest month (average driest month)	13.7 mm (July)	21.9 mm (August)

* Monthly precipitation below 0.1 mm is considered negligible.

Some aspects of fractal and multifractal theories

The invariance of properties manifested across scales can be investigated mathematically using fractal and multifractal theories. These theories apply to processes and systems that do not have a characteristic scale. Scale-invariance leads to a class of scaling rules (power laws) characterised by scaling exponents. This allows the relationship of variability between different scales to be quantified. Statistical properties of scale-invariant systems at different scales (i.e. on large and small scales) are related by a scale-changing operation that involves only scale ratios.

Scaling theories are developed in a non-dimensional framework, because one is looking for features that are independent of the physical size of the study-object. To non-dimensionalise time measurements, one assumes that the duration of the longest period of interest is equal to 1. If this period has a duration T, then the magnitude of any time interval τ should be divided by T. Any time scale corresponding to τ can then be characterised by a scale ratio λ, with $1/\lambda = \tau / T$. To non-dimensionalise the precipitation intensity on a time scale of resolution λ, the intensity can be divided by the ensemble average intensity of the process. For precipitation this means the climatological average precipitation. Nevertheless, in practice, one generally uses the average intensity of the sample, which corresponds to the largest scale of interest ($\lambda = 1$). Let the (average) precipitation intensity for $\lambda = 1$ be $< R_1 >$, where the angular brackets $< \ >$ mean (ensemble) average. So if the precipitation intensity in a time interval λ^{-1} is R_λ, the corresponding non-dimensional intensity is $\varepsilon_\lambda = R_\lambda / < R_1 >$, hence $< \varepsilon_\lambda > = 1$.

Using fractal theory (Mandelbrot 1977, 1982) one is dealing with simple scaling. Fractal behaviour is determined by one parameter. Fractal studies of the temporal structure of precipitation deal only with the oversimplified binary question of occurrence and non-occurrence of the process. The binary question (i.e. the definition of rainfall occurrence) is generally associated with a precipitation-intensity threshold. The geometric structure that is the 'support' of the precipitation process can be regarded as a fractal object embedded in the 1-dimensional space of time and is defined as the set of precipitation periods observed in a particular location. Its fractal dimension, D, is between 0 and 1. Very roughly, the fractal dimension of a set tells how densely the set occupies the metric space in which it lies. More generally, it tells how frequent a phenomenon is. The fractal dimension of a set can be determined with the box-counting method (e.g. Feder 1988, Hastings and Sugihara 1993).

The need to generalise the scaling properties of physical processes has led to the development of multifractal theory (Hentschel and Procaccia 1983, Grassberger 1983, Schertzer and Lovejoy 1983), dealing not with simple scaling but with multiscaling. It can handle the different intensity levels of processes. Multifractal behaviour is determined not by one, but by an infinity of scaling exponents.

One way to investigate the multifractal temporal structure of the precipitation process is by studying the (multiple) scaling of the probability distributions of the precipitation intensity (e.g. Schertzer and Lovejoy, 1987). The precipitation intensity threshold level is evaluated with the order of singularity γ of the intensities $\varepsilon_\lambda \sim \lambda^\gamma$ (e.g. Frisch and Parisi 1985, Halsey et al. 1986, Schertzer and Lovejoy 1987). The scaling of the probability distributions is given by the exponent function $c(\gamma)$:

$$\Pr(\varepsilon_\lambda \geq \lambda^\gamma) \approx \lambda^{-c(\gamma)} \qquad (1)$$

In literature, the function $c(\gamma)$ is called the codimension function. Eq. (1) holds for proportionality constants varying slowly with λ and depending weakly on γ (e.g. Schertzer and Lovejoy 1989, Lovejoy and Schertzer 1991). This statistical characterisation of multifractals arises directly from multiplicative cascade processes (see e.g. Schertzer and Lovejoy 1987). The scaling (power-law) behaviour can be tested with log-log plots of the probability of exceeding different levels of the precipitation intensity ε_λ, observed on scales of differing levels of resolution λ, against the scale ratio λ (e.g. Lavallée et al. 1991).

Another (equivalent) way to investigate the multifractal temporal structure of precipitation is with the statistical moments of the precipitation intensity (Schertzer and Lovejoy 1987). The scaling of the moments of precipitation intensity is described by the exponent function $K(q)$. The notion of moment can be generalised to any real value q. The moments' scaling function $K(q)$ satisfies:

$$< \varepsilon_\lambda^{\,q} > \approx \lambda^{K(q)} \qquad (2)$$

where $< \varepsilon_\lambda^{\,q} >$ is the (ensemble) average q^{th} moment of the precipitation on a scale specified by λ. The scaling of the moments can be tested with log-log plots of the average q^{th} moment of precipitation intensity ε_λ, observed on scales of different levels of resolution λ, against the scale ratio λ. The two multifractal scaling exponent functions $c(\gamma)$ and $K(q)$ are (theoretically) non-linear increasing functions (concave functions).

One can also use standard spectral methods and analysis to test for scale-invariance. The most familiar consequence of scaling is the power-law behaviour that is expected in the energy (power) spectra of scaling processes (e.g. Mandelbrot 1982, Schertzer and Lovejoy 1987, Ladoy et al. 1991, Lovejoy and Schertzer 1995):

$$E(\omega) \approx \omega^{-\beta} \qquad (3)$$

where ω is the wave-number, $E(\omega)$ is the energy, and β is the spectral exponent. For temporal processes, the wave number can be approximated by $\omega \sim 1/\tau$, with τ being the magnitude of any time interval. Thus, in this application, ω is a frequency. The power-law behaviour of the spectra is expected to occur over a range of wave numbers and might not be observed for small samples.

Scaling and multifractal analysis of daily precipitation data

This Section deals with the analysis of the daily precipitation, over a period of 54 years, from Coimbra and Penhas Douradas, recorded from 1941 to 1994. In the different analyses, given below, the statistics are accumulated for the 54 years covered by the data.

The energy spectra of the daily precipitation at these two locations are plotted in Figure 5. The spectra have been smoothed for high frequencies. The spectral peaks at $\omega \approx 0.0027$ day^{-1} correspond to the annual cycle frequency. The spectra exhibit power-law behaviour that extends from 1 day up to at least one month. The spectral exponent ß (Eq. 3) is estimated as 0.28, for the data from Coimbra, and 0.31, for the data from Penhas Douradas. Parameters ß were estimated from the absolute values of the slopes of the regression lines fitted to the right-hand side scaling regions of the spectra, plotted in log-log axis. For scales larger than one to two months, up to roughly one decade, one can distinguish a rather flat section in the spectra. The scaling regime associated with the range of scales characterised by such a spectral plateau is expected to govern inter- and intra-seasonal variability (Fraedrich and Larnder 1993). This plateau is followed by another section (i.e., for even larger scales), indicating large-scale climatic variability. For other locations, e.g. Ladoy et al. (1991), Fraedrich and Larnder (1993), Tessier et al. (1996) and Svensson et al. (1996) have reported similar results.

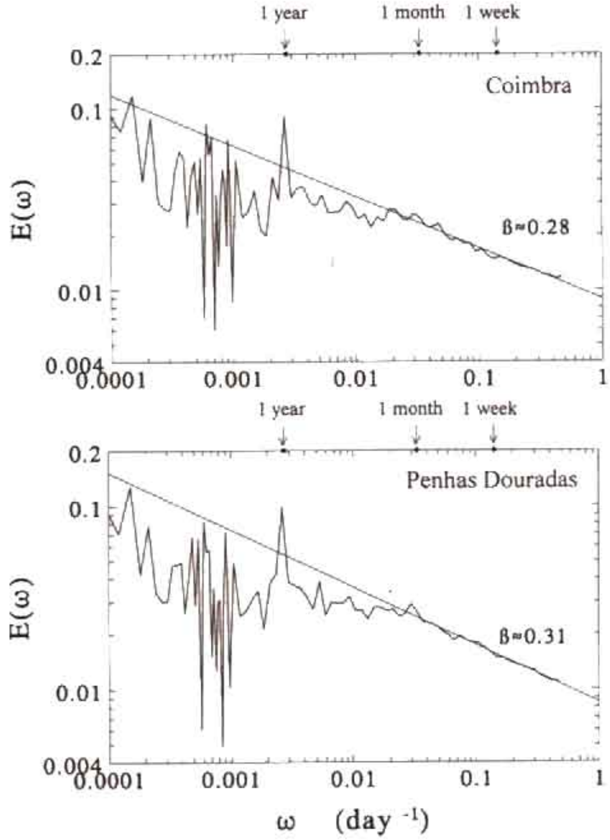

Figure 5. Energy spectra obtained for daily precipitation from Coimbra and Penhas Douradas, from 1941 to 1994.

The box-counting method can be also used to test the scale-invariant temporal structure of precipitation. The application of the box-counting method to precipitation, shown below, is based on a zero-precipitation threshold definition of precipitation occurrences. Figure 6 shows the box-counting plot obtained for the daily precipitation, for time scales from 1 day up to 8.5 months. A fractal dimension, 0.64, characterises precipitation occurrences in Coimbra, on the range of scales from 1 day up to about 13 days. A similar fractal dimension, 0.65, characterises precipitation occurrences in Penhas Douradas, on the range of scales from 1 day up to about 14 days. The fractal dimensions are estimated from the absolute value of the slopes of the regression (heavy) lines fitted to the left-hand side sections of the plots in Figure 6. Analysis of larger time scales is affected by 'saturation', which is a practical problem encountered when applying the box-counting method to precipitation occurrences. The regression (broken) lines fitted to the right-hand side sections of the plots in Figure 6 have (trivially) slope -1.

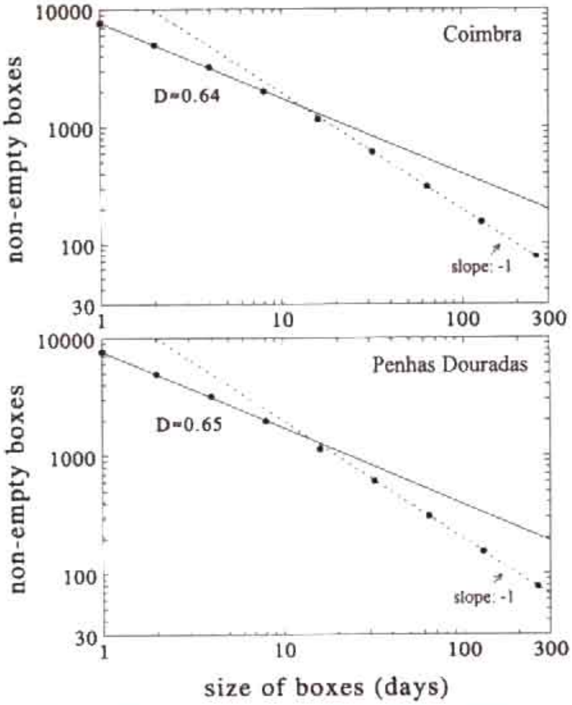

Figure 6. Box-counting plot obtained for daily precipitation from Coimbra and Penhas Douradas, for the period 1941 to 1994. The plot displays time scales from 1 day up to 8.5 months.

Figure 7 shows, for the data from Coimbra and Penhas Douradas, the log-log plot of the probability of exceeding precipitation intensity levels of singularity γ, observed on time scales from 1 day ($\lambda = 256$) up to 8.5 months ($\lambda = 1$), against the scale ratio λ. The orders of singularity γ of the precipitation intensity plotted in Figure 7 are indicated in the legends. The scaling behaviour observed in Figure 7 is maintained from one day up to more than one month. The scaling range observed for Penhas Douradas seems to be larger than for the data from Coimbra. The probability plots in Figure 7 show regression lines fitted to the data that clearly indicate the presence of scale invariance in the temporal structure of precipitation.

Also relative to the data from Coimbra and Penhas Douradas, Figure 8 shows the log-log plot of the average q^{th} moments of precipitation intensity ε_λ on time scales from 1 day ($\lambda = 256$) up to 8.5 months ($\lambda = 1$), against the scale ratio λ. Figure 8(a) shows moments larger than 1 and Figure 8(b) moments smaller than 1. The moments q plotted in Figure 8 are indicated in the legend. For moments larger than 1, the scaling range seems to extend from one day up to about one month, for the data from Coimbra, and up to about 2 months, for the data from Penhas Douradas. One expects that the scaling behaviour for some small moments, which highlight the small intensities of the data, would be affected by an incorrect description of the precipitation process by the data,

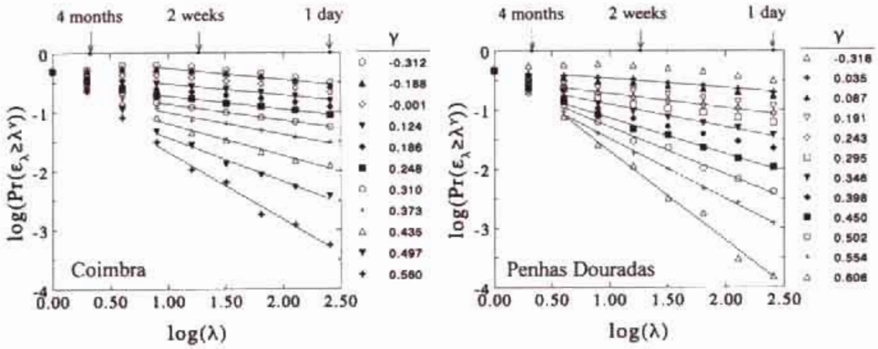

Figure 7. Log-log plot of the probability of exceeding precipitation intensity levels of singularity γ, observed on scales from 1 day ($\lambda = 256$) up to 8.5 months ($\lambda = 1$), against the scale ratio λ. The legend indicates the order of singularity γ of the precipitation intensity ε_λ. The plots are for the data from Coimbra and Penhas Douradas.

over this range of the precipitation dynamics. This could result from the inability of the measuring devices to record precipitation intensities smaller than a characteristic value.

The scaling functions $c(\gamma)$ and $K(q)$, in Eqs. (1) and (2) respectively, which would describe the statistics of precipitation in Coimbra and Penhas Douradas are obtained from the regression lines fitted to the probability plots in Figure 7 and moments' plots in Figure 8, over the relevant range of orders of singularity γ, and moments q of precipitation intensity.

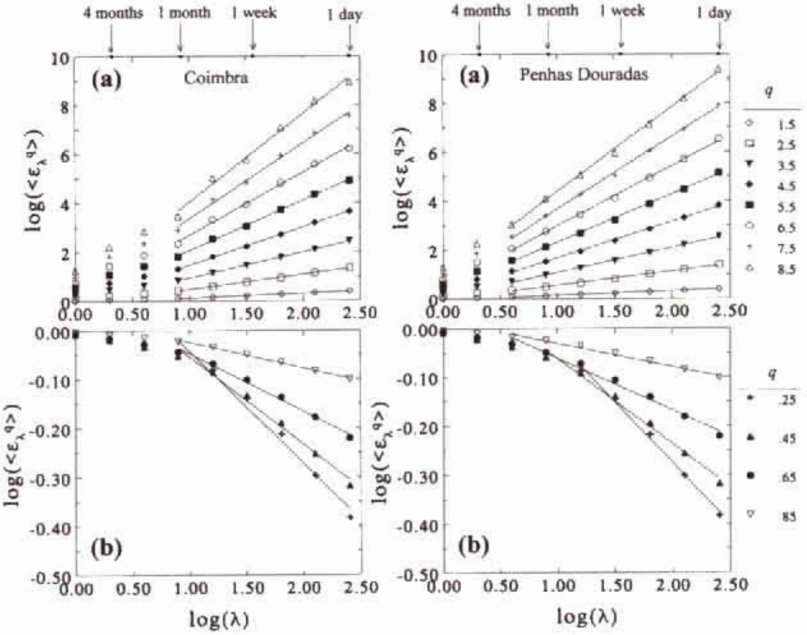

Figure 8. Log-log plot of the average q^{th} moments of the precipitation intensity ε_λ on scales between 1 day ($\lambda = 256$) and 8.5 months ($\lambda = 1$), against the scale ratio λ: (a) for moments larger than 1; and (b) for moments smaller than 1. The plots are for the data from Coimbra and Penhas Douradas.

Concluding remarks

This study shows the presence of scale-invariant and multifractal properties in the temporal structure of precipitation in the Mondego drainage basin. The study used daily precipitation from Coimbra and Penhas Douradas. Scale-invariant and multifractal properties are maintained from one day up to roughly one month. Results obtained for the data sets investigated are consistent with results reported by other researchers, for different precipitation data sets. One can refer to e.g. Ladoy et al. (1991), Hubert and Carbonnel (1991), Hubert (1992), Tessier et al. (1992), Ladoy et al. (1993), Tessier et al. (1993), Hubert et al. (1993), Lima et al. (1993), Olsson and Niemczynowicz (1994), Hubert (1995), Lima and Bogardi (1995), Harris et al. (1996), Svensson et al. (1996), Bendjoudi et al. (1997), Lima (1998, 1999) and Lima and Grasman (1999).

The scale-invariant and multifractal approach to the study of precipitation in the Mondego basin has still not been fully explored. There is still insufficient knowledge about the dependency of the multifractal behaviour of precipitation on climatological and geographical factors (i.e. on precipitation-generating mechanisms). Thus, more research is needed involving the study of precipitation from other locations. Further research should include the analysis of precipitation data from more locations in the Mondego catchment, and also the analysis of higher resolution precipitation time series (e.g. hours, minutes). This will allow us to characterise the precipitation process better. It will also allow us to fully explore the invariance of precipitation properties across scales. Some studies have already shown that this scale invariance is observed down to scales of the order of minutes (e.g. Lima 1998). Such properties permit us to use the statistics of precipitation derived, for example, from daily data to infer the statistics of precipitation at smaller time scales. The expectation is that the multifractal theory and its application in models (e.g. Schertzer and Lovejoy 1987) may be tools that help produce high-resolution synthetic precipitation data that can be used in many hydrological applications and studies (e.g. rainfall-runoff transformation, soil erosion, spread of pollutants, urban drainage). For the Mondego basin, this could greatly assist in water resources studies and management.

Acknowledgements

The authors are grateful to Dr. M. F. Espírito Santo Coelho, of the Portuguese Institute of Meteorology (IM), who kindly provided the precipitation data used in this work, within the framework of a joint research project PRAXIS/P/ECM/12018/1998. This project is funded by the Portuguese Foundation for Science and Technology (FCT).

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HYDRODYNAMIC MODELLING FOR MONDEGO ESTUARY WATER QUALITY MANAGEMENT

Abstract

The south arm of Mondego estuary, located in the central western Atlantic coast of Portugal, is almost silted up in the upstream area. So, tides, wind and the tributary river Pranto discharges mostly drive the water circulation in this system. Annual freshwater inflow, regulated by precipitation and by sluice management practices, has a significant impact on flow velocity, salinity, N:P ratios and light extinction coefficients, which control biomass growth and loss processes. Eutrophication has been taking place in this ecosystem during last twelve years, where macroalgae reach a luxuriant development covering a significant area of the intertidal muddy flat.

A sampling program was carried out from June 1993 to January 1997. Available data on River Pranto flow discharges, salinity profiles, precipitation and nutrients loading into the south arm were used in order to get a better understanding of the ongoing changes.

Since hydrodynamics strongly affects the occurrence of macroalgal blooms, residence time can be a key parameter to characterise this influence. Integral formulations are typically based on assumptions of steady state and well-mixed systems and thus cannot take into account the space and time variability of estuarine residence times, due to river discharge flow, tidal coefficients, discharge(s) location and time of release during the tidal cycle.

This work presents the hydrodynamic modelling (1D and 2D-H) of this system in order to estimate some kinetic variables and the residence times variability to assess the main factors that control opportunistic macroalgal blooms, contributing to better environmental management strategies selection.

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Introduction

The physical and chemical dynamics and the ecology of shallow estuarine areas are strongly influenced by the freshwater runoff and the adjacent open sea. The freshwater input influences estuarine hydrology by creating salinity gradients and stratification and assures large transport of silt, organic material and inorganic nutrients to the estuaries. The open marine areas determine large scale physical and chemical forcing on the estuarine ecosystem, due to tide and wind generating water exchange (Berner and Berner 1996). Due to all this characteristics, inside the estuarine system efficient water column mixing and frequent re-suspension events ensure fast vertical transport of organic and inorganic matter (Pardal 1998) integrating the pelagic and benthic food webs and the biogeochemical processes (Lillebø 2000).

In the South Arm of the Mondego estuary eutrophication has increased due to massive nutrient loading from urbanised areas and diffusive runoff from intensively agricultural areas. This increased nutrient loading has severe consequences for the ecology of the system, due to changes in plant composition (Pardal 1998, Flindt et al. 1999), which consequently affects heterotrophic organisms, specialised in living on this production (Pardal 1998, Pardal et al. 2000, Lillebø et al. 1999). As a consequence of nutrient enrichment, opportunistic macroalgae growth was strongly stimulated allowing the occurrence of macroalgae blooms and the extinction of seagrass in more shallow areas. This situation may result in anoxic system collapse, with the development of hydrogen-sulphide conditions, lethal to rooted macrophytes such as *Zostera noltii*. The consequence is a structural change of the ecosystem, from a grazing controlled system to a detritus/mineralisation system (Pardal 1998), where the turnover of oxygen and nutrients is much more dynamic (Lavery and McComb 1991), and macroalgae play an important role in the nutrient pathways of the ecosystem.

Thus, due to the importance of their impacts in the ecosystem, it becomes crucial to obtain information on the mechanisms that regulate the abundance of opportunistic macroalgae. Advective transport may be the most important process that controls the spatial and temporal distribution of macroalgae. Depending on the tidal amplitude, depth, cohesiveness of plant material, current velocity, wind and wave-induced vertical turbulence, plants growing in shallow areas are suspended in the water column and transported out and eventually settled in deeper areas (Sfriso et al. 1992). In this system, available data analysis allows to conclude that the occurrence of green macroalgal blooms is strongly dependent on the hydrodynamic conditions, precipitation and salinity gradients (Martins et al. 2001).

A wide range of mathematical models has been developed and applied to predict water quality changes in surface waters, being a useful tool for systems eutrophication vulnerability assessment (Vieira et al. 1998). Residence times estimation, allowed by mathematical modelling, can provide essential information about estuarine hydrodynamic behaviour, considering different scenarios, in order to select better water quality management practices (Duarte et al. 2001).

The aim of this study is to assess the role of estuarine hydrodynamics on the macroalgae biomass control, because the quantitative aspects of this phenomenon are not well known. Hydrodynamic models (1D and 2D-H) of Mondego estuary south arm were implemented in order to estimate residence times, current velocity and salinity distribution at different simulated scenarios, considering average tidal conditions. The conclusions of an early study about the macroalgae growth behaviour and the monitoring program results analysis are also presented. In further research works, useful tools for an estuarine integrated management will be developed in order to support the decision making process (Vieira and Lijklema 1989).

The present work was based on previous published results, namely Pardal (1998) and Duarte et al (2001).

Study area

The Mondego river basin is located in the central region of Portugal. The drainage area is 6,670 km^2 and the annual mean rainfall is between 1,000 and 1,200 mm. This estuary (40°08'N 8°50'W) has a considerable regional importance due to the Figueira da Foz mercantile harbour, but is under severe environmental stress, namely an ongoing eutrophication process, due to human activities: industries, aquaculture farms and nutrients discharge from agricultural lands of low river Mondego valley (Fig. 1).

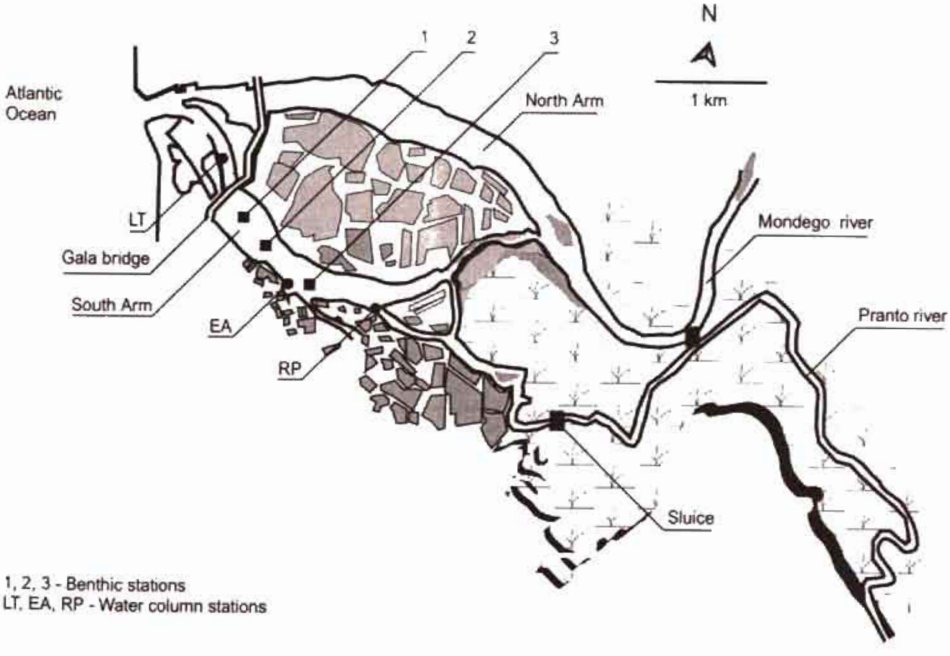

Figure 1. River Mondego estuary and sampling stations location

This estuarine system is divided into two arms (north and south) with very different hydrological characteristics, separated by the Murraceira Island. The north arm is deeper and receives the majority of freshwater input (from Mondego river), while the south arm of this estuary is shallower (2 to 4 m deep, during high tide) and is almost silted up in the upstream area (Fig.2). Consequently, the south arm estuary water circulation is mainly due to tides, wind and the usually small freshwater input of Pranto River, a tributary artificially controlled by a sluice, located about 6 km from the mouth.

Figure 2. Bathymetry of the Mondego estuary south arm

Methods

Monitoring program

Most of the estuarine processes (physical, chemical and biological) are related to salinity, because its variation is one of the major characteristics of an estuarine system. For the water sampling location and benthic stations (Fig. 1), an indication of the variability in the local salinity regime over a tidal period (for little and high river discharges) can facilitate future interpretation of the data.

A sampling program was carried out from June 1993 to January 1997 at three benthic stations (1, 2 and 3) during low water tide, and, from June 1993 to June 1994, at three other sites: river Pranto sluice, Armazéns channel mouth and Gala bridge for water column monitoring. In this period, the river Pranto freshwater input was estimated (68 times during sluice openings), measuring current velocities immediately after the sluice. The current velocity was also measured immediately after the Gala Bridge, at different depths and along cross section. The field data were used to define the boundary conditions of the hydrodynamic model.

The choice of benthic stations was related with the observation of an eutrophication gradient in the south arm of the estuary, involving the replacement of eelgrass, *Zostera noltii* by green algae such as *Enteromorpha* spp. and *Ulva* spp.. There is a non-eutrophicated zone (site 1), where a macrophyte community *(Zostera noltii)* is present, up to a strongly eutrophicated zone (site 3), in the inner and shallower areas of the estuary, where the macrophytes disappeared while *Enteromorpha* spp. blooms have been observed during the last 15 years. This is probably a result of excessive nutrient release into the estuary, coupled with longer persistence of nutrients (nitrogen and phosphorous) in the water column (Marques et al. 1997, Pardal et al. 2000) and the silting up of upstream area. Nevertheless, such macroalgae blooms may not occur in exceptionally rainy years (e.g. year 1994) due to low salinity for long periods, as a result of the Pranto river discharge (Pardal 1998, Pardal et al. 2000, Lillebø et al. 1999, Martins et al. 2001). As general pattern *Enteromorpha* spp. biomass normally increases from late winter up to July, when an algae crash usually occurs due to anoxia and most of the biomass is washed out to the Atlantic.

Simultaneously, attending to the relationships between external abiotic variables and macroalgae growth in this system, temperature, salinity, dissolved oxygen, pH, and dissolved nutrients, like orthophosphate, nitrites, nitrates and ammonium were measured (Fig. 3).

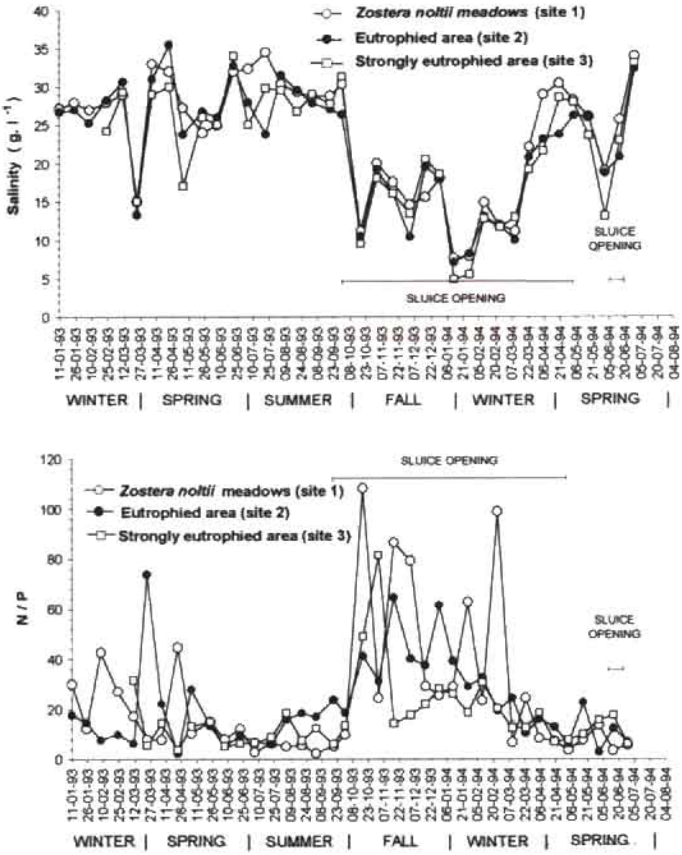

Figure 3. Sampling data (Salinity and N:P ratio)

Nowadays a sampling program is being done, at stations LT, EA and RP, in order to a better understanding of south arm hydrodynamic behaviour, namely the smooth sinusoidal variation over the tidal cycle of the tide-induced velocities due to coastal zone and estuary geometry.

Sampling data analysis

The data was analysed and discussed in early works (Pardal 1998, Martins et al. 2001) in order to understand how the main biochemical variables can be affected by estuarine hydrodynamics. Based on the present knowledge important conclusions were established:

- Fresh water input is controlled by precipitation and sluice management practice (related with the upstream water deficit of rice fields, that can determinate to keep the gates closed even if it is raining). Sometimes there is a time lag between the occurrence of precipitation and sluice gates opening, or the sluice gates remain open for a longer period even without precipitation;
- There is a direct relationship between maximal current velocities and tidal amplitude, being higher when sluice gates are opened. Additionally, turbidity will be higher due to sediments re-suspension increasing;
- Salinity is negatively correlated with freshwater discharges, remaining high (> 20 psu) for low river flow values, but it may reach extremely low values (< 1 psu), such as in January 2001);
- *Enteromorpha* spp. represent more than 85% of the total green macroalgae biomass and present important inter-annual variations. Its production depends on the amount of freshwater input in system during late winter and spring. *Enteromorpha* spp. growth increases with salinity, being the optimum range 17-22 psu. (Martins et al. 1999);
- Nitrites and nitrates concentrations, higher in autumn and winter, are strongly correlated with River Pranto freshwater discharge and are not always directly related with precipitation. Ammonium is the major contributor to total inorganic nitrogen;
- The highest orthophosphate concentration occurs mostly in summer, because the increase on efflux of phosphorus from sediments to water column is related with the temperature;
- Macroalgae nutrient uptake rates depend on the difference between internal nutrient concentration and external nutrient concentration in the water. So, primary production can not always be P-limited as suggested by the occurrence of high N:P ratios in the water column;
- Despite of *Enteromorpha* spp. fixing ability, strong current velocities erode its attachment or tear away their fronds once they reach a critical length (Lowthion et al. 1985).

The dependency of opportunistic macroalgae blooms on precipitation and on river management practices was suggested the usefulness of mathematical modelling application to characterise estuarine hydrodynamics and to estimate residence times, related with nutrients and macroalgae exportation or remaining periods in this ecosystem.

Tidal prism

Tidal movement is one of the major driving forces of estuarine circulation in this system and it is also visible in several variables like salinity, turbidity, re-suspension/ deposition of the sediment and transport of pelagic organisms. Tide effects are almost exclusively due to *M2* tidal cycle with a full tidal period covering about 12.4 hours. For modelling purposes, the tide characteristics considered are summarised in Table 1.

Based on the south arm bathymetry, tidal prism estimation was performed for several tidal ranges, according to Table 1. Results are presented in Table 2 (Duarte 1997).

Flow distribution in an estuary over a tidal cycle shows that the flood current velocities slowly decrease during flood tide and that after slack the ebb flow velocities increase in the opposite direction. Contrarily, after ebb slack the flood tide velocities increase rapidly and the incoming flood tide reaches high flow velocities very quickly.

Table 1. Tidal average levels (m) at Figueira da Foz harbour (June 1993-June 1994)

Maximum level		3.7
Mean level		2.0
Minimum level		0.3
Spring tide	Flood tide	3.5
	Ebb tide	0.3
Neap tide	Flood tide	2.7
	Ebb tide	1.8
Selected tide	Flood tide	3.3
	Ebb tide	0.7

Table 2. Tidal prism (m^3) of south arm Mondego

Ebb tide **Flodd tide**	0.0	0.5	1.0	1.5	2.0	2.5
2.0	960,495	890,985	723,055			
2.5	1,615,075	1,545,565	1,377,635	1,099,195		
3.0	2,414,600	2,345,090	2,177,160	1,898,720	1,454,105	
3.5	3,262,790	3,193,280	3,025,350	2,746,910	2,302,295	1,647,715
4.0		4,067,405	3,899,475	3,621,035	3,176,420	2,521,840

Figure 4 presents smooth sinusoidal variation based on sampling data at LT and RP sites for two (spring and neap) tidal amplitudes.

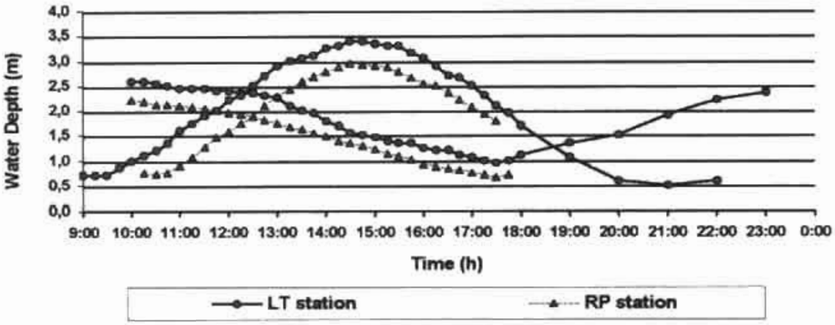

Figure 4. Water level variation over a spring tide and a neap tide

Model description

DUFLOW Model (1 D)

The *DUFLOW* model was designed to cover a large range of applications, such as propagation of tidal waves in estuaries, flood waves in rivers, operation of irrigation and drainage systems and water quality problems. As the relationship between quality and flow gets special attention nowadays and this package is suitable for modelling both, it becomes a useful tool in water quality management.

The package is based on the one-dimensional partial differential equations that describe non-stationary flow in open channels, which are the mathematical translation of the laws of conservation of mass and of momentum. These equations are discretized in space and time using the four point implicit Preissmann scheme. This scheme is unconditionally stable, shows little numerical dispersion and allows non-equidistant grids. It computes discharges and elevations at the same point.

The quality part of the *DUFLOW* package is based upon the one-dimensional transport equation. This partial differential equation describes the concentration of a constituent in a one-dimensional system as function of time and place. The production term of the equation includes all physical, chemical and biological processes to which a specific constituent is subject to. The user can supply the process descriptions.

$$\frac{\partial(AC)}{\partial t} = -\frac{\partial(QC)}{\partial x} + \frac{\partial}{\partial x}(AD\frac{\partial C}{\partial x}) + P \qquad (1)$$

Where:

C Constituent concentration [$L^{-3}M$];
Q Flow [L^3T^{-1}];
A Cross-sectional flow area [L^3T^{-1}];

D Longitudinal dispersion coefficient $[L^2T^{-1}]$;
t Time [T];
P Production of the constituent per unit length of the section $[L^{-1}MT^{-1}]$.

This package was applied to south arm Mondego estuary, as a first approach, to estimate the annual nutrient balance, based on current velocities obtained from model results, after model calibration with field data.

Figure 5 presents the increase (always about 60 %) on average flow velocity due the Gala bridge pillars contracting effect, comparing several sampling values of flow velocity with the model results obtained immediately upstream Gala bridge section (Duarte 1997). The downstream boundary condition used by hydrodynamic model was the water level recorded at this point.

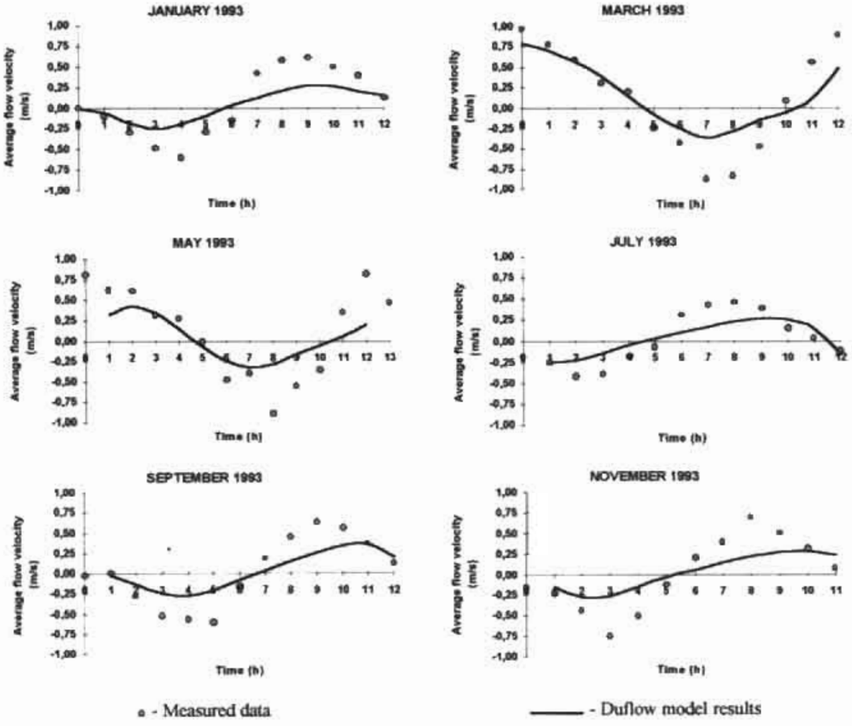

Figure 5. Average flow velocity at Gala bridge: pillars effect

SMS Model (2D-H)

SMS package was designed as a comprehensive hydrodynamic modelling system being a pre- and post-processor for two-dimensional finite element and finite difference models. Interfaces have designed to be used with the programs *RMA2* and *RMA4* (US WES-HL, 1996).

The hydrodynamic model *RMA2* is used to compute water surface elevations and flow velocities for shallow water flow problems. This program solves the depth-integrated equations of fluid mass and momentum conservation in two horizontal directions by the finite element method using the Galerkin Method of weighted residuals.

The elements may be one-dimensional lines or two-dimensional quadrilaterals or triangles, even with parabolic sides (Pinho et al. 1999). The shape functions are quadratic for velocity and linear for depth. Integration in space is performed by Gaussian integration and derivatives in time are replaced by a non-linear finite difference approximation. It is not applicable to supercritical flow problems.

The quality model *RMA4* is an interface used to assess the migration and dissipation of a constituent, describing its concentration in two horizontal directions as a function of time and place. It uses the hydrodynamic solution from *RMA2* to define a flow velocity field for a given mesh and also reads a set of user-specified point loads as input. It was applied with success for longitudinal dispersion coefficient estimation in a Mondego river water quality study (Duarte et al. 1999).

Residence times estimation

Residence times (RT) are broadly recognised as important descriptors of estuarine behaviour, but no real consensus exists on its definition. Traditionally, a single value has been used to RT evaluation in estuaries to characterise the whole system (Officer & Kester 1991). This procedure, assuming steady state and well-mixed conditions is attractive to establish comparisons among systems and to estimate ecological quantities. Local analysis is necessary to address important ecological local problems or resulting from local physical processes (e.g. turbidity maximum). Time variability of the environmental forcing also makes RT strongly dependent on the release time (Oliveira and Baptista 1997).

In this work, a sensitivity analysis on RT spatial variability was performed. The effect of various factors (river flow discharge, release time, discharge tracer duration, and tracer mass) was anticipated for invariable tidal conditions. For each point of the physical system, RT was considered as the time period in which the conservative and once-through tracer concentration remains higher than its initial concentration in the system. This concept can be considered as a simplified approach methodology provided that baroclinic and wind forcing were not accounted for, as well as river Pranto flow and tidal cycle time variability were also neglected. Re-entrant and non-conservative tracer behaviour and the effect of other factors like injection point location and river Pranto sluice opening regime will be considered in further research work.

Results and conclusions

The estuary hydrodynamic model based on *RMA2* program was implemented to compute water levels and mean velocity fields, resulting from the tidal and the river Pranto flow forcing. This system, with a total area of about 1.89 km^2, was discretized using a 2D mesh (Fig. 6) composed by 3,371 triangular finite elements with 7,020 nodes. Two open boundary conditions were considered: the water surface elevation, defined according the

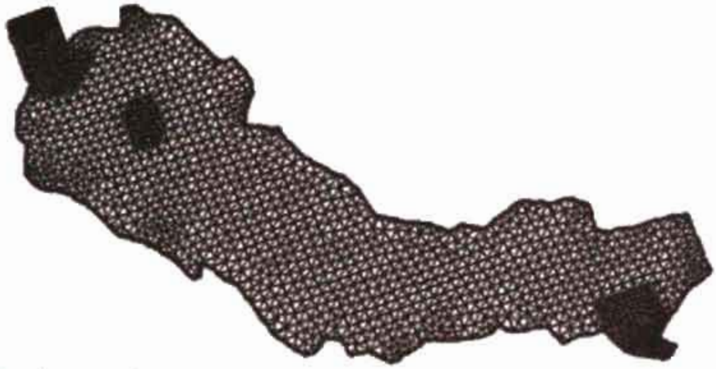

Figure 6. Finite element mesh

characteristics of a semi-diurnal *M2* selected tide, imposed downstream Gala bridge and a constant flow boundary condition upstream at the river Pranto entrance.

An important intermediate hydrodynamic result is the pattern and the magnitude of estuarine velocity currents. Indeed, for estimation of the potential drift of the opportunistic macroalgae a simultaneous experimental study is being carried out in order to relate the mean current velocities with the critical bottom stresses for

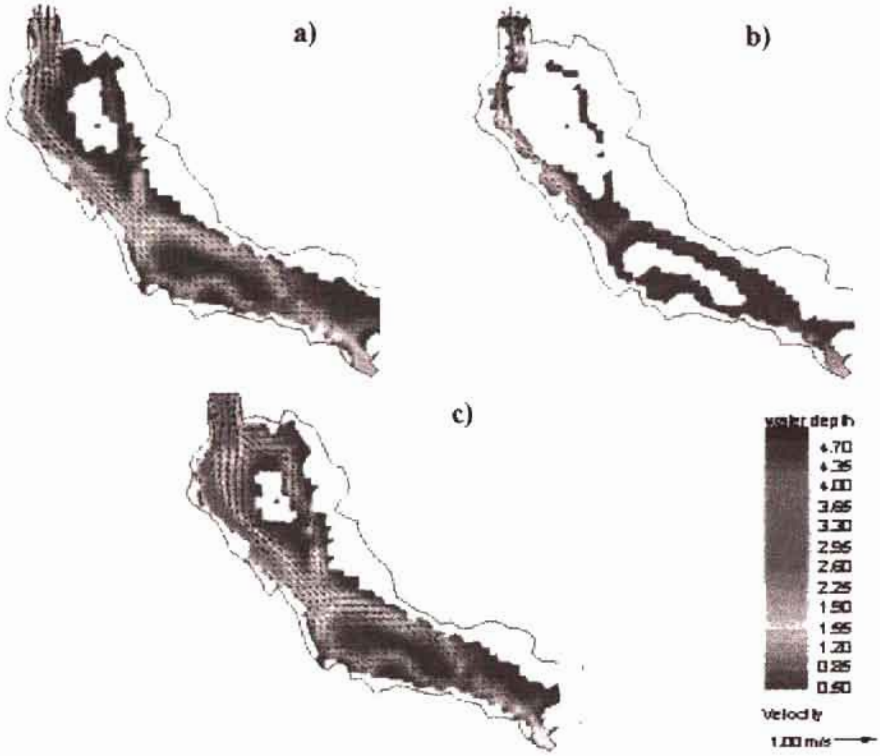

Figure 7. Water depths and velocity fields: a) half; b) three-quarter; c) tidal period

Enteromorpha drifting. Figure 7 presents the simulated results of water depth and velocity field for the three most representative instants during tidal cycle.

Calibration procedure was performed comparing measured values of velocity and salinity in a few stations with simulated model results. Calibration will be improved when more field data became available.

The simulated scenarios worked out are summarised in Table 3. River Pranto flow variation, mass of tracer injected, time release and tracer discharge were considered in order to assess their effects on spatial estuary residence times variability.

Table 3. Simulated scenarios

River Pranto flow [m^3/s]	5				10			
Tracer mass [kg]:	1,800				3,600			
Release Time (h)	6	9	6	9	6	9	6	9
Tracer discharge duration (h)								
1	S 1	S 2	S 6	S 7	S 11	S 12	S 16	S 17
3	S 3	S 4	S 8	S 9	S 13	S 14	S 18	S 19
6	S 5	---	S 10	—	S 15	---	S 20	—

RT obtained from the different simulated scenarios allow to establish the most sensitive zones to estuarine eutrophication process, assuming that nutrients enrichment can be simulated by the tracer presence in most vulnerability zones of the system. Samples of the obtained results for different effects of simulated factors, considering the river Pranto flow value of 5 m^3/s, are depicted in Figure 8.

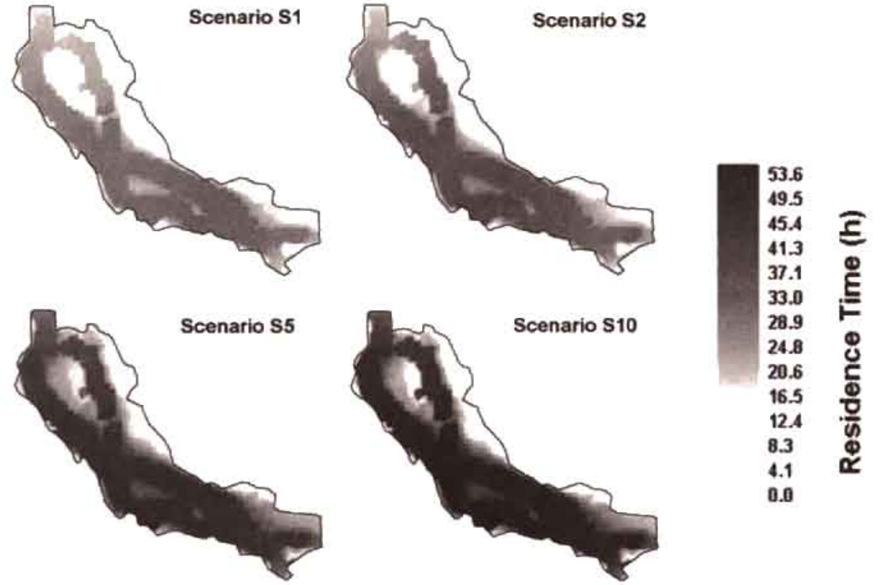

Figure 8. Residence times spatial variation

The effect of tracer discharge duration can be seen comparing scenarios S1-S5. Scenarios S1-S2 illustrate time-release effect during ebb tide. Tracer mass variation discharge effect can be analysed by means of scenarios S5-S10. In these conditions, discharges tracer duration appears to be a key parameter for residence time variation. So, when necessary, it is preferable to do more sluice openings, but during a short period of time.

Being a particularity of each ecosystem, the identification of which variables control macroalgal abundance and regulate their biomass, and mostly the quantification of their influence contribute to a deeper knowledge of estuarine eutrophication, helping to find suitable solutions for this environmental problem.

Although these results can be taken as a first approximation, they represent an important step for time scale definition of eutrophication processes in estuarine systems. Modelling results confirm the eutrophication gradient measured in the south arm of the river Mondego estuary, validating the methodology applied.

Since river Pranto is artificially controlled by sluices, the results obtained in this work and its future developments will constitute an important input for optimised operation policy of those hydraulic structures, in order to reduce the negative impact of nutrients discharges from this river in estuarine system.

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SEDIMENTARY DYNAMICS OF THE MONDEGO ESTUARY

Abstract

The Mondego estuary is composed of two subsystems with contrasting sedimentary, hydrodynamic, physical and chemical characteristics. Is affected by a mesotidal, semi-diurnal, regime. The Mondego subsystem, currently including a navigation channel, is exclusively bounded by artificial banks. The Pranto subsystem is more shallow and less affected by anthropogenic interventions. The aims of this study are the recognition of freshwater/saltwater circulation and the analysis of surface sediments in order to propose a pattern of sediment circulation. Studies on grain-size, mineralogy and quartz-grains surface textures and roundness of surface sediment samples were conducted and a detailed grain-size map was obtained. Most of the data was acquired in 1994 to 1996, at low and high tide of neap and spring tides, including measurements in selected locations, at several depths, of conductivity, salinity, temperature, current velocity/direction, turbidity, concentration of silt/clay, pH and Eh. The Mondego subsystem is under significant tidal control, being well mixed with reduced fluvial flow and stratified during seasonal floods. The hydrodynamic pathways (tidal vs. fluvial, this prevailing in the northern margin) causes clear contrast in sediment transport. The Pranto subsystem is mainly brackish, well mixed, with strong tidal hydrodynamic, physical and chemical fluctuations; fluvial floods rarely have high discharges. The well developed Gala flood tidal delta, and the extensive saltmarsh and intertidal mudflat, dissected by tidal creeks, are the main features of this subsystem. The Pranto subsystem is tidal dominated, while the Mondego subsystem is both tidal or fluvial dominated.

Introduction

The sedimentary dynamics is one of the main controls in an estuarine ecosystem, a complex transitional environment with a delicate balance. This work is focused on the sedimentology and hydrodynamics of the Mondego estuary and adjacent shore (Fig. 1). As discussed in this work, the Mondego estuary is composed of two subsystems – the Mondego and the Pranto – with contrasting sedimentary, hydrodynamic, physical and chemical characteristics.

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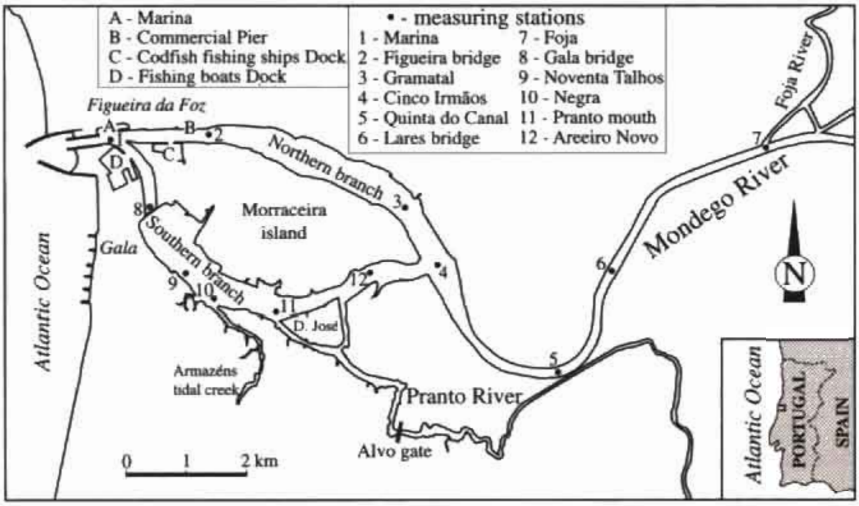


Fig. 1. Location and map of the Mondego estuary (except a small portion of the upstream sector), with the position of water properties measurements stations.

Estuarine deposits are controlled by periodic phenomena, at different time and space scales, as tides (Allen 1993) and seasonal floods. In this study, the surface sediments after a 125-years return flood were characterised, allowing the estimation of water and sediment flow and provenance. The water mixing and circulation pattern was evaluated through the study of the hydrodynamic, physical and chemical parameters variation.

The role of natural or human processes is discussed, as well as the long-term and long-distance impacts of changes in the main controls of the sedimentary system dynamics. In particular, the morpho-sedimentary evolution can assess the impact of the extensive human modification and occupation, and contributes for the acquisition of fundamental elements in the decision-making, towards a sustainable and integrated management of natural resources in coastal zones.

Regional Setting

Geological and geographical setting

The Mondego River has a catchment basin of 6671 km^2, draining granitoids, metamorphic and sedimentary rocks. Downstream of Coimbra the river flows in a floodplain up to 4 km wide. The estuary location is fixed just south of an uplifted relief, the Serra da Boa Viagem. Despite the significant fluvial and tidal flows (specified bellow), the strong southwards longshore current built an important spit, such that the estuary can be morphologically classified, after Fairbridge (1980), as bar-built. About 6.5 km upstream of its mouth the river is divided in two branches (northern and southern), corresponding to the above mentioned subsystems, that converges again defining the

Morraceira Island (Fig. 1). The sedimentary areas reaches 4.64 km^2 in the Mondego subsystem and 2.20 km^2 in the Pranto subsystem.

Fluvial regime

Before the construction of several dams during the decade of 1970, the fluvial regime was directly controlled by the seasonal precipitation in the catchment basin (Tab. 1), with a large bed-load inflow to the estuary and coastal areas. However, essentially with the construction of the Aguieira and Fronhas dams, the fluvial regime was smoothed, reducing slightly the water discharge, but most of bed-load remains trapped (compare Tabs. 1 and 2). Notice also that the average discharge for the days of each year with highest discharge was quite reduced. For instance, the 10 days with highest discharge of a wet year reached a mean of 825 m^3/s before the construction of several dams, and only 700 m^3/s after the construction.

TABLE 1. Water and solid discharges (m^3/s) of the Mondego measured in the Coimbra streamflow station, before the construction of several dams (DIRECÇÃO GERAL DE RECURSOS E APROVEITAMENTOS HÍDRICOS 1987).

Days/year (total 360)	Average year	Wet year	Dry year
10	490	825	245
50	175	325	75
100	85	210	40
200	20	25	5
Annual mean waterflow	84	155	37
Dominant waterflow	370	490	230
Solid flow	0.0075	–	–
Estimation for a 100-years return flood: peak flow of 3663 m^3/s			

TABLE 2. Estimated water and sediment discharges (m^3/s) of the Mondego measured in the Coimbra streamflow station, after dams construction, embankment and canalisation of the river (DIRECÇÃO GERAL DE RECURSOS E APROVEITAMENTOS HÍDRICOS 1987).

Days/year (total 360)	Average year	Wet year	Dry year
10	500	700	160
50	175	360	58
100	85	240	30
200	18	40	5
Annual mean waterflow	72	145	27
Dominant waterflow	340	440	150
Solid flow	0.0015	–	–
Peak flow (100-year return period) in Coimbra considered for the main river channel: 1200 m^3/s in Coimbra (corresponding to about 2955 m^3/s in the mouth)			

The total freshwater inflow of the tributaries downstream of Coimbra is very reduced (Tab. 3), including in the right margin the Ançã and Foja Rivers, and in the southern margin the Cernache, Ega, Arunca and Pranto rivers. The freshwater in the Pranto subsystem is usually limited to the flow of the Pranto River and the Esteiro dos Armazéns.

The solid inflow to the estuary from the Mondego tributaries downstream of Coimbra is about 26.5 × 10^3 m^3/year (after Tab. 3 data).

The Mondego discharge measured in Coimbra during the field work period shows that, despite the construction of the dam system upstream, the flow regime is irregular (Tab. 4) and quite seasonal.

TABLE 3. Water and sediment discharges (m^3/s) of the Mondego tributaries downstream of Coimbra, after dams construction, embankment and canalisation of the river (DIRECÇÃO GERAL DE RECURSOS E APROVEITAMENTOS HÍDRICOS 1987).

Tributary	Streamflow annual mean	Solid flow annual mean	Streamflow dominant mode	Flood flow (25 years return period)
Ançã	1.1	0.00016	2.8	325
Foja	1.3	0.00017	2.6	135
Cernache	0.5	0.00002	1.8	175
Ega	1.9	0.00004	7.1	70
Arunca	4.9	0.00018	15.9	895
Pranto	1.9	0.00027	5,1	315
Total	11.6	0.00084	35.3	1915

TABLE 4. Extreme Mondego streamflow daily mean discharge (m^3/s) measured in Coimbra, during the considered field work.

Year	Minimum	Maximum
1994	12.6 (April, 3)	694.5 (January, 7)
1995	8.6 (November 4)	1013.0 (December, 26)
1996	6.0 (November, 8)	1034.6 (January, 7)

Tidal regime

The tidal regime, coupled with the fluvial inflow, controls the shape and sedimentary dynamics of the Mondego estuary. It is affected by a mesotidal regime (Hayes, 1975), with semi-diurnal tides and small diurnal inequality. In the predicted tidal levels for 1996, the most frequent range is 2.4 m, with a mean of 2.2 m, a minimum of 0.9 m and a maximum of 3.6 m.

During seasonal low fluvial flow and spring tides, measurable tidal level change reaches 26 km upstream of the mouth (Montemor-o-Velho). For instance, in the summer of 1989 in the Ereira bridge (18 km upstream), a range of 2 m was recorded in spring tide and 0.7 m in neap tide (Baptista 1990), with fluvial discharge of 8 to 24 m^3/s in Coimbra (Açude measuring station). According to Duarte and Reis (1994), during fluvial floods the tidal wave penetrates only 16 km, but these authors do not provide the correspondent fluvial discharge. The Pranto subsystem is nowadays limited upstream by the Alvo gate (Fig. 1), avoiding the propagation of the tidal wave.

Methods

The field work was performed between 1994 and 1996 (details in Cunha et al. 1997a). Measurements of conductivity, salinity, temperature, current velocity and direction, turbidity, concentration of silt/clay, pH and Eh were carried out in selected locations (Fig. 1). The field analysis of water properties were carried with fluvial discharges lower than the annual mean, at low and high tide of neap and spring tides, in order to recognise the freshwater and saltwater circulation pattern. Separate longitudinal profiles were recorded in each subsystem, in an upstream direction (Fig. 1). A total of 23 profiles were obtained: 12 in the Pranto subsystem and 11 in the Mondego subsystem, corresponding to 12 tidal cycles (2 in 1994, 6 in 1995 and 4 in 1996). The collected data of water properties is considered as representative of frequent situations of annual and inter-annual variability. All the stations were located in the thalweg, and the measurements were made at each meter of depth, from the surface to the bottom.

The field description of surface sediment samples, collected directly in the intertidal and supratidal areas and by dredging in the channels (164 of the Mondego subsystem and 63 of the Pranto subsystem), were used to elaborate a detailed grain-size map (Udden-Wentworth scale; see the Mondego Estuary grain-size map in appendix). A study on grain-size, mineralogy and quartz-grain roundness and surface textures was performed on selected samples – 114 of sand and 15 of mud.

Surface sediment was sampled just after the important fluvial winter flood of 1995-1996, with a maximal discharge of 1210 m^3/s in Coimbra corresponding in the estuary to an inflow near 3000 m^3/s (Tab. 2). This flood is estimated as having an 125-year return period, considered here as a situation of maximal fluvial control in the estuary.

Water properties

The obtained physical, chemical and hydrodynamic information, combined with previous data, allowed the characterisation of the estuarine freshwater and saltwater, and contributed to recognise the way in which they interact in space and time during seasonal and tidal cycles. Furthermore, these informations are relevant contributions to understand the sedimentary dynamics. In fact (e.g. Perillo 1995), the bed load deposition is strongly controlled by the velocity and pathway of tidal and fluvial currents, the suspended load deposition depends on the margins hypsometry and the maximum flocculation occurs in the zone of sharp salinity change (halocline).

Currently, the salinity increase induced in the Mondego subsystem by tidal propagation reaches the Foja station, 15 km upstream of the mouth (Fig. 1; salinity of 7 ‰ in the spring high tide of 96/07/02, with a discharge of 16.7 m^3/s in Coimbra; Cunha et al. 1997), but a salinity of 5 ‰ was already recorded near the Ereira bridge (18 km upstream) in September of 1989 (Baptista 1990), with a fluvial discharge of 8 m^3/s in Coimbra. According to Duarte and Reis (1994), the brackish water can penetrate only 8 km in the Mondego subsystem during fluvial floods, but these authors do not provide quantitative discharge data.

During the field work of March to July 1996 the discharge in Coimbra oscillated from 13 to 182 m^3/s, covering a wide range of water mixing and variation of physical and chemical parameters. The presented longitudinal profiles (Figs. 2 to 4) exemplifies the main interpretations.

The saltwater temperature is frequently different from the freshwater, specially during the hot and dry season (spring and summer). Eh and pH can be directly correlated with the salinity – saltwater average: pH=8,1 and Eh=65 mV; freshwater average: pH=7,4 and Eh=30 mV – and presence of contamination can be related with abnormally high values of those parameters (Fig. 2). The turbidity, in general lower then 10 MTU, has a complex distribution, attaining peaks in the narrow zones of sharp salinity change (halocline) or near the main bottom morphologies. The concentration of silt and clay was always lower than 0.1 g/l.

Mondego subsystem

With neap tides this subsystem presents a salt-wedge during low tide, changing to partially-mixed during high tide. Fig. 2 shows the salt-wedge and the upstream current, even with the upstream stations under a dominant fluvial influence; the pH, Eh and turbidity values indicates water contamination in the Quinta do Canal station.

Contrasting trends were recorded in spring tides: slightly partially-mixed in low and well-mixed in high tide. Fig. 3 is an example of a fully mixed situation, since there is no vertical differences on salinity. The Pritchard's (1955, see also Dyer 1995) approach points to a stratified situation in the Mondego subsystem with high ratio between the fluvial flow (Tabs. 2 and 3) and the tidal volume (spring tides: 12.5×10^6 m^3, average tides: 11.5×10^6 m^3 and neap tides: 8.0×10^6 m^3; Consulmar 1973), frequent during the rain season.

A moderate increase of the fluvial discharge affects mainly the salt-wedge thickness, rather then its upstream extension. The 30 ‰ isohaline swings about 9 km in a spring tide cycle and near to 4 km in a neap tide cycle.

The flow velocity clearly decreases to the bottom. During flood, fluvial and tidal currents merges in the Cinco Irmãos area. Mainly in neap tides, the flood current frequently continues after the highest water stage, creating a salt-wedge. The maximum ebb velocity is attained in the mouth, where the flow of both subsystems converge. The bottom morphology and the water quality of the small tributaries can be better evaluated during low tide. Due to the fluvial current, the maximum velocity of flood current is located about half depth (Fig. 2).

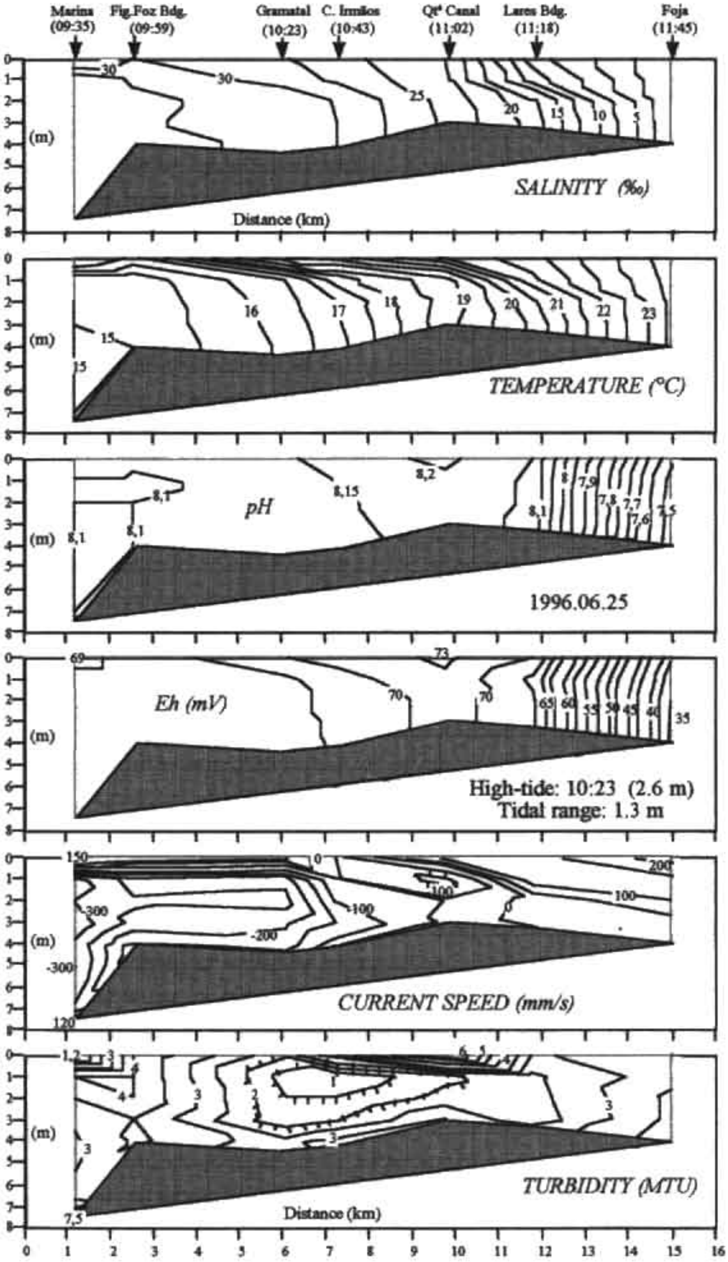


Fig. 2. Physical and chemical parameters of the Mondego subsystem water in a high neap tide, with a fluvial inflow about 20 m^3/s (17.2 m^3/s measured in Coimbra and 1.6 m^3/s in the Ponte de Mocate-Arunca station). The high tide value is referred to the Portuguese Hydrographical Zero. Negative current values indicate upstream displacement, and positive downstream.

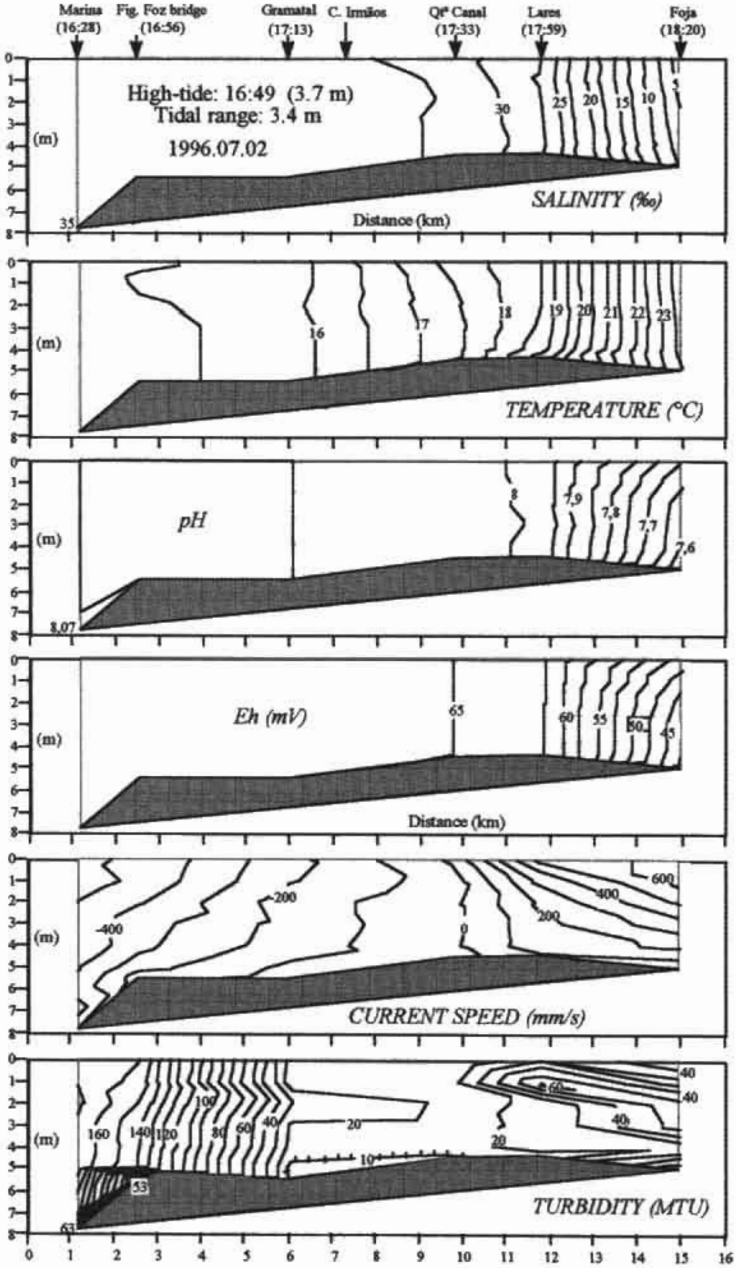


Fig. 3. Physical and chemical parameters of the Mondego subsystem in high spring tide, with fluvial inflow about 20 m³/s (16.7 m³/s measured in Coimbra station).

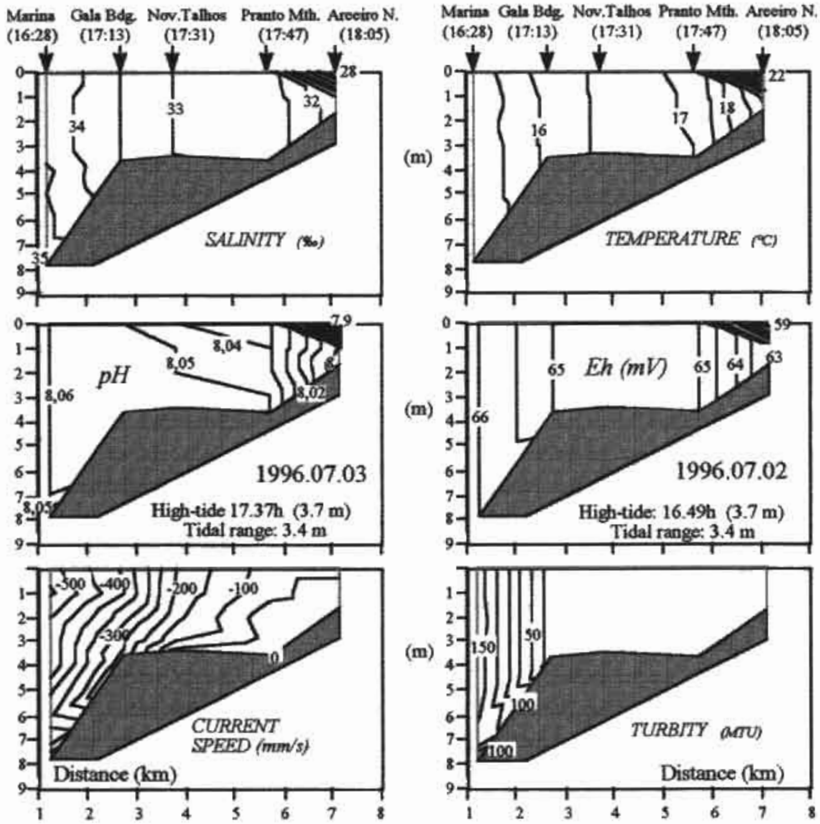


Fig. 4. Physical and chemical parameters of the Pranto subsystem in a high spring tide.

The ebb velocity in the northern estuarine branch during a 100-year return period fluvial flood (1200 m^3/s in Coimbra) is estimated in 2.7 m/s, decreasing to about 1.6 m/s with a 1000 m^3/s discharge in Coimbra (Consulmar et al. 1991). With a low river discharge (13.6 m^3/s in Coimbra), the maximum ebb velocity recorded reached 0.86 m/s in the Marina station.

Under weak fluvial discharge, the spring tide flood can have a significant control even in the upper estuary. Field experiments of 95/04/12 and 96/07/02 had a low fluvial flow and, therefore, considered as representative of such conditions. In 95/04/12, under a 14.1 m^3/s Mondego discharge measured in Coimbra, upstream velocities of -0.36 m/s in the Foja station and -0.60 m/s in the Ponte de Lares station were recorded. In the same conditions, the maximum flood velocity was measured in the Marina station at the surface (-0.70 m/s). Near the bottom, upstream velocities of -0.25 m/s were recorded in Ponte de Lares (spring tide flood of 95/04/12) and -0.3 m/s in the Marina, about 20 minutes before the high spring tide of 96/07/02.

The high and homogeneous turbidity of the seawater inflow shows a clear upstream dilution. The over-flowing fresh water turbidity has its maximum at the surface with a gentle decrease to the bottom (Figs. 2 and 3). The ebb flow friction over the bottom increases significantly the turbidity (Kappenberg et al. 1995).

Pranto subsystem

During low tide this subsystem is almost completely exposed, with small fluctuations of several parameters probably due to local damming of brackish water by the large sandy-muddy bars between Ponte da Gala and the mouth of the Pranto River. Usually fully mixed, it can be partially mixed during the rare fluvial floods, as also suggested by the ratio (after Pritchard 1955) between the fluvial flow (Tabs. 2 and 3) and the tidal volume.

The subsystem attains high salinity in each tidal cycle – even during a spring low tide, values of 23-25 ‰ were recorded at the bottom near the Pranto mouth. During high tide all the area is filled with salt water (33 ‰ in spring and 25 ‰ in neap tides at the Areeiro Novo station).

The temperature can show significant fluctuations, specially with spring tides. For instance in the Noventa Talhos station variations of 8 °C were recorded during a tidal cycle (16-24 °C).

The upstream decrease of turbidity reflects the mixing of salt water, typically with high values, but during neap tides the variation of this parameter is more irregular, with high values related to the friction of fast current in channels over the sandy-muddy bottom (Kappenberg et al. 1995). The maximal speed of spring tide flood is attained in the downstream zone, at the surface, but throughout neap tides it was recorded in the narrow channel at Ponte da Gala station.

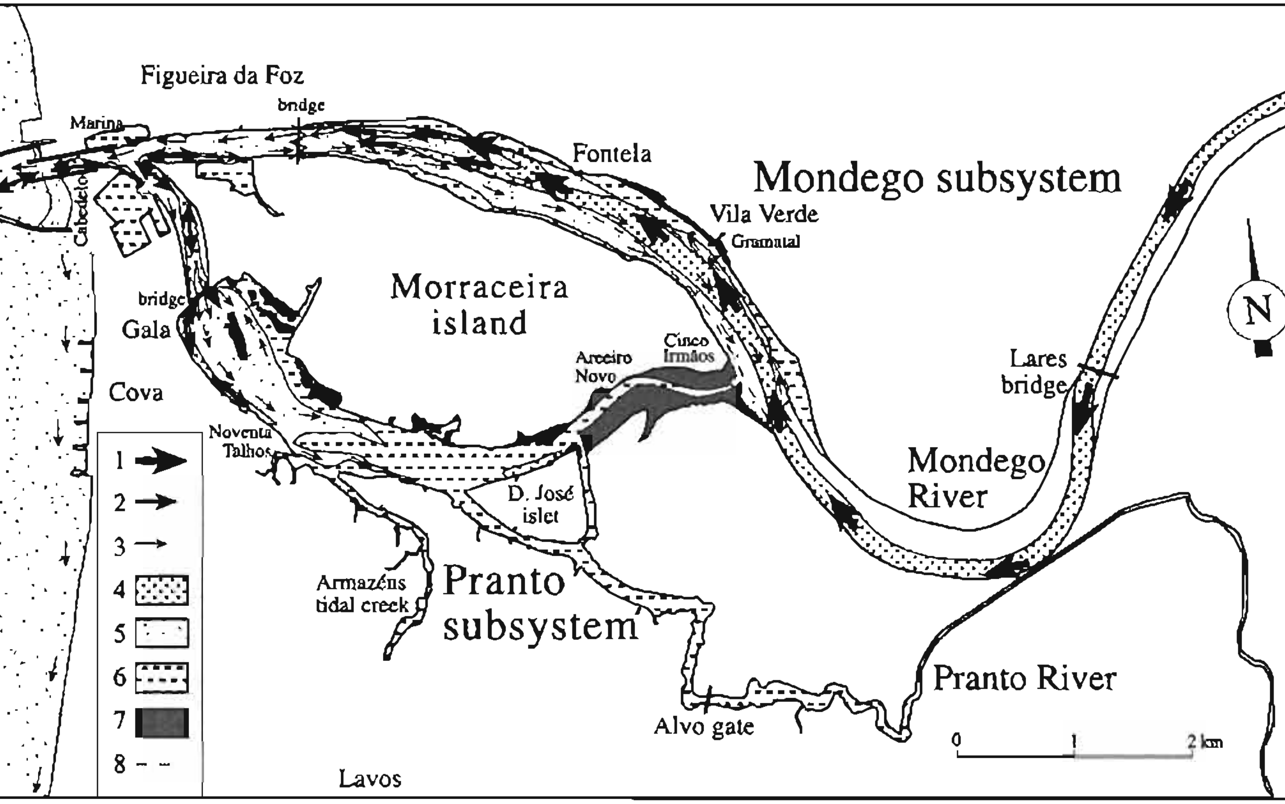
In high spring tides, the upstream brackish water inflow from the Mondego River is expressed by several parameters recorded in the Areeiro Novo station. On Fig. 4 we can see that salinity, turbidity, pH and Eh documents brackish water inflow from the Mondego River and negligible fresh water inflow from the Pranto, in a typical fully mixed situation.

Surface sediments description

Mondego subsystem grain-size distribution

Sector I (Montemor-o-Velho to the Lares bridge)

The sector from Montemor-o-Velho (26 km upstream of the estuary mouth) to the Lares bridge is dominated by the fresh water flow, but records also the variation of water stage over a tidal cycle. It consists of a channel with hard bank protection (Fig. 5) with a very coarse sand and gravel bed, frequently organised in isolated longitudinal and point bars.



hematic summary of the sediment circulation pattern of the Mondego estuary and adjacent beaches - maximal transport capability of tra
rrents (1 - up to granules, 2 - up to very coarse sands, 3 - up to medium sands); bottom sediments mean grain-size (4 - granules to c
nd, 5 - medium to fine sand); tidal mudflats and muddy bottoms (6); saltmarshes (7); navigation channel boundary (8).

Sector II (Lares bridge to Cinco Irmãos)

Includes part of the sector channelised with a bank protection wall finished in 1984, increasing the agricultural use of the muddy floodplain. The thalweg has granules and is bordered by very coarse to medium sand (Fig. 5), but some marginal locations are covered with fine sand to mud.

Sector III (Cinco Irmãos to the Figueira da Foz bridge)

In this section, dredged and totally bounded by hard bank protection after 1995, the water is usually brackish, except during fluvial floods. The morphology and dynamics are slightly dominated by fluvial processes. The channel bottom has granules to medium sand and, in some marginal areas with reduced hydrodynamics, fine sand to sandy mud (Fig. 5). The longitudinal belt of coarse bottom sediments bifurcates downstream in response to the interconnection of tidal and fluvial dynamics. Intertidal margins consist in medium sand (near the channel), grading laterally to sandy mud. Large sectors were filled with dredged material in 1995-1996, later mixed with overbank flow sediments. Small areas of saltmarshes occur in the right margin, upstream of Fontela, representing what is left of very extensive marshes, now largely reclaimed.

Sector IV (Figueira da Foz bridge to the branches confluence)

Salt water and tidal dynamics predominate in this section. A straight channel with rockwalls and dredge filled margins was finished in 1984, were the Commercial Pier, the Bacalhoeiros (codfish fishing ships) Dock and the Marina are located.

The thalweg follows closely the southern margin and is covered with coarse sand, grading laterally to medium sand (Fig. 5). Near the margins there are small areas of fine sand to sandy mud sedimentation, namely at the commercial pier. Settling out of black organic mud is the only sediment accumulation in the Bacalhoeiros dock and in the Marina.

Sector V (branches confluence to the seaward end of external jetties)

Corresponds to the estuary mouth and the associated estuarine beach/uncovered aeolian dune complex, and is the only sector of the estuary influenced by wave dynamics. A belt of coarse and very coarse sands reflects the sediment transport during tidal floods along the southern external jetty, following along the margin of the Cabedelo beach and the southern margin of the navigational channel. The channel axis is covered mainly with medium sand, but a medial ribbon with fine sand probably reflects the different pathways of salt water flood and freshwater flow during high fluvial discharge. The Cabedelo beach, between the internal and the external southern jetties (Fig. 5), is composed of micaceous fine sand and feeds the adjacent aeolian dune field.

Pranto subsystem grain-size distribution

Sector I (branches bifurcation to the D. José islet)

The area from the Cinco Irmãos station to the small channel east of the D. José islet is mainly composed of saltmarsh and intertidal mudflats, dissected by narrow, meandering, muddy tidal creeks (Fig. 5). It must be stressed that, even during the seasonal floods, the Mondego supplies no bedload sediments to this subsystem.

Sector II (D. José islet to the Noventa Talhos station)

Intertidal mudflats and sinuous creeks cover most of this sector, associated to minor amounts of fine to very fine sand downstream of the Pranto mouth (Fig. 5). The main tidal channel, near the southern margin, is covered with medium to coarse sand, remains of recent times when this subsystem had a significant upstream connection with the Mondego River. The Pranto River feeds exclusively suspended material (silt and clay).

Sector III (Noventa Talhos station to the Gala bridge)

The margin of the Morraceira Island is composed of saltmarshes and intertidal mudflats, dissected by meandering tidal creeks, among which many connected with fish farms and salt-pans. The sudden widening upstream of the Gala bridge created a flood tidal delta – composed chiefly by coarse to medium sands – with a major south-western ebb channel covered with bioclast rich medium sand. Fine sand covers the shallower north-east ebb channel.

Sector IV (Gala bridge to the branches confluence)

The downstream sector of the Southern branch, already fully limited by rockwalls by 1934, is dominated by medium sand, but the thalweg, close to the right margin nearby the confluence, is covered with coarse sand (Fig. 5). Downstream of the Fishing-boats Dock entrance, a zone of fine sand to sandy mud deposition occurs, and upstream of the entrance a medium sand intertidal bar is formed. Black organic mud accumulates in the Fishing-boats Dock.

Grain-size of the beaches adjacent to the Mondego mouth

The influence of wave dynamics inside the estuary is reduced. However, it is the main control on beaches and on estuary mouth grain-size, morphology and evolution (Carvalho and Barceló 1966, Almeida and Santos 1991, Santos et al. 1991, Cunha and Dinis 1998). In the beach north of the jetties, nourished by the southward prevailing longshore current, the intertidal sector has essentially coarse sand, grading offshore to medium sand and to micaceous fine sand. The narrow beach south of the river mouth (Fig. 5) is mainly constituted by medium sand and nourished by the longshore current and the local erosion of the aeolian dune field.

Composition of the coarse fraction (0.5-1 mm) of estuarine sands

Colourless quartz grains have marine and fluvial provenance. Upstream of the branch bifurcation (Cinco Irmãos) the proportion of colourless quartz, feldspars and milky quartz are high, but just downstream, in the northern branch, the percentage of quartz diminishes due to high proportion of micas and estuarine bioclasts. The colourless quartz content increases again from the Fontela to the mouth, but downstream of the Figueira bridge it occurs mainly near the southern margin by the mixing with the marine provenance, also rich in bioclasts. The highest contents of quartz are coincident with the energy axes, as also happens in the southern branch with an upstream dissipating axis.

Feldspars grains are concentrated along the northern margin of the northern branch (Mondego subsystem), reflecting the fluvial input in this concave sector. In the Pranto subsystem, feldspars found at some places (trapped within depressions of the main channel) are considered as remains of a formerly wider upstream connection with the Mondego River.

High amounts of bioclasts (mainly of calcium carbonate) occur bellow the tidal level in the beach, as well as concentrations of estuarine molluscs shells, namely in the southern branch upstream of the Gala bridge.

Micas are concentrated in several zones with reduced hydrodynamic, as in the convex sector of the northern branch, along the right margin of the southern branch (just upstream of the Gala bridge) and in the internal beach of Cabedelo.

Quartz-grains roundness and surface textures

The roundness and surface textures of quartz-grains were studied through binocular microscope examination of the coarse fraction of sands (0.5-1 mm).

Sands with fluvial provenance are predominantly angular and very angular, shiny, but minor amounts of any other arrange of roundness and shininess can occur. Considering all the estuary, the roundness decreases upstream, as well as, less clearly, the shininess increases.

All across the northern branch, upstream of Fontela, the dominant population has characteristics that clearly points to a fluvial provenance, except in the southern margin upstream to Gramatal, where two modes of roundness correspond to the mixing of fluvial and marine sand. These were carried by tidal floods over an intertidal margin (formerly used as dredge disposal area). The roundness and shininess distribution shows a dominance of the marine provenance over the fluvial sub-population between the Marina and the Figueira da Foz bridge, in particular near the southern margin. A minor population of angular and frosted grains found upstream of Gramatal, quite rare between Fontela and Gramatal, is interpreted as redeposited from Cretaceous and/or Tertiary deposits. The mixed population of the mouth sector, up to the Marina, has a major component of fluvial (angular) sands, but the high proportion of more rounded sand in the Cabedelo beach reveals the dominant transport of marine sands by tidal floods and the protection effect of the internal jetty in relation to the fluvial transport.

In the Pranto subsystem, the sector between the branches confluence and the Gala bridge has sands with a bimodal roundness distribution, more obvious in the talwegue where rounded and sub-rounded grains are the more frequent types. In the same sector, the bar linked to the western margin is mainly composed by rounded sands coupled with a minor mode of sands with fluvial provenance. Around the flood delta, the marginal ebb channels between Gala and Noventa Talhos, and in particular the main one (southwest), are covered essentially by angular and shiny grains, but including sands with marine provenance. Most of those sands with fluvial characteristics were probably injected is this subsystem before the enormous reduction of the upstream connection with the Mondego River.

The flood tidal delta located upstream of Gala bridge is composed of sands with a high mean roundness and reduced shininess (trend more pronounced within the well rounded class) allowing its attribution to a marine provenance. The delta is created by the loss of competence when the tidal flood reaches the sudden widening of the channel. Also a significant aeolian provenance can be inferred by the high content of frosted grains, quite probable considering the short distance to the uncovered aeolian dune field (Fig. 5) and the dominant winds with an eastward component.

On the beaches adjacent to the estuary, despite the wide range of roundness, the sub-rounded class is the dominant mode and well rounded grains are rare. Shiny grains are dominant, but the classes of better roundness include high contents of frosted grains, probably reflecting the fact that the intense beach erosion has already reached the aeolian foredune (Cunha et al. 1997b).

Sediment circulation pattern

The integration of hydrodynamic data (contrasting salinity mixing types and flows) and the analysis of surface sediments (grain size, composition and quartz-grains surface texture) supports the proposed pattern of sedimentation on both estuarine subsystems (Fig. 5). In detail, the figured situation corresponds to a boundary situation, after a major fluvial flood and with a reduced sediment supply. The map of provenance and transport potential synthesises a qualitative model of the sedimentary dynamic and its controls. These data, linked with the morpho-sedimentary units distribution, clearly emphasises the differences between the two estuarine subsystems.

In a long-term scale (years to decades), the bottom morphology and the sediments of the Mondego subsystem are dominated by fluvial processes related to floods (Cunha et al. 1997b), as expected in a laterally restricted estuary fed by a large river (Cooper 1993). The studied situation shows that the fluvial flow was able to transport granules as far as Fontela, nearby the right margin in this sector, coarse to very coarse sands untill the Figueira da Foz bridge, and medium sands down to the mouth. The tidal flood flows, essentially, along the southern margin of the mouth channel, transporting coarse to very coarse sands up to the commercial pier and carrying medium sands up to the Cinco Irmãos station, mixing gradually with fluvial sands. In the area between the northern internal and external jetties, the hydrodynamic protection from the prevailing waves (WNW) and from fluvial flow favours deposition.

In the Pranto subsystem the flood tidal delta of Gala is similar to those created by a narrow inlet (Dalrymple et al. 1992). Flood follows mainly the south-western marginal channel and the delta ramp, whereas the ebb sedimentary transport is essentially along both marginal channels. In the small scale sandy bay located south-east of the Gala bridge, the sand grain-size and the total sand content shows a clear increase towards west, probably as a result of the Coriolis effect (e. g. Van den Berg et al. 1996). This subsystem shows a clear trend to an almost complete infilling, mainly by fine-grained sedimentation in marshes and mudflats, accelerated, as expected (Patchineelam 1999), by the artificial constraining of the Mondego branch connections (Cunha et al. 1997b, Cunha 1998, Dinis and Cunha 1998). In fact, most of the mechanisms operating in this subsystem corresponds to a saltmarsh estuary, characterised by tidal regime dominance and little or no fluvial input (Frey and Howard 1986). The location of the limit between saltmarshes and mudflats reflects the average wind waves above the erosion threshold (Pethick 1996). The dominance of north and north-western winds (Arroteia 1985), coupled with the flow hydrodynamic asymmetry, explains the larger area occupied by saltmarshes in the Morraceira Island margin of this subsystem (Fig. 5).

The most intense anthropogenic impact on the sedimentary processes of the Mondego estuary results, probably, from dredging and sand mining. For purposes of harbour maintenance and enlargement, large volumes are removed from the sedimentary system (see data from the Port Authority of Figueira da Foz and environmental agencies in Cunha et al. 1995, 1998; Fig. 6), reaching values higher than the sum of fluvial and littoral sediment inputs. Even if the direct quantification of the marine sediments entrance is not available, it can be considered as similar to the total of sand mining at the mouth and in the adjacent beach (around 400 × 10^3 m^3/year during 1995 and 1996). The evaluation of the solid longshore drift is still under discussion (see Cunha and Dinis 1998), but the value of 1 500 × 10^3 m^3/year seems a reasonable estimate.

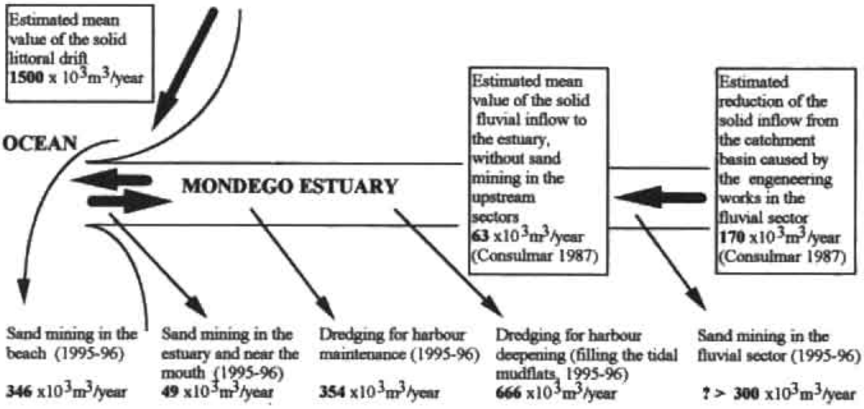


Fig. 6. Estimated volumes of inflow, solid littoral drift and artificially removed sediments in the Mondego estuary, during 1995-96 (adapted from Cunha et al. 1998).

The previous natural situation was characterised by an intense deposition of fluvial bed-load (Loureiro 1882, Oliveira 1967, Cunha et al. 1997b) and this estuary could be classified as river dominated (sensu Cooper 1993). Currently, the fluvial sediment inflow to the estuary is probably less than predicted by Consulmar (1987) for the situation after fluvial damming (63×10^3 m^3/year), due to the huge volumes removed by sand mining in the fluvial sector between the downstream dam and the estuary (at least 300×10^3 m^3/year). Dredging and sand mining are particularly intense in the Mondego subsystem, leading to its deepening. The tidal hydrodynamics play a major role in the natural trend to re-establish the equilibrium depth (Simmons and Herrman 1972), in particular in a situation where the mouth sand bar, coupled with the narrow navigation channel, constitutes an hydraulic constrain acting like an inlet. This effect creates a depositional trend similar to the occurring in a flood-tidal delta (Dalrymple et al. 1992, Perillo 1995). As a consequence, and as expected (Dyer 1973), this subsystem presents an upstream displacement of the sedimentary domains. In fact, the sampled surface sediments reflect a significant upstream penetration of marine sands along the Mondego subsystem, the huge decrease of fluvial bedload and the reduced transport capability of fluvial floods.

In the sector between the Figueira da Foz bridge and Cinco Irmãos the disposal of dredged spoils was made mostly in intertidal areas (Fig. 5). This led to reduction in the tidal prism, and, hence, a general decrease of the discharge and velocities of tidal currents (O'Brien and Dean 1972).

Synthesis and conclusions

The Mondego Estuary comprises two subsystems, with different hydrodynamics, morphologies and sedimentary characteristics, which have limited connections in space and time. In the hydrodynamics of the Mondego subsystem the river inflow is more important than the tidal control. The Pranto subsystem is clearly dominated by the tidal dynamic.

In the Mondego subsystem partially stratified to fully mixed situations were recorded with low fluvial inflow, and it can most probably be stratified with intermediate to high fluvial discharge. The deepening by dredging and the change to an artificial channel improved the upstream penetration of saline water and marine sands. In the channel the fluvial sediment transport is expressed by a grain-size decrease towards the mouth (gravel to fine sand), but an inverse variation along the southern margin results from the tidal flood currents. Fine sediments, like mud and muddy very fine sand, accumulated on areas of reduced hydrodynamics of the channel margins and in the tidal flats.

The Pranto subsystem is typically fully mixed, but during high fluvial discharges, rare nowadays, it probably grades to stratified. In a tidal cycle the salinity variation is high, mainly in the central sector (a small scale sandy bay), and an important local retention of waters was documented, caused by the muddy sand bars morphologies. The seaward area of marine influence is dominated by sand with some shell gravel, whereas the upstream area is mainly muddy. The Mondego River inflow of low salinity waters and sediments in this subsystem only occurs during seasonal floods.

Successive anthropogenic interventions led to an important artificialisation of the landscape. In particular, embankment and reclamation greatly reduced the natural areas, and changes on the hydrodynamics were also promoted by landfills of intertidal flats and dredging. Synthesising the last decades evolution of this estuarine system, several capital consequences must be stressed. A severe reduction of sediments in the estuary results from the upstream capture by dams and removal by sand mining and dredging. On the other hand, the dynamic of the system was reduced after stabilisation of the fluvial discharge, and tidal prism decrease due to successive landfills of intertidal flats. The fluvial and coastal heavy engineering works had major environmental impacts, namely the drastic reduction of the sedimentary inflow from the Mondego River to the littoral. This inverted the coastal progradation caused by man-driven increase of river sediment discharge during the last centuries (Ludwig and Probst 1998) and recorded in many estuaries of similar context in Iberia (e.g. Morales 1997, Dabrio et al. 2000). The intense harbour dredging induced the rapid trap of sandy sediments with marine and fluvial provenance. The interventions in the Pranto subsystem accelerated the silting, but in the Mondego subsystem the infilling trend resulting from energy decrease is not able to balance the fluvial sediment starvation and the removal of sediments.

Acknowledgements

The research was financially supported by the "Programa Específico para o Ambiente" of the JNICT/DGA (project PEAM/C/GEN/247/93), with logistic support of the Instituto do Mar. We thank German Flor (Univ. Oviedo), Juan Morales (Univ. Huelva) and J. Antunes do Carmo (Univ. Coimbra) for the fruitful discussions and an anonymous referee for improvements of an earlier version.

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DIVERSITY OF FRESHWATER ALGAE

Abstract

In this paper we present an historical review of the published information on freshwater algae collected within the Mondego Basin and report on the algae identified in a survey covering the Basin area. The reviewed publications concern predominantly taxonomy and go back to the beginnings of the 19th century. In the last thirty years, the possibility of combining taxonomic and ultrastructural studies led to the establishment of a culture collection of microalgae at the Department of Botany, University of Coimbra (ACOI). Only a few ecological studies have been conducted in the area, quite recently, concerning the structure and dynamics of the phytoplankton.

A survey of the phytoplankton composition in 21 sites located from the highland areas to the coast revealed the presence of 388 taxa, the great majority being diatoms (232 taxa). Green algae, euglenophytes and silica-scaled chrysophytes were other well represented groups, with 60, 48 and 35 taxa, respectively. The remaining algal groups were poorly expressed by this survey. Higher algal variety was found in the eutrofic waters of the lowland rice-field areas of the Basin when compared with the oligotrophic waters of Serra da Estrela. However, a remarkable flora develops in the small ponds of this area in spring, due to the occurrence of rare specimens of chrysophytes and desmids.

Introduction

Biodiversity has recently become the focus of international debates, but algae have been largely omitted and neglected even by institutions such as the United Nations (Norton et al. 1996). Among the reasons for this neglect are certainly their microscopic size, the time consuming methods for identification of some species (such as electron microscopy or molecular biology), a reduction in the number of algal taxonomists and a reduction in funds to support systematic research. These reasons act synergistically to hamper the research on algal biodiversity (Andersen 1992).

However, the algae include the dominant primary producers of our planet and the main producers of oxygen. Finally, algae include at least seven distinct phylogenetic lineages

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of organisms that arose independently during geological time. Therefore, the biodiversity of algae in terms of species and phylogenetic groups seems to be unusually high.

In Portugal several valuable contributions for the knowledge of the algal flora have been made since the 19th century, but the algal diversity is still very far from being properly estimated. A Portuguese algal flora simply does not exist, has not been published so far, and experienced taxonomists are a very few. Also, in order to develop ecological algal studies, a strong taxonomic basis is required. As a consequence, only a small number of studies have been carried out.

Despite the Mondego River importance as one of the major rivers in central Portugal and the largest entirely Portuguese River, little published information is available on the diversity and ecology of microalgae present within the drainage area. To investigate the information available on algae for this Basin is equivalent to investigate the evolution of the phycological studies at the Department of Botany, University of Coimbra. Most of that information has been produced by academics working at this Institution and is scattered throughout publications made over almost two centuries. Contributions written by academics or naturalists of other main Botanical Institutions (in Lisboa or Porto) were most often based on material provided by collectors or researchers from Coimbra.

The purposes of this paper are (1) to compile this scattered information on freshwater microalgae of the Mondego Basin; (2) to present data from collections made in the area recently.

Historical review

The 19th century contributions

To our knowledge, the first references to algae in Portugal go back to Brotero's work Flora Lusitana (Brotero 1804), where a small list of marine and freshwater species was published. Specifically for the Basin area it was reported *Chara vulgaris* (in Quinta das Lágrimas, Coimbra and surroundings) and two brown macroalgae (in Buarcos, Figueira). However, under the generic designations of *Byssus* and *Conferva*, Brotero reported three other "algae verae", collected from the surroundings of Coimbra and from the Mondego (Mundae) margins.

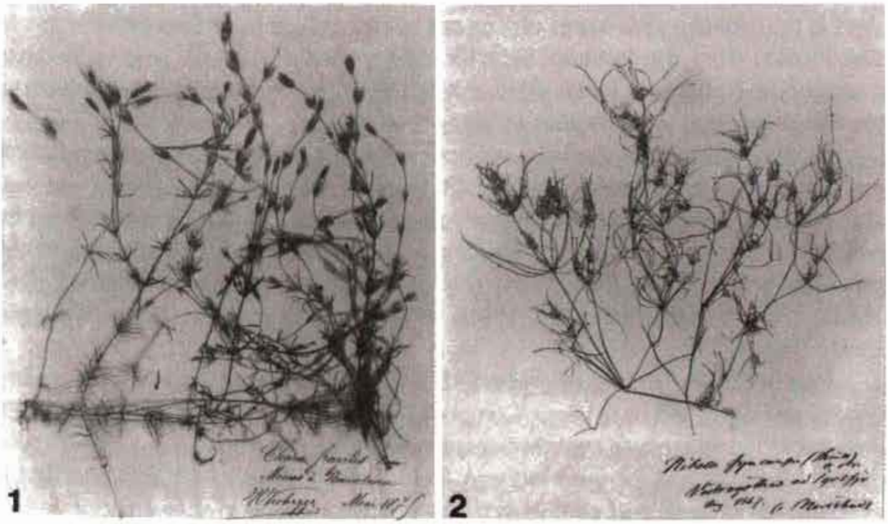
Brotero was one of the first directors of the Botanical Garden of Coimbra. A botanical society in his honour, Sociedade Broteriana, was founded at the end of that century (in 1880) by initiative of Júlio Henriques, director of the Institution at the time. The society still exists and produces three different publications, the most important in terms of scientific contributions being the Boletim da Sociedade Broteriana (Bol. Soc. Brot.), which first number goes back to 1880.

Within the scope of the society, an increase of phycological studies occurred at the turn of the century. The collection and interchange of botanical specimens (including algae) were promoted, with posterior identification by international experts such as Hauck, Kützing, Nordstedt (Santos and Mesquita 1981), several new taxa for science being described. Much of this material became part of the valuable Herbarium of Coimbra and is still available, although losses have occurred. Lists summarising some of these studies were published or translated by Júlio Henriques (Henriques 1881, 1889) and report a total of 69 freshwater taxa, mainly chlorophytes (about 37), but also diatoms, cyanophytes, xanthophytes and rhodophytes. Most of these algae were collected from tanks or moist soil in Coimbra or around by the renowned A. F. Moller, supervisor of the Garden at that time.

The first half 20th century

Several important contributions for the taxonomical knowledge of the Portuguese algal flora, mentioning material from the Mondego Basin, were made during this period. Diatoms were studied by Carvalho and Zimmermann, the first reporting about 118 taxa for Serra da Estrela (Carvalho 1913) and the second about 28, mostly from Coimbra (tanks, the Botanical Garden, the Mondego River and some of the irrigation channels) (Zimmermann 1906, 1909, 1910, 1914).

About twenty years later, new references appear in the literature. A survey of green flagellates (order Volvocales) was done in 18 sampling points in Coimbra and 16 species were found, 3 being described as new for science (Moewus 1935). A study of charophytes, based mainly on material from the Herbarium of Coimbra (Figs 1 and 2), but also on some collections made by the author (working at the University of Lisbon) lists a total of 15 taxa, including 5 varieties of *Chara vulgaris* for the central region of the Mondego Basin (Cunha 1934, 1935, 1942, 1943).



Figs 1, 2. Two specimens of the genera *Chara* (Fig. 1) and *Nitella* (Fig. 2) from the Coimbra Herbarium, with at least 130 years.

Desmids and cyanophytes were collected and studied in the North of Portugal for more than twenty years by Sampaio, a naturalist at the Instituto de Botânica (Porto). In his synopsis that includes about 200 known desmids for Portugal, he mentions only two species of *Closterium* and one of *Cosmarium* for the Mondego Basin, collected in the Botanical Garden of Coimbra (Sampaio 1943, 1944). These species had been recorded by the author for the garden (Sampaio 1923). His studies of cyanophytes mention a total of 24 taxa, the majority for Coimbra and a few for Serra da Estrela (Sampaio 1933, 1934, 1947a,b).

Other studies of green algae include a revision of literature on Zygnemataceae (Alte 1945) and a first survey of desmids in Serra da Estrela (Rozeira 1947). The first mentions 8 taxa of *Spirogyra*, all collected by A. F. Moller from Coimbra and surroundings; the second reports 15 taxa from ponds near Lagoa Comprida.

After an interruption of about forty years, a renewal of phycological enthusiasm from academics at the Department of Botany, Coimbra, started in the late forties and afterwards, with no further interruption till the present. A major contribution for the knowledge of the green algae, in particular Oedogoniaceae, was made by Lacerda (Lacerda 1946, 1949). The author reported 32 taxa belonging to that family (28 to the genus *Oedogonium* and 5 to the genus *Bulbochaete*) based on specimens collected mainly in Coimbra and near Figueira da Foz. He also reported a few taxa belonging to other algal groups (Lacerda 1945, 1948).

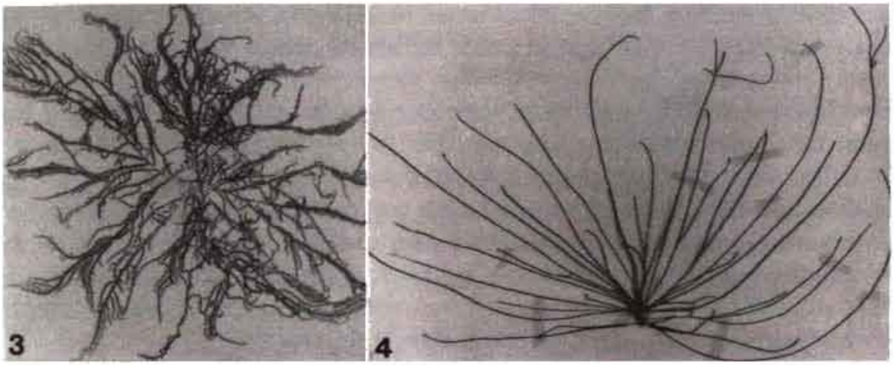
The second half 20th century

Taxonomical and ultrastructural studies

In this half century there was an increase in taxonomical studies of freshwater algae in the Mondego Basin area carried out by researchers from Coimbra and also by Vasconcellos from the Instituto Superior de Agronomia (Lisboa), who reported 6 macroscopic filamentous green algae and one rhodophyte infesting the rice fields of the Mondego Basin (Vasconcellos 1956).

In the 1950s and during a period of about thirty years, the freshwater rhodophytes were studied by the naturalist priest M. P. Reis, first at Coimbra and later at Aveiro. He described about 20 taxa new for science. For the Mondego Basin he reported a total of 26 taxa (not counting forms and varieties), 15 belonging to the genus *Batrachospermum* (Reis 1974) (Fig. 3) and 11 to the genera *Lemanea* (Reis 1961a) (Fig. 4), *Audouinella* (Reis 1961b, 1966), *Pseudochantransia* (Reis 1962) and *Thorea* (Reis 1957).

In the 1960s, Rodrigues reported on algae collected mainly in Coimbra (Rodrigues 1961) and near Lagoa Comprida (Serra da Estrela) (Rodrigues 1963) and Rino published four major contributions describing and illustrating more than 70 taxa new for Portugal and for the Mondego Basin (Rino 1967a,b, 1969; Rino and Santos 1968). Posterior published contributions from this author for the region are punctual (Rino and Pereira 1989), since he moved first to Mozambique, later to Aveiro and became engaged in the study of the algal flora of those regions. However his published



Figs 3, 4. Representatives of the genera *Batrachospermum* (Fig. 1) and *Lemanea* (Fig. 2), studied by M. P. Reis and kept at the Coimbra Herbarium.

and unpublished data and his knowledge on freshwater algae would certainly be enough for publication of a first Portuguese Flora of Freshwater Algae, a need that he mentioned in his first publication as an incentive to study algae.

Since the seventies, a similar taxonomic work on microalgae from the region has been continued at Coimbra by M. F. Santos. About 94 taxa were recorded as new for Portugal and the Basin in her six contributions (Santos 1970, 1971, 1973a,b, 1976; Santos and Morgadinho 1987). In order to have algal material to perform cytological studies, conditions were created at the Department for the establishment of algal cultures. The result was a collection of more than 900 (see below). Aspects of the cytology and ultrastructure of a few taxa collected from sites within the Basin have been studied and published (Mesquita and Santos 1976a,b, 1981) or presented at conferences (e.g. Santos et al. 1997; Osório et al. 1999). A survey of the algal flora in the Natural Park of Serra da Estrela was also prepared and waits publication. It lists a total of 574 taxa (125 new for the park), with illustrations of most.

Taxonomical studies of groups requiring the use of electron microscopy, such as the silica-scaled chrysophytes, were done more recently. The first published survey of these organisms in Portugal (and the Iberian Peninsula) reported 23 species from lakes, reservoirs and ponds of Central Portugal, 16 for the Basin area (Santos and Leedale 1993). Posterior studies (Calado and Craveiro 1995; Santos et al. 1996) added 11 new species for the region and included information on their ecology.

Phytoplankton

The first major attempted survey of phytoplankton in Portuguese waters is Nauwerck 's work (Nauwerck 1962). It refers to samples collected from lakes, rivers and reservoirs throughout the country; it provides lists and drawings of the algae found in those sites and information on the water physical and chemical parameters measured as well as biomass. Within the Mondego Basin, he sampled Lagoa Comprida, Covão do Vidoal, springs and moist stones near Lagoa Escura and the Mondego River at Coimbra (since we are revising the information on freshwater algae found within the Basin, we include here the algae found on moist stones by the author, although "moist stones do not give phytoplankton"). The same author had already done a previous shorter report on the phytoplankton found in spring at two sites near Coimbra (Nauwerck 1959).

To our knowledge, only thirty years later another study was published, dealing with the dynamics of cyanophyte blooms in the Aguieira reservoir (Oliveira and Monteiro 1992). The blooms were followed during four months, in summer 1992, to evaluate their effects on water treatment processes, drinking water quality and zooplankton community. It was found that, under such cyanophyte biomass, used water treatment processes could not remove algae from drinking water, including some toxic species. It was also found that changes in dominant zooplanktonic species were related to the type of cyanophyte species responsible for the bloom.

More information on diversity, density and toxicity of cyanophytes in the Mondego reservoirs, Aguieira in particular, is given by Vasconcelos in his contribution for this book.

A study of silica-scaled chrysophytes from three shallow water bodies rich in organic matter, in the lowland region of the Basin, reported on the eutrophic taxa found and suggested the use of the saprobity system to a better delimitation of the ecological characteristics of eutrophic taxa (Santos et al. 1996).

The most comprehensive available study on composition and dynamics of the phytoplankton in the Mondego Basin has been done in the Coimbra reservoir (Craveiro and Santos 1997, 1998). The phytoplankton assemblages were studied during one year and compared to those present in the Mondego and Ceira rivers, before their confluence. Several physical and chemical parameters of the water were also measured. It was found that the phytoplankton community in this small reservoir was mainly conditioned by the flow. Only in late spring and summer, when the retention times increased to two or tree days and the mean values of flow became lower than 20 $m^3.s^{-1}$, a phytoplanktonic community different from the one entering with the tributaries could develop in the reservoir. During most of the year, the diatoms were the dominant group, but diversity increased in summer, a maximum value of biomass being due to *Aulacoseira ambigua, Peridinium umbonatum* and some Cryptophyceae.

The ACOI culture collection

The Culture Collection of Algae at the Department of Botany, University of Coimbra (ACOI) (http://www.uc.pt/botanica/ACOI.htm) was built in 1972 for institutional research purposes. The first list of strains included 167 taxa, mostly isolated from central Portugal (Santos and Mesquita 1986). Two posterior supplements added 88 taxa (Santos 1988) and 194 (Santos et al. 1993) to the collection. The present list is available on the web and includes more than nine hundred cultures, although the total number of strains in culture is about 4 thousand. They are mostly freshwater microalgae isolated from field samples collected across the country by many different persons. The ACOI collection provides experimental organisms for research and didactic purposes within the Department and other national and international Institutions.

A rather tiring and time-consuming process is needed to establish these cultures. Aquatic, free-living organisms are collected with a 20-30 µm mesh phytoplankton net whereas algae attached to aquatic plants, stones or other substrates are removed with the help of a knife or a spoon. The samples must be kept alive, at low temperatures, until they reach the laboratory.

Isolation is performed by pipetting the cells directly from the field sample to a tube, a flask or a Petri dish with culture medium, under an inverted microscope. Some taxa, due to the small dimension or the low number of cells present in the field sample, may require to develop first on a Petri dish with solidified culture medium (streak cultures) (Venkataraman 1969). The isolation procedure usually has to be repeated several times, before a unialgal culture is established. Identification of the culture to species level (or lower) always requires the expertise of an experienced algal taxonomist.

Since the establishment of the ACOI, many types of culture media recipes, previously developed and published by authorities on this subject, were tried, modified or mixed in order to suit the algal growth requirements. Also, physical space and staff limitations to maintain the increasing number of cultures made it necessary to reduce the culture media to the fewer possible number. A total of only six different media (see the web site for recipes), are used to grow the nine hundred identified species and the other thousands waiting for identification to species level. Therefore, the cultures are maintained on media that are not necessarily the best for their growth.

Qualitative phytoplankton survey

Since the first reports on phytoplankton of Portuguese waters (Frade 1951; Nauwerck 1962), several studies were made throughout the country (e.g. Oliveira 1982, 1984, 1987; Coutinho 1990; Vasconcelos 1990), but only a very few included reservoirs or sites in the Mondego River or Basin, as mentioned.

Here we report on a recent qualitative survey aimed to assess the diversity of the freshwater microalgae in the Mondego Basin. We also provide taxonomical lists and illustrations of some taxa.

Methods

Phytoplankton samples were collected with a 20-30 µm mesh net, in a total of 21 sites within the Mondego Basin. Eighteen were sampled once in April or June 1996, a few of these were sampled again every two months from March 1997 to March 1998 and the remaining three were sampled once or twice during that period. The sampling sites include ponds and reservoirs in Serra da Estrela Natural Park, main river and tributaries in the central area of the Basin and ponds, marshes and irrigation channels in the lowland region (Fig. 5).

In the field, each sample was divided in two replicates: one was kept in ice for posterior observation and isolation purposes, the other was immediately fixed with 5% formalin. Some physical and chemical water parameters (such as pH, conductivity, dissolved oxygen, ammonia, nitrates, nitrites, orthophosphates, silica and calcium) were determined, either in the field using WTW portable meters or in the laboratory by

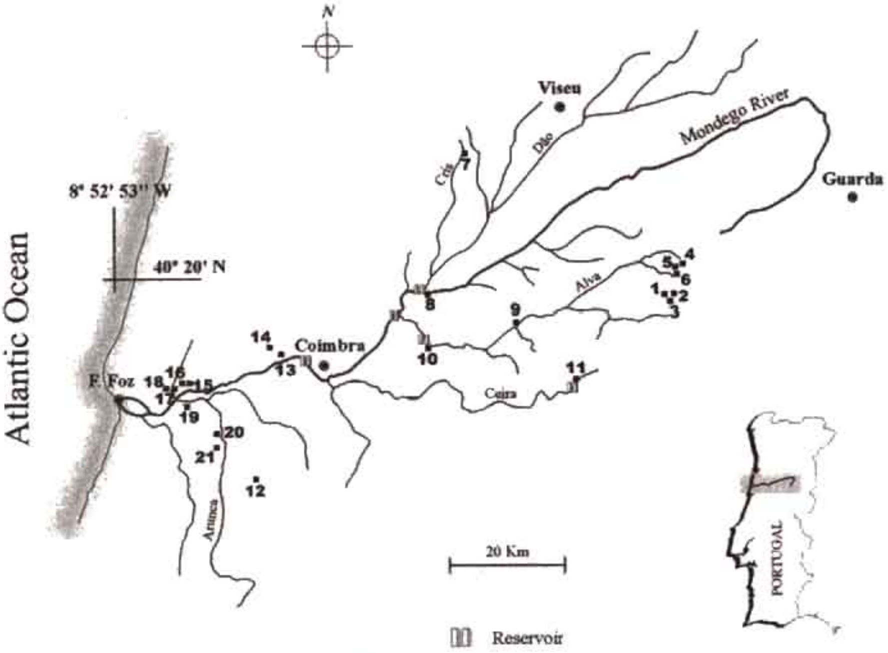


Fig. 5. Location of the sampling sites within the Mondego Basin. Serra da Estrela Natural Park: 1, Covão do Curral; 2, Pond near Lagoa Comprida; 3, Lagoa Comprida; 4, Vale do Rossim Reservoir; 5, Erva da Fome Reservoir; 6, Lagoacho Reservoir. Mondego River and tributaries: 7, Cris River, near Tondela; 8, Aguieira Reservoir; 9, Alva River; 10, Fronhas Reservoir; 11, Alto Ceira Reservoir. Lowland areas: 12, Pond near Sr^{a} da Estrela; 13, Poço dos Albinos; 14, Ribeira de Ançã; 15, Marsh near Montemor; 16, Irrigation channel near Montemor; 17, Pond near S.^{ta} Olaia; 18, Irrigation channel near S.^{ta} Olaia; 19, Pond near Marujal; 20, Irrigation channel near Soure; 21, Ribeira near Casal da Rola, Soure.

standard methods, but this information will be presented elsewhere, since it is not within the scope of this work.

The samples were generally examined with a Leitz – Biomed or Leica DMRB light microscope. Isolation and culture were performed as previously described (see the ACOI section), from samples collected in 1996 and 1997/1998. The whole phytoplankton was studied in eleven samples out of the eighteen collected in 1996 (sites 1 – 4, 6, 7, 10, 11, 15, 16 and 20). To study the diatoms, subsamples were oxidised with concentrated nitric acid for one hour, then washed in distilled water and mounted with Naphrax resin for preparation of permanent slides.

To identify the silica-scaled chrysophytes (or particular details of other algae), the samples collected in 1996 were examined by transmission or scanning electron microscopy. For transmission (TEM), one small drop of material was placed on formvar and carbon coated grids, air-dried and directly examined with a Siemens Elmiskop 101. For scanning electron microscopy (SEM), a larger drop was dried onto aluminium foil glued to a stub, sputter – coated with gold for five minutes and observed with a Jeol JSM 5400.

Results and discussion

The qualitative examination of the samples allowed the identification of 387 taxa (see Tables 1 and 2, Appendix 1), 231 being diatoms (class Bacillariophyceae) (Table 1).

Forty diatom genera were found, *Navicula* and *Nitzschia* being the most represented, followed by *Eunotia, Fragilaria* and *Cymbella*. The majority of the species are considered of broad geographical distribution and occurrence, either in Portugal or in central Europe, such as *Achnanthes minutissima, Eunotia minor, Frustulia rhomboides, Navicula angusta*. They usually occur in oxygenated waters, with a pH slightly acidic to moderate and relatively poor in nutrients (nitrates, nitrites and orthophosphates). Taxa that markedly prefer less oxygenated waters but higher nutrient concentrations, such as *Cyclotella meneghiniana, Melosira varians, Navicula phyllepta* and *Nitzschia acula*, occurred and were dominant in lentic waters of the irrigation channels, marshes and ponds. Rich and diverse communities were found in lowland water systems such as sites 15 and 16 when compared with sites at higher altitude, such as 3 and 4 (Serra da Estrela Natural Park) (see Table 3, Appendix 1). Low silica concentrations in these sites (less than 1.5 $mg.L^{-1}$) may explain the reduced number of taxa registered. We should bear in mind, however, that many of the diatom taxa listed in Table 1 are not phytoplanktonic organisms, only temporarily being found in suspension.

A hundred and fifty-six taxa, belonging to other algal groups, were identified during this general phytoplankton survey in the Mondego Basin (Table 2, Appendix 1). The list is particularly rich in Euglenophyta of the genera *Trachelomonas* and *Phacus* and in Chlorophyta, especially Chlorococcales and Desmidiaceae. The presence of these euglenophytes is worth mentioning; they usually appear in lentic waters during spring, summer and autumn, in particular when there is an enrichment in organic matter, the conductivity is medium to high (from 250 to 1900 $\mu S.cm^{-1}$), the pH around 6.0 to 7.5, and several inorganic ions are present (Pereira 1999). These conditions therefore existed at several of the sampled water bodies, mainly in the lowland areas. The occurrence of *Trachelomonas* further indicates significant levels of iron, because this element is often found in the mineralised lorica of such organisms (Rino and Pereira 1991, Pereira 1999).

Fifty-two species were isolated into culture, mainly from the samples collected in Serra da Estrela, and are now part of the ACOI collection (Table 2, marked with an asterisk). The great majority belongs to the green algal genera *Staurastrum, Staurodesmus, Euastrum* and *Closterium*. Representatives of the other groups, although frequent and sometimes abundant in the sampled areas, are not well represented in the culture collection, either because they are quite common and therefore not selected for isolation or because they do not survive in culture or need special conditions and constant surveillance to keep alive.

A total of 35 species of silica-scaled chrysophytes was registered in this study (Table 2). The most frequent were *Paraphysomonas vestita, Mallomonas acaroides, M. punctifera, M. tonsurata, Synura curtispina* and *S. petersenii*, all quite common in our freshwaters and other parts of the world. Organisms rare from other surveys, such as *Mallomonas calceolus* (Calado and Craveiro 1995), *M. retifera* (Santos and Vicente

1999) and *Spiniferomonas breaknecki* (Santos and Leedale 1993), were found in Serra da Estrela. Reported here for the first time for Portugal is *Paraphysomonas capreolata*; it was found in site 18, an irrigation channel of eutrophic water, with a conductivity of about 500 $\mu S.cm^{-1}$ and a pH of 6.5. Four other taxa are new for the Basin area. The present survey and others conducted in the region (published or unpublished) show the presence of a rich flora of silica-scaled organisms, particularly in the marshes, irrigation channels and small ponds of the rice-field area. Forty-two species, mainly belonging to the genus *Mallomonas*, are now known for the Mondego Basin from a total of 53 reported for Portugal.

Final remarks

We may say, in conclusion, that a very rich, diverse and interesting flora of freshwater algae is already known for the Mondego Basin but that it still waits to be revealed to its full splendour. Illustrations of some taxa, that may give an idea of this richness, are compiled on eight plates (see Appendix 2). Higher variety can be found in the marshes, irrigation channels, small ponds and lagoons of the lowland rice-field areas. On the other end, in highland water bodies, an attractive flora usually develops in spring and some ponds are worth to preserve, due to the occurrence of quite rare algal species.

A few groups, such as the rhodophytes, some greens and the silica-scaled chrysophytes have deserved particular attention from phycologists and have been reasonably searched for and taxonomically investigated in the surroundings of Coimbra, the rice-field area and easily accessible water bodies in Serra da Estrela. The present survey gives a good insight on diatoms (more than 200 taxa) and euglenophytes (about 50). Further estimation of the diversity of these groups is still required and less explored areas should be investigated.

Other groups in particular the cyanophytes and the yellow-green representatives, need special attention in future surveys. It is of great importance and public health service to improve our taxonomic knowledge on cyanophytes, since this is indispensable to quickly determine the presence of potentially toxic species in water supply systems and to take adequate measures to prevent the formation of toxic blooms.

Further progresses in the study of freshwater algae require the urgent publication of a Portuguese Algal Flora that integrates existing disperse information on taxonomy (descriptions and iconography), distribution, ecology and other relevant data. It is essential that easily reading and consulting guides become available to teachers and students, as well as to common people, simply interested in the preservation of Life and Nature; these guides would help them to program field trips, to collect and identify the most frequent species and to apprehend the otherwise inconceivable biodiversity of these freshwater microscopic organisms.

It is recognised that algae have the capacity to integrate and reflect the environmental conditions they are submitted to. This is particularly true for diatoms, since more studies with this group have been performed. The evaluation of water quality in rivers, lakes and other water systems, based on the profound knowledge of the species auto-ecology is today a current practice, world-wide applied (see Descy and Coste 1991). This survey allowed us to recognise exclusively from a taxonomical point of view the algal flora dominant in the Basin; however, the extension and patrimonial value of this region would certainly deserve and require a more detailed investigation in the future. It is essential to characterise the auto-ecology of the dominant species, to recognise the diversity of their environmental requirements, in order to be able to use that information for water quality evaluation and monitoring purposes. That knowledge is essential to apply and improve the existing biological indices to Portuguese waters, namely the diatomological indices (Descy and Coste 1991; Coste 1992) and to compare results with other studied areas (see Gil et al. 1989; Almeida 1998; Pereira 1999).

The isolation of algal strains into culture is one way of preserving their genetic potential for the future, now that the aquatic habitats are massively destroyed and their biodiversity lost. Furthermore, in culture they are more easily accessible for use and exchange as experimental organisms, either by academics or for commercial purposes. These reasons justify future work aiming the conservation and expansion of the ACOI collection and the improvement of its available database.

Many similar remarks and topics of needed research on freshwater algae in the Mondego Basin area or the whole country could be given. As a final remark, we may say that collaboration among institutions and people dealing with algae is fundamental for further phycological progresses.

Acknowledgements

Financial support for this study was provided in part by the Foundation for Science and Technology (FCT) (Project n° PBIC/C/BIA/2051/95). Permission to reproduce some drawings was given by the Sociedade Broteriana and/or by the authors. We thank Margarida Vicente for her assistance in sample collection and water analyses, José Dias for his help during scanning electron microscopy of samples, Carlos Morato, Arlindo Cardoso, Graça Carvalho and Mário Morais for their contributions during bibliographic survey, manuscript writing or micrograph preparation. We acknowledge the constructive review of the manuscript provided by Manuel Graça and Jorge Rino.

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APPENDIX 1

Table 1. List of diatoms found in the phytoplankton of the Mondego Basin freshwaters, during a 1996 survey.

Taxa	Sites
Achnanthes chlidanos Hohn & Hellermann	1,2, 7,15
A. *coartacta* (Brébisson) Grunow	4,7,11,15
A. *exigua* Grunow	1,15
A. *hungarica* (Grunow) Grunow	7, 15,16
A. *inflata* (Kützing) Grunow	15,16
A. *lanceolata* (Brébisson) Grunow	7,10,11, 15, 16, 20
A. *lanceolata* ssp. *dubia* (Grunow) Lange-Bertalot	7
A. lanceolata ssp. *rostrata* (Oestrup) Hustedt	7,10
A. *lapidosa* Krasske	7,10,11
A. *minutissima* Kützing	1,3,4, 7,10, 11, 15,16, 20
A. *minutissima* var. *affinis* (Grunow) Lange-Bertalot	4,15
A. *oblongella* Öestrup	7,11, 15,16
A. *subatomoides* (Hustedt) Lange-Bertalot	4, 7,10,15
Amphipleura pellucida (Kützing) Kützing	15,16, 20
Amphora fogediana Krammer	20
A. *libyca* Ehrenberg	4, 11,15
A. *montana* Krasske	20
A. *ovalis* (Kützing) Kützing	7,16, 20
A. *pediculus* (Kützing) Grunow	1, 7, 15, 16, 20
A. *veneta* Kützing	15,16
Anomoeoneis brachysira (Brébisson) Grunow	1,2,3,15
A. *sphaerophora* (Ehr.) Pfitzer	15
A. *sphaerophora* var. *sculpta* Ö Muller	6,15,16
A. *vitrea* (Grunow) Ross	1,15
Asterionella formosa Hassall	1, 10,15
Aulacoseira distans (Ehrenberg) Simonsen	1,15
A. *granulata* (Ehrenberg) Simonsen	7, 10, 15,1 6
A. *granulata* var. *angustissima* (O. Müller) Simonsen	10.15
A. *muzzanensis* (Meister) Krammer	3,15
A. *perglabra* (Oestrup) Haworth	3,15
A. *tethera* Haworth	1,3,6,15
Bacillaria paradoxa Gmelin	15.16, 20
Caloneis silicula (Her.) Cleve	20
Ceratoneis arcus (Ehrenberg) Cleve	1,15
Cocconeis neodiminuta Krammer	20
C. *placentula* Ehrenberg	1, 7,10,11, 15,16
C. *placentula* var. *euglypta* Ehrenberg	11
C. *placentula* var. *lineata* (Ehr.) Van Heurck	15,16
Cyclotella atomus Hustedt	6,15
C. *meneghiniana* Kützing	6, 7,10,15,
C. *Ocellata* Pantocsek	10
C. *pseudostelligera* Hustedt	10,11
C. *stelligera* Cleve & Grunow	10
Cymatopleura solea (Brébisson) W. Smith	15,20
Cymbella affinis Kützing	16,20
C. *aspera* (Ehr.) Cleve	7,10,16, 20
C. *caespitosa* (Kützing) Brun	16
C. *cistula* (Ehr.) Cleve	20
C. *cymbiformis* Agardh	16,20
C. *elginensis* Krammer	20

C. *gracilis* (Ehr.) Kützing	1,11
C. *helvetica* Kützing	16
C. *lata* Grunow	11
C. *mesiana* Cholnoky	7,16
C. *microcephala* Grunow	16
C. *minuta* Hilse ex Rabenhorst	7,10,11
C. *naviculiformis* Auerswald	1,7
C. *perpusilla* Cleve-Euler	1
C. *tumida* (Brébisson) Van Heurck	1,16,20
Denticula tenuis Kützing	16
Diatoma mesodon (Ehrenberg) Kützing	4,7,11
D. *vulgaris* Bory	11
Diploneis interrupta (Kützing) Cleve	15
D. *puella* (Schumann) Cleve	20
Epithemia adnata (Kützing) Brébisson	15,16
E. *sorex* Kützing	15
E. *turgida* var. *granulata* (Ehr.) Brun	15,16
Eunotia alpina (Naegeli) Hustedt	1,2,3,
E. *bilunaris* (Ehrenberg) Mills	1,2,4,6, 7,10,11,16,20
E. *bilunaris* var. *lineans* (Okuno) Lange-Bertalot	11,16
E. *diodon* Ehrenberg	2
E. *exigua* (Brébisson) Rabenhorst	1,10
E. *formica* Ehrenberg	7,11
E. *incisa* Gregory	1,2
E. *implicata* Nörpel &Lange-Bertalot	1,11
E. *minor* (Kützing) Grunow	6,7,15, 20
E. *paludosa* Grunow	2,10
E. *parallela* Ehrenberg	2
E. *pectinalis* (Dillwyn) Rabenhorst	1,3, 7,16, 20
E. *serra* Ehrenberg	1,2
E. *soleirolii* (Kützing) Rabenhorst	7, 11,16
E. *submonodon* Hustedt	11
E. *tenella* (Grunow) Hustedt	1,2,3,4,6,7
E. *veneris* (Kützing) De Toni	15
Fragilaria capucina Desmazières	4,6,7,10,11
F. *capucina* var. *gracilis* (Oestrup) Hustedt	10,11
F. *capucina* var. *vaucheriae* (Kützing) Lange-Bertalot	7,11
F. *construens* (Ehr.) Grunow	11
F. *construens* f. *subsalina* (Hustedt) Hustedt	1
F. *construens* var. *venter* (Ehr.) Hustedt	1
F. *crotonensis* Kitton	6,7,10,11,20
F. *dilatata* (Brébisson) Lange-Bertalot	16
F. *fasciculata* (Agardh) Lange-Bertalot	1
F. *parasitica* var. *subconstricta* Grunow	1
F. *virescens* Ralfs	7,11
Frustulia rhomboides (Ehr.) De Toni	1,2,6,7
F. *rhomboides* var. *crassinervia* (Brébisson) Ross	2,11
F. *rhomboides* var. *saxonica* (Rabenhorst) De Toni	1,2,11
F. *vulgaris* (Thwaites) De Toni	7,11,16
Gomphoneis clevei (Fricke) Gil	3,4,7,10,11
Gomphonema acuminatum Ehrenberg	1,3, 7,10,11,15, 16, 20
G. *acutiusculum* (O. Müller) Cleve-Euler	7
G. *affine* Kützing	10
G. *angustum* Agardh	4, 7,16
G. *anjae* Lange-Bertalot & Reichardt	11

G. augur Ehrenberg	20
G. clavatum Ehrenberg	20
G. gracile Ehrenberg	1, 10,11,16, 20
G. minutum (Agardh) Agardh	16
G. parvulum (Kützing) Kützing	1,4,6, 7.10.11,15
G. parvulum var. *parvulius* Lange-Bertalot & Reichardt	1,10
G. pseudoaugur Lange-Bertalot	6
G. truncatum Ehrenberg	7,10,11.15.16
Gyrosigma acuminatum (Kützing) Rabenhorst	6, 15,16
G. attenuatum (Kützing) Rabenhorst	16,20
G. macrum W. Smith	6, 15,16
G. scalproides (Rabenhorst) Cleve	20
Hantzschia amphyoxis (Ehrenberg) Grunow	7,10,11
Melosira varians Agardh	1,7,10,11,15,16,20
M. lineata Agardh	20
Meridion circulare var. *constrictum* (Ralfs) Van Heurck	4,7,11
Navicula angusta Grunow	1,7,11
N. aquaedurae Lange-Bertalot	11
N. bryophila Petersen	7
N. capitata Ehrenberg	16
N. capitata var. *hungarica* (Grunow) Ross	16
N. cincta (Ehr.) Ralfs	7,15,16
N. clementis Grunow	7
N. cohnii (Hilse) Lange-Bertalot	7
N. *contenta* Grunow	7,10
N. cryptocephala Kützing	6,7,10,11,20
N *cryptotenella* Lange-Bertalot	3
N *cuspidatu* (Kützing) Kützing	15,16, 20
N. cuspidata var. *ambigua* (Ehr.) Cleve	20
N *decussis* Östrup	7
N *elginensis* (Gregory) Ralfs	7,16, 20
N. exilis kützing	1
N. gregaria Donkin	7,10
N. halophila (Grunow) Cleve	20
N. lanceolata (Agardh) Ehrenberg	7
N. leptostriata Jörgensen	16
N. lundii Reichardt	11
N. margalithii Lange-Bertalot	16
N. mutica Kützing	10,20
N. oblonga Grunow	16,20
N. phyllepta Kützing	11
N *pupula* Kützing	1,4.6,7.11. 15,16
N *pusilla* W.Smith	7
N *pygmaea* Kützing	15
N *radiosa* Kützing	7,11,16.20
N. rhynchocephala Kützing	1,10
N. salinarum Grunow	16,20
N. seminulum Grunow	4,7
N. submolesta Hustedt	6
N. trivialis Lange-Bertalot	16.20
N. variostriata Krasske	16
N. veneta Kützing	6. 7,15.16. 20
N. viridula (Kützing) Ehrenberg	6.16,20
N. viridula var. *rostellata* (Kützing) Cleve	6
Neidium affine (Ehr.) Pfitzer	1.7

N. ampliatum (Ehrenberg) Krammer	7
N. dilatatum (Ehr.) Cleve	3
N. indis (Ehr.) Cleve	1,11
Nitzschia acula Hantzsch	20
N. amphibia Grunow	1,16
N. bacillum Hustedt	10
N. brevissima Hustedt	10
N. capitellata Hustedt	15
N. commutata Grunow	20
N. commutatoides Lange-Bertalot	20
N. constricta (Kützing) Ralfs	7,16,20
N. dissipata (Kützing) Grunow	1,7,11
N. dubia var. *latestriata* Öestrup	7
N. filiformis var. *conferta* (Richert) Lange-Bertalot	15.16
N. fruticosa Hustedt	10
N. graciliformis Lange-Bertalot & Simonsen	4,15
N. gracilis Hantzsch	11.15
N. granulata Grunow	16
N. hungarica Grunow	16
N. levidensis (W. Smith) Grunow	15.20
N. linearis (Agardh) W. Smith	7. 15.16.20
N. lorenziana Gregory	20
N. macilenta Gregory	20
N. nana Grunow	10.20
N. navicularis (Brébisson) Grunow	16
N. obtusa W. Smith	16
N. palea (Kützing) W. Smith	4.7.20
N. palea var. *debilis* (Kützing) Grunow	4.6
N. paleacea Grunow	4.6.7.10.11
N. perminuta (Grunow) M. Peragallo	10,11
N. recta Hantzsch	7.10
N. sigma (Kützing) W. Smith	15,16
N. sigmoidea (Nitzsch) W. Smith	16,20
N. thermalis (Kützing) Auerswald	16
N. tryblionella Hantzsch	15,16, 20
N. tryblionella var. *levidensis* (W. Smith) Grunow	15
N. vitrea Norman	4
Pinnularia borealis Ehrenberg	2,4.7,10,11
P. braunii (Grunow) Cleve	1.7
P. brebissonii (Kützing) Rabenhorst	7
P. divergens W. Smith	11
P. gibba Ehrenberg	2. 7.10.11.16,20
P. interrupta W. Smith	2.7
P. microstauron (Ehr.) Cleve	3,20
P. subcapitata Gregory	1,2.4.6.7.10.11
P. sudetica (Hilse) Peragallo	1,2.6
P. tabellaria Ehrenberg	11
P. viridis (Nitzsch) Ehrenberg	1.2.3.6.7.11.16.20
Rhoicosphaenia abbreviata (C. Agardh) Lange-Bertalot	1.16
Rhopalodia gibba (Ehr.) O. Müller	15,16
Stauroneis anceps Ehrenberg	1.10.11.16
S. anceps var. *gracilis* (Ehrenberg) Brun	1. 7.11.16
S. phoenicenteron (Nitzsch) Ehrenberg	7.16,20
S. smithii Grunow	20
Stenopterobia curvula (W. Smith) Krammer	16

S. delicatissima (Lewis) Brébisson	2, 11,16
Stephanodiscus minutulus (Kützing) Cleve & Möller	1
S. parvus Stoermer & Hakansson	10,15
Surirella angusta Kützing	7,10
S. brebissoni Krammer & Lange-Bertalot	20
S. linearis W. Smith	1,2,3,4,7,11,16,20
S. linearis var. *helvetica* (Brun) Meister	16
S. ovalis Brébisson	16,20
S. roba Leclercq	6,16
S. tenera Gregory	4,7,11,16
Synedra ulna (Nitzsch) Ehrenberg	1,4,7,10,11,16,15, 20
S. ulna var. *acus* (Kützing) Lange-Bertalot	4
S. ulna var. *biceps* (Kützing) Kirchner	11,16,20
S. ulna var. *oxyrhynchus* (Kützing) Van Heurck	11
Tabellaria fenestrata (Lyngbye) Kützing	6,10,11
T flocculosa (Roth) Kützing	1,2,6,7,10,11

Table 2. General list of freshwater phytoplanktonic algae found in the Mondego Basin (diatoms excluded). The taxa marked with an asterisk were isolated into culture and are maintained at the ACOI culture collection. The collecting year is indicated for the ACOI taxa, after each site, all the others referring to 1996 samples.

Taxa	Sites/Year
CYANOPHYTA	
**Anabaena flos-aquae* (Lyngb.) Bréb. fa. Lemmermanii (P. Richter) *Canabaeus*	*17/97
**Chroococcus turgidus* (Kütz.) Nägeli	*2/96
**Scopulonema fluviatilis* Lagerheim	*14/96
EUGLENOPHYTA	
Colacium cyclopicola (Gicklh.) Bourr.	15,16
Euglena acus Ehr.	6,15,16,20
**E. deses* Ehrenberg	*21/96
E. fusca Schmitz	15,16
**E. gaumei* Allorge et Lefèvre	*17/96
E. oxyuris Schmarda	12,15,16,20
E. spathirhyncha Skuja	15,16
**E. spirogyra* Ehrenberg var. *fusca* Klebs	*17/96
E. subehrenbergii Skuja	15
Phacus aenigmaticus Drez.	15,16
P. caudatus Swir.	15
P. curvicauda Hübn.	15,16,20
P. gigas Da Cunha	15
P. helicoides Pochm.	16
P. longicauda (Ehr.) Duj.	15,16,20
P. oscillans Klebs.	20
P. pleuronectes (O. F. M.) Duj.	15,20
P. pyrum (Ehr.) Stein	15
P. raciborskii Drez.	16
P. tortus (Lemm.) Skv.	15,16,20
P. triqueter (Ehr.) Duj.	15,16,20
Lepocinclis fusiformis (Carter) Lemm. emend. Conr.	15
**L. ovum* (Ehrenb.) Lemmermann	*17/96
L. ovum (Ehr.) Lemm. var. *dimidio-minor* Defl.	20
L. ovum (Ehr.) Lemm. var. *ovum*	15,16
L. salina Fritsch	15,16,20
Strombomonas fluviatilis (Lemm.) Defl.	15
S. urceolata (Stokes) Defl.	15
S. verrucosa (Daday) Defl. var. *zmiewika* (Swir.) Defl.	15
Trachelomonas abrupta Swir. emend. Defl. var. *minor* Defl.	15
T. acanthostoma Stokes emend. Defl. var. *acanthostoma*	12
T. acanthostoma Stokes emend. Defl. var. *europaea* Drez.	12,15,20
T. armata (Ehr.) Stein var. *indica* Skv. fa.	16,20
T. cervicula Stokes	16
T. curta Da Cunha emend. Defl.	16,20
T. hispida (Perty) Stein emend. Defl. var. *hispida*	16
T. lefevrei Defl.	12,15
T. nigra Swir.	15
T. oblonga Lemm. var. *oblonga*	12, 15,16
T. planctonica Swir. var. *planctonica*	15,16,20
T. robusta Swir. emend. Defl.	15,16
T. stokesiana Palmer	12

T. stokesii Drez. emend. Defl.	16
T. superba Swir. emend. Defl. var. *spinosa* Prescott	12
T. varians Defl.	20
T. volvocina Ehr.	15
T. volvocinopsis Swir.	15,16
T. woycickii Koczwara var. *pusilla* Drez. fa.	15,16
CHROMOPHYTA	
Silica-scaled chrysophytes	
Chrysosphaerella brevispina Korshikov emend. Harris & Bradley	4
Paraphysomonas capreolata Preisig & Hibberd	18
P. imperforata Lucas	16,17
P. vestita (Stokes) de Saedeleer	15,16. 17,18,20
Spiniferomonas bilacunosa Takahashi	10
S. bourrellyi Takahashi	18
S. breaknecki Siver	4
S. trioralis Takahashi	15,18
Mallomonas acaroides Perty emend. Ivanov	4,15,16,17,18, 21
M. akrokomos Ruttner in Pascher	15
M. annulata (Bradley) Harris	4,15,16
M. calceolus Bradley	4
M. caudata Ivanov	18,20
M. crassisquama (Asmund) Fott	4,15
M. cratis Harris & Bradley	15
M. elongata Reverdin	20
M. intermedia Kisselev emend. Péterfi & Momeu	15,16
M. lelymene Harris & Bradley	9
M. mangofera Harris & Bradley	15,16
M. multiunca Asmund	15,16
M. oviformis Nygaard	16
M. papillosa Harris & Bradley	2,18
M. paxillata (Bradley) Péterfi & Momeu	11
M. peronoides (Harris) Momeu & Péterfi	18
M. portae-ferreae Péterfi & Asmund	15,16
M. punctifera Korshikov	11,15,16,18
M. rasilis Dürrschmidt	15
M. retifera Dürrschmidt	4
M. striata Asmund	20
M. tonsurata Teiling emend. Krieger	11,15,16, 18,17,20
Synura curtispina (Petersen & Hansen) Asmund	15,16,18,17,20
S. echinulata Korshikov	4
S. petersenii Korshikov	4,11,15,16,17,18,19,20,21
S. spinosa Korshikov	11
S. uvella Stein emend. Korshikov	15,16,17
Other groups	
Ceratium hirundinella (O. F. M.) Schrank	2
**Chlorobotrys regularis* (W. West) Bohlin	*2/97
Chrysococcus biporus Skuja	15
Dynobryon bavaricum Imhof	16
D. divergens Imhof	16,20
D. sertularia Ehr.	1,2,3,4,6,16, 20
**Ophiocytium parvulum* (Perty) A. Braun	*18/96
Peridinium cinctum (O. F. M.) Ehr.	16
**Tetraedriella regularis* (Kütz.) Fott	*18/96

CHLOROPHYTA	
*_Arthrodesmus. impar_ (Jacobs.) Grönblad	*2/97
*_Asterococcus superbus_ (Cienk.) Scherffel	*2/96
*_Chlorosphaeropsis alveolatum_ Herndon	*4/97
*_Closterium acerosum_ (Schrank) Ehrenberg	*14/96
*_C. acutum_ Bréb. var. _variabile_ (Lemm.) Krieger	*14/96
*_C. closterioides_ (Ralfs) Louis et Peeters var. _intermedium_ (Roy et Biss.) Ruzicka	*4/97
*_C. ehrenbergii_ Meneghini	*14/96
C. kuetzingii de Bréb. var. _kuetzingii_	1
C. moniliferum (Bory) Ehr. var. _moniliferum_	20
Coelastrum microporum Näg. in A. Braun var. _microporum_	10
Cosmarium botrytis Menegh.	12
C. margaritatum (Lund.) Roy & Biss.	4
C. umbilicatum Lütkem.	2
*_Desmidium baileyi_ (Ralfs) Nordstedt	*13/96
*_D. swartzii_ Agardh	*18/97
Dictyosphaerium pulchellum Wood var. _pulchellum_	2
*_Dispora speciosa_ Korchikoff	*2/97
*_Elakatothrix gelatinosa_ Wille	*2/96
*_Euastrum affine_ Ralfs	*2/96
*_E. ansatum_ Ehrenberg ex Ralfs	*2/96
*_E. bidentatum_ Näg. var. _speciosum_ (Boldt) Schmidle	*2/97
*_E. binale_ (Turp.) Ehrenberg var. _gutwinskii_ (Schm.) Homfeld	*2/96
*_E. humerosum_ Ralfs	*2/96
*_Gloeotila spiralis_ Chodat	*17/97
*_Gonatozygon brebissonii_ De Bary	*5/96
*_G. pilosum_ Wolle	*4/97
*_Gongrosira leptotricha_ Reineri	*14/96
*_Hyalotheca mucosa_ (Dillw.) Ehrenberg	*2/96
*_Micrasterias truncata_ (Corda) Brébisson	*2/96
*_Microspora quadrata_ Hazen	*14/96
Monoraphidium contortum (Thur.) Kom.-Legn.	15
Pandorina morum (Müller) Bory	12
Pediastrum duplex Meyen var. _duplex_	15,16
Scenedesmus acuminatus (Lagerh.) Chod.	15,20
S. acutus Meyen var. _acutus_	15
S. disciformis (Chod.) Fott & Kom.	12
S. protuberans Fritsch var. _minor_ Ley	15,16
*_Sphaerozosma aubertianum_ W. West	*1/97
*_Spirotaenia condensata_ Brébisson	*2/97;*1/97
*_Staurastrum brachiatum_ Ralfs	2,*2/97
*_S. gemelliparum_ Nordstedt	*13/96
S. histrix Ralfs	2
S. manfeldtii Defl. var. _manfeldtii_	20
*_S. paradoxum_ Meyen	*2/96
S. pilosum (Näg.) Arch.	2,4,6
* _S. striolatum_ (Näg.) Archer	12,*2/96
*_S. subscabrum_ Nordstedt	*2/96
*_S. teliferum_ Ralfs var. _ordinatum_ Borgessen	*2/96
*_S. tohopekaligense_ Wolle	*8/96
S. tohopekaligense Wolle var. _trifurcatum_ W. & G. S. Smith	20
*_Staurodesmus connatus_ (Lund.) Thomasson	*2/96
*_S. convergens_ (Ehrenb.) Teiling var. _laportei_ Teiling	*13/96
*_S. cuspidatus_ (Bréb.) Teiling var. _divergens_ Nordstedt	*2/96

*_S. glaber_ (Ehrenb.) Teiling var. _hirundinella_ (Messik.) Teiling	*2/96
*_S. spencerianus_ (Mask.) Teiling	*2/96
*_S. triangularis_ (Lagerh.) Teiling	*2/96
*_Tetmemorus brebissonii_ (Menegh.) Ralfs	*2/96
*_Tetraedron minimum_ (A. Braun) Hansgirg	*4/97
*_Tetrastrum komarekii_ Hindák	*1/97
*_Xanthidium brebissonii_ Ralfs var. _varians_ Ralfs	*2/97;*5/96

Table 3. Number of phytoplanktonic freshwater taxa found in the 21 sampled sites within the Mondego Basin. Samples from sites marked with an asterisk were only used for isolation purposes and/or the study of silica-scaled chrysophytes. The whole phytoplankton was studied in the remaining samples.

Site	Taxa
Serra da Estrela Natural Park	
1. Covão do Curral	58
2. Pond near Lagoa Comprida	55
3. Lagoa Comprida	16
4. Vale do Rossim Reservoir	42
5. Erva da Fome Reservoir*	2
6. Lagoacho Reservoir	31
Mondego River and tributaries	
7. Cris	78
8. Aguieira Reservoir*	1
9. Alva*	1
10. Fronhas Reservoir	53
11. Alto Ceira Reservoir	71
Lowland area of the Basin	
12. Pond near Srª da Estrela*	10
13. Poço dos Albinos*	3
14. Ribeira de Ançã*	6
15. Marsh near Montemor	114
16. Irrigation channel near Montemor	126
17. Pond near Sta Olaia*	12
18. Irrigation channel near Sta Olaia*	15
19. Pond near Marujal*	1
20. Irrigation channel near Soure	92
21. Ribeira near Casal da Rola. Soure*	3

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APPENDIX 2

Plates 1 to 8: Freshwater Flora of the Mondego Basin

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[1] Craveiro and Santos 1997; [2] Lacerda 1946; [3] Nauwerck 1962; [4] Rino 1967b; [5] Rino and Santos 1968; [6] Rodrigues 1961; [7] Rodrigues 1963; [8] Santos and Vicente 2000; [9] Santos 1970; [10] Santos 1971; [11] Santos 1973a; [12] Santos 1973b; [13] Santos 1976; [14] Santos and Morgadinho 1987.

Plate 1: Diatoms

1-3: *Achnanthes chlidanos* Hohn & Hellermann. 4,5: *Achnanthes oblongella* Öestrup. 6: *Achnanthes exigua* Grunow. 7,8: *Achnanthes minutissima* Kützing. 9,10: *Achnanthes lanceolata* (Brébisson) Grunow. 11-13: *Achnanthes hungarica* (Grunow) Grunow. 14: *Amphora libyca* Ehrenberg. 15: *Amphora veneta* Kützing. 16: *Anomoeoneis brachysira* (Brébisson) Grunow. 17: *Anomoeoneis sphaerophora* (Ehr.) Pfitzer. 18: *Aulacoseira granulata* (Ehrenberg) Simonsen. 19,20: *Cocconeis placentula* Ehrenberg. 21: *Cyclotella meneghiniana* Kützing. 22: *Cyclotella meneghiniana* Kützing (inside face). 23: *Cyclotella pseudostelligera* Hustedt. 24: *Diatoma mesodon* (Ehrenberg) Kützing. 25,26: *Eunotia bilunaris* (Ehrenberg) Mills. 27: *Eunotia pectinalis* (Dillwyn) Rabenhorst.

8 (SEM, x 5,100); 22 (SEM, x 13,600); 25 (x 1,020); all remaining figures (x 1,360)

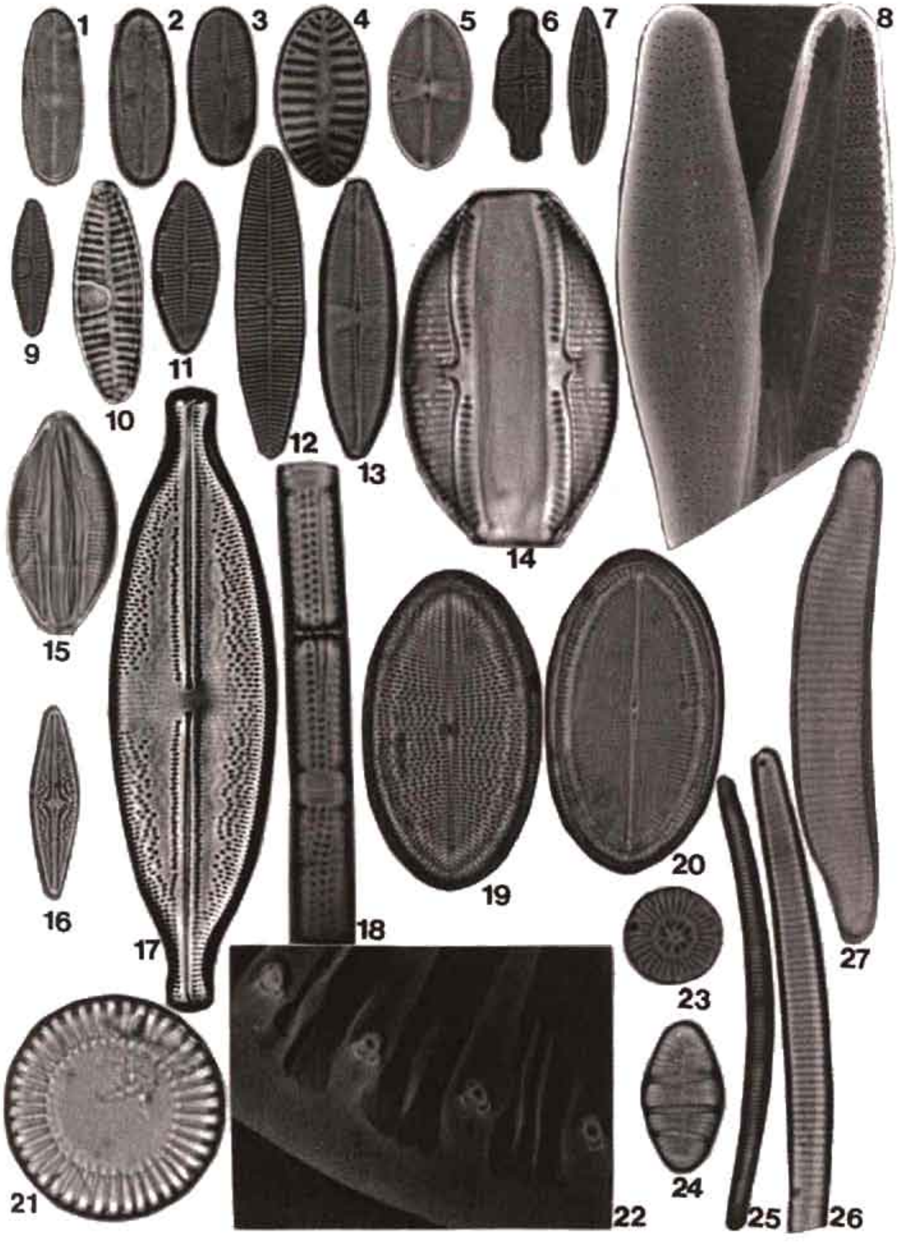
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Plate 2: Diatoms

1: *Cymbella gracilis* (Ehr.) Kützing. 2: *Cymbella mesiana* Cholnoky. 3,4: *Fragilaria capucina* Desmazières. 5: *Fragilaria crotonensis* Kitton. 6: *Frustulia rhomboides* (Ehr.) De Toni. 7: *Gomphonema acuminatum* Ehrenberg. 8: *Gomphonema gracile* Ehrenberg. 9: *Gomphonema parvulum* (Kützing) Kützing. 10: *Gomphonema truncatum* Ehrenberg. 11,12: *Navicula cryptocephala* Kützing. 13: *Navicula cryptotenella* Lange-Bertalot. 14: *Navicula pupula* Kützing. 15: *Navicula radiosa* Kützing. 16: *Navicula veneta* Kützing. 17: *Melosira varians* Agardh. 18: *Nitzschia dissipata* (Kützing) Grunow. 19: *Nitzschia linearis* (Agardh) W. Smith. 20: *Nitzschia paleacea* Grunow. 21: *Pinnularia gibba* Ehrenberg. 22: *Pinnularia subcapitata* Gregory. 23: *Synedra ulna* (Nitzsch) Ehrenberg. 24, 25: *Tabellaria flocculosa* (Roth) Kützing.

12 (SEM, x 2,240); 18 (SEM, x 3,060); 25 (SEM, x 6,800); 6,19 (x 1,020); all remaining figures (x 1,360)

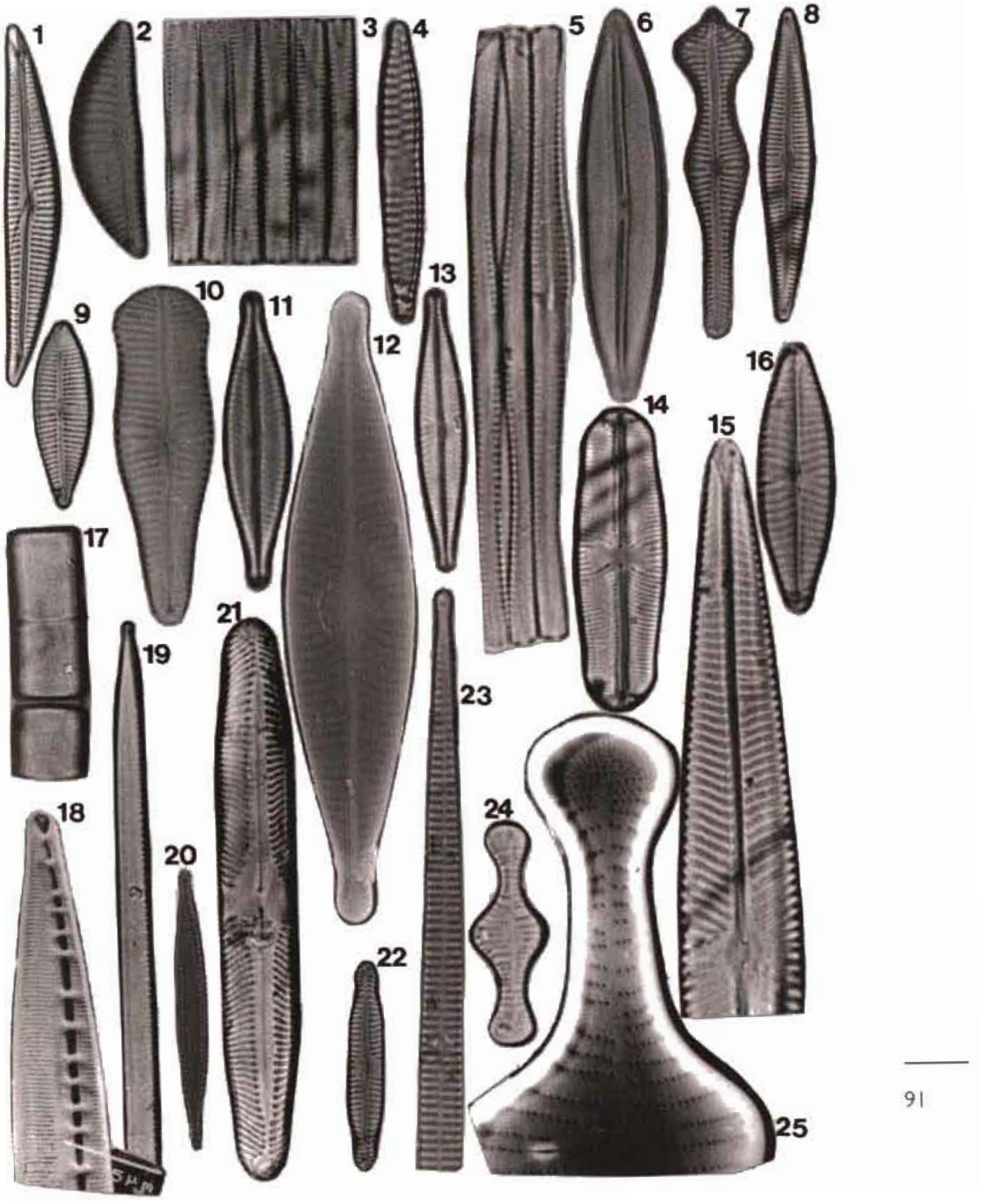
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Plate 3: Euglenophytes

1: *Phacus longicauda* (Ehr.) Duj.. 2: *Phacus helicoides* Pochm. 3: *Phacus gigas* Da Cunha. 4, 9: *Trachelomonas cervicula* Stokes. 5: *Trachelomonas woycickii* Koczwara var. *pusilla* Drez. fa. 6: *Trachelomonas hispida* (Perty) Stein emend. Defl. var. *coronata* Lemm.. 7: *Trachelomonas varians* Defl.. 8: *Trachelomonas stokesiana* Palmer. 10: *Strombomonas verrucosa* (Daday) Defl. var. zmiewika (Swir.) Defl..

5,9 (bar = 5 µm); all remaining figures (bar = 10 µm)

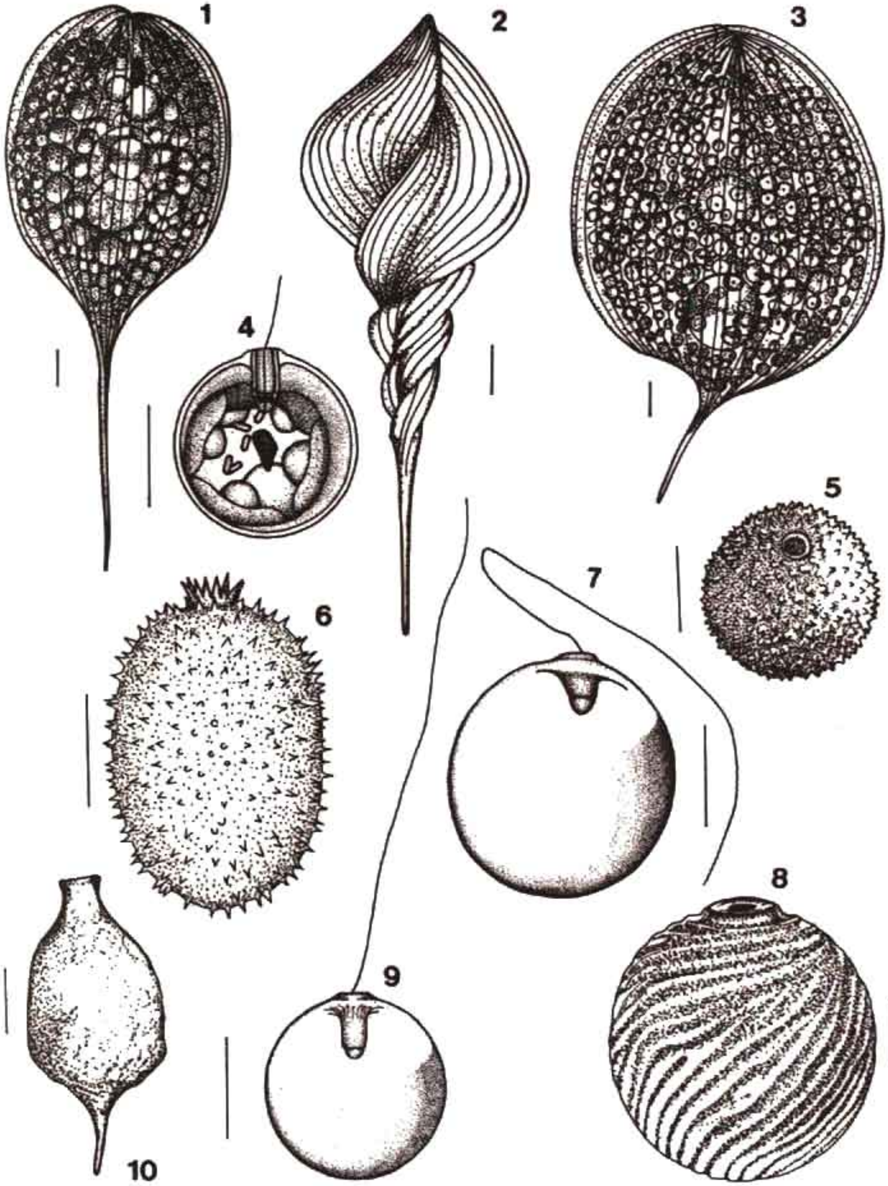
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Plate 4: Cyanophytes and some chromophytes

1: *Chroococcus turgidus* (Kütz.) Näg. var. *maximus* Nygaard[13]. 2: *Merismopedia elegans* A. Braun. 3: *Nostoc paludosum* Kützing[7]. 4, 5: *Cylindrospermum licheniforme* (Bory) Kützing[13]. 6: *Anabaena solitaria* Kleb. fa. *smithii* Komárek[11]. 7: *Anabaena cylindrica* Lemmermann[13]. 8, 9: *Tolypothrix tenuis* (Kütz.) fa. *lanata* (Wartmann) Kossinskaja. 10, 11: *Microcystis aeruginosa* Kützing emend. Elenkin / *Microcystis wesenbergii* Komarek (according to Jorge Rino). 12, 13: *Peridinium umbonatum* Stein[1]. 14: *Mallomonas* cf *alpina* Pascher[3]. 15, 16: *Cryptomonas marssonii* Skuja[3]. 17. *Cryptomonas erosa* Ehrenberg[3]. 18, 19: *Ceratium furcoides* (Levander) Langhans[1]. 20: *Dinobryon sociale* Ehrenberg, var. *americanum* (Brunthaler)[3]. 21: *Dinobryon bavaricum* Imhof. 22, 23: *Hydrurus foetidus* (Villars) Trevisan[3]. 24: *Synura* sp.

1,2,9 (x 544); 3,21 (x 270); 4,5,7,15-17 (x 680); 6 (x 730); 8 (x 100); 10 (x 48); 11 (x 820); 12,13 (bar = 10 µm); 14 (x 1,020); 18,19 (bar = 40 µm); 20,24 (x 410); 22 (x 290); 23 (x 666)

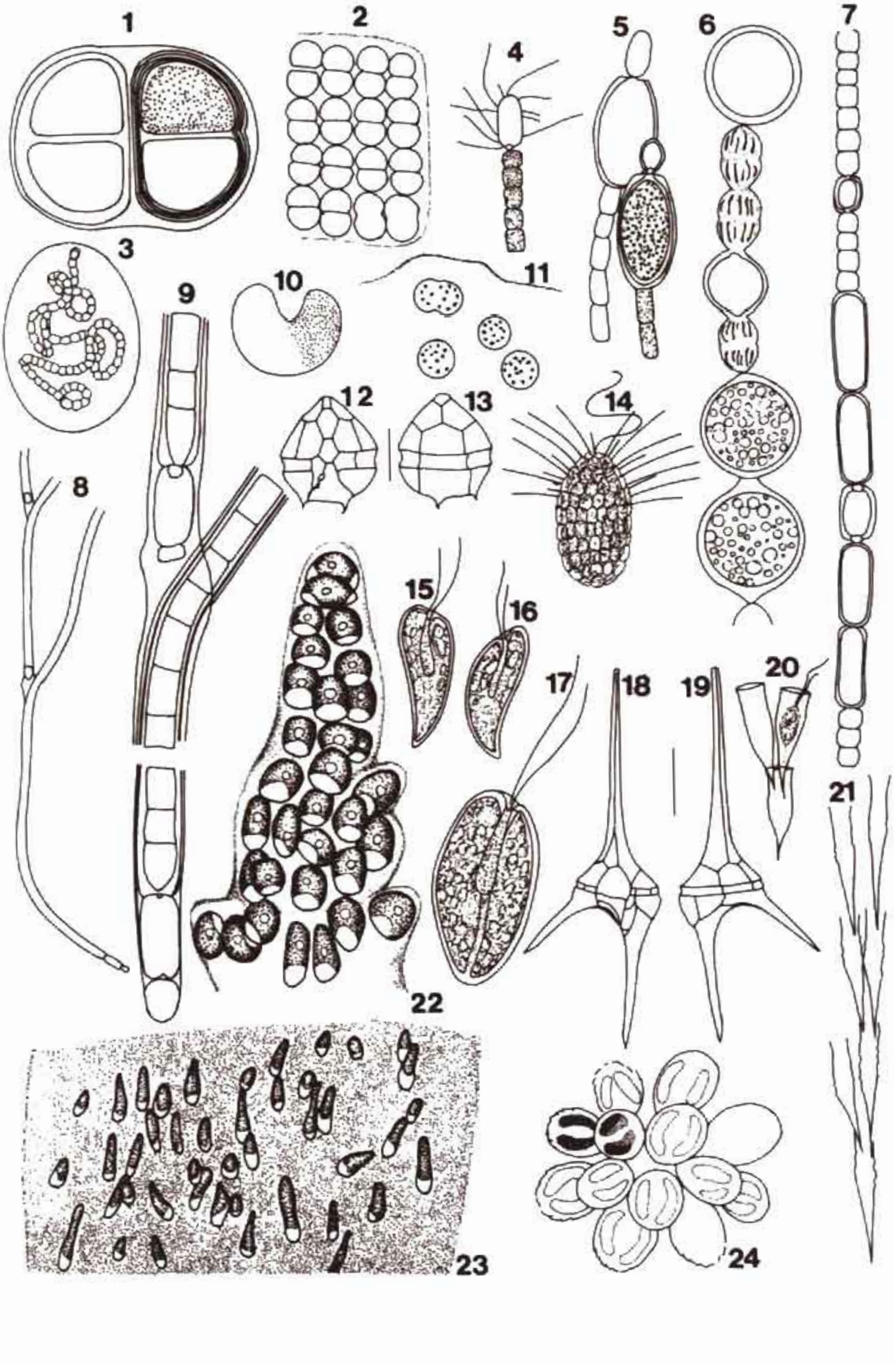
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Plate 5: Xanthophytes and green algae

1: *Tribonema regulare* Pascher. 2: *Ophiocytium mucronatum* (A. Braun) Rabenhorst[10]. 3: *Chlorobotrys gloeothece* Pascher. 4: *Characiopsis saccata* Carter. 5: *Vaucheria sessilis* (Vaucher) de Candolle. 6: *Volvox tertius* A. Meyer*. 7: *Haematococcus pluvialis* Flotow emend. Wille*. 8: *Chlamydomonas kleinii* Schmidle*. 9: *Asterococcus superbus* (Cienk.) Scherffel. 10: *Chlorella vulgaris* Beijerinck. 11: *Dictyosphaerium pulchellum* Wood[1]. 12: *Pediastrum boryanum* (Turp.) Menegh. var. *longicorne* Raciborski[5]. 13: *Pediastrum duplex* Meyen var. *gracillimum* W. et G.S. West. 14: *Scenedesmus quadrispina* Chodat. 15: *Scenedesmus acuminatus* (Lagerh.) Chodat. 16: *Ankistrodesmus gracilis* (Reinsch) Korshikov. 17, 18: *Monoraphidium griffithii* (Berk.) Komárkova – Legnerová1*. 19: *Coelastrum reticulatum* (Dangeard) Senn[10].

1,3,4 (x 1,088); 2,19 (x 734); 5 (x 102); 6 (x 75); 7 (x 782); 8 (x 1,700); 9,10 (x 540); 11,13 (bar = 10 μm); 14,15 (x 816); 16 (x 680); 17 (x 394); 18 (x 1,157)

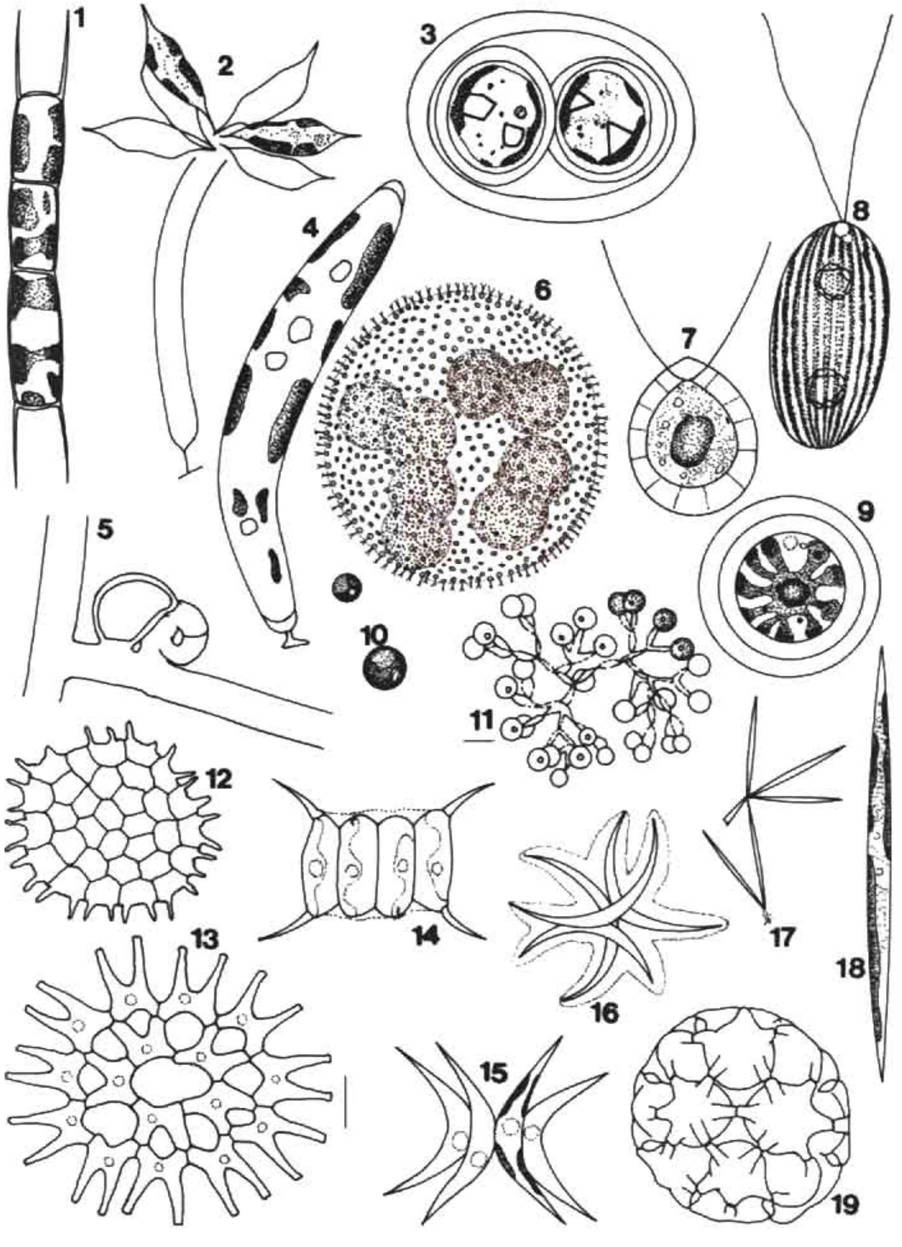
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Plate 6: Green algae

1: *Microspora crassior* (Hansgirg) Hazen[8]. 2: *Geminella minor* (Nägeli) Heering[14]. 3-5: *Spirogyra gracilis* (Hassal) Kützing[11]. 6, 7: *Spirogyra submaxima* Transeau[11]. 8: *Coleochaete orbicularis* Pringsheim[5]. 9-12: *Oedogonium borisianum* (Le Cl.) Wittrock[3]. 13, 14: *Oedogonium eriense* Tiffany. 15-17: *Bulbochaete insignis* Pringsheim[16]. 18, 19: *Bulbochaete crassiuscula* Nordstedt[12].

1,8 (x 442); 2 (x 775); 3,4 (x 558); 6,14 (x 270); 5,7 (x 190); 9-12 (bar = 50 µm); 13 (x 99); 15,18 (x 108); 16,17 (x 289); 19 (x 425)

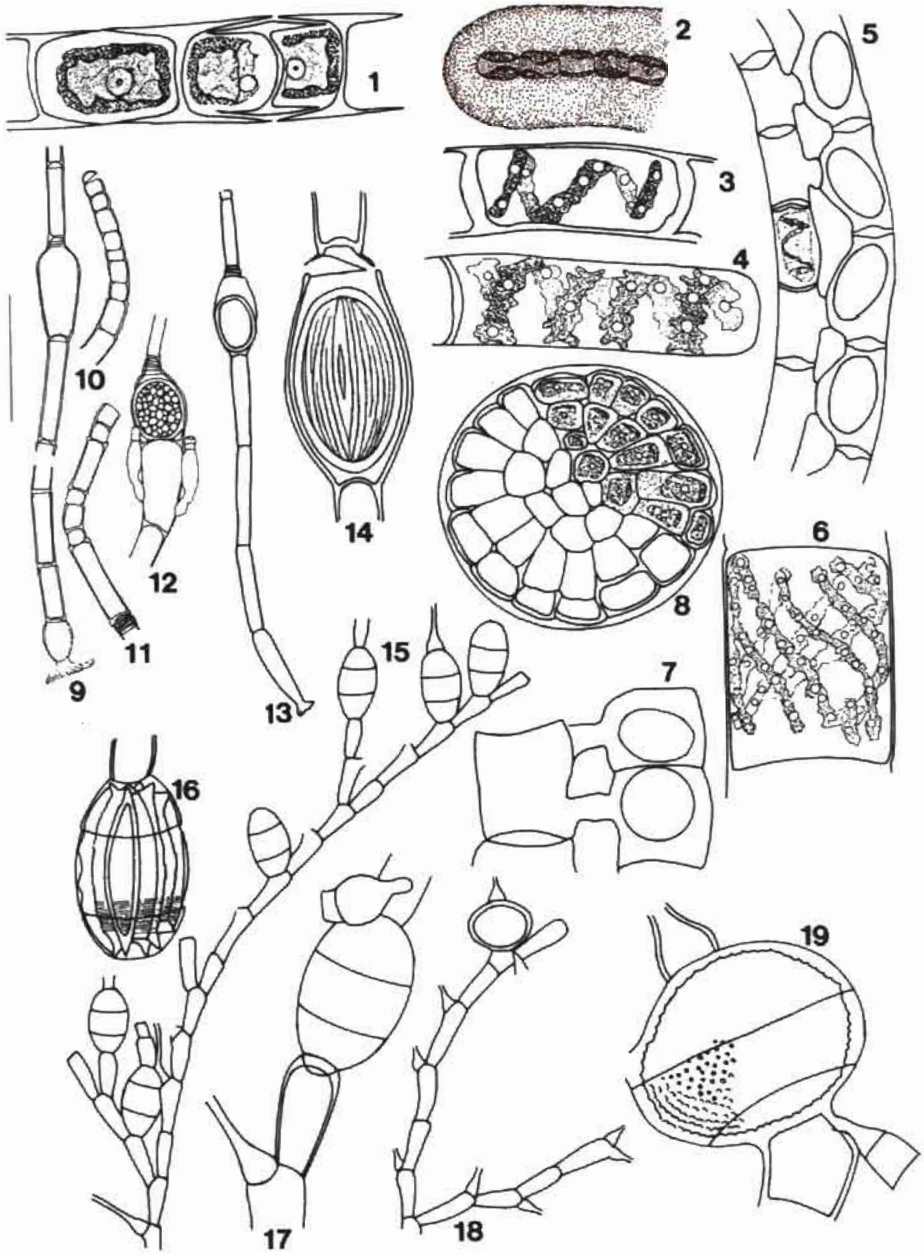
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Plate 7: Green algae

1: *Closterium moniliferum* (Bory) Ehrenberg. 2: *Closterium navicula* (Bréb.) Lutkemuller. 3, 4: *Cosmarium difficile* Lutkemuller. 5: *Cosmarium brebissonii* (Menegh) Ralfs. 6: *Cosmarium amoenum* (Bréb.) Ralfs. 7: *Euastrum ampullaceum* Ralfs. 8: *Euastrum spinulosum* Delponte var. *henriquesii* Sampaio fil[8]. 9, 10: *Staurastrum margaritaceum* (Ehrenb.) Meneghini[4]. 11, 12: *Staurastrum lusitanicum* Nauwerck[3]. 13, 14: *Staurastrum histryx* Ralfs. 15, 16: *Staurodesmus glaber* (Ehrenb.) Teiling var. *hirundinella* (Messik.) Teiling. 17, 18: *Staurodesmus triangularis* (Lagerh.) Teiling. 19: *Micrasterias papilifera* Brébisson[7]. 20: *Tetmemorus brebissonii* (Menegh.) Ralfs var. *minor* de Bary[7]. 21: *Hyalotheca mucosa* (Dillw.) Ehrenberg[3]. 22, 23: *Xanthidium brebissonii* Ralfs var. *varians* Ralfs[4]. 24.25: *Gonatozygon pilosum* Wolle. 26: *Spirotaenia condensata* Brébisson. 27, 28: *Cylindrocystis brebissonii* Meneghini.

1 (x 270); 2-4.6 (x 1,020); 5,7 (x 490); 8 (x 306); 9 (x 558); 10-12 (x 510); 13,14 (x 670); 15-18,27,28 (x 653); 19 (x 286); 20,21 (x 720); 22,23 (x 850); 24,26 (x 326); 25 (x 816)

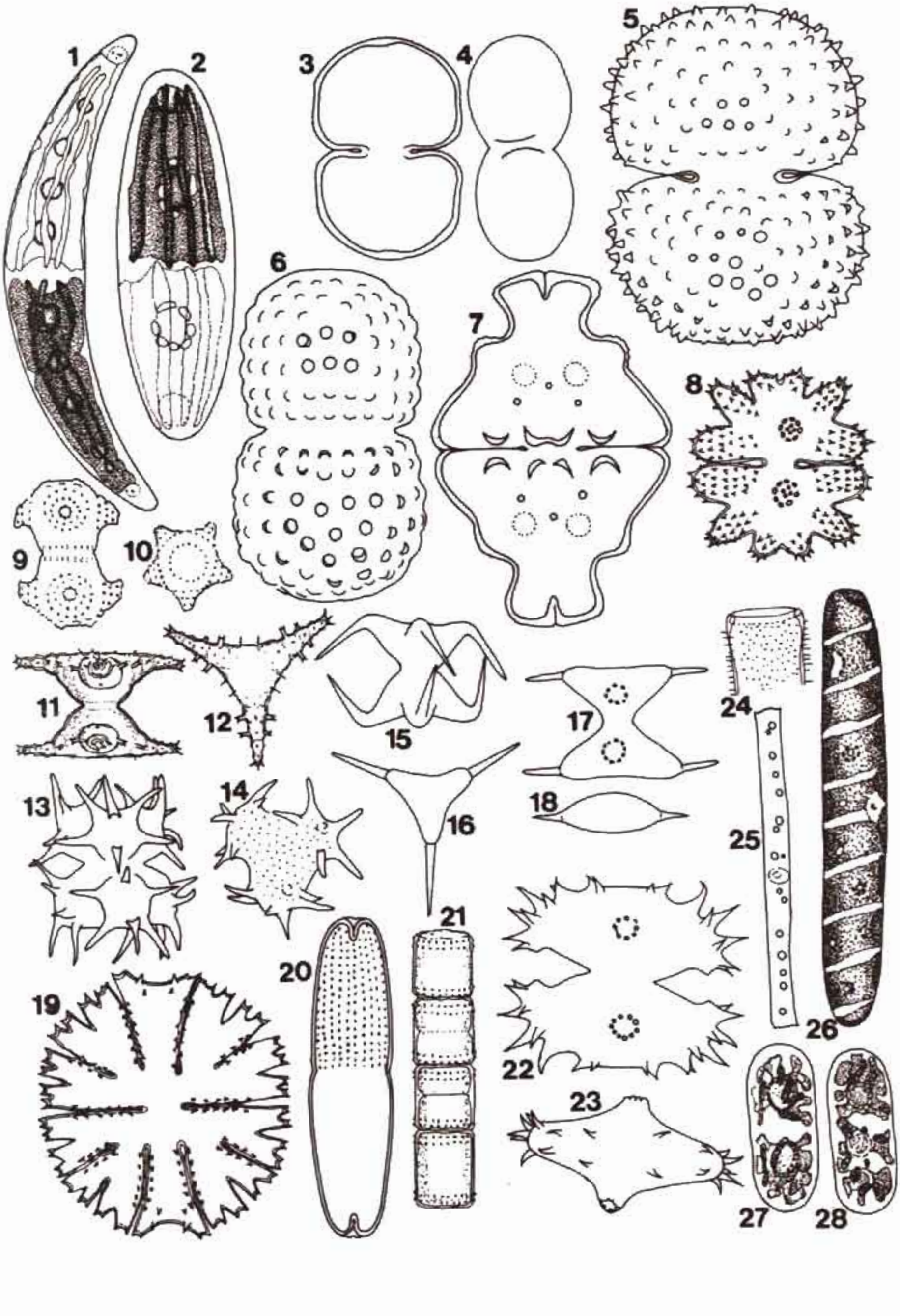
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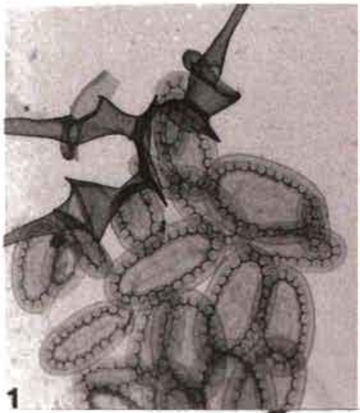
Plate 8: Silica-scaled chrysophytes

1: *Chrysosphaerella brevispina* Korshikov emend. Harris & Bradley[A]. 2: *Paraphysomonas vestita* (Stokes) de Saedeleer[B]. 3: *Spiniferomonas breakneckii* Siver. 4: *Mallomonas lelymene* Harris & Bradley. 5: *Mallomonas punctifera* Korshikov. 6: *Synura curtispina* (Petersen & Hansen) Asmund[B]. 7: *Synura petersenii* Korshikov.

1 (TEM, x 4,725); 2,3 (TEM, x 6,300); 4 (x 3,675); 5 (SEM, bar = 10µm); 6 (TEM, x 4,200); 7 (SEM, bar = 5 µm)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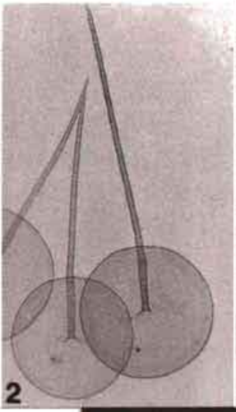
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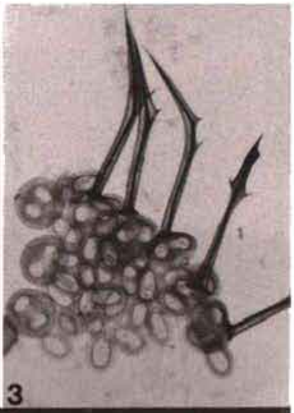
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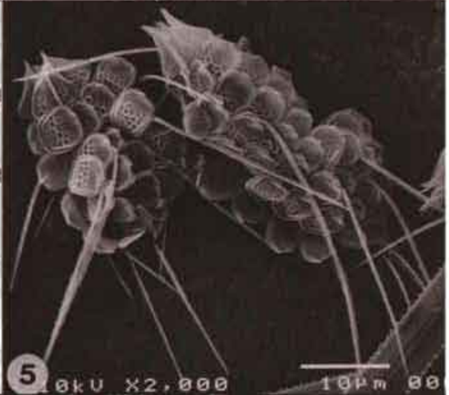
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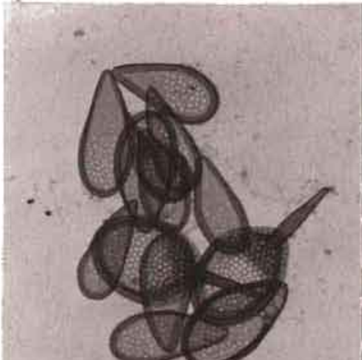
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VITOR VASCONCELOS [1]

TOXIC CYANOBACTERIA IN THE MONDEGO BASIN RESERVOIRS. AN OVERVIEW

Abstract

Toxic cyanobacteria in Portuguese freshwaters have been studied since 1989. Hepatotoxin producing species have been found to be the dominant contributors to bloom forming populations in a range of Portuguese lakes and reservoirs.

In the Mondego river basin reservoirs few studies have been carried out regarding phytoplankton ecology, and especially cyanobacteria. The eutrophication of this river basin, increases from upstream sites down to Aguieira reservoir but decreases downstream. The Carlson Trophic State Index for chlorophyll was used to assess eutrophication. The most eutrophic sites were Fronhas, Fagilde and Aguieira reservoirs with cyanobacteria blooms recorded during several years.

Microcystins are the only toxins found to date within bloom populations of cyanobacteria with microcystin-LR being the dominant. Other less common microcystins, such as MCYST-HilR and [MeSer^7]MCYST-LR have also been detected. Toxin concentrations within blooms vary with time. However samples collected in 1992 have been found to be the most toxic ones.

The use of the reservoirs of this river basin for drinking water withdraw, irrigation, recreation and for fishing may lead to human health hazard if no proper monitoring is performed.

Introduction

Freshwater cyanobacteria have been studied in Portugal since the early 30's. Initially, these studies focused on the taxonomy and diversity (Sampaio 1933) of potentially toxic species such as *Oscillatoria formosa* and *Lyngbya majuscula*. In 1959, Nauwerck published a paper on the occurrence of phytoplankton species in three locations close to Coimbra, identifying the cyanobacterium *Pseudanabaena catenata* in

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samples collected from Poço do Almegue, in the Mondego river (Nauwerck 1959). Later, Nauwerck (1962), gave an overview of the phytoplankton composition of several Portuguese waterbodies. This study highlighted the occurrence and dominance of cyanobacteria in some of these reservoirs. This author referred to the occurrence of the cyanobacteria *Lyngbya limnetica* and *Anabaena variabilis* in Lagoa Comprida, an oligotrophic lake located in the upper Mondego river basin, during the spring of 1960.

The first data on the toxicity of cyanobacteria in Portugal from the analysis of a *Microcystis aeruginosa* dominated toxic bloom, was recorded in the Douro river (Crestuma reservoir) in 1989 (Vasconcelos et al. 1993).

The Mondego river basin contains several reservoirs (Fig. 1) that are used for irrigation, hydroelectric power generation and also for human consumption and recreational purposes. In these reservoirs, cyanobacteria are now known to reach high concentrations, often forming thick surface scums. Such blooms represent a considerable human health risk. In this paper, an overview of the occurrence and toxicity of cyanobacteria in several reservoirs of the Mondego river basin is presented.

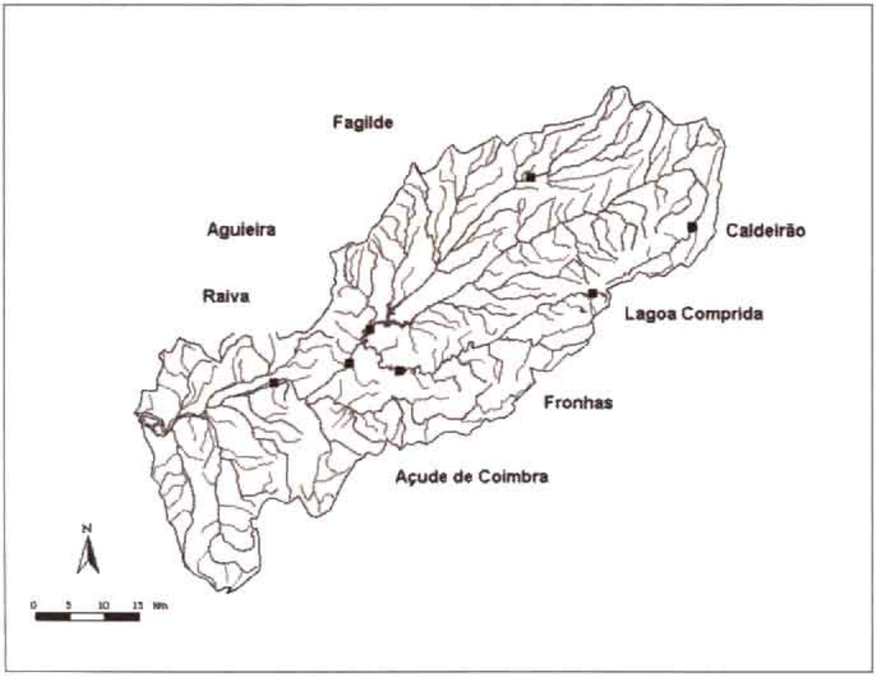

Figure 1. Location of the studied reservoirs of the Mondego river basin.

Eutrophication of Mondego river

Although the Mondego river basin is an important source for the supply of water for human consumption and also for industrial and agricultural purposes, there are few systematic studies concerning the ecology of phytoplankton within its reservoirs.

The eutrophication of two locations of Mondego river (Aguieira and Raiva reservoirs) measured by the Carlson Trophic State index for chlorophyll, is shown in Table 1. Data were obtained from the INAG site (Water Institute- Portuguese Ministry of Environment – www.inag.pt). and calculated according to Carlson (1977). This index uses chlorophyll, phosphorus or secchi disk depth. Higher values indicate more pronounced eutrophy. Taking into account the Carlson's classification of aquatic ecosystems in terms of eutrophy (Carlson 1977), both reservoirs may be classified as meso-eutrophic. In fact, the occurrence of cyanobacteria during summer months is a clear sign of eutrophication. Nevertheless, as it may be seen latter in this work, eutrophication varies considerably along the Mondego River Basin, from highly eutrophic reservoirs such as Aguieira Reservoir (located in an agricultural region) to relatively oligotrophic water bodies close to Coimbra and in the upper reaches of the basin (Lagoa Comprida).

Cyanobacteria and their toxins in Portugal

Freshwater cyanobacteria may produce different kinds of toxins having neurotoxic, hepatotoxic and dermatotoxic effects in humans and other animals (Carmichael 1997). These toxins may adversely affect aquatic animals and also terrestrial animals that drink water contaminated with cyanobacteria. Human populations may also be affected by (i) drinking water not properly treated, (ii) using it for recreation, or (iii) eating aquatic animals contaminated with cyanobacterial toxins (Chorus and Bartram 1999). Recently it was also shown that lethal human intoxication may occur by using contaminated water in dialysis systems (Jochimsen et al. 1998).

Table 1 – Trophic State Index (TSI) of Raiva and Aguieira reservoirs during 1988 to 1998 (x- average, m- minimum, M-maximum and VC- Variation coefficient).

Parameter Reservoir	Average	Minimum	Maximum	Variation Coefficient
Aguieira	49.2	35.2	66.0	15.7
Raiva	48.7	36.4	59.3	12.8

Table 2 – LD50 values (i.p mouse bioassay) of blooms and *Microcystis* strains collected from Portuguese freshwaters - maximum, minimum, average and standard deviation (SD).

	Blooms (n=18)				*Microcystis* strains (n=15)			
	Max.	Min.	Average	SD	Max	Min	Average	SD
LD_{50} SD(mg/kg)	700.0	20.0	136.0	175.0	75.0	15.0	37.5	23.5

A survey of the distribution of toxic cyanobacteria in Portuguese freshwaters began in 1989. Preliminary work surveyed 36 lakes, reservoirs and rivers during the period 1989-1992. At this time only hepatotoxic blooms were found and 60% of the blooms were found to be toxic. The main species present in toxic blooms were *Microcystis aeruginosa* (72%) and *Anabaena flos-aquae* (28%) (Vasconcelos 1994). The toxicity of the bloom samples evaluated by intraperitoneal (i.p.) mouse bioassay – LD_{50} – varied from 20 mg/kg to 700 mg/kg (dry weight of bloom sample to kg mouse body weight). *Microcystis* strains isolated from these blooms were found to be toxic, varying from 15 mg/kg to 75 mg/kg (Vasconcelos 1994) (Table 2). The main hepatotoxins in Portuguese freshwaters are MCYST-LR, MCYST-LA, MCYST-YR and [D-Asp^3]MCYST-LR (Vasconcelos et al. 1995; 1996).

Occurrence and abundance of cyanobacteria in the Mondego river basin

Cyanobacteria blooms are found only in some of the reservoirs in the Mondego river basin. In this work we review the information on cyanobacteria in this river basin from upstream sites (Lagoa Comprida) to the most downstream site (Coimbra reservoir).

Upstream sites, such as Lagoa Comprida are oligotrophic with few cyanobacteria present. Nauwerck (1962) classified this lake as oligotrophic describing *Anabaena variabilis* and *Lyngbya limnetica* as the only cyanobacteria present. Santos and Mesquita (1986) isolated several phytoplankton species from this lake and among them only one cyanobacteria species – *Fortiea crassa*. More recently, Boavida and Gliwicz (in press) analyzed the phytoplankton community during September and October 1993 and did not report any species of cyanobacteria.

Caldeirão reservoir is not well studied from a planktonic point of view but a sample analyzed in April 1997 revealed no cyanobacteria in a total of 7819 cells/ml of total phytoplankton (Vasconcelos et al. 1999).

In Fronhas reservoir, during the autumn of 1998 no cyanobacteria were found in spite of high chlorophyll concentrations. In the Spring 1999, cyanobacteria attained high density, constituting 87.0-98.4% of the phytoplankton biomass. This reservoir was reported as eutrophic with *Oscillatoria limnetica* as the dominant species (Vasconcelos et al. 1999). Total cyanobacteria density reached then 89,141 cells/ml during the spring of 1999.

Fagilde reservoir was found to be dominated by cyanobacteria. In a study performed in 1998 and 1999 cyanobacteria composed 70.9 to 90.6% in autumn and spring samples respectively (Vasconcelos et al. 1999). In the autumn 1998 dominant cyanobacteria species were *Anabaena flos-aquae*, *Microcystis aeruginosa* and *Phormidium mucicola* whereas during spring 1999 *Anabaena spiroides*, *Microcystis aeruginosa* and *Phormidium mucicola* dominated. These species are potentially toxic, being the most common in Portuguese freshwaters (Vasconcelos 1994). A TSI value of 52.3% during autumn confirmed the eutrophy of this reservoir.

In the Aguieira reservoir, bloom samples of *Microcystis aeruginosa* collected in September and October of 1992 showed strong hepatotocity, with a LD_{50} of 31-35 mg/kg (Vasconcelos 1995). This reservoir is the most eutrophic within the Mondego river basin. Oliveira and Monteiro (1992) studied the dynamics of cyanobacteria

Table 3 – Total phytoplankton density and percentage of cyanobacteria in surface samples of Aguieira reservoir – Santa Comba Dão (from Oliveira & Monteiro 1992).

Sampling date	Total phytoplankton (cells/ml)	Cyanobacteria (%)
14.07.92	120,895	80.1
18.08.92	554,744	98.7
20.09.92	164,835	97.8
21.10.92	7,064	58.8

Table 4 – Vertical distribution of total phytoplankton and of cyanobacteria (cells/ml) in Aguieira reservoir– at Santa Comba Dão in October 1992 (from Oliveira & Monteiro 1992).

Depth (m) group	0	1	3	6
Cyanobacteria (cells/ml)	4,154	6,102	18,483	20,181
Total phytoplankton (cells/ml)	7,064	7,286	20,889	22,099

blooms in this reservoir and found out that between July and October 1992, cyanobacteria were dominant in the phytoplankton community (Table 3)

The main cyanobacteria species present were *Raphidiopsis mediterranea, Anabaena cylindrica, Gomphosphaeria lacustris, Microcystis aeruginosa* and *M. flos-aquae* (Oliveira and Monteiro 1992). These authors also stated that cyanobacteria showed a sub-surface peak in abundance in October, with the highest concentrations detected at 3-6 m depths (Table 4). As they did not present temperature data we can not assume that this is due to the thermocline. Nevertheless, in these temperate systems thermocline tend to be deeper (Vasconcelos 1990). This is an important consideration when the reservoir is used for drinking water supply. Most programs monitoring the presence of cyanobacteria are based on surface sampling and in cases where the highest concentrations are found at greater depths, there may be a biased diagnosis of water quality with important consequences in terms of human health.

It was also shown that there is a longitudinal gradient in terms of cyanobacteria density in this reservoir. The density close to the dam wall was four times lower than that of Santa Comba Dão (Oliveira and Monteiro 1992) being a result of the influence of the agriculture runoff and municipal wastewater disposed from Santa Comba. In fact, due to its shape Aguieira reservoir has to be studied and sampled taking this into consideration.

In S. C. Dão, the main cyanobacteria species are *Raphidiopsis mediterranea, Anabaena cylindrica, Gomphosphaeria lacustris, Microcystis aeruginosa* and *M. flos-aquae* (Oliveira and Monteiro, 1992). Work done from 1994 to 1999, in collaboration with the health authorities of Santa Comba Dão, Mortágua, Tábua and Carregal do Sal, the phytoplankton dynamics, with special relevance to cyanobacteria was analyzed (Vasconcelos et al. 1999). The highest concentrations of cyanobacteria were found in the summer and autumn of 1995, with peak concentrations of 1 × 10^6 cells/ml.

Raiva reservoir is meso-eutrophic reservoir based on its TSI values (Table 1). The occurrence of potentially toxic species such as *Aphanizomenon* sp., *Microcystis aeruginosa* and *Oscillatoria* sp. indicates a clear potential for human health hazards. Nevertheless, maximum concentrations of cyanobacteria measured by Vasconcelos et al. (1999) are not yet very high, attaining only 2,250 cells/ml in the autumn 1998 and 1,244 cells/ml in the spring 1999.

Açude de Coimbra is a reservoir used for the production of drinking water for Coimbra municipality. In a study performed during 1993-1994, Craveiro (1994) analyzed the phytoplankton community of Coimbra reservoir. Chlorophyll attained a maximum concentration of 18.7 mg/m^3 in July 1993 and TSI values calculated with the whole data reached an average of 43.5% with a CV of 20.8%. This clearly indicates a mesotrophic state where cyanobacteria may increase their role. During this study, a total of 11 cyanobacteria species were found with *Aphanothece clathrata* and *Oscillatoria limnetica* as dominant in terms of occurrence. Although no density values measured as cells/ml are available, it was found that, the maximum densities were due to O. limnetica (Craveiro 1994). Data obtained by us (Vasconcelos et al. 1999) revealed that the concentration of cyanobacteria varied from 413 cells/ml in the autumn 1998 to 3,008 cells/ml in the spring 1999, with *Microcystis incerta, O. limnetica* and *Coelosphaerium kuetzingianum* as the main species.

Comparing all the sites described in this work in terms of maximum cyanobacteria density (Fig. 2), we may say that eutrophication of this river basin shows a pattern that increases from the upstream sites to Aguieira reservoir and subsequently decreases downstream.

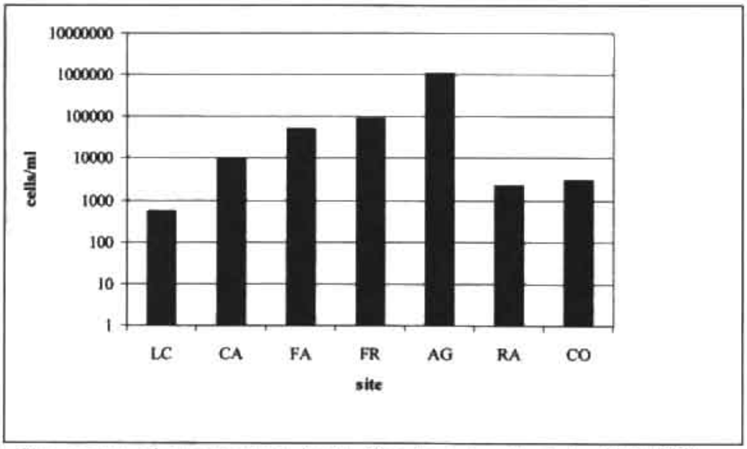

Figure 2. Occurrence of cyanobacteria in the Mondego river basin in 1998-1999 – maximum cyanobacteria values (cells/ml) (LC – Lagoa Comprida, CA – Caldeirão, FA – Fagilde, FR – Fronhas, AG – Aguieira, RA – Raiva, CO – Coimbra reservoirs) (from Vasconcelos et al. 1999)

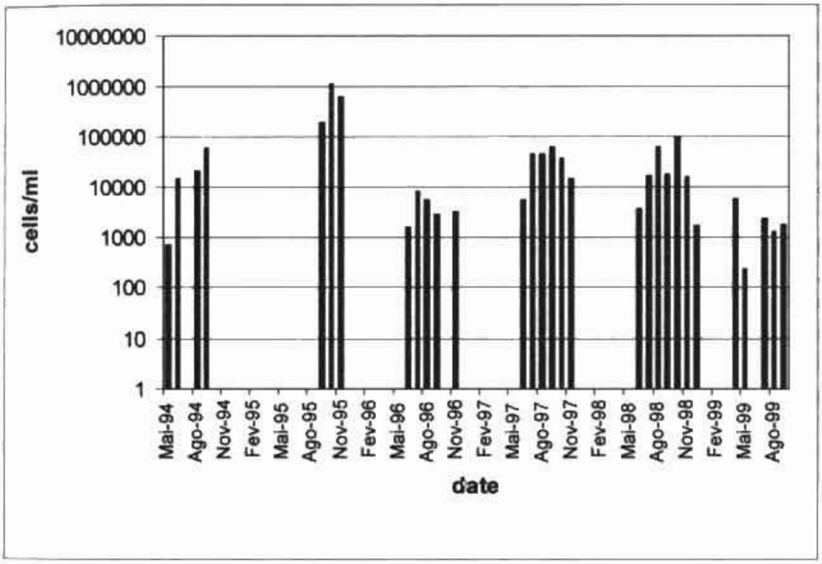

Figure 3. Dynamics of total cyanobacteria density (1994-1999) in the Aguieira reservoir (S.C. Dão) (from Vasconcelos et al. 1999).

Toxins in Mondego river basin

Initially, toxicity was measured by mouse bioassay - LD$_{50}$ (mg/kg animal weight). The results of toxicity (LD$_{50}$), amount of microcystins and isolated microcystins – MCYST - from blooms collected at Aguieira reservoir in 1992 (Santa Comba Dão) are presented in Table 5, revealing blooms with high hepatotoxicity.

Table 5. Toxicity (LD50), amount of microcystins and isolated microcystins from blooms collected at Aguieira reservoir (Santa Comba Dão) (from Vasconcelos 1995).

Date	Toxicity (LD$_{50}$ mg/kg)	Toxin amount (µg/mg)	Isolated microcystins
10/09/92	31	5,6	MCYST-LR, MCYST-RR, MCYST-HilR [Dha^7]MCYST-LR, [MeSer^7]MCYST-LR, [D-Asp^3]MCYST-LR
04/10/92	35	-	-

The September 1992 sample presented the highest MCYST diversity of the many analyzed from Portuguese cyanobacterial blooms. Seven different MCYST were found and isolated although MCYSTY-LR was the dominant – 64.2% of the total MCYST content. In this sample, it was also found MCYST-HilR (2.3%), a MCYST similar to MCYST-LR but that contains homoisoleucine instead of leucine (Vasconcelos et al. 1996). It was only found before in a *Microcystis* spp. bloom sample in Illinois, USA (Namikoshi et al. 1994). The toxin [L-MeSer^{7}]MCYST-LR has N-Methylserine instead of dehydroalanine was also present in this sample and it had been reported before only in the same Illinois bloom.

Later, from 1994 to 1997, the evaluation of toxicity revealed negative results in terms of mouse bioassay. This may be due to a decrease on toxicity but also to the fact that mouse bioassays have low sensitivity. Data obtained using a more sensitive method, the immunoassay ELISA (Ann and Carmichael, 1994) are shown in Table 6. Although cyanobacteria density were high during this period (Fig. 1), microcystin concentrations were low. This may be due to a low percentage of toxic strains among the blooms or to unfavorable environmental conditions for toxin production.

Table 6 Microcystin concentration (µg/l) in Agueira reservoir during 1997 and 1998 measured by ELISA.

Months	Year	1997	1998
August		1,6	0
September		0,3	1,2
October		1,7	1,3
November		0	0,5
December		-	0

Environmental and human health risks

Taking into account the data presented in this paper we may conclude that the occurrence of toxic cyanobacteria in Mondego river basin represent a potential human health hazard.

The variability in patterns of eutrophication along this river basin led us to focus our attention on three sites: Fagilde, Fronhas and Aguieira reservoirs which are most susceptible to blooms of cyanobacteria. Sites downstream of Aguieira should also be monitored regularly, since toxic cyanobacteria are detected in the phytoplankton. Cyanobacteria and their toxins may be transported downstream reaching Raiva and Coimbra reservoirs, so there is a clear need for monitoring microcystin concentrations as well as phytoplankton populations.

The presence of toxic cyanobacteria detected since 1992 in Agueira reservoir may cause human health problems due to the fact that this reservoir is used for drinking water withdraw and also for recreation and fishing. Aquatic animals, including fish, may accumulate microcystins (Amorim and Vasconcelos 1999, Vasconcelos 1999) and transfer them along the food chain.

Taking into consideration the WHO provisional guideline values for drinking water – 1 µg/l (Chorus and Bartram 1999), cyanobacteria toxins should be monitored regularly in Mondego river basin reservoirs during the whole bloom season.

Acknowledgements

I am indebted to Dr. Martin Saker for his comments on this manuscript and for English corrections.

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FRESHWATER MACROINVERTEBRATES IN THE MONDEGO RIVER BASIN

Abstract

Invertebrates in streams and rivers are diverse, abundant and perform important ecological functions, recycling organic matter, feeding upon algae and transferring energy for larger animals, including fish, birds, amphibians and others. The macroinvertebrate communities of 34 stream sites in the Mondego River basin were analysed to assess changes in taxa richness and percentage of shredders along river order / size gradient and to assess water quality by means of biotic indices. Taxa richness was higher in low order streams when compared with larger rivers. In some rivers, shredder abundance was higher in autumn, but in other rivers we did not observe changes in shredder abundance along the year. Water quality, measured by the application of the biotic index BMWP', revealed that many small low order streams can be considered as unpolluted. However, water quality decreased in larger rivers.

Introduction

In stream ecology studies, the definition of macroinvertebrates is subjective: invertebrates visible to the naked eye. In more practical terms, they are invertebrates captured by a 0.2 to 0.5mm sampling net (Hellawell 1978). Unlike in salt-water systems and soil, freshwater macroinvertebrates are uniform in size, with most of specimens ranging from fractions of mm to 30mm.' In streams and rivers, macroinvertebrates comprise mostly insects, but also include other arthropods (Acarina, Crustacea), worms and leaches (Annelida), flatworms (Plathelminthes), nematodes (Nemathelminthes), snails and bivalves (Mollusca) (Tachet et al. 1987). Among the insects, 4 orders have juvenile stages exclusively aquatic and adults with aerial life: Ephemeroptera, Plecoptera, Odonata and Trichoptera. Some Diptera also have a juvenile aquatic phase. Two additional orders may be abundant in the water either as juveniles and / or adults: Heteroptera and Coleoptera.

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Vannote et al. proposed in 1980 a general model – the River Continuum Concept (RCC) – to explain the functional link along rivers, from the source to the mouth. This framework establishes the relationships between stream order / size and energy sources of rivers. According to the RCC, streams receive most of their energy in the form of leaves and other plant detritus produced in the riparian area. This material will be used as food by a large proportion of aquatic macroinvertebrates. Macroinvertebrates are, in turn, the energy source for many species of fish, amphibians and aquatic birds and mammals.

Freshwater macroinvertebrates have been used for nearly a century as indicators of water quality (e.g. Kolkwitz and Marsson 1908, 1909). The reason is simple: It has been documented for long time that as the quality of water deteriorates, an increasing number of species no longer are capable of survive and the diversity of the community generally decreases. The first taxa to disappear are known as "intolerant". With the elimination of competitors and predators, the remaining species tend generally to grow to large numbers, especially if the food is abundant. These organisms are known as "tolerant". Decrease in water quality may therefore result in a decrease in diversity and change community structure and function.

Changes in (a) diversity of macroinvertebrates, (b) the structure of the community and (c) the proportion of taxa with known environmental tolerances have been therefore used as indicators of environmental quality in biomonitoring programmes (Metcalfe-Smith 1996). Biotic indices have also been used to assess water quality. Those indices are numerical expressions based on the presence and number of indicator taxa. Biotic indices have been used in Europe and North America for several decades for routine rapid assessment of water quality in rivers (e.g. De Pauw and Vanhooren 1983, Washington 1984, Metcalfe 1989).

Although several groups of taxa can be used for biomonitoring proposes (e.g. algae, macrophytes, bacteria, fish, protozoans), macroinvertebrates are the most popular. Three main reasons contribute to such popularity: (a) They have low mobility or are fixed to the substratum and, therefore, their composition is related to water conditions at the place where they occur. (b) Most taxa are ubiquitous; this is very convenient when trying to make comparisons among different areas. (c) There is a high diversity of forms, colonizing almost any environmental condition (Hellawell 1978).

In Portugal several research groups have been studying the distribution or the ecology of freshwater invertebrates. E.g. Fontoura (1984), Fontoura and De Pauw (1989) in Northern Atlantic rivers; Cortes (1990, 1992) in the Northeast; Moreira et al. (1988) in the Vouga Basin; Graça et al. (1989), Abelho and Graça (1996) in the Central Portugal and Graça and Coimbra (1998), Coimbra and Graça (1998), Graça et al. (1995), Coimbra et al. (1996), Malo et al. (1998) in the South.

The objective of this paper was to summarise the research carried in the Mondego River basin in terms of (a) richness of taxonomic invertebrate groups along a longitudinal gradient (b) proportion of shredders along river order gradients, and (c) classification of water quality by means of macroinvertebrate indicators. For this propose, we used published and unpublished data.

Materials and methods

We used data originally from Graça et al. (1989, R. Alva and R. Soure), Abelho and Graça (1996; rivers from Açor and Margaraça Mountains), Graça et al. (2001; Lousã mountain and Ceira basin). We also used unpublished material collected by (a) Carvalho and Coimbra in several rivers at the Lousã and Caramulo Mountains, and (b) Oliveira and Coimbra at the upper Mondego River and several tributaries.

Samples of invertebrates were generally collected with a hand net 0.3 × 0.3 aperture and 0.5 mm mesh size. When rigorous quantitative sampling was necessary (and possible) Surber samples were collected (same dimensions as hand net). At each sampling site, in general, six sample replicates were collected at the major macrohabitats, covering each a distance of approximately 1 meter. Samples were transported to the laboratory and the specimens sorted alive. Alternatively, samples were preserved in the field with 4% formalin, and the invertebrates sorted after washing with tap running water. For preservation we used 70% ethanol. Identification was done to the level of species (e.g. some Plecoptera) or, more frequently to genus (e.g., Coleoptera), but in many cases specimens were only identified to family (e.g. Diptera) or to sub-family (e.g. Chironomidae) levels.

Invertebrate sampling was generally accompanied of water sampling for chemical analysis. Several physical parameters of streams and rivers were also measured. In order to understand the distribution of macroinvertebrate taxa, a table was produced relating the 3 more abundant taxa with the environmental stream characteristics. For each biological sample, the proportion of shredders (i.e. invertebrates feeding on decaying leaves) was computed, related with the stream order and compared with the previsions of the river continuum model. Finally, for each invertebrate collection, a biotic index was computed: the BMWP' adapted for the Iberian Peninsula (Alba-Tercedor 1996, Alba-Tercedor and Sanchéz-Ortega 1988) in order document water quality.

Results

We analysed more than 30 stream sites sampled once to four times. Most of the sites corresponded to low order (≤ 4), narrow rivers (width < 5m; Table 1). In terms of discharge and width, the larger rivers were Alva, Soure and Ceira. In general, those were also the rivers with higher water velocities. The Mondego River was not sampled in a correspondent larger section. All rivers had high content of dissolved oxygen, probably as a result of the continuous flow and/or lack of strong organic load. However, in terms of water chemistry, there was a high variation with pH values ranging from acidic (pH < 5) to basic (pH > 8) and conductivity ranging from < 30 µS to > 300 µS (Table 2). Nutrient content was generally low, except in some sites were the higher values denote organic pollution (Table 2).

In general, the number of invertebrate families was low in larger rivers, when compared with streams of low to intermediate order (≤ 5; Table 3). As an example, along a longitudinal transept, the maximum number of invertebrate families sampled in

Table 1. Selected physical characteristics of the studied stream / river sites. Subst.= mean size of the dominant substrate particles

Stream / River	Subst. (cm)	Width (m)	Depth (m)	Current ($m\ s^{-1}$)	Flow ($m^3\ s^{-1} \times 10^{-3}$)	Temp. (°C)	O_2 $mg\ L^{-1}$	Source
Rib. da Fonte de Espinho	19	0.4-1.7	0.1-0.2	0.13-0.22	9-49	8-13	9.3-9.5	Graça et al. 2001
Rib. do Cabeço	16	0.5-1.1	0.1	0.10-0.17	9-11	8-13	9.5-9.6	Graça et al. 2001
Rib. do Candal	17	1.3-1.6	0.1	0.27-0.29	48-52	8-14	9.5-9.6	Graça et al. 2001
Rib. de Cerdeira	21	0.6-0.6	0.2	0.44-0.46	38-58	8-15	9.4-9.5	Graça et al. 2001
Rib. Pé da Lomba	>30	0.3-0.7	0.1-0.2	0.10-0.11	3-16	9-15	9.5-9.7	Graça et al. 2001
Rib. de Espinho (Cadaixo)	12	2.1-2.2	0.1	0.39-0.41	94-114	11-18	10.1	Graça et al. 2001
Rib. da Sra da Piedade (Pereira)	16	0.9-2.3	0.1-0.3	0.13-0.45	59-75	10-18	10.2-10.5	Graça et al. 2001
Rib. da Avessada	8	1.2-1.2	0.1	0.31-0.55	39-40	11-18	9.5-9.8	Graça et al. 2001
Rib. de S. João (Lousã)	10	1.5-6.8	0.2-0.4	0.07-0.22	58-200	9-17	9.9-10.2	Graça et al. 2001
Rio Sotão (Ponte do Seladinho)	>30	0.7-11.1	0.3-0.4	0.07-0.46	94-295	9-18	9.8-10.2	Graça et al. 2001
Rio Ceira (Serpins)	18	17	0.2	0.38	1058	10	10.5	Graça et al. 2001
Rio Ceira (Azenha)	14	12.5-11.7	0.2-0.3	0.70-0.79	1888-2773	10-23	9.4-10.8	Graça et al. 2001
Rio Soure 1 (Alberg. dos Doze)	5	2	0.3	0.48	290	14-16	9.4-11.3	Graça et al. 1989
Rio Soure 2 (Vermoil)	15	7	0.3	0.25	380	16-22	9.3-11.9	Graça et al. 1989
Rio Soure 3 (Almagreira)	10	9	0.3	0.80	1780	6-25	9.8-10.1	Graça et al. 1989
Rio Soure 4 (V.N. de Anços)	<1	15	0.7	0.49	4730	17-27	9.6-10.0	Graça et al. 1989
Rio Alva 1 (Sabugueiro)	>30	5	0.2-0.5	0.30-1.60	100-5200	9-12	8.5-11.4	Graça et al. 1989
Rio Alva 2 (Sandomil)	10	13	0.3-0.9	0.20-1.05	500-12300	1-14	8.6-12.4	Graça et al. 1989
Rio Alva 3 (Coja)	9	15	0.6-1.1	0.40-1.40	3600-20600	3-21	8.8-12.4	Graça et al. 1989
Rio Alva 4 (Pombeiro da Beira)	2	14	0.8-1.0	0.10-0.50	1000-7500	4-26	9.0-12.2	Graça et al. 1989
Rio Alva 5 (Foz do Alva)	<1	28	0.5-1.6	0.90-1.20	16200-27400	4-27	9.1-12.3	Graça et al. 1989
Rio Ceira (Foz de Arouce)	7	-	0.3	1.00	-	16.5	7.9	Oliveira & Coimbra unp.
Rio Ceira (Vendas de Ceira)	8	-	0.4	0.60	-	15.9	7.4	Oliveira & Coimbra unp.
Rio Dueça (Sobral)	5	-	0.3	0.43	-	16.0	8.6	Oliveira & Coimbra unp.
Rio Dueça (Tremôa)	5	-	0.4	0.80	-	15.8	7.7	Oliveira & Coimbra unp.
Rio Alva (Coja)	8	-	0.5	0.22	-	5.2	9.9	Oliveira & Coimbra unp.
Rio Alva (Sabugueiro)	19	-	0.5	0.14	-	6.8	10.5	Oliveira & Coimbra unp.
Rio Mondego (Trinta)	13	-	0.4	0.34	-	4.6	11.5	Oliveira & Coimbra unp.
Rio Mondego (P. da Rainha)	-	-	-	-	-	7.6	9.4	Oliveira & Coimbra unp.
R. da Mata da Margaraça 1	7	-	-	0.24-0.62	5-15	10.0-13.6	10.4-14.1	Abelho & Graça 1996
R. da Mata da Margaraça 2	6	-	-	0.15-0.32	4-28	9.0-14.2	10.3-17.1	Abelho & Graça 1996
R. da Fraga da Pena	6	-	-	0.10-0.67	21-71	10.0-16.0	10.2-16.3	Abelho & Graça 1996
R. da Mata da Margaraça 1	7	-	-	0.24-0.62	5-15	10.0-13.6	10.4-14.1	Abelho & Graça 1996
R. da Mata da Margaraça 2	6	-	-	0.15-0.32	4-28	9.0-14.2	10.3-17.1	Abelho & Graça 1996
R. da Fraga da Pena	6	-	-	0.10-0.67	21-71	10.0-16.0	10.2-16.3	Abelho & Graça 1996
R. Sótão (Ponte do Seladinho)	7-8	6-9	0.2-0.4	0.11-0.34	346-392	13.6-14.1	10.8-11.0	Carvalho & Coimbra unp.
R. S. João (Lousã)	9-10	5-8	0.2-0.4	0.01-0.10	24-91	14.2-15.7	9.6-9.8	Carvalho & Coimbra unp.

Table 2. Water chemistry of the studied stream / river sites.

Stream / River	pH	Cond. µS cm^{-1}	Alk mg L^{-1}	NO_3 mg L^{-1}	NO_2 µg L^{-1}	PO_4 mg L^{-1}	Source
Rib. da Fonte de Espinho	5.9-6.4	34-35	2.8-3.3	1.35-2.66	<2	2	Graça et al. 2001
Rib. do Cabeço	5.8-6.4	27-28	2.3-3.4	0.44-0.53	<2	4-10	Graça et al. 2001
Rib. do Candal	6.2-6.5	31-33	4.2-4.6	0.09-1.32	<2	7-21	Graça et al. 2001
Rib de Cerdeira	6.2-6.7	36-40	5.2-7.3	0.50-0.52	<2	4-11	Graça et al. 2001
Rib. Pé da Lomba	6.4-7.0	51-53	9.1-9.5	1.52-1.66	<2-4	7-15	Graça et al. 2001
Rib de Espinho (Cadaixo)	6.5-7.1	67-83	9.7-13.3	3.41-7.51	3-4	10-34	Graça et al. 2001
Rib. da Sra da Piedade (Pereira)	6.3-6.7	44-46	5.3-6.3	1.34-2.09	<2-3	4-5	Graça et al. 2001
Rib. da Avessada	6.6-7.2	100-112	11.0-13.6	3.16-3.32	<2-5	3-19	Graça et al. 2001
Rib. de S João (Lousã)	6.3-6.9	45	5.1-7.6	1.67-1.86	2	6-16	Graça et al. 2001
Rio Sotão (Ponte do Seladinho)	6.0-6.6	33-34	3.7-5.9	1.94-2.13	<2-2	3-13	Graça et al. 2001
Rio Ceira (Serpins)	6.3	65	7.9-13.0	5.62	15	12	Graça et al. 2001
Rio Ceira (Azenha)	6.6-7.1	79-92	13.9	4.40-6.68	17-25	7-21	Graça et al. 2001
Rio Soure 1 (Alberg. dos Doze)	6.6-7.8	75-205	22-61	-	-	-	Graça et al. 1989
Rio Soure 2 (Vermoil)	7.6-7.9	117-335	69-145	-	-	-	Graça et al. 1989
Rio Soure 3 (Almagreira)	7.6-8.1	129-420	79-205	-	-	-	Graça et al. 1989
Rio Soure 4 (V.N. de Anços)	7.8-8.2	210-440	96-235	-	-	-	Graça et al. 1989
Rio Alva 1 (Sabugueiro)	5.0-5.8	9-15	1-2	-	-	-	Graça et al. 1989
Rio Alva 2 (Sandomil)	4.8-6.1	10-15	2-4	-	-	-	Graça et al. 1989
Rio Alva 3 (Coja)	5.2-6.3	12-23	2-7	-	-	-	Graça et al. 1989
Rio Alva 4 (Pombeiro da Beira)	5.4-6.5	15-39	2-9	-	-	-	Graça et al. 1989
Rio Alva 5 (Foz do Alva)	5.9-6.9	17-78	3-10	-	-	-	Graça et al. 1989
Rio Ceira (Foz de Arouce)	-	72	14	6.3	10	142	Oliveira & Coimbra unp
Rio Ceira (Vendas de Ceira)	-	-	6	5.6	15	157	Oliveira & Coimbra unp
Rio Dueça (Sobral)	-	355	-	-	-	-	Oliveira & Coimbra unp
Rio Dueça (Tremôa)	-	356	55	10.0	99	398	Oliveira & Coimbra unp.
Rio Alva (Coja)	-	37	9	1.4	5	10	Oliveira & Coimbra unp.
Rio Alva (Sabugueiro)	-	19	8	1.2	3	35	Oliveira & Coimbra unp.
Rio Mondego (Trinta)	-	22	7	2.2	3	64	Oliveira & Coimbra unp.
Rio Mondego (P. da Rainha)	-	76	16	3.7	15	18	Oliveira & Coimbra unp.
R. da Mata da Margaraça 1	6.3-6.8	40-50	20-24	-	-	-	Abelho & Graça 1996
R. da Mata da Margaraça 2	6.1-6.5	30-58	17-23	-	-	-	Abelho & Graça 1996
R. da Fraga da Pena	6.2-7.2	20-45	9-11	-	-	-	Abelho & Graça 1996
R. Sótão (Ponte do Seladinho)	7.1-7.3	23-38	-	0.09-0.14	3-7	9-10	Carvalho & Coimbra unp.
R. S. João (Lousã)	6.1-6.2	44-59	-	0.16-0.23	<2-2	7-10	Carvalho & Coimbra unp

the Alva River decreased from 27 in the uppermost site to 21, 18, and 16 just before the confluence with the Mondego. Low number of families were also observed in the Ceira (8-11) and in the Mondego itself (18-19), when compared with low order streams (generally > 20 taxa; Table 3).

In low order streams, shredders were the dominant feeding group in terms of percentage of taxa or percentage of total invertebrate AFDM (Tables 4 and 5). The importance of shredders tended to decrease downstream. Patterns of seasonal (summer vs. winter) variation of shredders differed among rivers. In the Lousã streams, the variation was low. However, in the Alva River the shredder taxa varied from 0% to 37% (Graça et al. 1989). The longitudinal and seasonal variation in shredders was in agreement with the River Continuum (Vannote et al. 1980).

In terms of water quality, as judged by the application of the biotic index BMWP', the rivers and streams from the Lousã mountain can be considered unpolluted (Table 3). Scores above 200 were recorded at ribeira do Espinho (Cadaixo) and Ribeira da Sra. da Piedade (Pereira). According to data from 1989, the Soure River had an intermediate situation. In some cases it revealed clean conditions, whereas in other occasions it revealed pollution stress. At Soure 4 (V. N. Anços) the low score of the index revealed a clear pollution stress. The situation was similar for the Alva River. However, the lower Alva River had a predominance of a sandy substrate and it has frequently demonstrated that this substrate is unfavourable for invertebrate colonisation and therefore, the low score could reflect, in this case, not only a decrease in water quality, but also the substrate characteristics.

Water quality in the Ceira rivers varied from good – acceptable (BMWP' = 122-152) to bad (BMWP' = 48 and 75). The same occurred in the Mondego River (BMWP' = 89-114). In general, in the Mondego River there are streams with a clear high quality of waters and severely polluted sites.

Discussion

Higher taxa richness was observed in low order streams (≤ 4) when compared with intermediate / larger rivers (≥ 5). This pattern was postulated by the river continuum concept (Vannote et al. 1980), based on the spatial and temporal environmental heterogeneity of intermediate rivers. Although we measured richness in terms of number of families, some studies have shown a strong correlation between the number of species and the number of higher taxonomic groups (e.g. Graça et al. 1995, Chessman 1995).

As predicted by the river continuum concept, shredders were the dominant group in the low order streams but decreased downstream. This can be explained by the expected differences in the amount of coarse particulate organic matter. However, the differences in seasonal changes are more challenging. Although litter inputs are higher in autumn than in other seasons (Abelho and Graça 1998), organic matter accumulation in the stream bed may not be correlated with litterfall due the hydrologic regime and the occurrence of spates causing an increased transport of benthic organic

Table 3. Biological parameters and water quality according with the BMWP' index for several stream / river sites in the Mondego river basin.

Stream / River	Order	Nº Families	BMWP'	Year	Representative Families	Source
Rib. da Fonte de Espinho	2	14-23	112-160	1998/1999	Leuctridae, Nemouridae, Chironomidae	Graça et al. 2001
Rib. do Cabeço	2	18-26	125-159	1998/1999	Leuctridae, Chironomidae, Nemouridae	Graça et al. 2001
Rib. do Candal	3	27-28	181-185	1998/1999	Leuctridae, Goeridae, Chironomidae	Graça et al. 2001
Rib. de Cerdeira	3	27-35	168-237	1998/1999	Leuctridae, Chironomidae, Hydropsychidae	Graça et al. 2001
Rib. Pé da Lomba	3	19-27	109-175	1998/1999	Chironomidae, Sphaeridae, Sericostomatidae	Graça et al. 2001
Rib. de Espinho (Cadaixo)	4	30-33	229-240	1998/1999	Chironomidae, Sphaeridae, Limnephilidae	Graça et al. 2001
Rib. da Sra da Piedade (Pereira)	4	34-42	210-270	1998/1999	Baetidae, Elmidae, Tricladida	Graça et al. 2001
Rib. da Avessada	4	33-34	188-212	1998/1999	Chironomidae, Baetidae, Limnephilidae	Graça et al. 2001
Rib. de S. João (Lousã)	5	27-29	166-181	1998/1999	Chironomidae, Hydropsychidae, Leuctridae	Graça et al. 2001
Rio Sotão (Ponte do Seladinho)	5	30-33	180-242	1998/1999	Baetidae, Chironomidae, Leuctridae	Graça et al. 2001
Rio Ceira (Serpins)	6	24	152	1998/1999	Baetidae, Simuliidae, Philopotamidae	Graça et al. 2001
Rio Ceira (Azenha)	6	21-22	122-127	1998/1999	Hydropsychidae, Philopotamidae, Baetidae	Graça et al. 2001
Rio Soure 1 (Alberg. dos Doze)	-	14-22	80-108	1984/1985	*Potamopyrgus*, *Baetis*, *Caenis*	Graça et al. 1989
Rio Soure 2 (Vermoil)	-	18-25	88-159	1984/1985	*Potamopyrgus*, Chironomidae, *Baetis*	Graça et al. 1989
Rio Soure 3 (Almagreira)	-	15-24	61-101	1984/1985	Simuliidae, *Baetis*, Chironomidae	Graça et al. 1989
Rio Soure 4 (V.N. de Anços)	-	8-18	35-82	1984/1985	Chironomidae, Hydracarina, Simuliidae	Graça et al. 1989
Rio Alva 1 (Sabugueiro)	-	15-27	93-150	1984/1985	*Leuctra*, *Amphinemura*, *Hydropsyche*	Graça et al. 1989
Rio Alva 2 (Sandomil)	-	14-21	76-124	1984/1985	Chironomidae, *Baetis*, *Leuctra*	Graça et al. 1989
Rio Alva 3 (Coja)	-	14-21	82-121	1984/1985	Hydracarina, *Hydropsyche*, Chironomidae	Graça et al. 1989
Rio Alva 4 (Pombeiro da Beira)	-	12-18	75-96	1984/1985	*Caenis*, *Baetis*, Chironomidae	Graça et al. 1989
Rio Alva 5 (Foz do Alva)	-	7-16	37-98	1984/1985	*Baetis*, *Choroterpes*, Simuliidae	Graça et al. 1989
Rio Ceira (Foz de Arouce)	-	15	75	1999	*Baetis*, *Potamopyrgus*, *Chimarra*	Oliveira & Coimbra unp.
Rio Ceira (Vendas de Ceira)	-	8	48	1999	Lumbriculidae, *Hydropsyche*, *Boyeria*	Oliveira & Coimbra unp.
Rio Dueça (Sobral)	-	11	51	1999	*Baetis*, *Atyaephyra*, Simuliidae	Oliveira & Coimbra unp.
Rio Dueça (Tremôa)	-	15	60	1999	*Atyaephyra*, Lumbriculidae, *Caenis*	Oliveira & Coimbra unp.
Rio Alva (Coja)	-	9	48	1999	*Ephemerella*, *Baetis*, *Nemoura*	Oliveira & Coimbra unp.
Rio Alva (Sabugueiro)	-	14	81	1999	*Leuctra*, *Baetis*, Limnephilidae	Oliveira & Coimbra unp.
Rio Mondego (Trinta)	-	19	114	1999	Simuliidae, *Sericostoma*, *Baetis*	Oliveira & Coimbra unp.
Rio Mondego (P. da Rainha)	-	18	89	1999	Chironomidae, *Caenis*, *Baetis*	Oliveira & Coimbra unp.
R. da Mata da Margaraça 1	1	32-35	187-198	1991/1992	Chironomidae, *Leuctra*, Naididae	Abelho & Graça 1996
R. da Mata da Margaraça 2	3	31-34	201-216	1991/1992	*Leuctra*, Chironomidae, *Baetis*	Abelho & Graça 1996
R. da Fraga da Pena	3	28-29	181-187	1991/1992	*Leuctra*, Chironomidae, *Ephemerella*	Abelho & Graça 1996
R. Sótão (Ponte do Seladinho)	5	31-36	208-225	1996	Chironomidae, *Baetis*, *Caenis*	Carvalho & Coimbra unp.
R. S. João (Lousã)	5	39-48	242-294	1996	Chironomidae, *Baetis*, *Leuctra*	Carvalho & Coimbra unp.

Table 4 - Percentage of taxa classified as shredders in several studied sites of the Mondego basin

Name	Order	% of shredder taxa	Source
Rio Soure 2 (Vermoil)		0-3	Graça et al. 1989
Rio Soure 3 (Almagreira)		0	Graça et al. 1989
Rio Soure 4 (V. N. de Anços)		0-1	Graça et al. 1989
Rio Alva 1 (Sabugueiro)		9-33	Graça et al. 1989
Rio Alva 2 (Sandomil)		2-35	Graça et al. 1989
Rio Alva 3 (Coja)		0-37	Graça et al. 1989
Rio Alva 4 (Pombeiro da Beira)		1-21	Graça et al. 1989
Rio Alva 5 (Foz do Alva)		0-20	Graça et al. 1989
Rio Ceira (Foz de Arouce)		15	Oliveira & Coimbra unp.
Rio Ceira (Vendas de Ceira)		8	Oliveira & Coimbra unp.
Rio Dueça (Sobral)		11	Oliveira & Coimbra unp.
Rio Dueça (Tremôa)		15	Oliveira & Coimbra unp.
Rio Alva (Coja)		9	Oliveira & Coimbra unp.
Rio Alva (Sabugueiro)		14	Oliveira & Coimbra unp.
Rio Mondego (Trinta)		19	Oliveira & Coimbra unp.
Rio Mondego (P. da Rainha)		18	Oliveira & Coimbra unp.
R. da Mata da Margaraça 1	1	8-55	Abelho & Graça 1996
R. da Mata da Margaraça 2	3	19-35	Abelho & Graça 1996
R. da Fraga da Pena	3	28-37	Abelho & Graça 1996
R. Sótão (Ponte do Seladinho)	5	4-5	Carvalho & Coimbra unp.
R. S. João (Lousã)	5	6-9	Carvalho & Coimbra unp.

Table 5. Percentage of shredders in terms of biomass m^{-2} at 12 sites ranging from 2nd to 6th order (Lousã region) (after Graça et al. 2001)

Stream order	Shredder density (μg AFDM m^{-2})	% AFDM of shredder taxa
2	65	45
3	138	41-44
4	92	20-21
5	17	21-24
6	8	2

matter (Abelho and Graça 1998). Streams and rivers may differ in their retentiveness and hydrologic regime and these differences may condition the seasonal abundance of shredders. This relationship was not yet tested in our stream systems.

Some streams and small rivers had a high water quality, according to the application of biotic index. The BMWP' was adapted for the Iberian Peninsula, but based in studies carried out in Spain. Indices developed for a particular area have been applied, apparently with success, in other geographical areas (e.g. the BBI, developed for Belgium, applied in Portugal, Indonesia, Canada and other areas: Fontoura and Moura 1984, Krystiano and Kusjantono 1991, Barton and Metcalfe-Smith 1992, De Pauw et al. 1986). However, before the application of the indices developed for other areas, it is necessary first to test them according with the local ecological, hydrologic and geological conditions (e.g. Coimbra and Graça 1998, Graça and Coimbra 1998). The results of the application of the BMWP' were consistent with the chemical information of the studied sites and therefore, the index is likely to be a useful tool for the water quality assessment in Central Portugal.

For rapid biological monitoring proposals, several studies have shown that aquatic invertebrates identified to the family level are good indicators of water quality. (Graça et al. 1995) showed that reducing the identification of invertebrates to the family level instead of species / genus level saved 50% of identification effort with no information loss in terms of water quality. The BMWP score system used to evaluate water quality in British rivers (Armitage et al. 1983) rely on families of aquatic invertebrates. Hughes (1978) showed that the diversity (H') of species, genus, family and orders where highly correlate. Osborne et al. (1980) analysing macroinvertebrate samples identified to species, genus and family levels from contaminated and clean sites showed that identification to family level was sufficed to detect inter-site diversity differences.

Invertebrates in streams and rivers fill numerous ecological niches, feed on aquatic producers and on allochthonous organic matter and serve as food to other aquatic and terrestrial / flying organisms. They are therefore an important energetic component transferring energy and material from the producers and the detritus pool to upper levels in the food chains. Aquatic invertebrates have also been used to test numerous ecological theories (e.g. Allan 1984).

The information here provided resulted from several independent and small-scale investigations. We suggest that macroinvertebrates and environmental conditions of a larger set of clean sampling sites should be investigated in order to provide reference conditions to which impacted or potentially impacted areas could be compared for the propose of water quality evaluation.

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FACTORS AFFECTING THE DISTRIBUTION OF FISH COMMUNITIES IN THE RIVER MONDEGO AND MAIN TRIBUTARIES

Abstract

Fish assemblage structure and factors affecting the distribution of fish communities were examined for 23 sites in the River Mondego basin during the summer of 1998. The cyprinids *Rutilus macrolepidotus*, *Barbus bocagei*, *Chondrostoma polylepis*, *Rutilus alburnoides*, *Leuciscus carolitertii* and *Gobio gobio* were the most abundant species among the 25 *taxa* forming the freshwater fish community. Species richness was generally higher in impoundments and in the watershed downstream from the Açude-Ponte dam, at Coimbra. The anadromous species were restricted to the area downstream from the Açude-Ponte, except for a landlocked population of *Alosa alosa* in the Aguieira reservoir. In the river stretches less affected by the construction of dams, the two main factors responsible for the structure of fish communities were the altitude and the distance from the source. *R. macrolepidotus* and *R. alburnoides* were dominant closer to the source, at lower altitudes, whereas *L. carolitertii* dominated in upper reaches. As the distance from the source increases and the altitude decreases, the fish communities become dominated by *B. bocagei* and *C. polylepis*, larger fishes that take advantage of the increase in river depth.

Introduction

The available information on the ecology of freshwater fish is considerably less than that which exists for brackish and marine species in Portugal. This is a consequence of the poor economic and commercial interest that lies in freshwater fish, except for the diadromous species.

Very little research work has been published on the fish community of the River Mondego basin and most of the studies were conducted on the estuary (Jorge and

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Sobral 1989, Guimarães 1990, Jorge 1991, Ribeiro 1991a, 1991b, Domingos 1992, Ribeiro and Gonçalves 1993). The work developed on the freshwater zone was either restricted to genetical problems (Collares-Pereira 1983, Coelho 1987, Alexandrino 1996) or to one species in particular as it is the case of *Gambusia holbrooki* (Cabral et al. 1998, Cabral and Marques 1999) and *Petromyzon marinus* (Almeida et al. 2000). An ecological approach to the freshwater fish community has recently started to take place (Domingos et al. 1999).

With the recent construction of dams and weirs in the Mondego river basin, several environmental impact studies have been done, but most of these are general reports and, although gathering some information about the fish fauna of those specific river stretches, usually have little scientific value and never refer to the ecological aspects of the fish community as a whole.

The aim of this study was to describe the fish fauna occurring in the freshwater zone of the River Mondego basin, to perform a preliminary analysis on the distribution and abundance of the species present in this watershed, and to relate the structure and composition of the communities with some environmental parameters. Changes in the ecosystem due to anthropogenic modifications were also analysed.

Materials and Methods

A total of 23 sites on the freshwater zone of the River Mondego basin, including the main river, the most important tributaries and the major existing impounded areas were prospected (Fig. 1). All sites located on rivers or streams were sampled by electrofishing, but data for reservoirs were obtained by enquiries and/or bibliography.

Sampling took place during the summer of 1998 and for that reason site 7, a small stream, was dry during that period. For the choice of the sampling sites several aspects of river morphology namely: altitude, distance from the source and type of substrate, as well as size and location of dams were taken into consideration.

Electrofishing was carried out using a semi-portable apparatus (Hans Grassl EL 62, 600 V-DC, 10A). Upon capture, fish were identified to the species level, counted and released. Since the correct identification of *Lampetra fluviatilis* and *Lampetra planeri ammocoetes* required a detailed observation and the sacrifice of the animals, they were only identified to the genus level.

Data obtained from the electrofishing surveys were transformed into catch-per-unit-effort (cpue), defined as number of individuals sampled during a 30 minute fishing period.

An hierarchical grouping of the 23 sites, based on the presence/absence of fish species, was performed by program SPSS (version 9.0) using the Squared Euclidian Distance and the Ward linkage clustering method (Norusis 1999). A canonical correspondence analysis (CCA) (ter Braak and Smilauer 1998) was used to relate fish population parameters (structure and abundance) with the environmental variables (altitude, substrate type and distance from source).

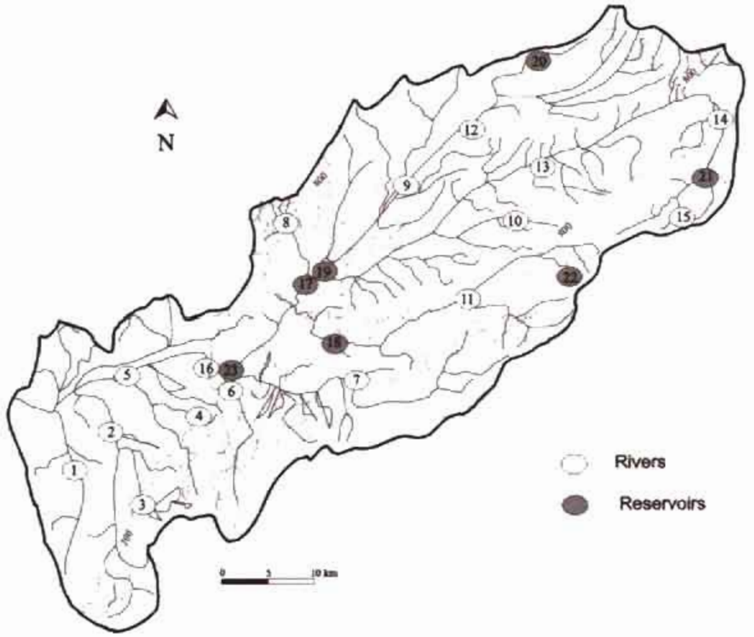


Figure 1. Location of sampling sites in the Mondego basin and corresponding altitude (m): 1 - river Pranto (4) ; 2 - river Arunca (7); 3 - river Anços (110); 4 - Alcabideque stream (179); 5 - river Mondego (6); 6 - river Corvo (103); 7 - river Sátão (247); 8 - Falheiros stream (214); 9 - river Dão (257); 10 - river Seia (401); 11 - river Alvôco (226); 12 - river Dão (168); 13 -river Mondego (300); 14 - river Mondego (420); 15 - Quêcere stream (846); 16 - Açude-Ponte dam (downstream) (25); 17 - Raiva dam (68); 18 - Fronhas dam (129); 19 - Aguieira dam (125); 20 - Fagilde dam (209); 21 - Caldeirão dam (502); 22 - Lagoa Comprida (1296); 23 - Açude-Ponte dam (upstream) (25). All sampling sites located on rivers or streams were sampled by electrofishing but the results for dams were obtained by enquiries and/or bibliography.

Results

Characterisation of the fish community

A total of 25 *taxa* of which eight are cyprinids, were recorded (Tab. 1). This family includes three Iberian endemisms *(Chondrostoma polylepis, Rutilus macrolepidotus* and *Leuciscus carolitertii)*, and three introduced species *(Carassius auratus, Cyprinus carpio* and *Gobio gobio)*. Besides these, there are four other exotic species: *Onchorhynchus mykiss*, especially in mountain areas, *Gambusia holbrooki*, and the centrarchids *Lepomis gibbosus* and *Micropterus salmoides* mainly in reservoirs where they can be the dominant species.

The analysis of fish general distribution shows that the most abundant species are the cyprinids, particularly *R. macrolepidotus, Barbus bocagei, C. polylepis* and *Rutilus alburnoides*, followed by *L. carolitertii* and *G. gobio*.

Table 1. Fish community in the Mondego watershed.

TAXA	ABBR	PHE	Abundance	BC	HD	PRDB
Family Petromizonidae						
Lampetra sp.	Lamp	-	●	-	-	-
Petromyzon marinus Linnaeus, 1758		AM	○	III	II	V
Family Clupeidae						
Alosa alosa (Linnaeus, 1758)		AM	○	III	II +V	V
Alosa fallax (Lacépède, 1803)		AM	○	III	II +V	V
Family Anguillidae						
Anguilla anguilla (Linnaeus, 1758)	Aang	CM	●	-	-	CT
Family Cyprinidae						
Barbus bocagei Steindachner, 1865	Bboc	F	●●●	III	V	NT
Carassius auratus (Linnaeus, 1758)	Caur	F(I)	●	-	-	-
Chondrostoma polylepis Steindachner, 1865	Cpol	F(Ib)	●●●	III	II	NT
Cyprinus carpio Linnaeus, 1758	Ccar	F(I)	○	-	-	-
Gobio gobio (Linnaeus, 1758)	Ggob	F(I)	●●	-	-	-
Leuciscus carolitertii Doadrio, 1988	Lcar	F(Ib)	●●	-	-	NT
Rutilus alburnoides (Steindachner, 1866)	Ralb	F	●●●	-	II	NT
Rutilus macrolepidotus (Steindachner, 1866)	Rmac	F(Ib)	●●●	III	II	I
Family Cobitidae						
Cobitis paludica (Debuen, 1930)	Cpal	F	●	-	-	NT
Family Gasterosteidae						
Gasterosteus aculeatus Linnaeus, 1758	Gacu	F	●	-	-	K
Family Centrarchidae						
Lepomis gibbosus (Linnaeus, 1758)	Lgib	F(I)	●	-	-	-
Micropterus salmoides (Lacépède, 1802)		F(I)	○	-	-	-
Family Poeciliidae						
Gambusia holbrooki Girard, 1859	Ghol	F(I)	●	-	-	-
Family Mugilidae						
Liza aurata (Risso, 1810)		E	○	-	-	-
Liza ramada (Risso, 1826)	Lram	CM	○	-	-	-
Mugil cephalus Linnaeus, 1758	Mcep	E	○	-	-	-
Family Atherinidae						
Atherina boyeri (Risso, 1810)		E	○	-	-	-
Family Pleuronectidae						
Platichthys flesus (Linnaeus, 1758)		E	○	-	-	CT
Family Salmonidae						
Onchorhynchus mykiss (Walbaum, 1792)		F(I)	○	-	-	-
Salmo trutta fario Linnaeus, 1758	Stru	F	●	-	-	NT

Abbreviation (ABBR) used in the correspondence canonical analysis. Phenology (PHE): F – freshwater; AM – anadromous migrator, CM – catadromous migrator, E – euryhaline, (I) – introduced, (Ib) – iberian endemism. (i) Electrofishing, (m) Bibliography and/or enquires. Levels of abundance (Very common ≥15 CPUE – (●●●), 5 CPUE ≤ Common <15 CPUE – (●●) and Rare <5 CPUE – (●) with CPUE being the number of individuals caught per 30 min of fishing per site) do not include impoundments. Conservation value – Bern Convention (BC), Habitats Directive (**HD**) and Portuguese Red Data Book of Vertebrates (**PRDB**): (**V**) – vulnerable, (**I**) –indeterminate, (**K**) – insufficiently known, (**CT**) – commercially threatened, (**NT**) – non threatened.

The migratory diadromous fish species identified were the anadromous *Petromyzon marinus*, *Alosa alosa*, and *Alosa fallax*, and the catadromous *Anguilla anguilla* and *Liza ramada*. The distribution of the anadromous fish species is restricted to the area downstream from the Açude-Ponte dam, at Coimbra, except for a landlocked population of allis shad (*A. alosa*) in the Aguieira reservoir. It is also downstream from the Açude-Ponte dam that euryhaline species such as *Platichthys flesus*, *Atherina boyeri* and some mugilids (*Liza aurata* and *Mugil cephalus*) occur.

At higher altitudes, the dominant species are the cyprinid *L. carolitertii* and the salmonids, especially *Salmo trutta*.

As for conservation value it should be stressed that the most threatened species are the diadromous fish, followed by *P. flesus*, *R. macrolepidotus* and *Gasterosteus aculeatus* (Vários 1991). Despite being considered, in their majority, as non threatened, except for *R. macrolepidotus*, the Iberian endemisms do also present an important conservation value which should be taken into consideration (Tab. 1).

A number of species of this fish community is included in the Annex III of the Bern Convention (Convention on the Conservation of European Wildlife and Natural Habitats) meaning that capture should be under restrictions. Some species are also included in the Annexes II and/or V of the Habitats Directive (92/43/CEE) (Tab. 1), requiring either the designation of special areas of conservation (for species included in the Annex II) or an exploitation which may be subject to management measures (for species included in the Annex V).

Hierarchical cluster analysis

The dendrogram constructed for the presence/absence data (Fig. 2) indicates a distinct grouping of the sites, clearly divided into two groups, *A* and *B*.

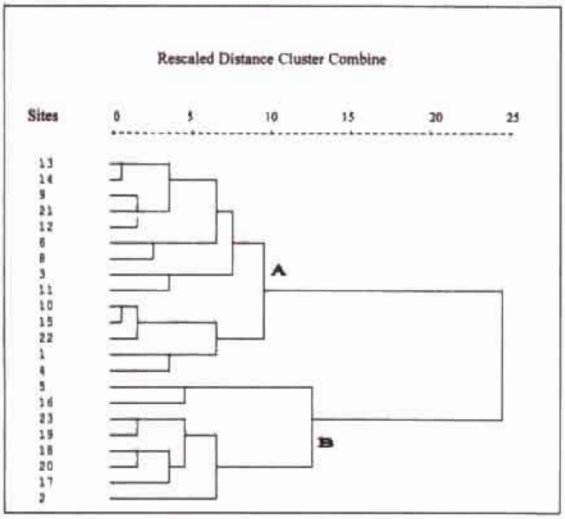


Figure 2. Dendrogram of the sampling sites, based on Squared Euclidian Distance and Ward linkage clustering. Names of the sampling sites are given in fig. 1.

Group A gathers the sites from the main river and tributaries upstream the Açude-Ponte dam at Coimbra which present a fish community with a lower species richness. Group B includes sites where a higher number of species is found, namely impounded areas, and the watershed downstream the Açude-Ponte dam, at Coimbra (Fig. 3). Considering a higher similarity level, each of the two previous groups can be divided into two different sub-groups. In group A, the sites with lower species richness (sites 1, 4, 10, 15 and 22) are separated from the rest of the group. This former sub-group includes mainly the sites located at higher altitudes except for site 1 (River Pranto) which despite of its low altitude presents an impoverished fish community.

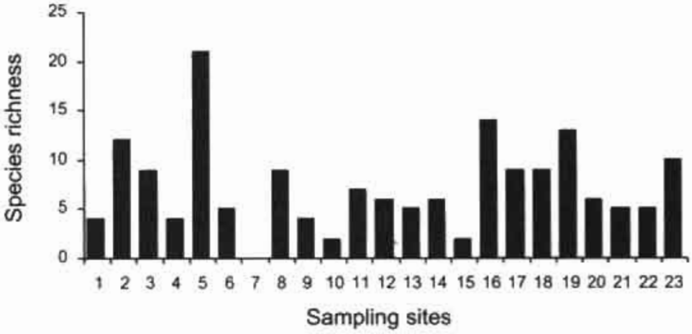


Figure 3. Species richness per sampling site.

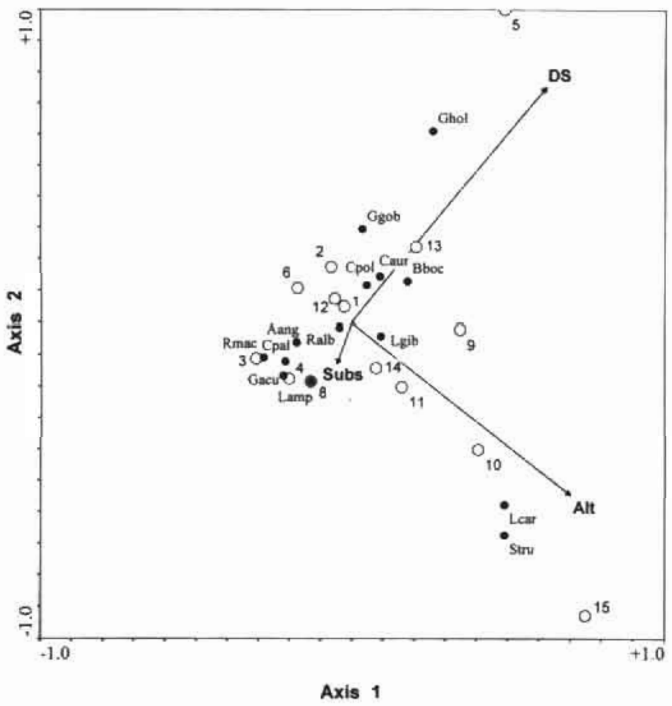
The same analysis applied to group B does also show evidence of two sub-groups. The first one grouping the two sites in the main river downstream from the Açude-Ponte dam (5 and 16) and the second including most of the impounded sites. Sampling site 2 (River Arunca), despite not being an impounded area, is also included in this sub-group, indicating a similar fish community.

Canonical Correspondence Analysis

The ordination diagram of the CCA (Fig. 4) explains 92% of the variance of species– environment relation. Table 2 shows that the variation in species composition in the first two axes is high ($\lambda_1 = 0.63$; $\lambda_2 = 0.51$) and that the environmental variables considered are highly related to the first two axes of the CCA, with the exception of substrate type, which is poorly related to the second axis.

The structure of the fish community in each sampling site is the result of the influence of two main environmental variables, the altitude and the distance from the source. The species *R. macrolepidotus*, *B. bocagei*, *C. polylepis*, *R. alburnoides*, *L. carolitertii* and *G. gobio* presented the highest weight in the CCA ordination diagram.

R. macrolepidotus and *R. alburnoides* dominate the communities closer to the sources located at lower altitudes (sites 3, 4 and 8). In the upper reaches, at higher altitudes (sites 10, 11, 14 and 15), *L. carolitertii* is the dominant species. As the distance from the source increases, at lower altitudes (sites 1, 2, 6 and 12) *B. bocagei* and *C.*



Subs - Substrate type; **Alt** - Altitude; **DS** - Distance to source

Figure 4. Species conditional triplot based on a CCA (canonical correspondence analysis) of the fish fauna from Mondego river basin. Sites are represented in fig. 1 and species abbreviations are given in tab. 1. The lenght of an arrow is relative to the importance of that environmental variable in the ordination.

Table 2. Results of the ordination by canonical correspondence analysis (CCA) of the River Mondego fishfauna data: eigenvalues, species-environment correlation coefficients, and intraset correlation of environmental variables with the first two canonical axes.

	Axis 1 ($\lambda_1 = 0.63$)	Axis 2 ($\lambda_2 = 0.51$)
Species – environment	0.90	0.88
Substrate	- 0.54*	0.15
Altitude	0.89*	- 0.73*
Distance from the source	0.56*	0.83*

(*) - $P < 0.05$; λ - eigenvalue *polylepis* are the species prevailing over the other fish species. In some of those sites (1 and 6) however, *G. gobio* can also be an abundant species. It is interesting to notice that this introduced species is the only exotic that can become almost dominating outside reservoirs.

Sampling sites 9 and 13, despite their medium altitude (257 m and 299 m, respectively), present fish communities very similar to those of the lower reaches, with great abundance of *B. bocagei* and *C. polylepis*, whereas sampling site 5 is clearly different from all the others, with a very poor community dominated by *G. gobio*.

Discussion

The fish fauna of the River Mondego, with 25 *taxa*, is slightly richer when compared with the 23 and 14 species, from rivers Vouga and Lis respectively (Domingos et al. 1999), and the 18 species from the Portuguese part of the River Guadiana watershed (Godinho et al. 1997).

The composition of the fish fauna in a river is diverse and, although a longitudinal pattern is usually expected along the river course (Lelek 1987, Schiemer and Zalewsky 1992, Cowx and Welcomme 1998), many factors, such as tributaries and presence of deteriorated habitats, mask or even change it in a way that the understanding of fish distribution becomes a difficult task. For example, dams divide rivers into more or less independent stretches and, for a given stretch, transverse interactions often dominate over the longitudinal ones (Decamps 1984).

In the river stretches less affected by the construction of dams, the two main factors responsible for the structure of fish communities are the altitude and the distance from the source. In areas close to the river source at a moderate altitude, the fish communities are dominated by *R. alburnoides* and *R. macrolepidotus*, but in the upper reaches the dominating species are *L. carolitertii* and the two salmonids, *O. mykiss* and *S. trutta*. As the distance from the source increases and the altitude decreases, the fish communities are dominated by *B. bocagei* and *C. polylepis*, larger fishes that take advantage of the increase in the river depth.

The presence of five diadromous fish species and three Iberian endemisms suggests that anthropogenic influences, namely the construction of dams and pollution levels have not yet been disastrous, and that in the River Mondego watershed there is an important potential in terms of conservation for this fish community. However, the existence of seven exotic species, and the fact that the anadromous species cannot pass the Açude-Ponte dam at Coimbra, which is equipped with a fish passage that does not function correctly (unpublished data), are indicative of the degradation of this ecosystem and of the reduction in the habitat available for those migratory species.

Other factors, such as pollution, are responsible for the reduction of the species richness, as can be concluded from the analysis of the dendrogram (Fig. 2), where sites 1 and 4, both polluted areas, mainly from agricultural practices, are linked quite close to each other.

The fact that some of the reservoirs have a relatively high species richness (Fig. 3), means that, in opposition to what has been happening in many reservoirs in our country, in the River Mondego watershed, probably due to the important tributaries that flow into the reservoirs, there are still conditions favourable to the maintenance of rheophilic species, such as *B. bocagei* and *C. polylepis*, and even a landlocked population of *A. alosa*. Despite all this, the fish communities in some of the Mondego impounded areas are dominated by introduced species such as *L. gibbosus*, *M. salmoides* and *C. carpio*.

One of the most serious impacts of the construction of dams is that they interrupt fish migration either by becoming impassable barriers to reproducers, or by decreasing the recruitment success. Before the construction of the Aguieira dam (1979), the diadromous fish migrated upstream reaching that area. After the construction of the Açude-Ponte dam, in 1981, the access of anadromous fish is limited to this point.

At the present situation, the most important stretch of the river, with a length of about 35 km, is located between Coimbra and Figueira da Foz, close to the river mouth and consequently an urgent conservation action is needed to promote the free circulation of diadromous fish up to the Raiva dam, as well as to implement measures for the protection of this part of the river. This protection could involve designating the river between Figueira da Foz and Raiva as a special area for conservation under the recommendations of the Habitats Directive since there are species from Annex II that would benefit by such an action.

If fish species are to be given adequate consideration, then appropriate management plans should be done in order to *(i)* make an inventory of the polluted areas and degradated communities, aiming at the rehabilitation of the aquatic ecosystem; *(ii)* increase the area available for diadromous fish, at least up to the Raiva dam, by introducing an effective fish passage in the Açude-Ponte; *(iii)* study the effect of river discharge on the fish community and make a careful regulation of discharge adjusted to biological needs of the several species along the year, similarly to what has been proposed by Almeida et al. (2000) for *P. marinus*, and Domingos (1992) for *A. anguilla;* and finally, (iv) carry out an active monitoring plan to be able to assess changes in the fish community.

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AMPHIBIAN POPULATIONS IN THE LOWER MONDEGO RIVER VALLEY: SPECIES OCCURRENCE AND DISTRIBUTION PATTERNS

Abstract

The lower Mondego River valley encompasses ca. 15,000 ha of fertile lowlands in central Portugal. Land-use is mostly agricultural. Projected infra-structural works aim to modify over 80% of the surface, including terrestrial and aquatic habitats, and may affect the occurrence and distribution of the local wildlife. We mapped the occurrence and distribution of amphibians in 1999. The following nine species were found: *Salamandra salamandra, Pleurodeles waltl, Triturus marmoratus, Discoglossus galganoi, Pelodytes ibericus, Bufo bufo, Hyla arborea, Hyla meridionalis* and *Rana perezi*. The highest species richness was found in the southern hills, adjacent to the valley. The core of the valley showed low amphibian diversity with only *Rana perezi* and *Hyla arborea* present.

Introduction

The lower Mondego River valley is located in the central part of Portugal. The valley is roughly east-west orientated and 40 km long (from the city of Coimbra to the estuary near Figueira da Foz, Fig. 1) and bordered by gently sloping hills. The floodplains cover ca. 15,000 ha of fertile land and have traditionally been used for agriculture. Currently, the main crops are rice and corn. Production is intensive. In consequence of the agricultural land-use, the floodplains form a monotonous landscape, with low habitat diversity. The most important places in terms of biodiversity are the numerous drainage ditches with, in some places, important settings of marginal and occasionally submerged aquatic vegetation. Many of these ditches are connected into a network, that, for some species, may function as corridors for dispersal (Andresen and Bóia 1999). The network is at places connected to the Mondego river with locally flourishing floral and faunal communities, underlining its importance (Andresen and Bóia 1999). The local nature reserves form part of the network also. The landscape of hills bordering the valley is structurally more complex, less intensively cultivated, with fair amounts of semi-natural

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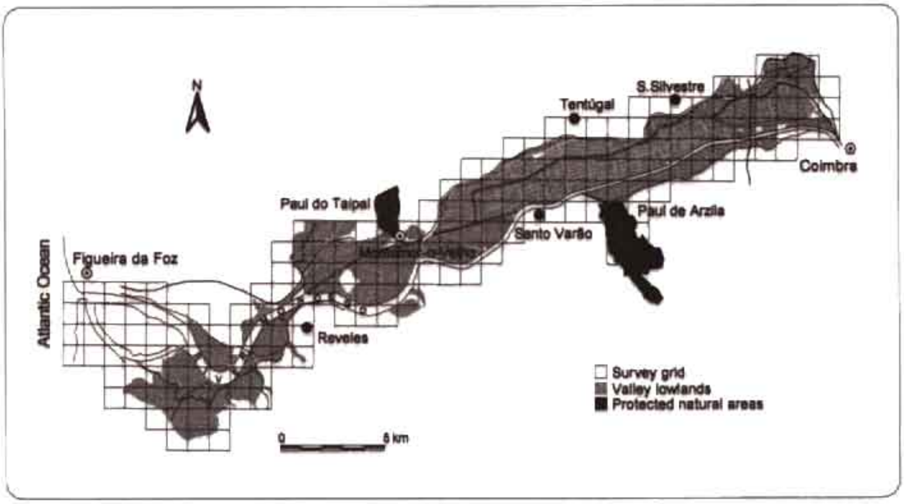

Fig. 1. Representation of the study area and survey grid used (1 km UTM squares).

vegetation and potentially presenting a mosaic of habitats suitable for amphibians. Altitude varies and with that, habitat types, including small forests, small agricultural parcels and a relatively high number of small water bodies with a more varied aquatic vegetation.

The near future will see the implementation of a re-allotment program in which the small agricultural parcels of the floodplains will be fused to form larger ones. The purpose of the project is to further increase the efficiency of production. The program will cover 67% of the surface and over 90% of the ditches (Andresen and Bóia 1999). These drainages, currently the main potential breeding sites for amphibians in the area, will either be transformed or disappear completely. Obviously, environmental changes of this magnitude will affect existing amphibian populations. Unfortunately, data on the distribution of amphibians in the lower Mondego River valley, such as in the national atlases (Crespo and Oliveira 1989, Malkmus 1995, Godinho et al. 1999) are limited – numerically, and also in terms of resolution. Detailed data are available for two of the protected areas of the valley only, namely Paul de Arzila and Paul do Taipal (Anastácio and Amaro 1989a, 1989b, Ferrand de Almeida et al. 1983, 1984, Ferrand de Almeida 1986, 1991, Ferreira et al. 1993). In order to be able to assess the response of amphibians to the imminent habitat change, it is important to now gather basic information on their distribution.

Materials and Methods

The study area was defined as the lowlands of the lower Mondego River valley, up to and including the slopes of surrounding hills and excluding the subsidiary rivers' basins. Surveys were made on the basis of the 1x1 km UTM grid system, marked on

1:25,000 military maps (Instituto Geográfico do Exército). The study area encompassed 192 grid cells. Each square was visited once over the period March – December 1999. The different surveyed areas of the valley were randomly visited during the study period. Every site of interest was searched. Special attention was given, during the breeding season from March to July, to the relatively few water bodies suitable for amphibian reproduction.

Water bodies were searched with the help of a dip net with a 4-mm mesh size, which allows detection of all stages of newts, frogs and toads (Griffiths and Raper 1994). Additionally, we searched for eggs that can be identified to the species, such as those of *Triturus marmoratus*, *T. boscai* and *Bufo bufo*. Larvae were identified following the tables in Arnold and Burton (1978) and Barbadillo (1987). Potential terrestrial habitats were searched, including the leaves and branches of small bushes, that may be used by *Hyla arborea* and *H. meridionalis* (Barbadillo 1987, Crespo and Oliveira 1989). Night searches (n=7) distributed across the valley were carried out during warm and humid nights and following rain, aiming for *Bufo bufo* and *B. calamita* in particular (Denton and Beebee 1992). Call recordings were also used as a survey tool, in particular for *Hyla arborea* and *Rana perezi* (Márquez and Matheu 1998).

Preliminary data vouch for *P. clarkii* (Girard 1852), an allochtonous crustacean, as an important predator on some amphibian species (J. W. Arntzen, pers. comm.). Its presence was surveyed from live individuals and, indirectly, from legs, carapaces and burrows.

Environmental data recorded included geographic location, weather conditions and a brief site description and its surroundings (e.g. nature of the habitat such as pond, lake, river, ditch or otherwise, vegetation, distance from water etc.). All biological material handled during the survey was released following identification. In face of the difficulty to obtain accurate population density estimates (Griffiths and Raper 1994) analyses were restricted to presence-absence data. Selected information is included in a Geographic Information System database of the Mondego valley, under the responsibility of I. C. N.

Results

Survey

The following nine species of amphibians were found: *Pleurodeles waltl*, *Salamandra salamandra* and *Triturus marmoratus* (order Urodela); *Discoglossus galganoi*, *Pelodytes ibericus*, *Bufo bufo*, *Hyla arborea*, *Hyla meridionalis*, and *Rana perezi* (order Anura). For further nomenclature see Table 1. All observations are in Figs 2 – 10, arranged according to UTM grid cells. *Rana perezi* and *Hyla arborea* were found across the valley, thus showing a more or less even distribution. The other seven species were found in the southern rim of the valley only. These differing distribution patterns are illustrated in Fig. 11. Fig. 12 shows amphibian species richness of the Mondego valley. Finally, the distribution of *P. clarkii* is shown in Fig. 13.

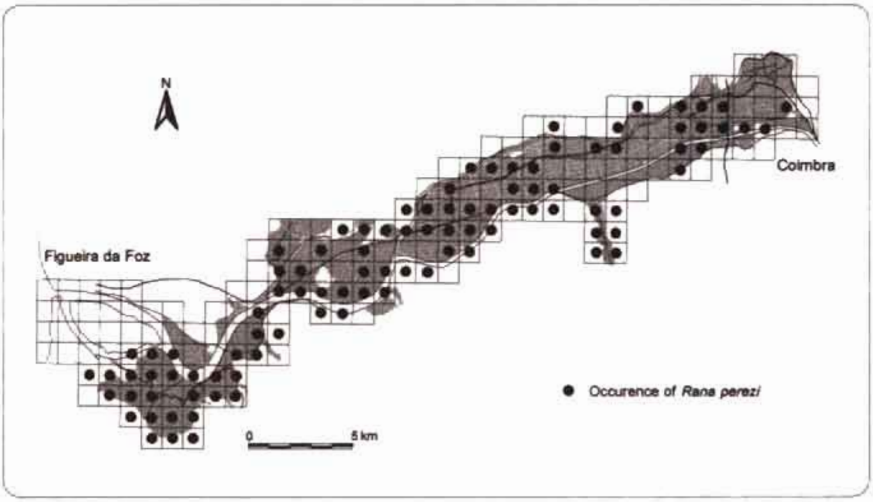

Fig. 2. Distribution of *Rana perezi* in the lower Mondego River valley.

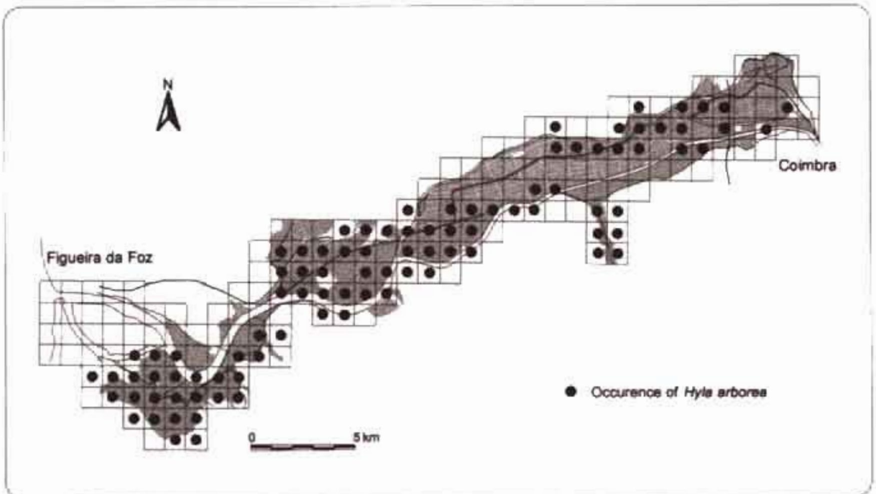

Fig. 3. Distribution of *Hyla arborea* in the lower Mondego River valley.

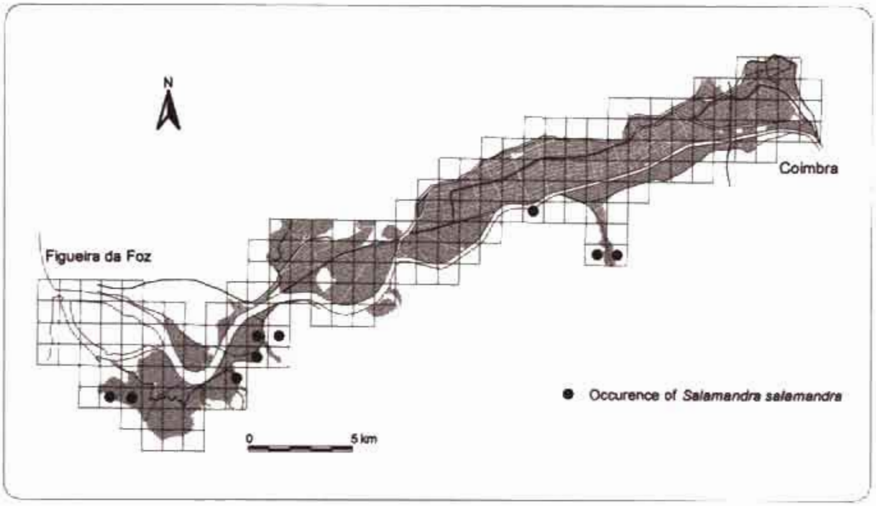

Fig. 4. Distribution of *Salamandra salamandra* in the lower Mondego River valley.

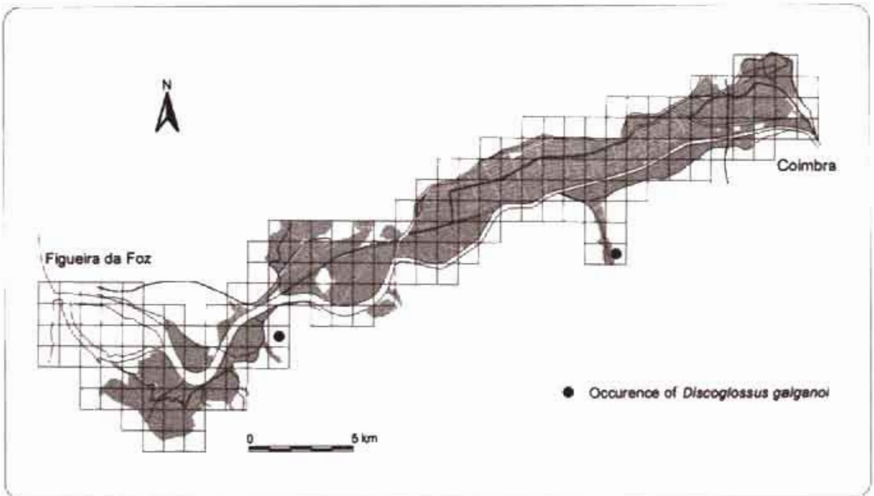

Fig. 5. Distribution of *Discoglossus galganoi* in the lower Mondego River valley.

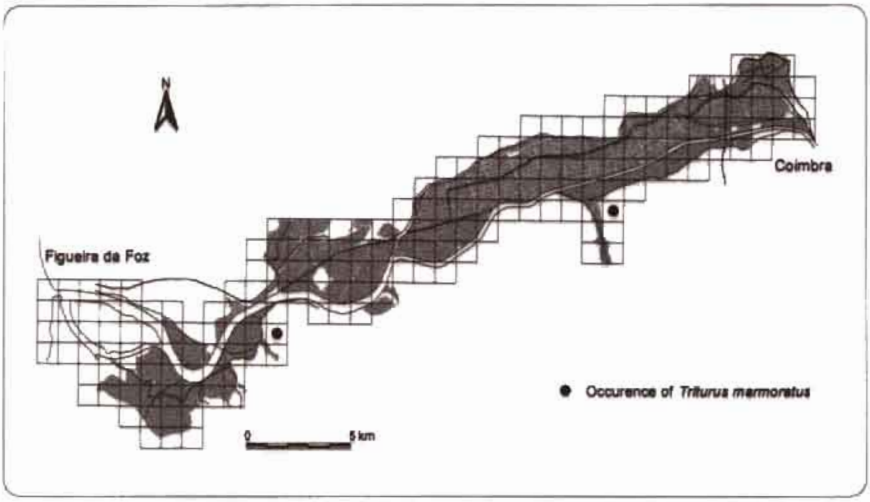

Fig. 6. Distribution of *Triturus marmoratus* in the lower Mondego River valley.

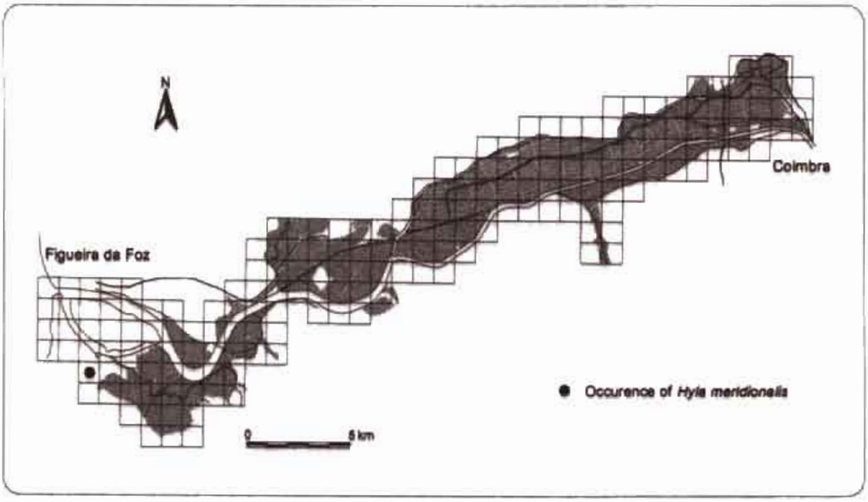

Fig. 7. Distribution of *Hyla meridionalis* in the lower Mondego River valley.

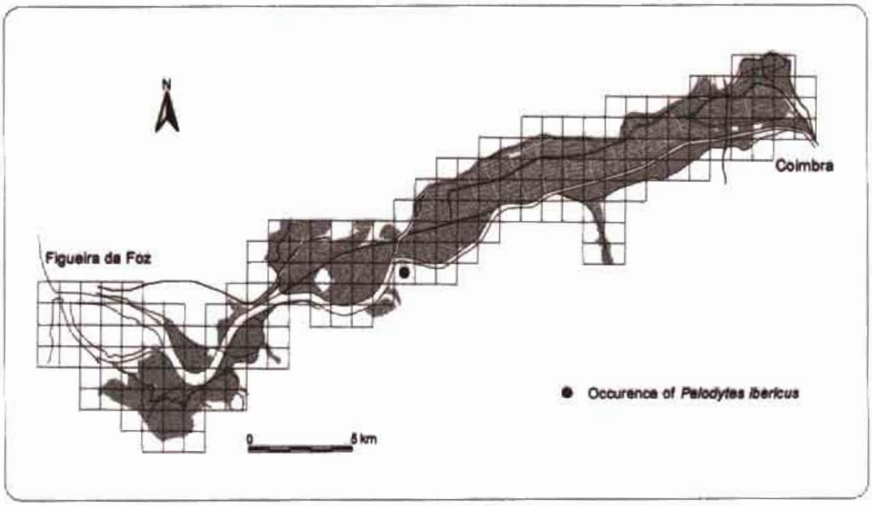

Fig. 8. Distribution of *Pelodytes ibericus* in the lower Mondego River valley.

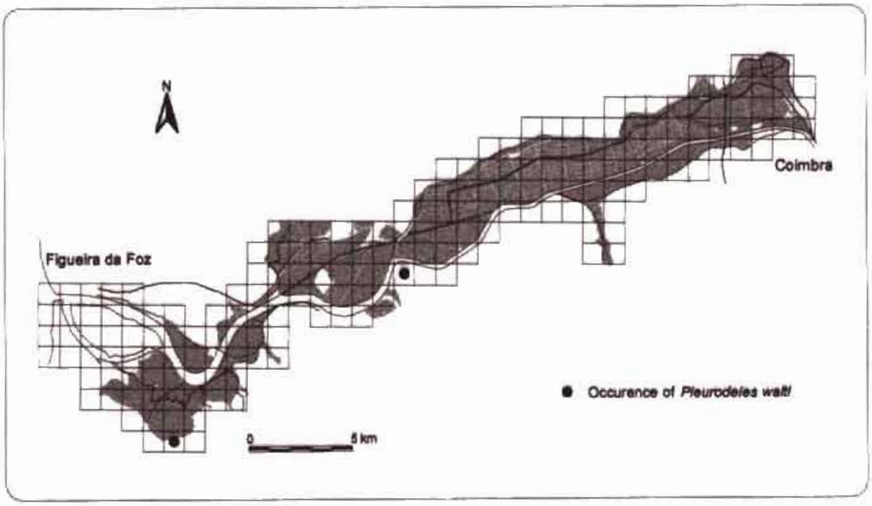

Fig. 9. Distribution of *Pleurodeles waltl* in the lower Mondego River valley.

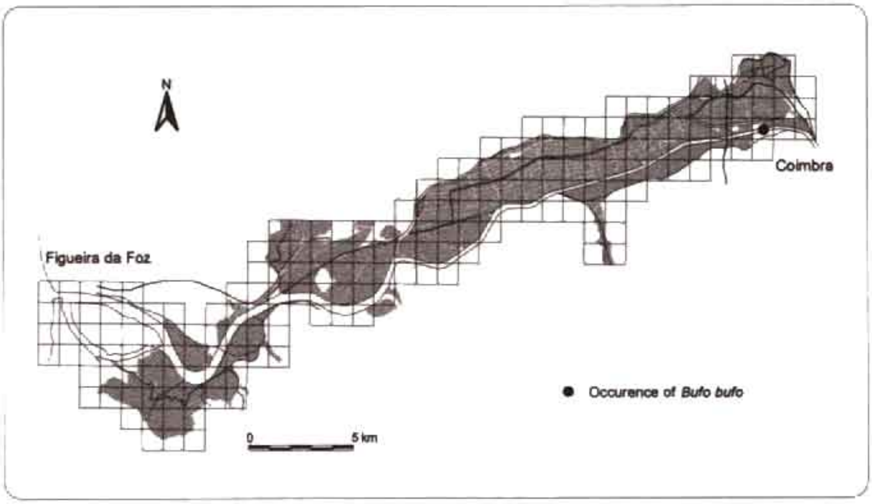

Fig. 10. Distribution of *Bufo bufo* in the lower Mondego River valley.

Table 1. Amphibian species found in the lower Mondego river valley in 1999, with their Portuguese and English vernacular names.

Species	English name	Portuguese name
Pleurodeles waltl Michahelles, 1830	Ribbed newt	Salamandra-de-costelas-salientes
Salamandra salamandra (Linnaeus, 1758)	Fire salamander	Salamandra-de-pintas-amarelas
Triturus marmoratus (Latreille, 1800)	Marbled newt	Tritão-marmorado
Discoglossus galganoi Capula, Nascetti, Lanza, Bullini and Crespo, 1985	Iberian Painted frog	Sapo-de-focinho-ponteagudo
Pelodytes ibericus Sánchez-Herráiz, Barbadillo, Machordom, Sanchiz, 2000*	Iberian Parsley frog	Sapinho-de-verrugas-verdes
Bufo bufo (Linnaeus, 1758)	Common toad	Sapo
Hyla arborea (Linnaeus, 1758)	European tree frog	Rela
Hyla meridionalis Boettger, 1874	Mediterranean tree frog	Rela-meridional
Rana perezi Seoane, 1885	Iberian waterfrog	Rã-verde

* *Pelodytes* from southern Spain and Portugal are no longer classified as *Pelodytes punctatus*, but, following the study on external morphology and allozymes by Sánchez-Herráiz et al. (2000), considered to belong to the newly recognised *P. ibericus*.

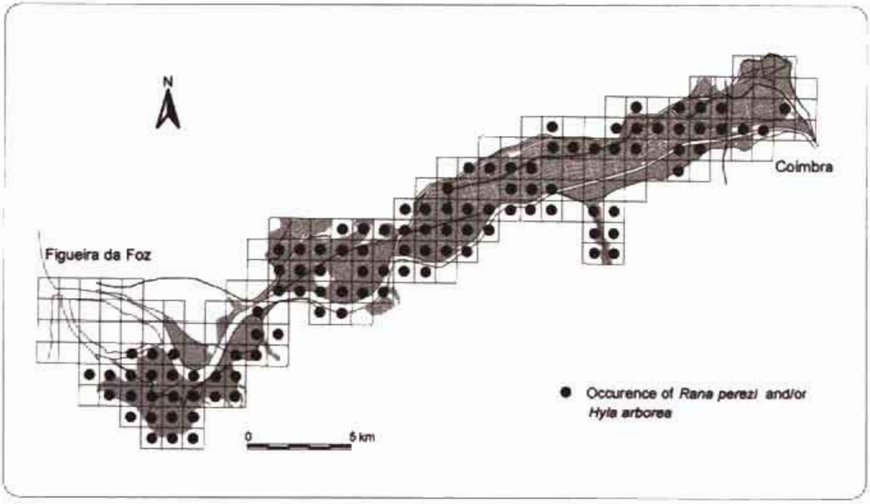

Fig. 11a. Distribution of *Rana perezi* and *Hyla arborea* in the lower Mondego River valley.

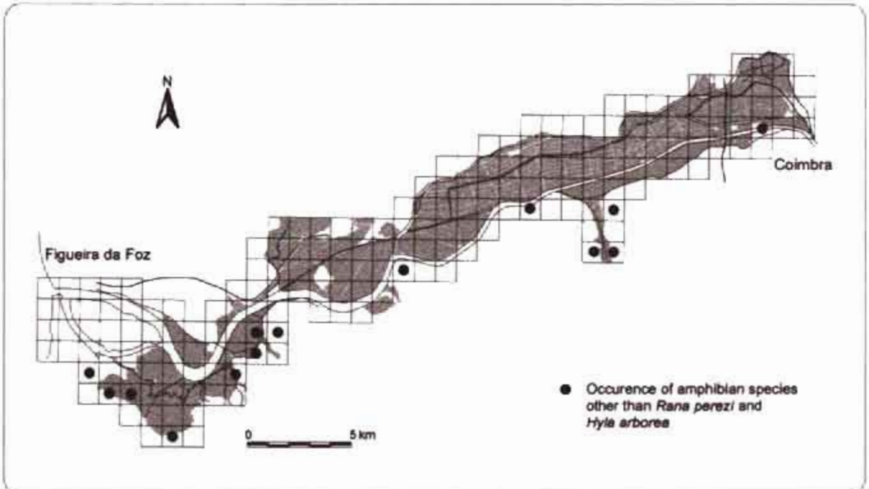

Fig. 11b. Distribution of amphibian species other than *Rana perezi* and *Hyla arborea*.

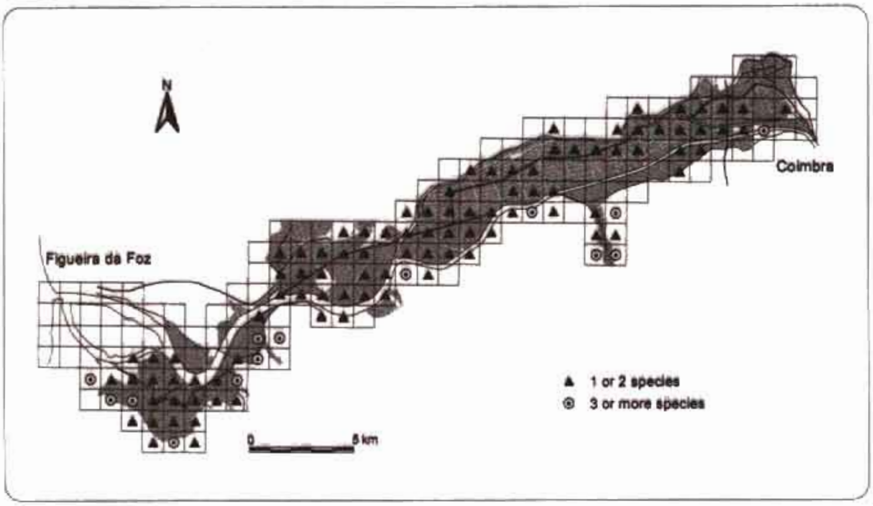

Fig. 12. Species Richness in the lower Mondego River valley.

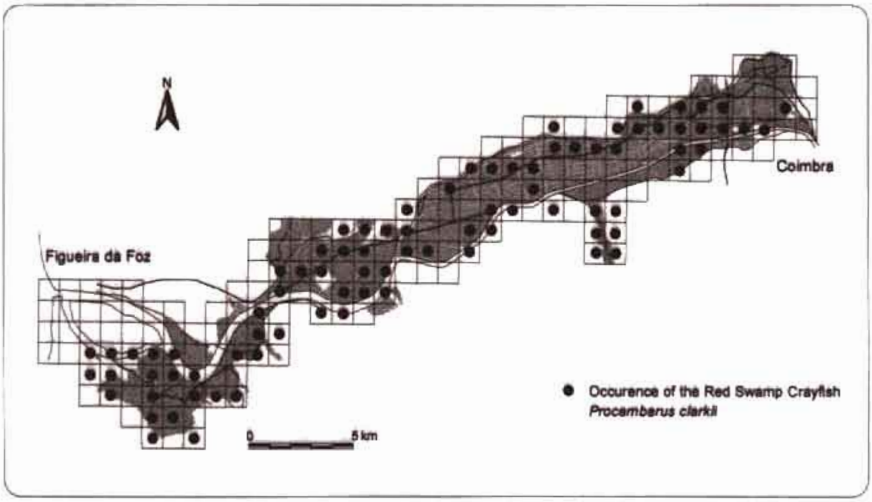

Fig. 13. Distribution of the Red Swamp Crayfish *Procambarus clarkii* in the lower Mondego River valley.

Discussion

Species accounts

The following accounts are arranged in decreasing order of observed species abundance.

Rana perezi was observed in all types of water bodies, including ditches, wells, ponds, rivers, quarries and channels, as well as on land, mostly in short vegetation near the water. With an observed presence in 96 of the 192 (50 %) grid cells *R. perezi* appears to be the most widespread amphibian of the lower Mondego with a wide ecological amplitude. It is probably the most widespread and abundant amphibian of Portugal (Godinho et al. 1999), and it is reported to tolerate strong pollution (Barbadillo 1987). Where crayfish density was high in shallow water, tadpoles frequently had damaged or cut tails, suggestive of crayfish attacks. However, direct predations of crayfish on the frogs were not observed.

Hyla arborea was observed in 92 out of 192 grid cells (48 %). From late spring onwards the species was easily detected by day, on the leaves and branches of small shrubs. In the Reveles village surroundings densities were estimated as up to six freshly metamorphosed froglets per m^2. While the species is in decline at the European scale (Stumpel 1997), it is as yet common and widespread in the Mondego valley. A close association was observed between *H. arborea* and the invasive umbelliferid *Eryngium pandanifolium* Cham. and Schlect. of South African origin. *Hyla arborea* was observed to often hide in between its spiky leafs, particularly so by daytime and in winter. This behaviour is similar to other tropical hylid frogs which rest in bromeliads and leave only the head exposed, potentially reducing evapotranspiration (Siebert et al. 1974, in Duellman and Trueb 1986). In Portugal *E. pandanifolium* is currently only known for the Mondego valley (Andresen and Bóia 1999), providing opportunities for a comparative study on the local adaptive behaviour shown by *H. arborea*.

All seven species with non-widespread distributions were limited to the southern rim of the valley. Adults and/or larvae of *Salamandra salamandra* were detected in nine grid cells (5 %). *Pleurodeles waltl*, *Triturus marmoratus* and *Discoglossus galganoi* were observed in two grid cells each (1 %). Observations on *P. waltl* concerned larvae. *T. marmoratus* were adults and eggs. No reproduction was observed for *D. galganoi*, for which species only adults were found. The remaining three species, *Bufo bufo*, *Hyla meridionalis* and *Pelodytes ibericus*, were observed in one grid cell each.

Bufo bufo was observed in the Mata Nacional do Choupal area as adults. The wide Eurasian range would suggest a wide ecological amplitude for this species and one might expect it to be widespread at a local scale also (Crespo and Oliveira 1989, Malkmus 1995, Borkin and Veith 1997). Perhaps we missed some occurrences due to inappropriate methodology. The survey method was carried mostly at daytime, while *Bufo*-adults are easier to detect at night (Denton and Beebee 1992). Also we may have missed the short period of breeding in early spring (Arntzen 1999). However, the night searches for other species were successful, and no *Bufo bufo* tadpoles were ever detected and we conclude that the species is genuinely rare locally. Honegger (1981) reported a decline of *Bufo bufo* on cultivated lowlands. In the absence of historical data we cannot establish the conservation status of the species in the lower Mondego valley.

Hyla meridionalis was detected in the southwestern part of the valley, which record constitutes one of the northernmost localities for this species in the western half of the country (cf. Godinho et al. 1999). Its close proximity to sites with *H. arborea* suggests their possible co-occurrence, which would provide the opportunity to study ecological and genetic species interactions, including hybridization (Oliveira et al. 1991).

Pelodytes ibericus is known to be difficult to observe, due to its weak voice, enigmatic ecological preferences and secretive behaviour. The recent recognition of this endemic taxon at the species level highlights our lack of basic knowledge on the species' natural history. The sister species *P. punctatus* was considered to be among the least known species of the Palaearctic herpetofauna (Toxopeus et al. 1993, Guyétant et al. 1999). The locality in the Mondego valley is a reproduction site, as evidenced by our observations on pairs in amplexus and tadpoles. Unfortunately, the locality may be under threat from future building activities. This is a serious concern, given the rarity of the species within its small, south-western Iberian range.

Amphibian landscape ecology

Two geographical patterns were observed for amphibian species in the lower Mondego valley. The first pattern arises from the distribution of *Rana perezi* and *Hyla arborea* that show a regular distribution across the valley (Fig. 11a). The coastal areas are not included, possibly due to a high level of salinity of the still waters. *Rana perezi* has a high reproductive capacity an account of an early sexual maturity, high female fecundity and the tolerance of tadpoles to conditions of poor water quality. The species thrives over a wide spectrum of environmental conditions and is tolerant to certain kinds of pollution (Barbadillo 1987, Nöllert and Nöllert 1995). The preponderance of *Hyla arborea* in the lower Mondego river valley suggests that good habitat conditions are available for this species also. Many of the ditches and some rice fields contained tadpoles of *Rana perezi* and *Hyla arborea*, whereas no other species were found.

The second distribution pattern arises from the shared distribution data on the other species, showing a distribution along the southern rim of the valley (Fig. 11b). Conversely, the northern margins of the valley do not seem to present similar species richness. A possible reason is that the area is more disturbed than at the south. It is also less accessible, which may have resulted in a survey bias.

Considering the known distribution and ecology of the 17 amphibian species of continental Portugal (Crespo and Oliveira 1989, Malkmus 1995, Gasc et al. 1997, Godinho et al. 1999), four more species could have occurred in the study area but were not detected. This involves Bosca's newt, *Triturus boscai* (Lataste 1879), the common midwife toad, *Alytes obstetricans* (Laurenti 1768), the Iberian spadefoot, *Pelobates cultripes* (Cuvier 1829) and the natterjack, *Bufo calamita* Laurenti 1768. Their apparent absence may be linked to a variety of factors, the most important ones probably are the scarcity of suitable breeding ponds and particular soil characteristics. Proper ponds are scarce in the valley. In large areas the drainage ditches are the only places available for breeding, but to several species these do not offer the right conditions. Similarly, soil characteristics are generally unsuitable for species with a fossorial mode of life. These species prefer sandy soils while most of the valley is dominated by acidic argillaceous substrates, often disturbed by agricultural machinery and periodic floods.

Conservation status and threats

The main potential factors influencing the local distribution of amphibians and their threats we assess as i) habitat availability and habitat change, ii) pollution and biocides, and iii) allochtonous species.

Habitat availability and change – The lower Mondego valley has been used for agricultural purposes for centuries. In former times, however, the land use was diverse, patchy and not intensive (Andresen and Bóia 1999), whereas at present agriculture is intensive, with a few main crops, and with little or no land remaining unoccupied. This suggests that the complexity of the ecosystem has been higher, potentially favouring species numbers and coexistence, in contrast with the present day situation of open, almost laser-leveled fields, with shelter of any sort, important to many species, and adequate breeding structures nearly absent. Only *Rana perezi* and *Hyla arborea* appear capable to use the ditches and temporary inundated rice fields for breeding. The almost absence of other types of standing water is the most likely single factor that prevents other species, now restricted to the margins, to occupy the core of the valley.

Pollution and biocides – Data on the effects of pollution on amphibians are scarce. Some evidence suggests that fungicides, herbicides and insecticides hinder reproduction and development (Blaustein and Wake 1995). Fourty-one different biocides were used in the Lower Mondego valley in 1998, for just corn and rice (Andresen and Bóia 1999). Soil disinfection is considered indispensable and common practice. In spite of legislation, insecticides based on organophosphates are still in use, alongside with pyrethroids and carbamates. Their application in April and May coincides with the breeding season of most amphibians. The use of liquid herbicides in rice cultivation extends in to July. Spread compounds such as chlorpyriphos are highly toxic. Moreover, the long half-life time of other used substances such as dimethoate may have serious environmental effects as to jeopardise human health (Andresen and Bóia 1999). In view of the intensive farming in the valley, the perceived need to increase production and known practices, the contamination of surface waters in the valley is more than likely and a certain impact on amphibians plausible.

Allochtonous species – Introduced species are increasingly recognised as serious threats to amphibian (Blaustein and Wake 1995), including known predators such as some decapod crustaceans (Axelsson et al. 1997). The red swamp crayfish, *Procambarus clarkii* is known to inhabit the Mondego valley since 1987. The density of this allochtonous crustacean rapidly increased and already by 1990 it was considered an agricultural pest (Marques et al. 1992), causing serious damage to the drainage systems through its borrowing activities (Anastácio and Marques 1995). The capacity of *P. clarkii* to settle in new habitats, its tolerance to a wide range of environmental conditions (Hobbs et al. 1989 in Anastácio and Marques 1995) and multiple recruitment periods in Portugal (Anastácio and Marques 1995) may have contributed to the success of the species in the Mondego valley.

The effects of this crustacean on the amphibian fauna might be either direct through e.g. predation on eggs and larval stages, or indirect, through alterations to the habitat. Axelsson et al. (1997) demonstrated with laboratory experiments the predatory activity of this group of crustaceans on amphibian eggs and larvae, including *Hyla arborea* and *Bufo bufo* (despite tadpoles of the latter species being unpalatable to many predator species, Duellman and Truebb 1986, Denton and Beebee 1991, Banks et al. 1993). Indirect effects include a decrease in the habitat complexity through a reduction in aquatic vegetation from crayfish grazing and burrowing, therewith reducing space available for shelter and for egg deposition. Indeed, Axelsson et al. (1997) showed that a reduced habitat complexity increased crayfish predation on *Hyla arborea* tadpoles. In 1990, massive amounts of xenobiotics (including dimethoate, endosulfan and parathion) were used in an attempt to eradicate the crayfish (Marques et al. 1992). The measures had no marked effect, and in 1991 the number of crayfish populations had further increased. Instead, the high doses that were applied caused serious risk to public health (Marques et al. 1992). The effects on the amphibian populations of the Lower Mondego valley remained unstudied.

Recommendations for future work

We assessed amphibian species presence and distribution across the lower Mondego river valley. The work constitutes a first and preliminary contribution to our knowledge of the local amphibian fauna. The Lower Mondego river valley shows an interesting diversity of amphibian species, which is surprising given the intensive human pressure in the area. However, future work is mandatory. Several species were only detected in one or two localities. Further surveying and the monitoring of populations is in order. In addition to mapping, it would be of importance for future reference to estimate the size of one or more populations for each of the species. Future surveying should include the secondary river basins. Several localities that harbour locally rare species deserve special attention in agricultural engineering and landscape planning. Habitat creation and restoration would be particularly beneficial for species that are locally abundant but rare and endangered elsewhere, such as *Hyla arborea*. Implementation of a program for pond creation could be an efficient and relatively inexpensive way of counteracting the negative impact of anthropogenic interventions on the amphibian populations of the valley. The potential effects of each of the threatening factors on the viability of amphibian populations in the valley require also careful assessment. For instance, the effects of the widely distributed *P. clarkii* on the amphibian populations of the valley remains unclear. In view of the potential regulatory role in amphibian ecosystems, a deeper look into crayfish - amphibian interactions seems warranted. Indeed, experimental confirmation and quantification of the effects of the mentioned factors on the regulation of the amphibian populations would be a first step towards the conservation of this group of vertebrates in the Lower Mondego river valley.

Acknowledgements

We thank Dr. J. W. Arntzen for encouragement and for critical comments on an earlier version of the manuscript and Manuel da Luz for assistance with the production of the figures. J.M.O. was supported by I.C.N. through a grant conceded by the "Programa Nacional de Estágios em Áreas Protegidas".

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ON THE STRUCTURE OF THE BENTHO-PELAGIC FAUNA FROM THE MONDEGO ESTUARY SOUTHERN ARM

Abstract

This work presents the investigations made on the bentho-pelagic zooplankton of the Mondego estuary southern arm. These communities were sampled from June 1996 to July 1997 using macrozooplankton and suprabenthic nets, with 335 µm and 500 µm mesh size respectively. The diversity of the samples and the distribution of the species along the main estuarine gradients were assessed. Diversity was highest in the marine zone where density and biomass were lowest. Diversity decreased upstream and was lowest in the brackish part where density and biomass reached maximal values. Communities were identified using a divisive classification multivariate statistical technique. Communities could be distinguished and their position along the unidirectional salinity-turbidity spatial gradient was described. The spatial patterns dominated over the temporal patterns. The species composition, density and biomass of the dominant species of each community were compared among communities. A general seasonal cycle was documented with a more abundant and diverse assemblage during the spring and autumn than summer and winter.

Introduction

In recent years, knowledge on the suprabenthic communities of the NE Atlantic has increased (Kaartvedt 1985, 1986, Macquart-Moulin 1985, Fossa and Brattegard 1990, Elizalde et al. 1991, Zouhiri and Dauvin 1996, Cunha et al. 1997 a, b) and several infralittoral and circalittoral areas have been studied (Sorbe 1982, 1984, 1989; Cornet et al. 1983, Wang and Dauvin 1984, Buhl-Jensen and Fossa 1991, Hamerlynck and Mees 1991, Dauvin et al. 1994, Zouhiri and Dauvin 1996, Azeiteiro and Marques 1999, Cunha 2000).

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Few systematic works have been carried out on the continental shelf off Portugal. Monteiro Marques (1979) and Sousa Reis et al. (1982) conducted biocoenotic studies off the southern and southwestern coast. Marques and Bellan-Santini (1985, 1991, 1993) studied the biodiversity and distributional ecology of amphipod crustaceans based on several benthic surveys carried out systematically along the coast. Recently the Southern Iberian margin suprabenthos was studied by Cunha et al. (1997 a, b) and estuarine suprabenthic communities are being studied by Azeiteiro and Marques (1999) in the Mondego estuary and by Cunha (2000) in Ria de Aveiro.

The suprabenthos (Beyer 1958, Brunel et al. 1978), tychoplankton (Kennish 1990) or tychopelagic compartment (Attlas and Bartha 1993), demersal zooplankton (Kennish 1990), benthopelagic zooplankton (Azeiteiro et al. submitted) and nectobenthic communities (Viitasalo and Rautio 1998) are known to be an important source of food for demersal fishes (Mauchline 1982, Sorbe 1981, 1984, Astthorsson 1985). These communities play an important role in the estuarine trophodynamics (Hamerlynck et al. 1990, Mees and Hamerlynck 1992, Sorbe 1981, 1984). A clear understanding of the energy and material fluxes in estuaries should always take into consideration the well established importance of these communities in those ecosystems (Boysen 1975, Wooldridge 1989, Buhl-Jensen and Fossa 1991, Hamerlynck and Mees 1991, Azeiteiro and Marques 1999, Cunha 2000).

Peracarid crustaceans of the suprabenthos, demersal zooplankton, benthopelagic zooplankton, nectobenthos and tychoplankton, or associated with floating macroalgae contribute to the pelagic community (Locke and Corey 1989). Their contribution to the plankton community is most important during night-time hours due to the migration patterns and then having a very important role in the trophodynamics of coastal and estuarine areas (Buhl-Jensen and Fossa 1991, Grabe 1996). Amphipods and cumaceans usually emerge from the sediment but do not commonly swim high up into the water column (Kaartvedt 1986, 1989). However some amphipods species are frequently collected in pelagic or hyponeustonic catches (Macquart-Moulin 1984). In contrast to amphipods and cumaceans mysids migrate upwards during the night and some of them are collected near the surface in high densities (Kaartvedt 1985). The migration of mysids constitutes an important mechanism for vertical and horizontal transfers of organic matter along coastal ecossystems (Macquart-Moulin and Maycas 1995). These transfers enable diel changes between the bottom and the surface. Mysids and some other benthopelagic crustaceans (especially the isopod *Eurydice* and decapod larvae), are the only vectors able to achieve diel transport of benthic matter directly up to the surface (Macquart-Moulin and Maycas 1995). Holopelagic animals can only ensure this upward transfer of benthic matter indirectly after predation of nectobenthic organisms (Macquart-Moulin and Maycas 1995).

Swimming activity and diel changes in the vertical distributions of suprabenthic species have been studied and described by several authors (Macquart-Moulin and Patriti 1993, Macquart-Moulin and Ribera Maycas 1995, Dauvin and Zouhiri 1996). On the whole these studies also documented that the swimming activity increases during the night. These diel activity patterns of the peracaridean crustaceans have been reported by several authors (e.g. Macquart-Moulin 1984, Sorbe 1984, Kaartvedt 1985, 1986, Wang and Dauvin 1994, Zouhiri and Dauvin 1996).

Our objectives for the present work were: (1) to investigate the density, biomass and diversity distribution of the faunal assemblage living in the vicinity of the bottom on the Mondego estuary southern arm and (2) to investigate the density, biomass and diversity distribution of the crepuscular peracarid macroplankton on the Mondego estuary southern arm.

Materials and Methods

Sampling

Five sampling stations were located along the southern arm of the estuary in order to represent the whole subsystem (Fig. 1). Station 1 is the closest one to the mouth of the estuary. The depth was 2 to 4m, the bottom consists of coarse to medium sand, low organic matter and carbonate content (Azeiteiro 1999, Azeiteiro and Marques 1999), and the influence of both the north arm and neritic waters is stronger. Station 2 is located in approximately the middle of the south arm, the depth ranges from 1 to 2m, and the bottom consists of fine sand (Azeiteiro 1999, Azeiteiro and Marques 1999). Station 3 is located in the inner area of the southern arm, close to the connection with the northern arm, whose key characteristics are very shallow waters and sandy mud bottoms, rich in organic matter and carbonate contents (Azeiteiro 1999, Azeiteiro and Marques 1999). Finally, stations 4 and 5 are located in the Pranto river, noted mainly for its very shallow waters and fine sediments, with large fractions of fine sand to clay, and the highest organic matter and carbonate contents (Azeiteiro 1999, Azeiteiro and Marques 1999).

Suprabenthic samples (samples collected with a suprabenthic sledge which consists of a heavy metal frame equipped with a net 500 µm mesh size; all the samples were quantitative) were collected monthly from the five stations (Fig. 1), which were visited in Spring tides, always following the same sequence. On 14 different occasions, between June 96 and July 97, a total of 70 daytime samples, during low tide, were collected (Azeiteiro and Marques 1999).

Monthly sunset plankton collections were made at the same five stations. Subsuperficial tows were made with a 0.5 m diameter 0.500 mm mesh plankton net. Each station was visited monthly for 12 months from July 1996 to June 1997, and on each visit various measurements were made and samples collected for analysis. All five stations were visited in spring tide waters in the same sequence and sampled in high tide. The volume of water filtered by the nets was measured with a HydroBios flowmeter mounted in the mouth of the nets. The zooplankton samples were preserved in 4 % neutralized formalin after collection.

Measurements of salinity, temperature, oxygen, pH and Secchi transparency were determined in situ each time faunal samples were collected. Water samples were collected for chlorophyll a biomass and phaeophytin (Azeiteiro and Marques 1999).

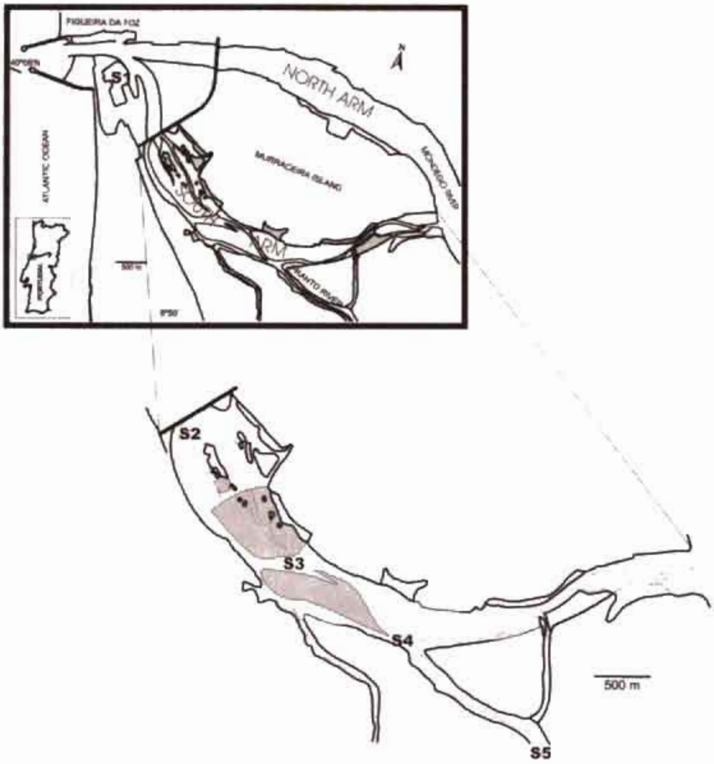

Fig. 1 Map of Mondego river estuary showing the locations of the five sampling stations in the southern arm.

Physico-chemical data analysis

Principal Component Analysis (PCA) was used to identify the major sources of variation in the physico-chemical data (Legendre and Legendre 1979). Data were organised into a matrix of 9 physico-chemical variables x 70 samples and standardised (Legendre and Legendre 1979). Correlation matrixes were calculated using the Pearson's coefficient. Data analysis was performed using the NTSYS-PC (Numerical Taxonomy and Multivariate Analysis) version 1.80 software package (Rohlf 1990).

Studies of the descriptive physico-chemical data and a description of the clorophyll a biomass related to this study have been published elsewhere (Azeiteiro and Marques 1999).

Laboratory procedures

In the laboratory all animals were identified, if possible to species level, and counted and weighed; all density and biomass data are presented as numbers of individuals (N) and mgrams ash-free dry weight (mgAFDW) per m^3 (Azeiteiro and Marques 1999).

Communities data analysis

The sampling sites were classified into clusters according to species composition using the classification program TWINSPAN (Hill 1979), which is a dichotomous divisive technique. TWINSPAN yields indicator species characterising the various groups (Azeiteiro and Marques 1999).

Diversity of the communities (Magurran 1988) was calculated with the H the Shannon-Wiener diversity index (Azeiteiro and Marques 1999).

Results

In the PCA analysis (Fig. 2), the first axis correlates strongly with the main estuarine gradients: salinity and Secchi disc depth. The largest vector, which, per definition, explains most of the variance, nearly parallels the firts axis and represents the salinity gradient. It is strongly and negatively correlated with the 1/Secchi vector. The first axis (with its correlated salinity-turbidity gradient) thus reflects the spatial longitudinal variation, whereas the second axis rather reflects the seasonal component. All marine samples are located on the positive side of the firts axis.

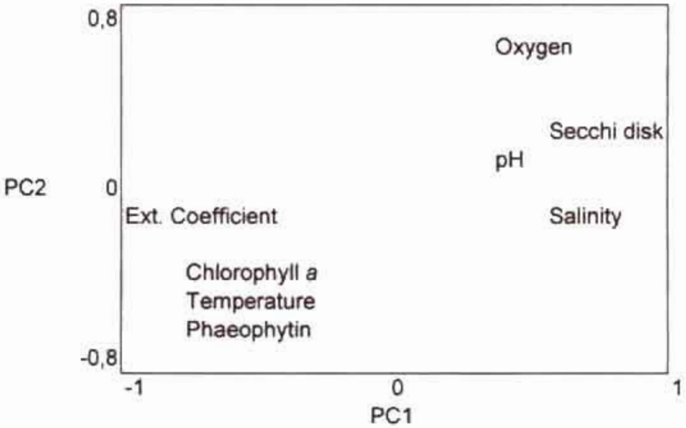

Fig. 2 Projection of the physicochemical and biological parameters and sites in the space defined by PC1 and PC2.

A total of 74 species were identified. The faunal assemblage living in the immediate vicinity of the bottom was dominated by crustaceans, especially mysids. Other important groups were amphipods, isopods, caridean shrimps, larval stages of brachyuran crabs and postlarval fish.

Seasonal variation of density (indm^{-3}) and biomass (mgm^{-3}) (AFDW) showed a clear bimodal variation pattern with highest values in October and May (Fig. 3). January with a lower density value than the other months showed the highest biomass values what is understood by the occurrence of Pisces post-larvae *(Anguilla anguilla)* (Fig. 4).

The highest diversity values where obtained in May and July to August (Fig. 5).

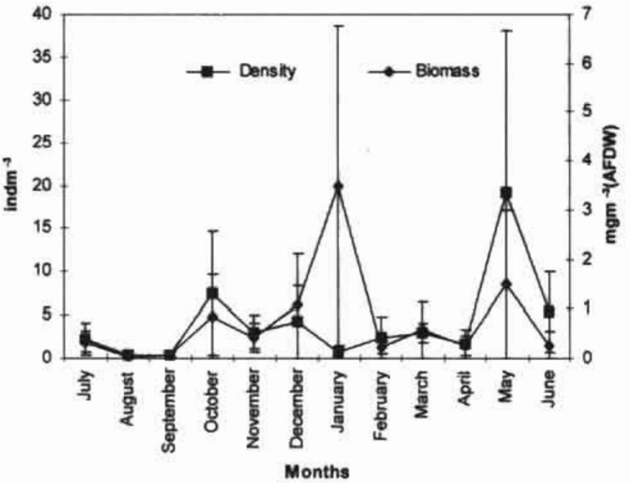

Fig. 3 Seasonal changes in total suprabenthic density (indm^{-3}) and biomass (AFDW) (mgm^{-3}), July 1996 – June 1997.

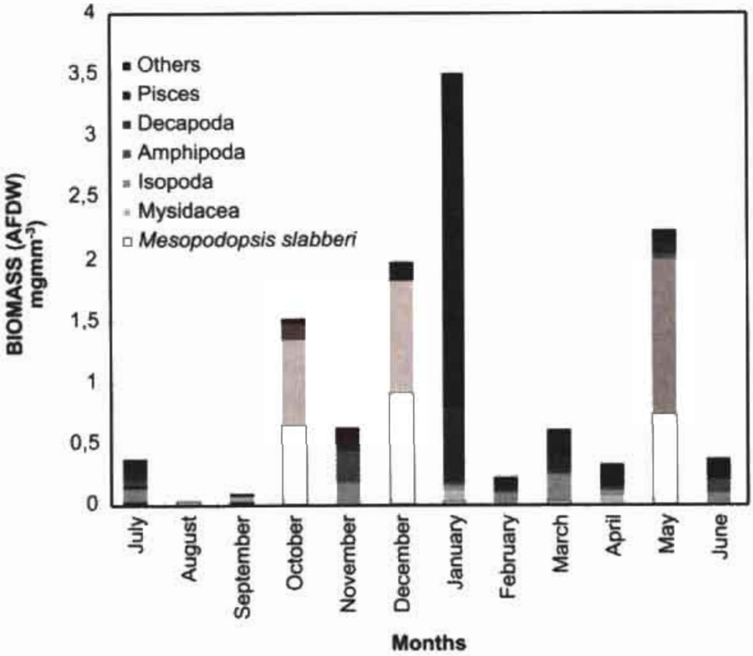

Fig. 4 Biomasses (AFDW) of the suprabenthonic groups and M. slabberi (mgm^{-3}), July 1996- June 1997.

Twinspan analysis of the 60 trawls shows a strong dominance of spatial structure, i.e. the similarities between the clusters grouping the samples of different months are high (Fig. 6). A first split divides the year in a cluster with the marine stations (mouth of

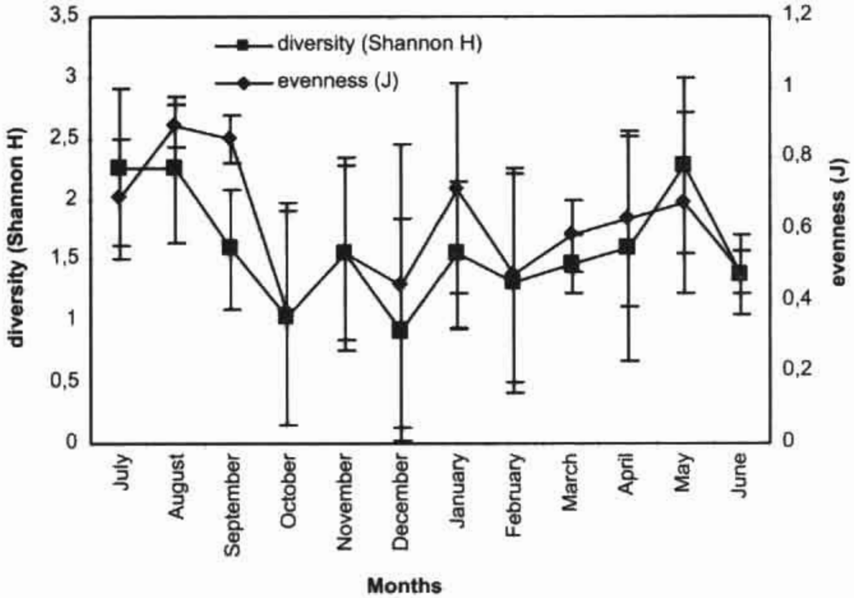

Fig. 5 Monthly changes in the species richness (Shannon H), July 1996- June 1997.

the estuary: sampling stations S1 and S2) and a cluster with the mid and inner stations (sampling stations S3, S4 and S5). Indicator species for the most seaward part are *Schistomysis spiritus*, *Gastrosaccus spinifer* and *Paramysis c.f. bacescoi* and for the inner estuary *Mesopodopsis slabberi* and *Paragnathia formica*. In the next division, in the mouth cluster Autumn and Spring with indicator species Mesopodopsis slabberi are separated from late Summer and Winter. The next split, in the second cluster, distinguishes Autumn from Spring with indicator species Engraulis encrasicolus, and, in the first cluster, distinguishes late Summer from Winter with indicator species *Anguilla anguilla*. The next split in the other main cluster of the mid and inner group divides the samples in Winter, late Summer and Autumn group with indicator species, *Gastrosaccus spinifer*, *Paragnathia formica* and *Melita palmata* from Summer and Spring with indicator species *Praunus flexuosos*. The next split, in the first cluster, isolates Winter from late Summer and Autumn, and, in the second cluster, Summer from Spring.

Figure 6 also shows the density values of the most important species in each cluster. The composition of species assemblages in the eight clusters differed substantially but mysids were dominant except for cluster 4 (Spring and early Summer inner and mid estuary) where temporary suprabenthos (merosuprabenthos) (Hamerlynck and Mees 1991, Azeiteiro and Marques 1999) dominated and cluster 5 (Winter mouth of the estuary) where temporary suprabenthos also dominated. The bottom fauna reaches higher numbers in the inner estuary (in all months). The brackish community is dominated by *Mesopodopsis slabberi* (Table 1).

Table 1. Biomass (%) spatial variation of the main taxonomic groups and *M. slabberi* within the Mondego southern arm sampling stations.

	S1	S2	S3	S4	S5
M. slabberi	0,74	7,76	8,05	18.27	40,60
Mysidacea	61,39	24,15	13,59	21.38	41,04
Isopoda	0,77	4,50	1,07	1.28	1,17
Amphipoda	3,99	8,39	0,56	0,37	0,05
Decapoda	23,91	12,01	13,49	6.88	5,70
Pisces	8,84	41,83	63,23	51.82	11,43
Others	0,37	1,37	0	0	0

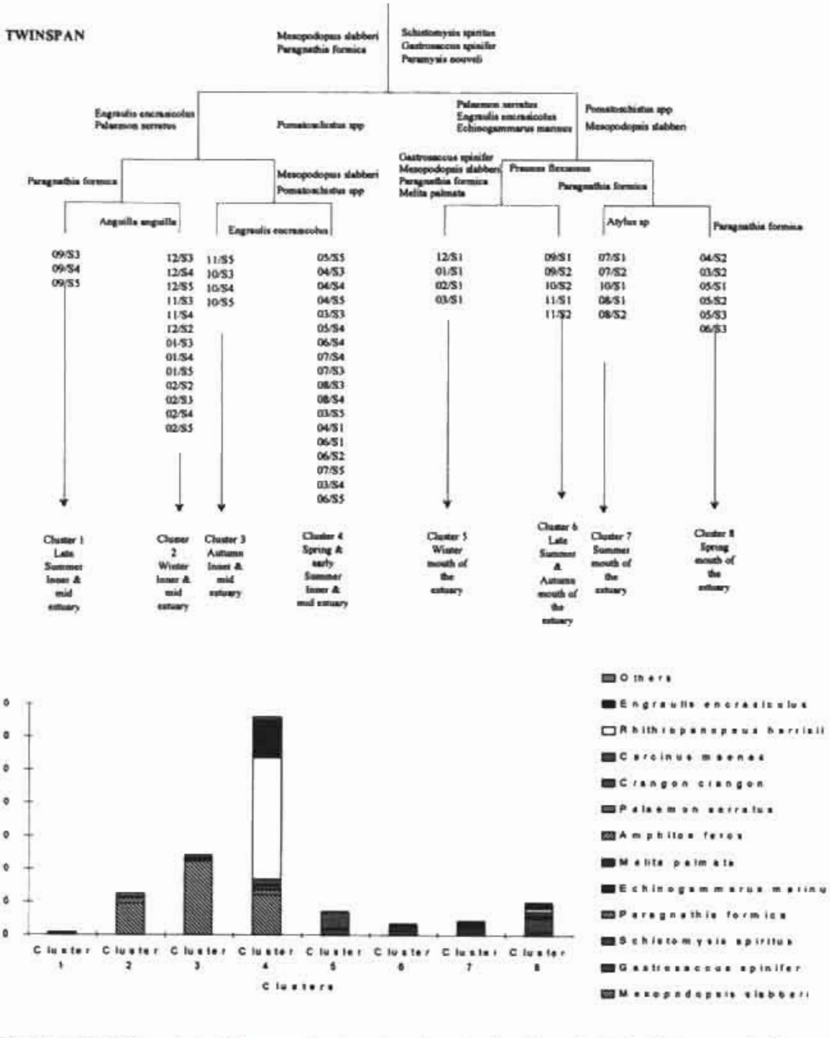

Fig. 6 The TWINSPAN analysis of the samples based on the density data with the indicator species for each division indicated and faunal composition for the identified clusters.

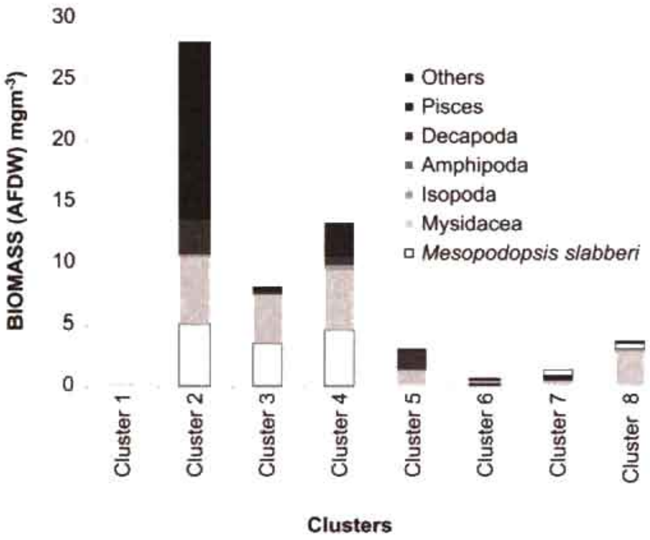

Fig. 7 Faunal composition, for the biomass values, of the communities identified by TWINSPAN of all density data.

The clusters also differed in biomass (Fig. 6). Figures 6 and 7 also illustrates the seaward decline of total density and biomass of the faunal assemblage living in the immediate vicinity of the bottom as well the distribution of the most important species and groups.

Mysids dominated the suprabenthos in all stations. *Neomysis integer* and *Paramysis bacescoi* appear always in mid estuary. *Praunus flexuosos* is also restricted to the mid zone. *Mesopodopsis slabberi* is a euryhaline species which occurs in higher numbers in the mid and inner estuary. *Schistomysis spiritus*, *Gastrosaccus spinifer* and *Siriella clausi* are the dominant mysids in the marine part of the estuary. *Schistomysis spiritus* is a typical marine species which enters the estuary in Winter, is still present in very low numbers in Spring but is completely absent in Summer.

Diversity Twinspan analysis showed the temporal structure (i.e. samples of a single month resemble one other more closely than samples from the same station in any other month) (Fig. 8) but also the spatial structure. The cluster analyses formed 4 clusters. Winter and late Summer are the poorest seasons. The most downstream stations have the highest diversities, where a high number of species are present and the individuals are distributed more evenly among them.

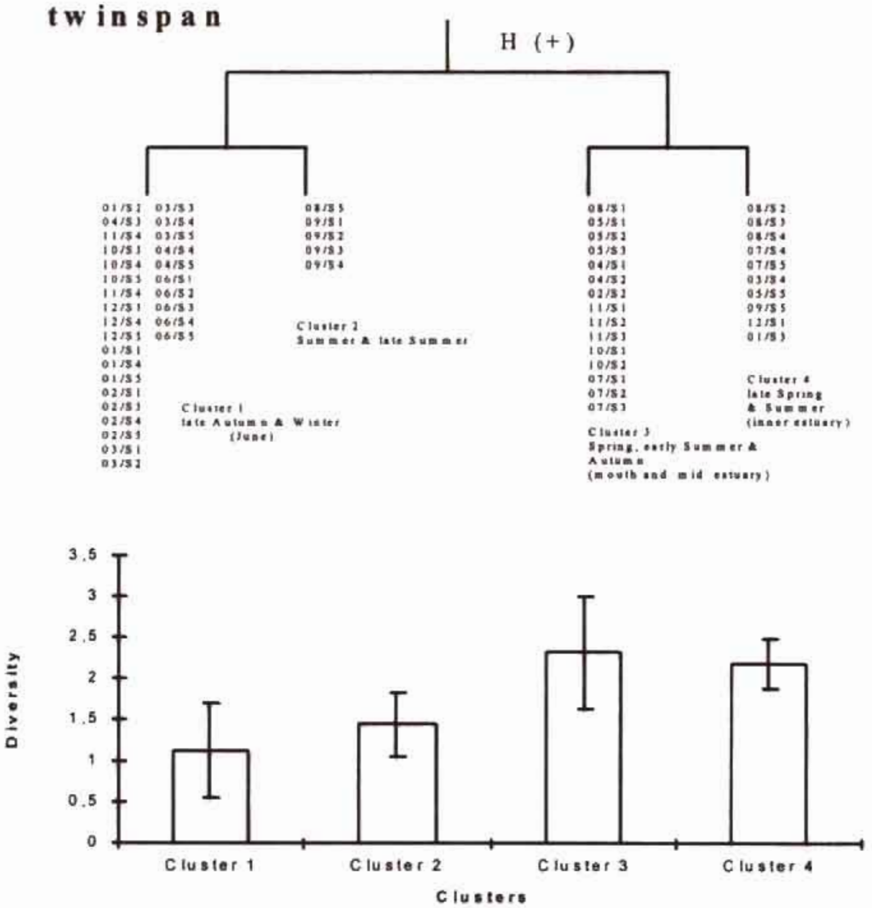

Fig. 8 The TWINSPAN analysis of the samples based on the diversity and formed clusters with the average diversity values.

In the crepuscular subsuperficial collections fifty species of peracarids were identified: isopods (14 species), mysids (14 species) and amphipods (18 species) were the most diverse taxocenes. Densities (ind100m^{-3}) and biomass (mg100m^{-3}) (AFDW) (Fig. 9) followed a similar pattern, with autumn-early winter and spring-early summer maxima.

Species richness (Margalef diversity index) (Magurran 1988) (Fig. 11) showed a pattern with a late summer-early autumn and spring maxima. April, with a low number of species and low number of individuals (the high diversity value only reflects the high equitability), doesn´t follow the regular pattern described in the last period.

The majority of the peracarids caught in the study area were estuarine species: mysids: *Mesopodopsis slabberi*, *Neomysis integer* (Leach), *Praunus flexuosos* (Muller), *Schistomysis spiritus* (Norman), and *Paramysis bacescoi*, *nouveli* and *helleri* (Labat), *Siriella* sp., *isopods: Idotea chelipes* (Pallas), *Lekanesphaera hookeri* (Leach), and *Paragnathia*

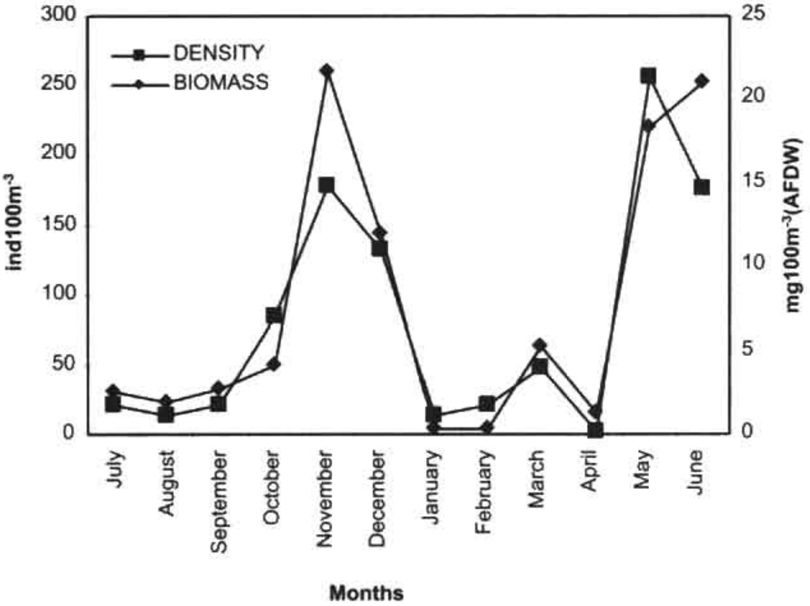

Fig. 9 Monthly changes in the total densitiy (ind100m^{-3}) and biomass (mg100m^{-3}) (AFDW) of peracarid zooplankton in crepuscular-time collections in the southern arm of the Mondego estuary, July 1996-June 1997.

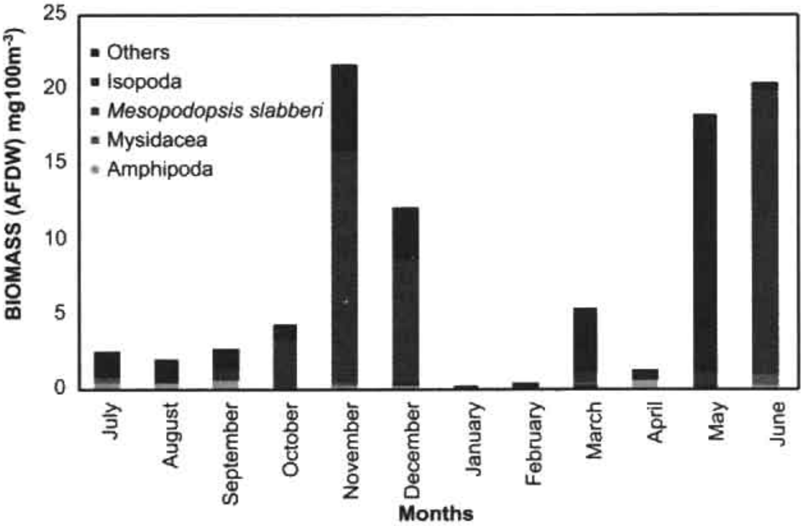

Fig. 10 Biomasses (AFDW) of peracarids (mgm^{-3}) in crepuscular-time collections in the southern arm of the Mondego estuary, July 1996- June 1997.

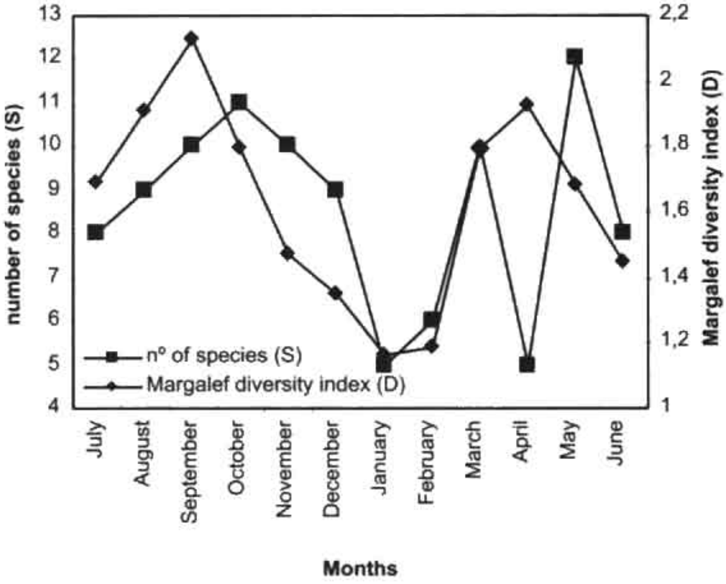

Fig. 11 Monthly changes in the species richness (Margalef diversity index) and number of species of peracarid zooplankton in crepuscular-time collections in the southern arm of the Mondego estuary, July 1996- June 1997.

formica and the amphipods: *Corophium multisetosum* (Stock), *Melita palmata* (Montagu), *Amphitoe* spp. and *Echinogammarus marinus* (Leach). The numerically dominant groups were the mysids and the isopods. In the autumn and late spring *Mesopodopsis slabberi* was the most abundant species and in the late winter and spring *Paragnathia formica* was the dominant species. The biomass dominant groups (Fig. 10) were also the mysids and the isopods. In the autumn *Mesopodopsis slabberi* was the biomass dominant species. Along the studied period, the mysid *Mesopodopsis slabberi* (van Beneden) and the isopod *Paragnathia formica* (Hesse) comprised about 93 % of the total density and biomass of the assemblage in the crepuscular peracarid macroplankton fauna.

Discussion

The suprabenthic communities have been studied for the last decade in several NE Atlantic areas (Bay of Biscay: Sorbe 1982, 1984, 1989, Cornet et al. 1983, English Channel: Dauvin et al. 1994, Wang and Dauvin 1994, Zouhiri and Dauvin 1996, North Sea: Fossa and Brattegard 1990, Hamerlynck and Mees 1991, Brattegard and Fossa 1991, Buhl-Jensen and Fossa 1991, West Portugal: Cunha 2000, Cunha et al. 1997 a, b).

Mysids dominated the suprabenthos. Mysids and decapods are well represented in coastal communities (Webb and Wooldridge 1990, Buhl-Jensen and Fossa 1991, Hamerlynck and Mees 1991, Dauvin et al. 1994, Brandt 1995, Azeiteiro and Marques

1999, Cunha 2000). The recorded densities are subject to several possible sources of bias. Net efficiency was assumed to be 100%. However, mysids are good swimmers which are known to actively avoid nets (Mauchline 1980). Thus, the reported densities may be a gross underestimate of the actual number of mysids present in the area. The species *Gastrosaccus spinifer* spends most of the daytime buried in the sand (Tattersall and Tattersall 1951 *in* Mees and Hamerlynck 1992) and was most certainly underestimated. The species *Praunus flexuosos* (Tattersall and Tattersall 1951 *in* Mees and Hamerlynck 1992) which are known to prefer shallow intertidal parts of estuaries, may be also underestimated due to problematic bed conditions and shipping. Another possible source of bias may be the vertical distribution and diel changes. Swimming activity and diel changes in the vertical distribution of suprabenthic species have been studied and described by several authors (Macquart-Moulin 1965, 1984, 1991, 1992, Macquart-Moulin and Patriti 1993, Macquart-Moulin and Maycas 1995, Dauvin and Zouhiri 1996). Species-specific behavioural patterns together with other factors such as light, currents or food availability determine the swimming activity of suprabenthic animals (Fossa 1985, 1986, Elizalde et al. 1991, Vallet et al. 1995).

Absence of a species from the samples does not necessarily mean it is not present in the estuary. This is certainly true for the rare species and for animals not efficiently caught with the sledge e.g. *Rhithropanopeus harrisi* (Wolff and Sandee 1971 and Van Damme et al. 1992 all *in* Mees et al. 1995), which are abundant in the estuary (Gonçalves 1991, Azeiteiro 1999).

The mysid fauna resembles that of other European estuaries (Sorbe 1981, Mees et al. 1993 a, 1995). The following within-estuary patterns were consistently found: diversity was highest in the marine zone, where density and biomass were lowest. Diversity decreased in an upstream direction, where density and biomass reached maximum values.

Multivariate analysis is a useful descriptive tool for an exploratory analysis of data. Multivariate analysis neatly summarises the structure in complex data sets and can help us to formulate hypothesis. The multivariate analysis showed that in the suprabenthic community spatial structure dominated over the seasonal patterns, i.e. the similarities between the clusters grouping the samples of different months are high. The main reason for this is the fact that the spatial gradient in species composition in estuaries is very steep: the communities of the marine and brackish parts are composed mostly of different species. Furthermore, most temporary and migratory suprabenthic species are not able to penetrate far into the estuary resulting in a species-poor community upstream, which is always dominated by the same few species. Despite the fact that strong temporal variations in abundances are observed for the main brackish water populations, community structure as a whole thus remains stable throughout the year (Mees et al. 1993 a, b, Azeiteiro and Marques 1999).

The sequential appearance, high abundance and disappearance of the different species of the temporary suprabenthos affects the community structure and the cluster analysis. Diversity values showed that temporal patterns remain important, but spatial patterns emerge in any case. In Winter, lower diversity values may also be due to the combined effect of tides and freshwater discharge, causing strong daily variations in physico-chemical factors. From Winter to Summer, the decrease in freshwater discharge, and consequently easier tidal penetration, seems to favour the incursion of suprabenthic marine species inside the estuary. Despite their sparse populations, the intrusion of these species may explain the observed increase in diversity (Azeiteiro and Marques 1999). Marques et al. (1993 a, b) observed the same pattern with epifaunal species. The hydraulic circulation in the southern arm depends essentially on tides and on the freshwater discharge of the Pranto river (Marques et al. 1993, 1994). Marques et al. (1993) claim, using the definition of McLusky (1989), that the southern arm presents favourable environmental conditions for true estuarine organisms. However, species diversity could be higher in Spring, Summer and Autumn seasons if there was not an increase of biological activity and the enhancement of estuarine populations, namely of *Mesopodopsis slabberi*, that decreased species evenness; thus the species diversity values may be underestimated in those seasons but not corresponding to a reduction in species richness. Marques et al. (1993) observed the same pattern with epifaunal species. Besides the behavioural and environmental factors, the life cycle features of suprabenthic species may determine density fluctuations that induce temporal changes in the community structure (Boysen 1975, Buhl-Jensen and Fossa 1991, Hamerlynck and Mees 1991, Dauvin et al. 1994, Azeiteiro et al. 1999). In summary, the estuary contained distinct communities along the unidirectional salinity-Secchi disc transparency gradient (Azeiteiro and Marques 1999).

In the Mondego estuary a first survey of peracarid crustaceans was done in the intertidal communities only on isopods and amphipods (Marques et al. 1988). In that study, using benthonic sampling methodologies on the intertidal, Marques et al. (1988) have made an inventory of 14 isopod species and 21 amphipod species.

The majority of the peracarids caught in the study area were estuarine species and most of the species are benthopelagic: all the mysids, isopods and same of the amphipods, e.g., *Atylus* and *Ampelisca*, (Macquart-Moulin and Maycas 1995) and *Dexamine* (Macquart-Moulin and Patriti 1981); the Oedicerotidae species (two species were caught: *Pontocrates arenarius* Bate and *Perioculodes longimanus* Bate and Westwood) are referred to (Macquart-Moulin and Patriti 1981) as pelagic species. The identified amphipods taken in the plankton nets were generally immature individuals, – a situation described by other authors (Macquart-Moulin and Patriti 1981). Cumaceans are not truly planktonic, but despite being sand-burrowers they do swim quite effectively. Males are especially prevalent in plankton samples taken at night (Todd and Laverack 1991). The euphausid *(Nyctiphanes couchi)* caught in the study area is a pelagic species, already refered for the Portuguese coast (Cunha et al. 1997 a, b) and the three cumaceans *(Pseudocuma longicornis* Bate, *Eocuma dollfusi* Calman and *Diastylis* sp.) are bentho-pelagic species (Macquart-Moulin and Patriti 1981). Water mixing and sediment resuspension mechanisms may also explain the presence of the two cumaceans caught in that study. Butman (1987 in Grabe 1996) claimed that that kind of mechanism may affect the biological communities associated with sediments. Sediments may be reworked and resuspended up from the bed and may disturb the infauna. Such a mechanism may contribute to the dispersal of infaunal and epifaunal peracarids.

The most abundant species were the euryhaline mysid *Mesopodopsis slabberi* found in almost all estuaries (Makings 1977, Wooldridge 1983 *in* Webb et al. 1987, Azeiteiro et al. 1999) and the isopod *Paragnathia formica* which is a very important species in the estuary (Gonçalves 1991, Azeiteiro 1999); *P. formica* adults are usually benthic but pranizas are often ectoparasitic on fish and often taken in plankton nets (Naylor 1972). Other species may originate from inshore and estuarine environments. Examples includes the mysids: *Siriella* and *Schistomysis* species and *Gastrosaccus spinifer* (Makings 1977); the isopods Ligia oceanica and *Idotea baltica* (Naylor 1972); and Oedicerotidae amphipods and certainly the euphausids.

The fact that the downstream stations present saline stratification (Gonçalves 1991) and the collections were made with sub-surficial tows probably led to a subapreciation of the results since the haloclin may determine the animals upward migration. The low abundances of amphipods and cumaceans, mostly mud and sand-dwelling and sand-burrowing animals, can be explained in light of their sensitivity to surrounding disturbance e.g., eutrophication (Pardal 1998) and pollution (Macquart-Moulin and Patriti 1981). On the contrary the mobile species of the diverse substrata can survive more easily. This is the case for the mysid *Mesopodopsis slabberi* and the isopod *Paragnathia formica*, which add their euryhaline character (Macquart-Moulin and Patriti 1981, Azeiteiro et al. 1999).

Mysids also dominated the crepuscular peracarid fauna. Mysids and decapods are well represented in coastal communities (Buhl-Jensen and Fossa 1991, Hamerlynck and Mees 1991, Dauvin et al. 1994, Azeiteiro and Marques 1999, Cunha 2000) and together with amphipods are usually the most abundant groups in coastal communities (Buhl-Jensen and Fossa 1991, Hamerlynck and Mees 1991, Azeiteiro and Marques 1999, Cunha 2000). That study brings a new importance to the isopod populations namely the contribution of immatures to the pelagic community.

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CHARACTERISATION OF THE MACROINVERTEBRATE BENTHIC COMMUNITIES IN THE MONDEGO ESTUARY

Abstract

The Mondego estuary is under severe environmental stress, but despite the increasing pressure, until 1985 there was no reference data on the Mondego estuary on which further studies on the impact of human activities over the structure and functioning of the ecosystem could be based. From 1985 to 1990 a reference study on the benthic communities was carried out, regarding both the intertidal and subtidal zones, aiming to characterise the macrobenthic communities structure in relation to physicochemical environmental factors and identify the most important species, which could play a key role in the ecosystem functioning. The intertidal communities were surveyed in December 1986 and July 1987, while the subtidal communities were seasonally studied from December 1989 to September 1990.

With regard to the intertidal area, the community's structure revealed differences between the two arms of the estuary for populations densities and diversity, which was consistent with results from the analysis of physicochemical data. The south arm appears to be less affected by human activities, presenting more favourable conditions for the development of abundant populations of typical estuarine species. Salinity was the most important factor controlling the distribution of hard substrates organisms, while particles size and organic matter contents of sediments, salinity, and dissolved oxygen are the most important factors for soft substrates organisms. *Spartina maritima* and *Zostera noltii* marshes, mainly located in the middle section of the south arm, exhibited the richest macrofaunal composition with regard to abundance and diversity.

The subtidal macrofauna in the Mondego estuary appears to be clearly impoverished. In the south arm, the macrobenthic community consists mainly of infaunal species and appears to be more stable and structured, presenting higher macrofauna abundance. On the contrary, sparse mobile epibenthic species populations mainly characterise the north arm community, exhibiting a lower biodiversity and an impoverished macrofauna, compared to the south arm. The subtidal communities appear to be physically controlled, with emphasis on the type of sediment, salinity, and currents, and biologically, due to their distinct physicochemical characteristics. The two

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arms of the estuary can be considered different sub-systems. Due to harbour facilities dredging takes place regularly along the north arm, and time intervals between dredging operations appear to be inadequate to allow macrofauna recovery.

As a whole, the south arm community appears to be structurally more stable, but due to the feeble water circulation may be more exposed to environmental changes. Monitoring of the Mondego estuary biological communities was considered clearly necessary to assess temporal trends and to establish if the ongoing environmental changes are reversible.

Results from these studies were published in two previous papers (Marques et al. 1993 a, 1993 b).

Introduction

The Mondego, due to a set factors previously described, may be considered under a severe increase of environmental stress. But despite the increasing pressure, until 1985 there was no reference data on the Mondego estuary on which further studies on the impact of human activities over the structure and functioning of the ecosystem could be based.

From 1985 to 1990 reference studies on the benthic communities were carried out, regarding the intertidal area, in December 1986 and July 1987, and the subtidal zone, from December 1989 to September 1990. The aim of these studies was:

a) To characterise the macrobenthic communities structure in relation to physicochemical environmental factors;

b) To identify the most important species, which could play a key role in the ecosystem functioning;

c) To provide reference information to assess afterwards the impact of human activities on the communities structure and functioning.

The results of these studies were previously published in two independent papers, regarding respectively the intertidal (Marques et al. 1993 a) and the subtidal communities (Marques et al. 1993 b).

Material and methods

Intertidal sampling programme

In December 1986 and July 1987 quantitative samples were carried out at 19 sampling stations (figure 1) to characterise the structure of the intertidal communities in winter and summer situations. Each time, sampling took place during five consecutive days, always in the morning and during a 3 hours period in low water. This allowed samples to be carried out in approximately uniform conditions.

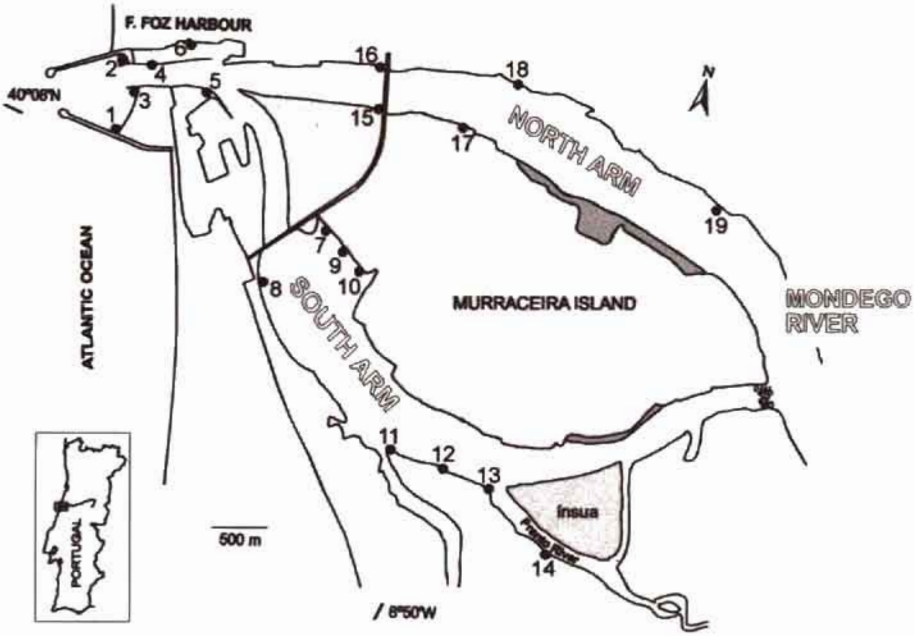

Figure 1. The Mondego estuary. Localization of the intertidal sampling stations.

Both hard and soft substrates were frequently found at the same sampling station, and depending on slope the area of the intertidal zone was quite variable. On soft substrates, *Spartina maritima* and *Zostera noltii* marshes could be present or not.

In order to establish a uniform sampling criterion, at each station the intertidal zone was stratified, taking into consideration different eulittoral levels, and the type of macroalgae or macrophytes covered areas. This criterion allowed considering three approximately equidistant levels between high water and low water levels. On hard substrates, depending on the sampling site, the two upper levels corresponded approximately to *Enteromorpha* spp. and *Fucus* spp. algal belts, whereas the lower level in stations located near the mouth of the estuary presented also a significant population of *Mytilus galloprovincialis* (mussels). On soft substrates with vegetal covered areas the two upper levels frequently corresponded respectively to the marsh-grass *Spartina maritima* belt and to the eelgrass *Zostera noltii* meadows, while the lower level corresponded mainly to sandy or muddy substrates without macrophytes.

Two different sampling techniques were used as a function of the type of substrate. On hard substrates three replicates of 625 cm^2 were randomly sampled in each level by scratching out organisms with a chisel. On soft substrates we adapted the technique described by Dexter (1979, 1983) for sandy beaches, and eight replicates were randomly sampled in each level by using a manual corer (each core corresponding to 141 cm^2 and approximately 3 litres of sediment).

All biological samples were sieved in situ using a 1 mm mesh size sieve, and then fixed in 4% neutralised formaldehyde. This mesh size was considered suitable for this study, regarding the types of sediment we expected to find along the estuary.

Each time and for each station, several physicochemical factors were determined, respectively salinity, temperature, pH, dissolved oxygen (measured *in situ*), nitrites, nitrates, and phosphates (analysed in the laboratory). The analysis of water samples followed the methods described in Strickland and Parsons (1968). Sediment samples were also collected and subsequently analysed for particles size, organic matter and carbonate contents.

For each sediment sample, particles were ranked into eight size categories (table I):

TABLE I Particle – size categories used to classify sediment types in the present study

Size class	Diameter (mm)	Sediment classification
1	> 2	Gravel
2	1 to 2	Coarse sand
3	0.5 to 1	Medium sand
4	0.250 to 0.5	
5	0.125 to 0.250	Fine sand
6	0.063 to 0.125	Silt
7	0.002 to 0.063	
8	< 0.002	Clay

The organic mater content in the sediments was calculated after destruction in a muffle furnace (8 hours at 500 °C).

In the laboratory the organisms were separated, preserved in 70% ethanol or in 4% neutralised formaldehyde, according to the presence or absence of calcareous parts, and identified and counted.

Subtidal sampling programme

In December 1989 and March, June, and September 1990 quantitative samples were taken at 13 sampling stations (A to M) (figure 2), to allow a seasonal characterisation of the subtidal macrobenthic communities. Each time samples were taken over a two days period, during high water of spring tides. At each station six replicates were sampled randomly, using a small Van Veen grab, capable of collecting up to 5 L of sediment, operated from a boat. The number of replicates per sample was settled by using the rank-frequency diagram method (Frontier 1983) for stabilising variability. Although the sampled area was approximately constant (496 cm^2), the amount of sediment collected was not, depending on bottom compactness. A certain degree of bias was therefore introduced into the sampling strategy.

The biological samples, both in the field and the laboratory, were treated the same way as indicated above, and physicochemical factors were also determined each time and for each station, following the same methodology.

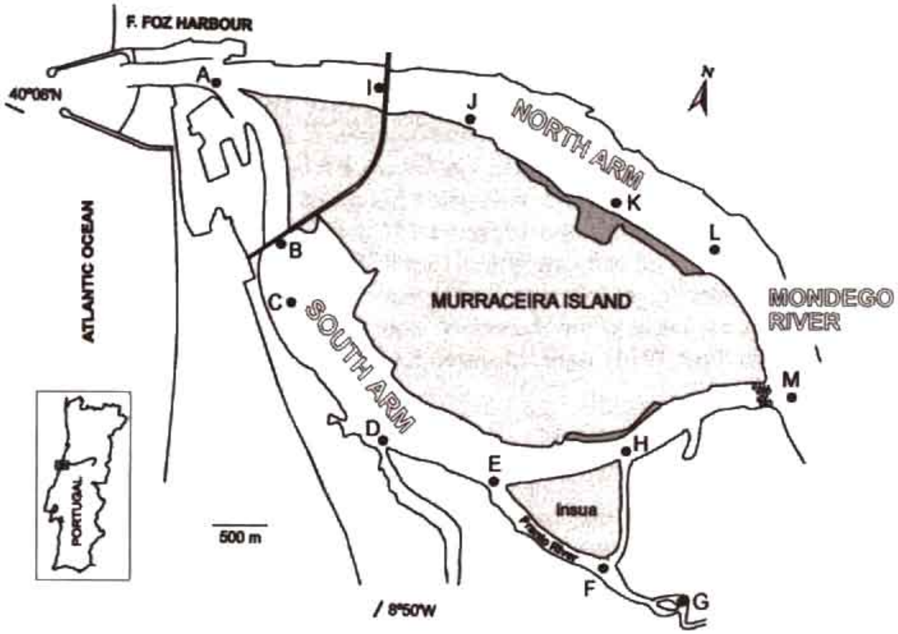

Figure 2. The Mondego estuary: Location of the subtidal sampling stations.

Data analysis

Intertidal communities

Data on both hard and soft substrates and on winter and summer situations were assumed to correspond to different ecological conditions, and therefore were analysed separately.

With regard to biological data, species X stations matrices were analysed, considering data on each sampling site as a whole. The goal of the analysis was to study the horizontal distributional ecology of the species along the estuary and to reveal differences between the two estuarine arms with regard to community's structure. A first analysis was achieved taking into consideration all the species, and a second one overlooking the species found only once (Legendre and Legendre 1984).

On hard substrates, since it was not possible to collect water in each sampling level, water samples for determination of physicochemical factors were always taken from the water column (one sample per station). On soft substrates, because of water retention in pools during low tide, it was always possible to get water and sediment samples in each sampling level. Consequently, in the first case, we analysed factors X stations matrices, while in the second case the analysis was based upon factors X samples matrices.

Data underwent principal component analysis (PCA), using the sampling stations or the samples as operational units in the space of biological or physicochemical variables. Sediment particles size fractions (expressed in %) and dissolved oxygen (% of saturation) were both submitted to angular transformation. Eigenvalues and eigenvectors of correlation matrices between variables were computed after centering and reduction to unit variance (Legendre and Legendre 1984). Correlation matrices were computed using the Pearson's correlation index. In addition, biological data was submitted to cluster analysis, using the Chi-Square distance coefficient (Lebart et al. 1984) (Q mode analysis) and the unweighted pair group mean of analysis (UPGMA) clustering method (Legendre and Legendre 1984). Data treatment was effectuated with the NTSYS-PC 1.60 software system (Rohlf 1990).

Finally, in order to get information on species richness and evenness in different estuarine areas, the values of the Shannon-Wiener heterogeneity index (Legendre and Legendre 1984, Peet 1974) were calculated for each sampling station in winter and summer situations.

Subtidal community

It was also assumed that data for each season should correspond to distinct ecological conditions, and were therefore analysed separately.

With regard to the biological data, seasonal matrices of taxa X stations were analysed, considering data from each station (a series of six replicates) as a whole. In this case, biological data underwent Correspondence Analysis (CA). The Chi-Square Distance coefficient (Lebart et al. 1984) was used to calculate the association matrices for the column (stations) variables. The eigenvalues and eigenvectors for the columns were then computed, followed by the computation of the row (taxa) vectors by projection.

Like before, the Shannon-Wiener heterogeneity index was used to assess biological diversity. Moreover, and as described above, physicochemical data on water and sediments underwent principal component analysis (PCA). The same software was used to perform multivariate data analysis.

Results

A – Intertidal zone

We identified 90 macrofaunal species from samples carried out in winter and summer situations (table II). A first look to data confirmed our primary assumptions for data analysis, showing that 34 taxa (38%) were found only in the winter, while 19 (21%) were found exclusively in the summer, reflecting a seasonal variation in the species composition. Moreover, 36 taxa (40%) were found exclusively on hard substrates, while 24 (27%) occurred only in soft substrates, exhibiting a different species composition as a function of the type of substrate.

Table II – List of the taxa identified in winter and summer situations, and on both hard and soft substrates. For each taxa, the average density (number of individuals.m-^{2}) is given.

Taxa	Winter		Summer	
	Hard substrates	Soft substrates	Hard substrates	Soft substrates
TURBELLARIA				
Convoluta sp		0.7	0.4	0.6
NEMERTINI				
Lineus sp	4.2			
Oerstedia sp		2.8	0.4	
Tetrastemma sp		3.5	2	0.6
Palaenemertea				0.6
OLIGOCHAETA		0.7		
POLYCHAETA				
Eteone picta		6.3		
Glycera convoluta		4.2		
Lepidonotus clava			0.4	
Nephthys cirrosa		0.7		
Hediste diversicolor	10.8	661	13.2	890
Neanthes irrorata		2.8	0.4	
Phyllodoce sp	1.4			
Polydora sp			2.9	
Amage adspersa		34.6	2	128
Amphictheis gunneri		17		
Capitella capitata		4.8	0.8	30.3
Cirratulus cirratus	0.5			
Heteromastus filiformis		72.9		
Lagis koreni		2.8		
Mercierella enigmatica			4.4	
Pomatocerus triqueter	9.4	0.7		
Pseudomalacocerus cantabra		8.4		
Pygospio elegans		0.7		
Sabellaria alveolata	2.4			
Spio filicornis		3.5		3.7
Streblospio dekhuyzeni		24.5	2.4	23.9
Sabellidae			0.4	
POLYPLACOPHORA	0.5			
Lepidochitona cinereus	0.5			
GASTROPODA				
Bittium reticulatum				0.6
Cerithium vulgatum				0.6
Gibbula umbilicalis				0.6
Haminea hydatilis	30	200		0.6
Hydrobia ulvae	52.2	1980	181	859
Littorina littorea	0.5	15.4		10
Littorina neritoides	2.8			
Littorina saxatilis				0.6
Murex trunculus	0.9			
Nassarius reticulatus				5

Nucella lapillus			0.4	
Odostomia unidentata	0.9			
Patella aspera	0.5			
Patella lusitanica	1.4			
Rissoa membranacea	0.5			
Rissoa parva	0.9			1.3
Cerastoderma edule		66.6	2.8	36
Montacuta ferruginosa	32.9	0.7		
Mytilus galloprovincialis	5790	4.9	1390	
Scrobicularia plana	1.9	283	4.8	103
ANOSTRACA				
Artemia salina		0.7		
CIRRIPEDIA				
Ballanus perforatus	11.8			
Chthamalus stellatus	764		1470	
ISOPODA				
Cyathura carinata	0.9	322	11.2	128
Dynamene bidentata	0.5		2.4	
Gnathia vorax	0.5			
Eurydice pulchra				0.6
Eurydice spinigera				0.6
Idotea chelipes	3.8	0.7		
Idotea granulosa	3.3		1.6	0.6
Idotea pelagica	64.9	0.7	37.6	
Jaera forsmani	9.9		63.6	
Sphaeroma hookeri	24.4	0.7	10.4	1.8
AMPHIPODA				
Amphithoe valida	1.4			
Amphithoe ramondi	3.3			
Amphithoe rubricata	3.4			
Bathyporeia sarsi		1.4		1.3
Corophium insidiosum	33.1		2.8	
Corophium multisetosum		3.4		5.7
Echinogammarus marinus	196	12.6	951	3.1
Echinogammarus stoerensis			68.4	
Gammarus chevreuxi	1.4	1.4		
Gammarus locusta	2.6		6.4	
Haustorius arenarius		0.7		
Hyale crassipes	3.3			
Hyale perieri	2.8			
Hyale stebbingi	184		117	
Jassa marmorata			0.4	
Leptocheirus pilosus	44.7		88.4	
Melita palmata	88.9	19.1	66.8	11.9
Talorchestia sp			8.4	
MYSIDACEA				
Paramysis helleri				1.9
DECAPODA				

Carcinus maenas	13.2	13.3	80	15
Crangon crangon		2	7.6	11.3
Pachygrapsus marmoratus	6.6		1.2	
Palaemonetes varians	0.9	0.7	2.8	1.8
INSECTA				
Diptera larvae	4.2	9.1	86.4	3.7
Lepidoptera larvae				1.6
PISCES				
Blennius sp 1	0.5			
Blennius sp 2	0.5			
Pomatoschistus sp	0.5	0.7		1.9

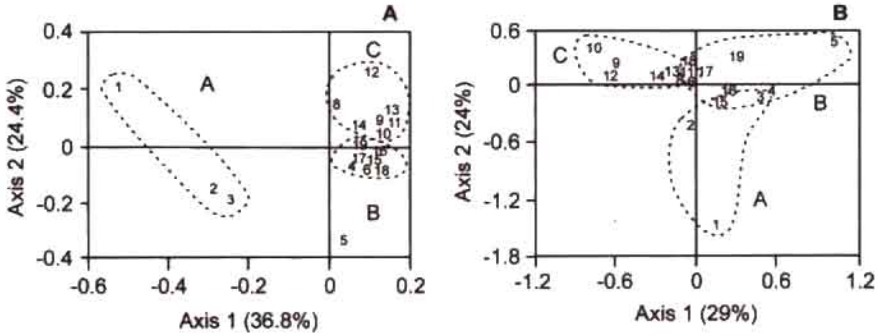

Figure 3. Analysis of hard substrates community structure from PCA of species X stations matrices overlooking species found only once. A - winter situation: Projection of stations against the first two axis, r = 0.88934. B - summer situation: Projection of stations against the first two axis, r = 0.85206. The percentage of variability explained by the principal axis is given. Groups of stations pointed out are discussed in the text.

Hard substrates community

Winter situation

PCA of species X stations data (figure 3-A) shows a clear separation between stations located near the mouth (group A) and stations located inside the estuary (groups B and C) along the first axis. A separation between stations from the south arm (group C) and stations from the north arm, together with a few stations located near the mouth (group B), is evident along the second axis. Near the mouth, sessile marine species like *Chthamalus stellatus* and *Mytilus galloprovincialis* are very abundant, and significant populations of *Montacuta ferruginosa, Idotea pelagica,* and *Hyale stebbingi,* all marine species, together with less important populations of *Littorina neritoides, Ballanus perforatus, Idotea chelipes, I. granulosa, Jaera forsmani, Amphithoe ramondi, A. rubricata,* and *Pachygrapsus marmoratus* are also found. In the inner areas of the estuary, *Mytilus galloprovincialis* and *Chthamalus stellatus* populations become much less

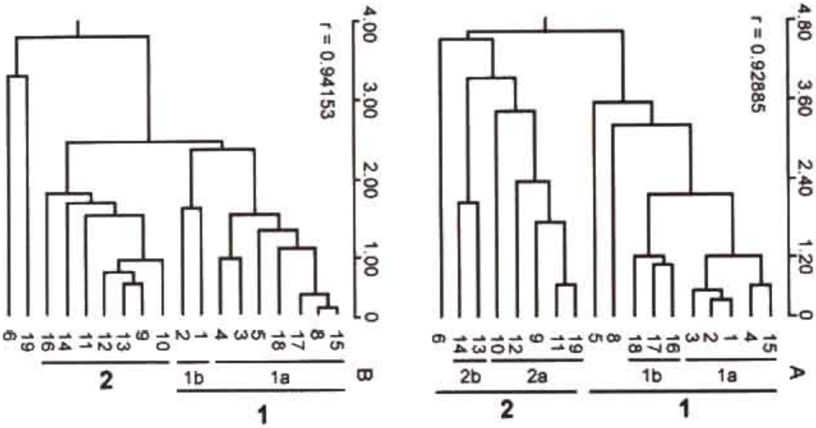

Figure 4. Hard substrates community structure: Cluster analysis of species X stations matrices overlooking species found only once. Data analysed using the Chi-Square distance coefficient (Q mode analysis) and the UPGMA clustering method. A - winter situation; B - summer situation. Values of cophenetic correlation coefficients are indicated.

abundant, and the presence of other marine species is inconspicuous. Station 1, which exhibits the strongest marine influence, (typical estuarine species are represented only by sparse populations of *Echinogammarus marinus* and *Carcinus maenas*), presents dense populations of *Mytilus galloprovincialis*, *Chthamalus stellatus*, and *Hyale stebbingi*. The separation of stations from both estuarine arms along the second axis is mainly due to the preferential occurrence of *Leptocheirus pilosus* and *Melita palmata*, followed by *Sabellaria alveolata*, in stations from the north arm, and of *Echinogammarus marinus*, *Sphaeroma hookeri*, and Hediste diversicolor (frequently found in sediment deposits over rock), followed by *Idotea chelipes*, *I. pelagica*, *Amphithoe ramondi*, *A. rubricata*, and *Carcinus maenas*, in stations from the south arm.

Station 5, located near the connection of the two arms, appears to be peculiar, exhibiting significant densities of *Melita palmata* (704 individuals/m^2) and *Leptocheirus pilosus* (437 individuals/m^2) populations. Typical estuarine species like *Hydrobia ulvae*, *Echinogammarus marinus*, *Sphaeroma hookeri*, and *Carcinus maenas* show higher abundances in the south arm, while *Mytilus galloprovincialis* and *Chthamalus stellatus* populations are significant in the north arm (although less abundant than in stations located near the mouth) and very scarce in the south arm.

Cluster analysis of species X stations data (figure 4-A) allows to recognise a structural discontinuity in the communities from both arms and near the mouth, corroborating therefore the results from ordination. Group 1 consists of stations located near the mouth (basically sub-group 1a) and inside the north arm (sub-group 1b), together with stations 8 and 5, located in the downstream section of the south arm. Group 2 consists primarily of stations from inner areas of the south arm, despite station 19 (upstream section of the north arm) being included in sub-group 2a, and station 6 (near the mouth) is still comprised in the group.

Summer situation

PCA of species X stations data (figure 3-B), show an opposition between stations located in the north arm and near the mouth (group B), and stations located in the inner areas of the south arm (group A) along the first axis. Stations from group B are characterised by the presence of several marine species, with a clear dominance of *Mytilus galloprovincialis* and *Chthamalus stellatus*, followed by significant populations of *Echinogammarus stoerensis*, *Leptocheirus pilosus*, and *Melita palmata*. Stations located in the south arm present *Hydrobia ulvae* and *Echinogammarus marinus* dense populations, exhibiting also a typical estuarine fauna with regard to other species.

The opposition between stations 1 and 2 (more exposed to marine influence), and the other stations is evident along the second axis. These two stations are characterised by a very strong abundance of *Mytilus galloprovincialis* and by the occurrence of typical marine species like *Hyale stebbingi*, *Dynamene bidentata*, *Idotea pelagica*, and *Jaera forsmani*. Station 5, like in the winter situation, is found to be peculiar, presenting relatively abundant populations of *Leptocheirus pilosus* (901 individuals /m^2) and *Melita palmata* (267 individuals /m^2). It must be emphasised that *Echinogammarus marinus* shows a quite abundant population all over the estuary in the summer situation.

Cluster analysis of species X stations data (figure 4-B) shows again a discontinuity within the hard substrates community structure in both arms and near the mouth. Group 1 consists basically of stations located in the north arm and near the mouth despite station 8 (downstream area of the south arm) being comprised in sub-group 1a. Stations 1 and 2 (sub-group 1b), located very close to the mouth appear to be distinct from stations inside the north arm (sub-group 1a). Group 2 consists of all stations from the inner areas of the south arm and station 16 (north arm). Stations 19 and 6 appear as outsiders.

Diversity

In the winter situation, the Shannon-Wiener index values calculated for each station (table III) demonstrate that the distance relatively to the mouth is not related with a diversity gradient. However, stations from the south arm show higher diversity values than stations located in the north arm and near the mouth, which may be due to the combined effects of tides and stronger freshwater discharge along the north arm, creating a significant daily environmental stress for environmental organisms.

Table III Values of the Shannon-Wiener index calculated for each station in winter and summer situations and for hard and soft substrates communities

	SAMPLING STATIONS																		
	Hard substrates																		
	Near the mouth						South arm								North arm				
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
Winter	0.98	0.61	0.81	1.24	2.54	1.23	-	0.99	1.07	1.21	1.26	2.31	1.16	1.94	1.11	2.27	0.35	0.62	1.2
Summer	1.55	2.09	2.22	2.12	1.58	2.2	-	0.41	0.78	1.21	1.4	0.73	1.12	1.07	0.78	0.99	0.97	1.5	3.0

Soft substrates

	Near the mouth						South arm								North arm				
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
Winter	1.0	1.39	-	-	-	1.52	2.32	1.5	2.69	2.19	1.96	1.43	1.91	1.71	2.13	3.08	1.6	2.01	1.52
Summer	0.92	0.76	-	-	-	-	2.65	1.83	2.3	1.6	2.08	1.36	1.19	1.62	1.51	1.45	0.66	1.38	0.54

On the other hand, in the summer situation, the Shannon-Wiener index values calculated for each station (table III) revealed several differences as compared to the winter situation. In the summer, the highest values for diversity are found near the mouth of the estuary, while the lowest values are found inside the south arm.

With regard to hard substrates community, a decrease in diversity was observed in the south arm from winter to summer, while an increase occurred in the north arm and near the mouth. The decrease in diversity observed in the south arm may be explained by the change in biological activity of *Echinogammarus marinus*, which becomes extremely abundant in the summer situation (average about 3000 individuals /m^2 in the south arm on the *Fucus* sp. covered areas), affecting species evenness.

Soft substrates community

Winter situation

PCA of species X stations data (figure 5-A) shows the opposition between stations 7, 9, 10, and 11 (group A), located in the south arm, corresponding to *Spartina maritima* and *Zostera noltii* marshes, and stations without vegetal covered areas (groups B and C) along the first axis. These stations differ from the others by the fact that several species (e. g. *Amage adspersa*, *Heteromastus filiformis*, *Hediste diversicolor*, *Hydrobia ulvae*, *Cerastoderma edule*, *Scrobicularia plana*, and *Cyathura carinata*) present considerably higher population abundances. Along the second axis we can distinguish

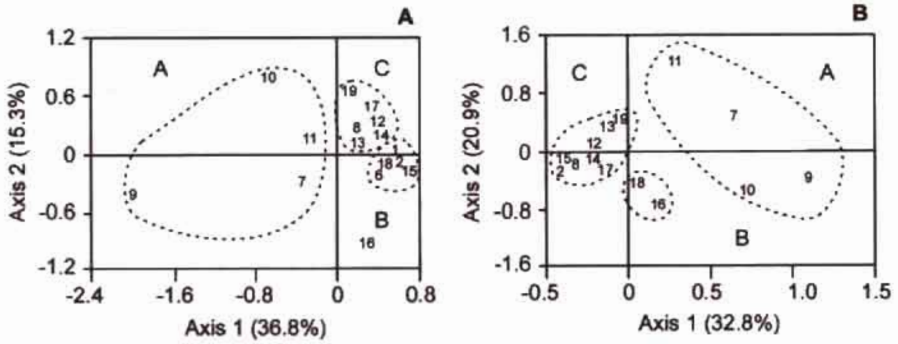

Figure 5. Analysis of soft substrates community structure from PCA of species X stations matrices overlooking species found only once. A - winter situation: Projection of stations against the first two axis, r = 0.80878. B - summer situation: Projection of stations against the first two axis, r = 0.87681. The percentage of variability explained by the principal axis is given. Groups of stations pointed out are discussed in the text.

between stations located in the south arm (8, 12, 13, and 14), followed by stations 17 and 19 (north arm) (group C), and stations located in the north arm and near the mouth of the estuary (group B). Stations from the south arm, even those located in areas without vegetal cover, present higher population abundances than stations from the north arm, namely with regard to common species like Hediste diversicolor, *Hydrobia ulvae, Scrobicularia plana,* and *Cyathura carinata.* Station 16, located in the north arm, is clearly separated along the second axis, which is explained by the sporadic occurrence of several rare species in the estuary like *Eteone picta, Glycera convoluta,* and *Spio filicornis.*

Cluster analysis of species X stations data (figure 6-A) does not reveal a clear discontinuity within the soft substrates community. Actually, a single main group of stations is recognisable (group 1), consisting of stations from both estuarine arms, while stations 1, 2, and 6, located near the mouth appear as outsiders. Nevertheless, stations 9, 11, 10, and 7, located in *Spartina maritima* and *Zostera noltii* marshes, are clearly assembled (sub-group 1b), which agrees with results from ordination.

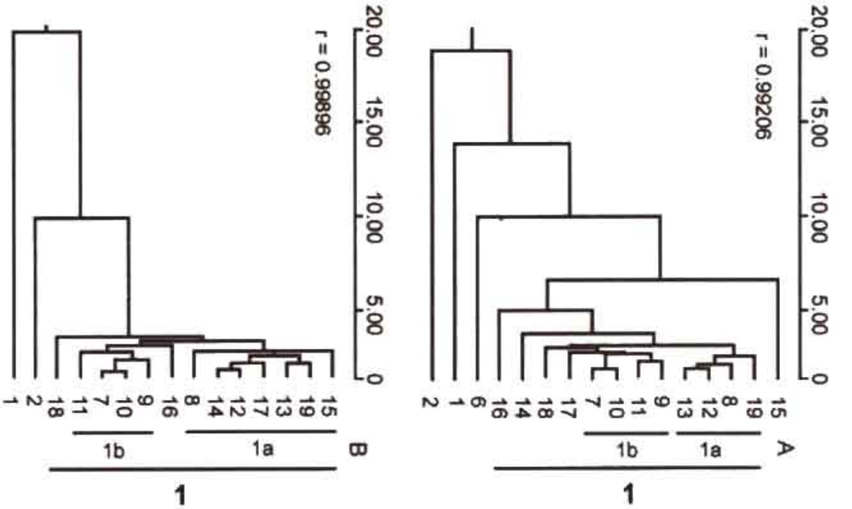

Figure 6. Soft substrates community structure: Cluster analysis of species X stations matrices overlooking species found only once. Data analysed using the Chi-Square distance coefficient (Q mode analysis) and the UPGMA clustering method. A - winter situation; B - summer situation. Values of cophenetic correlation coefficients are indicated.

Summer situation

PCA of species X stations data (figure 5-B) shows once more the opposition between stations corresponding to *Spartina maritima* and *Zostera noltii* marshes (group A) and stations without vegetal covered areas (groups B and C) along the first axis. Like in the winter situation, the most important species contributing to the observed variability are *Amage adspersa, Heteromastus filiformis, Hediste diversicolor, Hydrobia ulvae, Cerastoderma edule, Scrobicularia plana* and *Cyathura carinata* (positive side of factor 1), which populations are much more abundant in stations from group A as compared to other areas. Contrarily to the winter situation, differences between stations located in the south arm and stations located in the north arm are not evident. This may be due to the increase of marine influence inside the estuary in the summer, determining the occurrence of more uniform conditions.

Cluster analysis of species X stations data (figure 6-B), like in the winter situation, does not bare a discontinuity within the soft substrates community, and again a single group of stations is recognisable (group 1), consisting of stations from both estuarine arms, Stations 1 and 2 located very close to the mouth appear as outsiders. Again like in the winter situation, stations 9, 10, 7, and 11, located in *Spartina maritima* and *Zostera noltii* marshes, are assembled (sub-group 1b), corroborating results from ordination.

Diversity

The Shannon-Wiener index values calculated for each station in both winter and summer situations (table III) are consistently higher in stations located in *Spartina maritima* and *Zostera noltii* marshes, which emphasises their favourable conditions for the development of abundant populations and higher biodiversity. However, differences between other estuarine areas and seasonal variations in diversity are not outstanding.

Physical and chemical parameters

With regard to the winter situation, PCA of water physicochemical factors X stations matrices (figure 7-A) reveals a clear separation between stations from the north and south arms (groups A and B respectively) along the first axis, and a gradient from the mouth (group C) to inner areas of the estuary along the second axis. The variability along the first axis is mainly explained by the distribution pattern of dissolved oxygen and nitrates concentration values (negative side of factor 1), and of salinity and temperature values (positive side of factor 1). Along the second axis, variability is mainly explained by the distribution of salinity, temperature and nitrites values (negative side of factor 2), and of pH (positive side of factor 2). Actually, it is very clear the opposition along the first axis between stations from the north arm, presenting lower salinities (20.8 ± 6.8 ‰) (average ± standard deviation), more stable temperatures (12 ± 0.5 oC), higher concentrations of dissolved oxygen (76.5 ± 11.6 % of saturation) and nitrates (0.32 ± 0.18 mg.l^{-1}) during low tide, and stations from the south arm, presenting higher salinities (22.9 ± 6.7 ‰), more variable temperatures (12.9 ± 2.4 °C), lower dissolved oxygen (70.6 ± 7.1 %) and nitrates concentrations (0.16 ± 0.09 mg.l^{-1}).

These results can be explained taking into consideration the hydraulic circulation in the estuary. In the north arm, the water circulation depends on tides and on the freshwater discharge, determining a faster renewal of the water mass, and consequently higher values of dissolved oxygen. Moreover, since samples were taken during low tide, the river discharge (transporting nutrients from agricultural areas) determined the occurrence of lower salinities and higher nitrate concentrations in the north arm and areas near the mouth. The smaller depth may explain larger

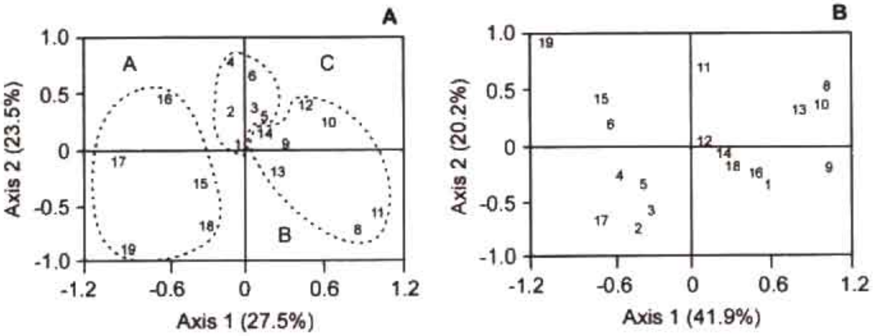

Figure 7. Analysis of physicochemical factors of the water from PCA of factors X stations matrices. A - winter situation: Projection of stations against the first two axis, r = 0.94057. B - summer situation: Projection of stations against the first two axis, r = 0.93314. The percentage of variability explained by the principal axis is given. Groups of stations pointed out are discussed in the text.

temperature ranges found in the south arm. Finally, the lower concentration of nitrates in the south arm may be a function of the smaller freshwater discharge.

Due to marine influence, temperature and pH values (7.4 ± 0.4) seem to be more uniform near the mouth of the estuary, and nitrite concentration to be low (0.006 ± 0.002 mg.l^{-1}) (probably as a function of stronger oxygenation of the water column).

In the summer situation, the analysis of physicochemical factors of the water does not show conspicuous differences between stations located in both estuarine arms and near the mouth (figure 7-B). Stations 2, 3, 4, 5, 6, 15, 17, and 19 (in the north arm and near the mouth) are opposed to stations 1, 8, 9, 10, 11, 12, 13, 14, 16, and 18 (in the north arm, south arm, and near the mouth) along the first axis. The variability along the first axis is mainly explained by lower salinities (25 ± 2.1 ‰), higher values of dissolved oxygen (92.8 ± 6.1 %), pH (7.8 ± 0.3) and nitrites (0.01 ± 0.002 mg.liter-1) found in stations from the negative side of factor 1, and by higher salinities (27.3 ± 2.4 ‰) found in stations from the positive side of factor 1.

Along the second axis, stations from the inner areas of both arms (8, 10, 11, 12, 13, 15, and 19) are partially separated from stations located in the downstream section of the north arm and near the mouth. Temperature is the factor that contributed the most for this partial separation. Actually, the smaller depth as compared to areas near the mouth may explain higher temperatures of the water found in estuarine inner areas.

PCA of water and sediments physicochemical factors X samples matrices shows similar results with regard to winter (figure 8-A) and summer (figure 8-B) situations. In both cases, projection of samples against the first two axis of variability allows to consider three distinct equivalent groups. Groups A1 and A2 correspond mainly to samples obtained on fine or medium sand bottoms with small organic matter contents (0 to 1.5%), proceeding from the lower limits of the eulittoral zone (low water level) in the downstream sections of both arms and near the mouth (sand pole). Groups B1, B2, C1, and C2 correspond to samples from bottoms with large fractions of fine

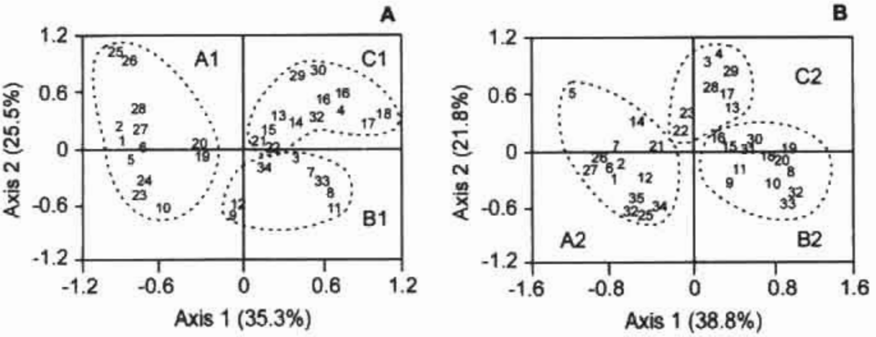

Figure 8. Analysis of physicochemical factors of water and sediments from PCA of factors X samples matrices. A - winter situation; Projection of samples against the first two axis, r = 0.91314; B - summer situation; Projection of samples against the first two axis, r = 0.94261. The percentage of variability explained by the axis is given. Groups of samples pointed out are discussed in the text.

particles (clay or silt) and higher organic matter contents (2 to 4.5%), proceeding from the inner areas of both estuarine arms. Groups B1 and B2 consist of samples from *Spartina maritima* and *Zostera noltii* covered areas in the south arm, characterised by fine sandy mud sediments with high organic matter contents (3.5 to 4.5%). Groups C1 and C2 consist essentially of samples from muddy bottoms with no vegetal cover, mainly characterised by clay and silty sediments mixed with medium to coarse sand (10 to 40%) (originated mainly by dredging activities), and significant organic matter contents (2 to 4%). Additionally, in the summer situation (figure 8-B) oxygen dissolved levels and salinities are higher in samples from group C2 as compared with samples from group B2.

B – Subtidal zone

52 samples, corresponding to 306 replicates distributed over the year, provided 7554 macrofaunal individuals and allowed the identification of 58 taxa (Table IV).

The relative frequencies of taxa and the average population densities (Table IV) show that only a few species appear consistently well represented through the year. The most frequent and abundant are *Amage adspersa*, *Scrobicularia plana*, and *Cyathura carinata* but other species are also well represented over the year: *Hediste diversicolor*, *Streblospio shrubsolii*, *Cerastoderma edule*, *Hydrobia ulvae*, *Saduriella losadai*, *Neomysis integer*, and *Carcinus maenas*.

Higher abundances of *Amage adspersa*, *Scrobicularia plana*, and *Cyathura carinata* populations (although values are expressed in number of individuals and not biomass, which may introduce a significant bias), suggest that these species play a key role in the ecosystem. However, *Scrobicularia plana* is primarily represented by juveniles (adults are typical of the intertidal zone), which is probably related to the planktonic larvae colonisation process.

Table IV List of taxa identified. The number assigned to each taxa correspond to numbers plotted in figures 9 – 12. The relative frequencies (F: number of replicates in which the taxa was found/total number of replicates) and average population density (AD: individuals.m-2 for the total surface of all the samples) found in the Mondego estuary each season are given.

		December		March		June		September	
	Taxa	F(%)	AD	F(%)	AD	F(%)	AD	F(%)	AD
1	*Turbellaria sp*	2.7	0.5	0	0	0	0	0	0
	Nemertini								
2	*Oerstedia sp*	4.1	1.3	0	0	7.7	2.1	0	0
3	*Tetrastemma sp*	1.4	0.3	1.3	0.3	5.1	1.6	4	0.9
4	sp 1	1.4	0.3	0	0	0	0	0	0
	Oligochaeta								
5	sp 1	6.8	1.2	0	0	2.6	0.5	2.7	0.8
	Polychaeta								
6	*Amage adspersa*	24.7	22.6	32.9	88.2	42.3	565	37.3	138
7	*Capitella capitata*	1.4	0.5	2.6	0.5	11.5	4.9	6.7	1.6
8	*Chaetozone setosa*	1.4	0.3	1.3	0.3	1.3	0.5	0	0
9	*Chone collaris*	0	0	0	0	1.3	5.4	2.7	0.8
10	*Eteone picta*	2.7	0.5	0	0	0	0	1.3	0.3
11	*Eulalia sp*	1.4	0.2	0	0	0	0	0	0
12	*Glycera convoluta*	0	0	0	0	0	0	4	0.8
13	*Hediste diversicolor*	13.7	3.4	6.6	2.1	7.7	4.7	10.7	30.3
14	*Heteromastus filiformis*	1.4	0.9	5.3	1	3.8	1.6	4	0.8
15	*Lagis koreni*	0	0	0	0	0	0	1.3	0.3
16	*Neanthes succinea*	0	0	1.3	0.3	0	0	0	0
17	*Nephtys cirrosa*	2.7	1.3	0	0	1.3	0.3	5.3	1.5
18	*Nephtys hombergii*	1.4	0.3	0	0	0	0	1.3	0.3
19	*Nephtys longosetosa*	1.4	0.3	0	0	0	0	0	0
20	*Nephtys paradoxa*	2.7	1.0	0	0	1.3	0.3	0	0
21	*Perinereis cultrifera*	0	0	0	0	0	0	1.3	0.3
22	*Polydora ciliata*	4.1	0.9	1.3	1.8	34.6	55.0	8	2.3
23	*Oriopsis sp Spio decoratus*	0	0	0	0	1.3	0.3	0	0
24	*Spio decoratus*	0	0	0	0	3.8	1.3	6.7	2.6
25	*Streblospio shrubsolii*	11	12.9	25	31.8	35.9	19.5	38.7	38.9
	Mollusca								
	Bivalvia								
26	*Abra nitida*	0	0	0	0	0	0	2.7	1
27	*Cerastoderma edule*	5.5	1.5	0	0	16.7	5.4	33.3	50.2
28	*Scrobicularia plana*	42.5	147	34.2	103	24.4	10.3	52	88.9
29	*Solen marginatus*	0	0	0	0	0	0	2.7	0.5
30	*Spisula subtruncata*	0	0	0	0	1.3	1.6	1.3	0.3
31	*Spisulla elliptica*	1.4	0.5	0	0	0	0	0	0
32	*Tellina tenuis*	6.8	2.1	0	0	0	0	6.7	4.4
	Gastropoda								
33	*Hydrobia ulvae*	8.2	2.7	11.8	7.8	43.6	37.2	33.3	14.3
34	*Nassarius reticulatus*	1.4	0.2	0	0	0	0	0	0
	Isopoda								
35	*Cyathura carinata*	41.6	55.6	39.5	41.4	33.3	35.9	49.3	59.1
36	*Eurydice pulchra*	1.2	0.2	0	0	0	0	0	0
37	*Idotea chelipes*	0	0	0	0	1.3	0.3	1.3	0.3
38	*Paragnathia formica*	0	0	0	0	0	0	1.3	0.3
39	*Sadunella losadai*	6.8	1.8	1.3	0.3	9	5.4	6.7	4.3
40	*Sphaeroma hookeri*	5.5	2.7	0	0	6.4	1.3	10.7	3.2
	Amphipoda								
41	*Bathyporeia sarsi*	4.1	3.1	0	0	7.7	2.3	2.7	1.3
42	*Corophium multisetosum*	2.7	0.6	0	0	1.3	0.3	2.7	1.6
43	*Haustorius arenarius*	0	0	1.3	0.3	0	0	0	0

44	*Melita palmata*	1.4	0.2	0	0	7.7	5.6	10.7	2.6
	Cumacea								
45	*Eocuma dollfusi*	0	0	0	0	1.3	0.3	0	0
	Mysidacea								
46	*Mesopodopsis slabberi*	0	0	0	0	1.3	0.3	0	0
47	*Neomysis integer*	16.4	4.3	11.8	17.2	2.6	0.5	1.3	0.3
	Decapoda								
48	*Carcinus maenas*	5.5	1	3.9	0.8	6.4	1.3	6.7	1.7
49	*Crangon crangon*	0	0	0	0	10.3	2.6	13.3	3.8
50	*Palaemonetes varians*	0	0	0	0	1.3	0.5	0	0
	Echinodermata								
51	*Marthasterias glacialis*	1.4	0.2	0	0	0	0	0	0
52	*Ophiuroidea sp*	1.4	0.2	0	0	0	0	0	0
	Insecta								
53	*Chironominae larvae*	2.7	0.5	0	0	3.8	2.6	1.3	1
54	*Diptera larvae*	0	0	0	0	0	0	4	1
	Gomphus pulchellus	1.4	0.3	0	0	0	0	0	0
	Pisces								
55	*Ammodytes tobianus*	0	0	1.3	0.3	0	0	0	0
56	*Engraulis encrasicolus*	1.4	0.3	0	0	0	0	0	0
57	*Platichthys flesus*	0	0	0	0	0	0	1.3	0.3

Seasonal variation of community structure

A seasonal variation of the total macrofaunal abundance was observed, with the lowest values for total macrofaunal abundance being found in December (972 individuals of 38 species were collected). Until March, although total macrofaunal abundance increased (1556 individuals were collected), the number of species found was much lower (only 17), probably as a repercussion of the effects of winter. From March to June, the spring influence was clearly discernible in the increase of total macrofaunal abundance and number of species (2981 individuals of 33 species were collected). Finally, from June to September, a small decrease in total macrofaunal abundance was observed (2045 individuals collected), although the number of species collected (37) was slightly higher.

The analysis of matrices of taxa X stations revealed clear differences between the macrofaunal community structure of the two arms of the estuary. Projection against the first and second axis of variability, based on December, March, June, and September data (figures 9: A, 10: A, 11: A, and 12: A), and despite seasonal variations, show a consistent pattern of structural discontinuity between stations D, E, F, G, and H, located in the inner areas of the south arm, and stations J, K, L, and M, located in the middle and upstream sections of the north arm.

Station A, located close to the mouth of the estuary, stations B and C, located in the downstream area of the south arm, and station I, located in the downstream section of the north arm, appear to be structurally more similar to each other, although seasonal variability in the macrofauna composition seems to be stronger, which can explain their irregular pattern of assemblage through the year. On the other hand, stations located in the upstream section of the north arm (L and M) appear to be relatively different from other stations in the north arm with regard to macrofauna, which is particularly evident in March situation (figure 10: A), where these two stations are clearly separated from the rest.

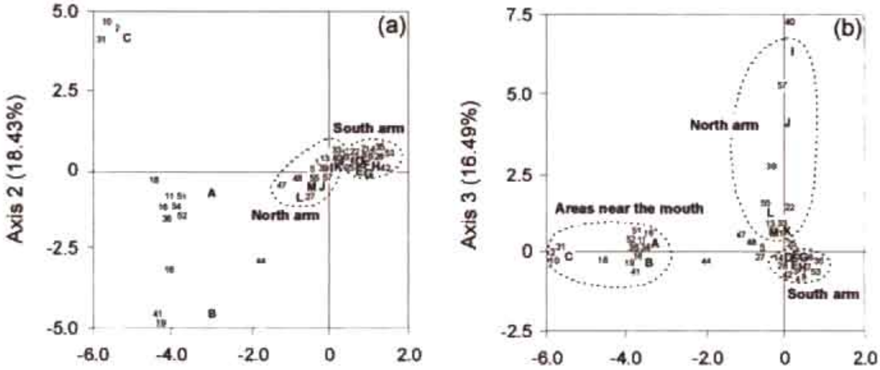

Figure 9. December situation: Results from Correspondence Analysis of benthic macrofaunal data. Projection of stations (A to M) and taxa (corresponding to numbers assigned in table 2) against the first and second (A) and first and third (B) axis of variability. The percentage of variability associated with each axis in indicated in parentheses.

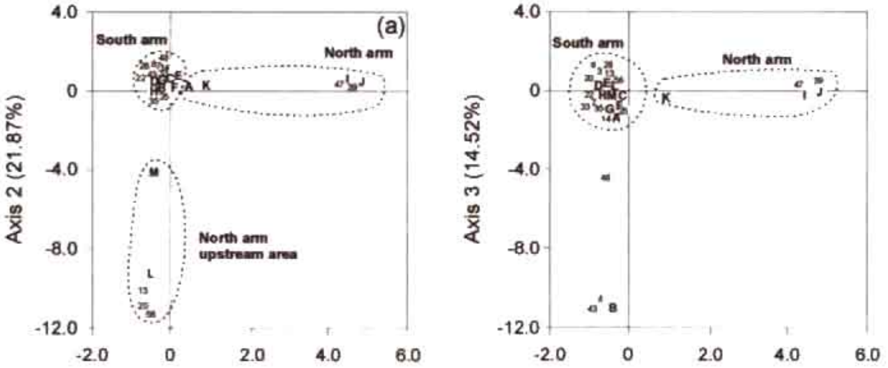

Figure 10. March situation: Results from Correspondence Analysis of benthic macrofaunal data. Projection of stations (A to M) and taxa (corresponding to numbers assigned in table 2) against the first and second (A) and first and third (B) axis of variability. The percentage of variability associated with each axis in indicated in parentheses.

Projection against the first and third axis of variability (figures 9: B, 10: B, 11: B, and 12: B) reveals a roughly comparable structural organisation, showing nevertheless more clearly the higher similarity between stations located closer to the mouth, with the exceptions of station I in December and station B in March.

Stations from the inner areas of the south arm (D, E, F, G, and H) are mainly characterised by the occurrence of abundant populations of *Amage adspersa*, *Scrobicularia plana*, and *Cyathura carinata*, true estuarine species, followed by more sparse populations of *Capitella capitata*, *Heteromastus filiformis*, and *Polydora ciliata*, and depending on the time of the year, by the less frequent or occasional occurrence of other species, like *Tetrastemma* sp. and other nemertines, oligochaetes, *Chaetozone setosa, Chone collaris, Oriopsis sp., Spio decoratus, Streblospio shrubsolii, Hydrobia ulvae, Idotea chelipes, Corophium multisetosum, Haustorius arenarius,* and tolerant *Chironominae* and *Diptera larvae.*

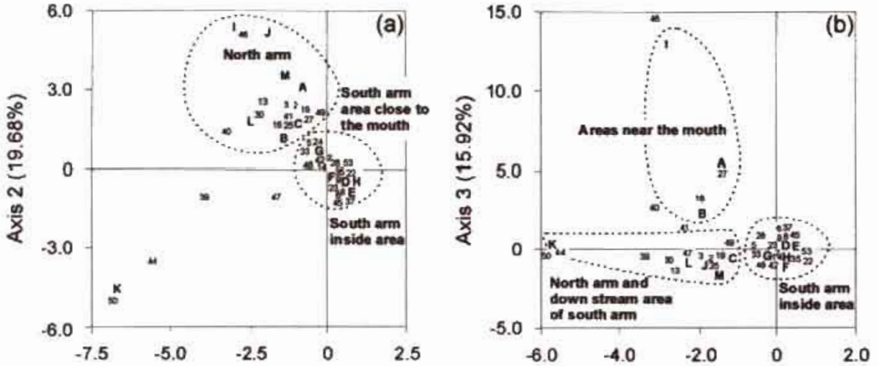

Figure 11. June situation: Results from Correspondence Analysis of benthic macrofaunal data. Projection of stations (A to M) and taxa (corresponding to numbers assigned in table 2) against the first and second (A) and first and third (B) axis of variability. The percentage of variability associated with each axis in indicated in parentheses.

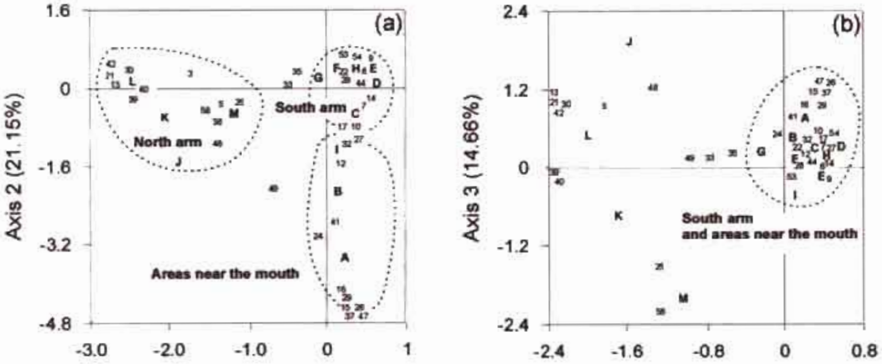

Figure 12. September situation: Results from Correspondence Analysis of benthic macrofaunal data. Projection of stations (A to M) and taxa (corresponding to numbers assigned in table 2) against the first and second (A) and first and third (B) axis of variability. The percentage of variability associated with each axis in indicated in parentheses.

Stations located along the north arm (J, K, L, and M), which present an impoverishment of the benthic populations, are mainly characterised by the presence of sparse populations of *Hediste diversicolor, Saduriella losadai, Sphaeroma hookeri,* and *Neomysis integer.* Through the year other species can be found more or less sporadically in these stations, like turbellarians, *Oerstedia sp., Tetrastemma sp.,* oligochaetes, *Nephthys paradoxa, Perinereis cultrifera, Streblospio shrubsolii, Cerastoderma edule, Hydrobia ulvae, Spisula subtruncata, Paragnathia formica, Bathyporeia sarsi,* *Corophium multisetosum*, *Melita palmata*, *Mesopodopsis slabberi*, *Carcinus maenas*, *Crangon crangon*, *Palaemonetes varians*, *Gomphus pulchellus*, *Ammodytes tobianus*, and *Platichtys flesus*. The presence of infaunal species, like *Hediste diversicolor* or *Streblospio shrubsolii* is nevertheless almost limited to stations L and M, located in the upstream section of the north arm. On the other hand, the occurrence of *Gomphus pulchellus* (a freshwater insect) in station M in December was surely related to the river freshwater discharge.

Finally, stations located closer to the mouth (A, B, C, and I), despite strong seasonal variations in macrofaunal composition, can be primarily characterised by the presence of sparse populations of *Cerastoderma edule* and *Bathyporeia sarsi*, followed by the irregular or sporadic occurrence of *Oerstedia sp.*, *Eteone picta*, *Eulalia sp.*, *Glycera convoluta*, *Lagis koreni*, *Nephthys spp.*, *Nereis succinea*, *Spio decoratus*, *Streblospio shrubsolii*, *Abra nitida*, *Nassarius reticulatus*, *Solen marginatus*, *Spisula eliptica*, *Tellina tenuis*, *Eurydice pulchra*, *Idotea chelipes*, *Saduriella losadai*, *Sphaeroma hookeri*, *Neomysis integer*, *Marthasterias glacialis*, and ophiuroids, which obviously reflects a stronger marine influence.

Influence of environmental factors on biodiversity and total macrofauna abundance

In order to understand the influence of physicochemical factors on the macrofauna distribution it was firstly necessary to characterise the estuary with regard to these factors.

PCA of matrices of physicochemical factors X sampling stations (figure 13) also reveal a consistent pattern over the year. From the projection against the first two axis of variability, stations appear distributed along a physical and chemical gradient, with stations located in the downstream areas of the north arm in one of the edges, stations located in the upstream section of the north arm and downstream areas of the south one in the middle, and stations located in the inner areas of the south arm in the other edge. This is clearly the situation in December (figure 13: A) and June (figure 13: C), while in March and September (figure 13: B and 13: D) station A, located near the mouth, appears to be separated.

Stations from inner areas of the south arm (D, E, F, G, and H) and stations from the downstream areas of the south arm, north arm and from near the mouth (A, B, C, I, J, K, L, and M) are almost always opposed along the first axis of variability. Stations located in the inner area of the south arm are mainly characterised by more fine sediments, with larger fractions of fine sand to clay, higher organic matter (from 3.2% in station D to 9% in station G) and carbonate contents (from 3.3% in station D to 8.7% in station G) and, in December, higher nitrite concentrations in the water column (from 1.28 $mg.l^{-1}$ in station D to 2.07 $mg.l^{-1}$ in station F). Stations located in downstream areas of the south arm, along the north arm, and near the mouth are mainly characterised by more coarse sediments, which tend to present larger fractions of gravel and coarse to medium sand, while the water column tends to present higher salinities (during high water), higher dissolved oxygen levels (the minimum observed was 86% of saturation in station A in March) and, in December, higher pH (from 6.7 in station A to 8 in station M).

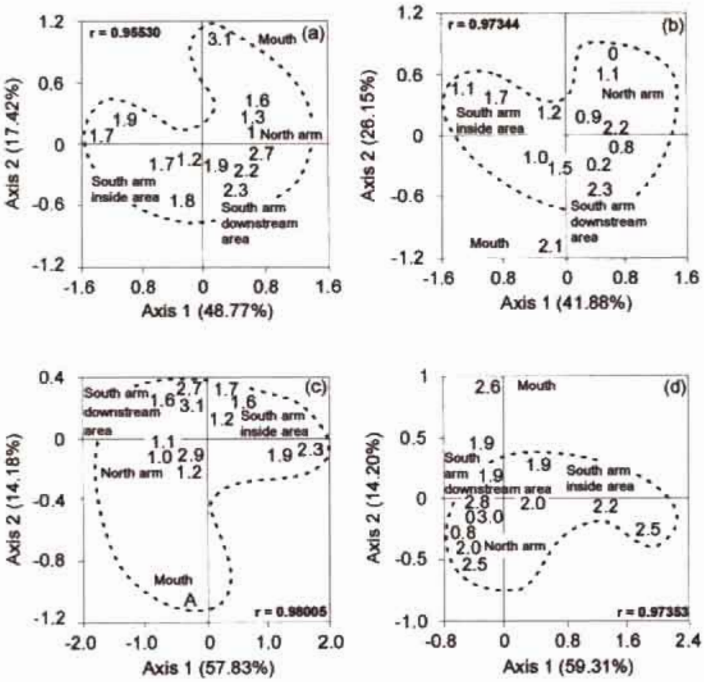

Figure 13. Results from PCA of physicochemical data of water and sediments. Projection of stations against the first two axis of variability: A - December; B - March; C - June; D - September. The percentage of variability associated with each axis in indicated in parentheses.

Along the second axis of variability station A, located near the mouth of the estuary, is opposed to the other stations, especially as a function of the characteristics of the water column, reflecting also the seasonal variations. In relation to water factors, resemblance between stations located inside both estuarine arms and station A, located close to the mouth, clearly changes trough the year. It is nevertheless impossible to go further in the analysis of the seasonal variation of water factors, because it depends on changes in the river freshwater discharge and on water circulation. Since the available data are prompt measures, they cannot be considered very significant. Nevertheless, closer to the mouth of the estuary salinity tends to be higher, which is normal, as well as dissolved oxygen levels, while in the inner areas temperature tends to be higher, as well as nitrogen concentrations.

The projection of the Shannon-Wiener index values, seasonally calculated for each station, over the ordination obtained from PCA of matrices of physicochemical factors X stations (figure 14), show a roughly regular pattern for the distribution of diversity values through the year. Despite seasonal variations, biodiversity tends to reach the highest values near the mouth and in the downstream area of the south arm, remaining approximately stable with relatively high values in the inner areas of the south arm. On the contrary, strong seasonal changes in biodiversity are evident in the north arm, although there is a certain pattern over the year. Diversity values tend to

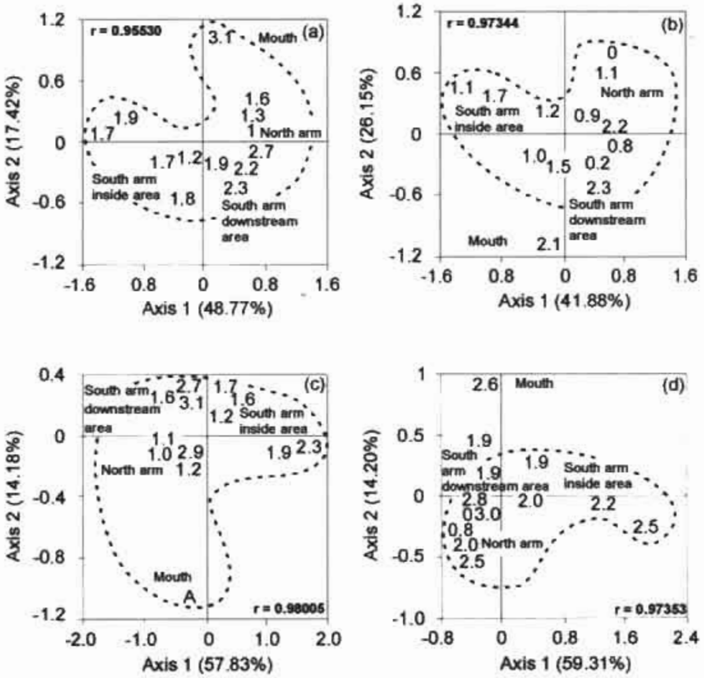

Figure 14. Plot of the Shannon-Wiener index values, calculated for each station in each time of the year, over the projection of stations against the first two axis of variability, obtained from PCA of physicochemical data of water and sediments: A - December; B - March; C - June; D - September. The percentage of variability associated with each axis in indicated in parentheses.

be higher closer to the mouth, decreases in the middle section of the north arm, and increases again in the upstream section.

With regard to total macrofauna abundance (individuals.m^{-2}), despite seasonal variations and the bias introduced by the sampling method, a pattern of distribution through the year is also recognisable (figure 15). Macrofauna is consistently more abundant in the inner areas of the south arm, although in the Pranto river values are comparatively lower (figure 15), and also significantly elevated in the downstream area of the south arm and upstream section of the north arm. In the north arm, there is a clear rarefaction of macrofauna from the upstream areas to the mouth, which is particularly evident in the middle section.

Summarising, it is possible to distinguish several areas in the estuary with regard to biodiversity and total macrofauna abundance:

Stations from the inner areas of the south arm, characterised by fine sediments, richer in organic matter and carbonate contents, and by higher concentrations of nitrogen in the water column, present a relatively stable and high biodiversity and by far the highest macrofauna abundances.

Comparatively, the downstream area of the south arm, characterised by sediments with significant fractions of coarse to medium sand, more poor in organic

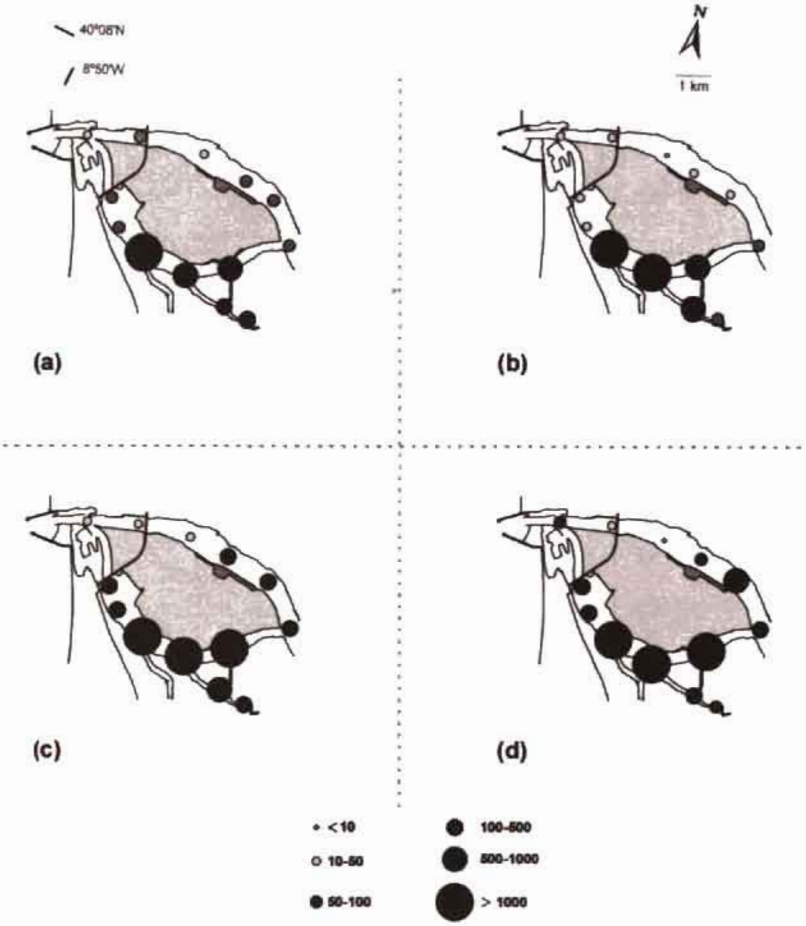

Figure 15. Spatial and temporal variation of total macrofauna abundance (individuals.m-2) in the Mondego estuary: A - December; B - March; C - June; D - September.

matter and carbonate contents, and by higher salinities and dissolved oxygen levels, presents an higher biodiversity but a lower macrofauna abundance.

Areas near the mouth and along the north arm are characterised by sandy bottoms, poor in organic matter and carbonate contents, although the fine sand fraction is more important near the mouth and in the upstream section, while the gravel to medium sand fractions are predominant in the middle section. Salinities and dissolved oxygen levels are also consistently higher along the north arm, although salinity tends to decrease from the mouth to the upstream areas. However, due to tidal currents and freshwater discharge, daily salinity fluctuations are by far more significant in the north arm (Marques 1989), especially in rainy periods. Near the mouth, biodiversity presents regularly the highest values found in the estuary, while the lowest ones and the strongest changes over the year occur in the north arm, particularly in the middle section. Total macrofaunal abundance is low along the north arm, with the exception of its upstream section, and therefore the middle section of the north arm constitutes the poorest area in the estuary for both biodiversity and macrofaunal abundance.

Discussion

Intertidal zone

The analysis of both hard and soft substrates communities structure showed clear differences between the two arms of the Mondego estuary, namely with regard to populations abundance and biodiversity. In both cases a good agreement was found between results from the analysis of biological and physicochemical data.

The observed differences are most probably due to very dissimilar hydrographic characteristics of the two arms. The south arm is still less affected by human activities and presents more favourable environmental conditions for the development of enhanced populations of true estuarine species. Nevertheless, the south arm is also shallower than the north arm, and water circulation depends widely on tides, especially in the summer. For these reasons, we consider that the south arm appears potentially much more exposed to environmental changes.

Salinity appears to be the most important factor controlling the hard substrates community structure, while sediments granulometry is the most important factor controlling the distributional ecology of soft substrates macrofauna, followed by organic matter contents, salinity, and dissolved oxygen. Other studied factors seem to play a less important role with regard to macrofauna distribution.

Spartina maritima and *Zostera noltii* marshes appear to be the richest areas with regard to macrofauna abundance and biodiversity. However, occasional blooms of *Enteromorpha spp.* have been observed in the south arm, probably as a function of excessive nutrients release into the estuary. Since macrophytes have roots and are only able to take up nutrients from the sediments, it seems possible that macroalgae like *Enteromorpha*, which is able to take up nutrients directly from the water, can take advantage from this situation. Therefore, it seems also likely that an eutrophication process might take place in the south arm, and in such a case a shift in the benthic primary producers could occur, affecting the structure and functioning of the trophic chain and ultimately the species composition in the community.

Subtidal zone

The subtidal benthic macrofauna of the Mondego estuary appears to be clearly impoverished. The Mira estuary, located in the Portuguese southwest coast, which is approximately identical to the Mondego in size, have been considered as relatively unaffected by human impacts, and can therefore provide a reasonable basis for comparison. Moreover, data on the Mira estuary (Andrade 1986) was collected according to a relatively similar sampling strategy and using identical sampling devices. With regard to subtidal benthic organisms, 151 taxa were identified for the Mira, while only 58 (about 38%) were identified for the Mondego. Furthermore, only 21 species were found in both estuaries, which appear to indicate a considerable contrast in the species composition. Differences observed with regard to total macrofaunal abundance were not so important (an average of 624 individuals.m^{-2} per sample in the Mira, and 466 individuals.m^{-2} per sample in the Mondego). Despite any conceivable bias in sampling, the observed differences must be considered highly significant.

The analysis of benthic macrofauna community structure through the year shows that, biologically, the two arms of the Mondego estuary constitute different sub-systems. This structural discontinuity is quite obvious between the inside areas of both arms, although closer to the mouth, due to the marine influence, differences become less apparent. This fits well with results from the independent analysis of environmental factors, and additionally the present results are consistent with those from the study on the intertidal communities. With regard to the community structure, biodiversity, and total macrofaunal abundance it is therefore possible to recognise different estuarine areas in relation to physicochemical environmental factors, respectively the inner areas of the south arm, the mouth of the estuary and downstream areas of both arms, and the middle and upstream sections of the north arm.

Since the water circulation in the south arm is mostly dependent on tides, current velocities are inferior and conditions are more favourable to fine particles and organic matter deposition (McLusky 1989). This tends to bring about a biological improvement, since subtidal fauna usually depends on sediments stability and organic matter contents (Gould et al. 1987). This can explain the relatively high and stable biodiversity values found through the year, and the higher abundances for total macrofauna, as observed in the inner areas of the south arm.

On the contrary, current velocities are higher along the north arm, due to both the river discharge (during low water) and a fast tidal penetration. This can explain the change in bottom characteristics, and although the species-sediment relationship is not always a simple linear function of grain size and organic matter contents (Jones et al. 1986), this bottom change is certainly one of the most important reasons for biological differences observed between both estuarine arms. Additionally, due to the river discharge and strong tidal current, daily salinity fluctuations in the north arm are higher than in the south arm (Marques 1989), which is probably a second major cause of faunal impoverishment (Barr et al. 1990). This agrees with the direct relationship between faunal type and tidal stress, as observed by Warwick and Uncles (1980).

The granulometric structure of the inhabitat and salinity fluctuations seems therefore to be the most important factors conditioning the subtidal macrofauna distribution in the Mondego estuary.

On the other hand, infaunal species are dominant in the south arm, especially in the inner areas (e. g. *Amage adspersa*, *Capitella capitata*, *Heteromastus filiformis*, *Polydora* *ciliata*, and *Scrobicularia plana*), while a clear dominance of epifaunal species (e. g. *Saduriella losadai*, *Sphaeroma hookeri*, *Neomysis integer*, and *Carcinus maenas*) is evident in the north one. This is probably related with shifting sediments, caused by a faster water circulation, which tend to prevent the colonisation and long-term establishment of a permanent infauna, determining the occurrence of typically sparse benthic communities, mainly constituted by mobile epibenthic species (Barr et al. 1990). Nevertheless, in the upstream section of the north arm, where dredging operations do not take place, infaunal species (e. g. *Hediste diversicolor* and *Streblospio shrubsolii*) can be found through the year. It appears therefore that the strong changes in biodiversity and the extreme macrofaunal impoverishment in the middle section of the north arm are also a function of regular dredging. Actually, dominant species decimation following disturbance of the bottom as been observed in other case studies. In Long Island Sound, for instance, polychaete populations of *Nephthys* strongly decreased at or near the disturbance site, although little or no effects on the populations were detected at more than 400 m from the impacted area (Zajac and Whitlatch 1988).

It has been observed that the recovery of dredged zones in number of species is practically obtained six months after the completion of dredging operations, although biomass takes longer to reach values similar to those found in unaffected areas (López-Jamar and Mejuto 1988). In the Mondego estuary, time intervals between dredgings (approximately twice a year) are likely to be too short, and do not allow macrofauna recovery, which surely contributes to the obvious instability of the north arm community. However, there are no other indications on the effects of dredging besides the absence of infaunal species and macrofaunal impoverishment.

Conclusions

The Mondego estuary is under severe environmental stress, and it is difficult to establish the benthic community temporal trends and if the ongoing changes are reversible. The benthic communities in estuarine environments are generally characterised by wide fluctuations in the abundance of constituent species, although they present a more persistent qualitative composition (Boesch et al. 1976). Moreover, benthic organisms, namely infauna, are especially important components in estuarine ecosystems, because most of them have limited mobility and respond to environmental stress (Bilyard 1987). It seems therefore necessary to monitor the Mondego estuary communities, probably with emphasis on benthic macrofauna, although such studies need labour-intensive sample sorting and taxonomy. This monitoring study should take all species into consideration, once using only the most abundant ones for characterising communities, or as indicators of physicochemical conditions, may be unreliable because of variation in both time and space in dominant species, and the lack of stress-response knowledge for local species (Jones 1990). It will provide valuable information that cannot otherwise be obtained, since the dynamics of estuarine benthos is very complex and strongly limits the usefulness of short-term baseline and impact studies.

Acknowledgements

The present paper was supported by FCT (Portuguese National Foundation for Science and Technology). The authors are indebted to all the colleagues from the University of Coimbra who assisted in field and laboratory work.

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FISH COMMUNITY OF MONDEGO ESTUARY: SPACE-TEMPORAL ORGANISATION

Abstract

Sampling fish community and abiotic characteristics of water was done in five stations each month, during 1988, 1991 and 1992. The structure of the community is organised in four components status: occasional marine, occasional freshwater, sedentary and migrant. Calendar of presence of this last group is given. Three habitats: marine, brackish, and freshwater were characterised with discriminant function analysis. AFC and communities indexes gave results about the space temporal variability of ichthyofauna. Interpretations about functioning ecosystem are given in relation with habitat characteristics and migrant status of population that represents the maximum abundance.

Introduction

The estuary of Mondego river is composed of two arms (north and south) limiting the Morraceira island (Fig. 1). Its upstream communication is silted up, allowing the change of water only in high tide or in a overflow situation. As a consequence of important hydraulic works carried out upstream, the freshwater affluence to the estuary proceeds partly from the artificial streams of the bridge-dam of Coimbra and Bizorreiro sluice. Whereas the regime of the latter is related with agriculture activity and is opened when there is too much water upstream, causing sometimes sharp disturbances in downstream system, the former has a continuous but variable flow, along the year. These two water control systems contribute to the different hydrological conditions of the two arms: northern one has a strong hydrodynamism affected by the tides and by the Mondego river flow, and the southern arm shows a lower water circulation, mainly affected by tidal currents (Duarte and Pena dos Reis 1993).

The estuary has been the place for an important fishing activity involving different sorts of gears and about one hundred of local boats, distributed by two small natural

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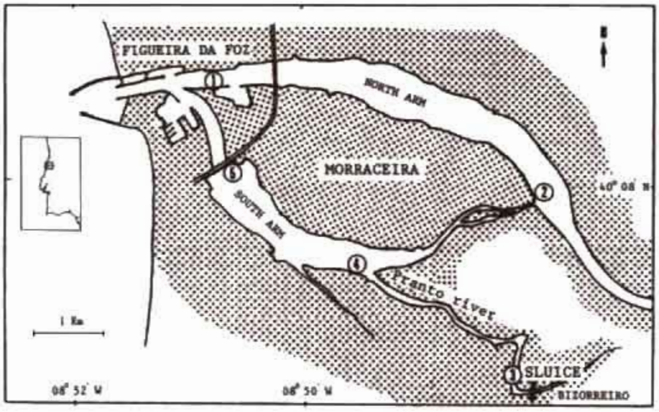

Fig. 1. The estuary of Mondego river and the ichthyofauna sampling places: 1 - Porto Comercial (C); 2 - Vila Verde (V); 3 - Pranto (P); 4 - Braço Sul (S); 5 - Gala (G).

harbours, Vila Verde and Gala. The local fishing is directed to a variety of resources like flatfish (flounder and soles), sea-bass, eel, mullets, green crab, bivalves, etc.

The exploitation of the seasonal migrants like lamprey, shad and glass-eel are very important estuarine activities owing to its high demand, providing important profits to the local fishermen. This is the main reason for the use of illegal gears and nets, of high efficiency, like the typical case of the fyke net for glass-eel and lamprey. In this system it has been traditionally developed the salt exploration, an activity that is in deep declining. For that reason the salt-marsh are being transformed into fish farming units where the seabass and gilthhead seabream are the most important cultivated species being eel farming supplied with glass-eel from the estuary.

A gradual decrease of landings from littoral coast of Figueira da Foz reflects a certain depletion of some resources mainly, due to the intensive fishing, not only in the coast but in estuaries too. The great importance of estuaries in fish production is a strong reason to protect them from human impact. This role emphasises the necessity of understanding the environment of fish and its interrelationships.

In the scope of the Integrated Programme for the Regional Development of Baixo Mondego, a study was carried out in 1988, 1991 and 1992, before the dredging of north arm, for characterising the ichthyological fauna and understanding the space-temporal variability of fish community and its relation with abiotic factors.

Methodology

Sampling stations

Information concerning some abiotic variables such as temperature and salinity was registered in ebb tide conditions for posterior environmental characterisation in the following five stations, selected by its different potential environmental conditions and distance from the sea (Fig. 1):

Porto Comercial (C) – on the left edge of north channel, at about 2 km from the entrance and near the sewage effluent of Figueira da Foz, is daily submitted to a marine influence save when a great freshwater discharge takes place.

Vila Verde (V) – in northern channel near the upstream division of the estuary in two arms is located in a permanent freshwater region, at about 7 Km from the sea.

Gala (G) – in the downstream region of south arm, at about 3 km from the sea is exposed to the marine influence except in overflow conditions.

Braço Sul (S) – in the inner part of south channel and submitted to marine influence except when the sluice of Pranto is open. It is the station with lowest depth and greatest water transparency.

Pranto (P) – situated in a tributary (Pranto river) of south channel, at about 8 km from the entrance and near Bizorreiro sluice.

Environmental variables

The 1991 and 1992 temperature and salinity values, with another complementary abiotic information like tide coefficient, monthly distribution of rain, flow in Coimbra bridge-dam in three days before sampling, percentage of sand in substratum, depth and distance from the sea, were organised in matrix and then transformed by centrage and reduction using a commercial computer program (NTSYS-pc). Based on the principles of cluster analysis the stations where assembled according similarities, using correlation coefficient. A discriminant analysis was after applied to research the capacity of environmental variables in discriminating stations assemblages identified by the above classification method.

Fish fauna

Fish fauna population study was based on sequential monthly sampling, referred to three hauls of beach seine in each of the three estuarine habitats identified by the analyse of abiotic parameters: marine, brackish and freshwater represented respectively by Gala/Porto Comercial, Pranto and Vila Verde stations.

Community index like specific richness represented by species number, Shannon-Wiener diversity index (Legendre and Legendre 1984) and evenness were used to characterise in a global way the fish populations. They were calculated by sampling zone and month to point out space-temporal variations.

Cluster analysis based on Jaccard coeficient were used to find similitudes between lagoons and estuaries based in fish fauna lists.

A correspondence factor analysis was applied to identify changes in fish community structure in a space temporal scale and to relate those variations with species, sampling regions and seasons. In the graphics the species are represented by a numerical code and the fish samples by a mixed code with a letter identifying the zones.

Environmental characteristics

Surface temperature in all years and stations had the same trend with the lowest values in winter and the greatest in summer or beginning of autumn, with extreme values upstream (Fig. 2).

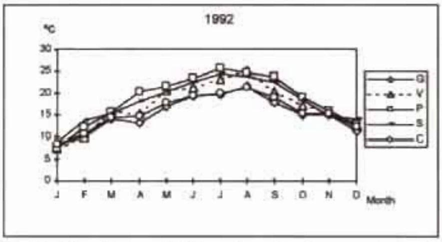

Fig. 2. Monthly evolution of water temperature at surface for each different stations.

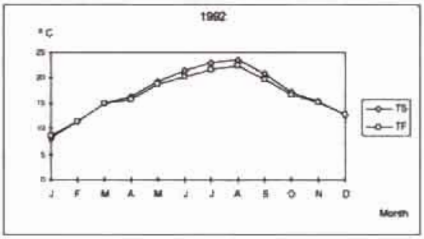

Fig. 3. Yearly cycle of temperature at surface (TS) and near the bottom (TF) averaged over all stations.

From spring till autumn the lowest values correspond to the stations nearest the sea, and the most disperse values were observed in Pranto station. The yearly average of bottom water temperature was similar to the correspondent at surface and its monthly values at the bottom follow closely the seasonal fluctuation of those at surface (Fig. 3).

The salinity had an irregular variation from year to year with an evolution closely related with the atmospheric precipitation and flow of Coimbra bridge-dam (Fig. 4). In

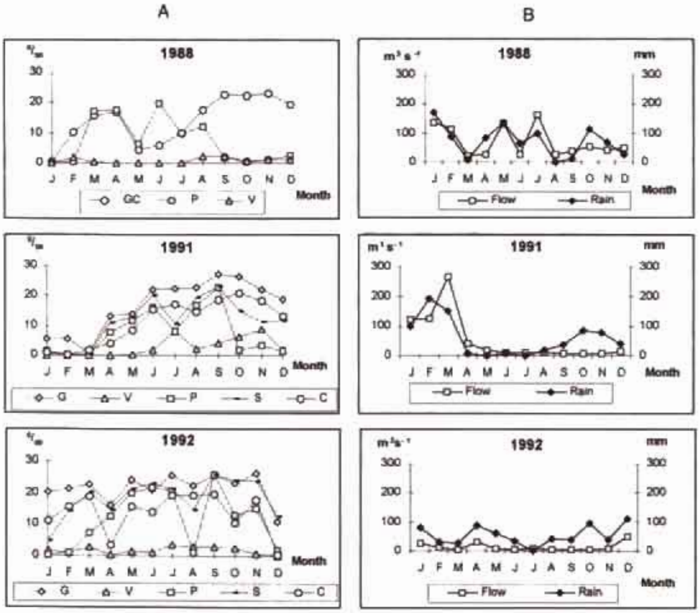

Fig. 4. A - Monthly water salinity at surface for each different stations; B - Monthly distribution of rain and average of flow at bridge-dam of Coimbra in three days before sampling.

1991 the monthly evolution of surface salinity showed very low values in winter, especially in March in all stations. The decreasing observed in July in Pranto and Braço Sul stations is related with the Pranto sluice regime. The 1992 year was drier than 1991, but some rain noted in January, April, October and December, together with greater flows in the dam of Coimbra, led to a diminution in salinity in all stations. In August the salinity in Pranto had a very accentuated decreasing whose influence was propagated as far as Gala station. The vertical gradient of salinity (Fig. 5) had only some importance in Porto Commercial area, where the depth is the greatest. In south arm the depth is small and the water column is homogeneous. The salinity decreases when the distance from the sea increases, being higher in south arm for stations almost equidistant from the entrance.

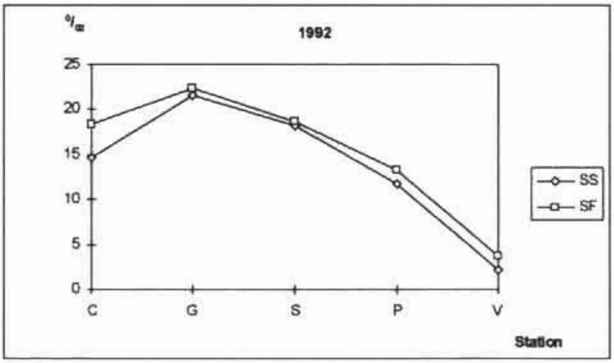

Fig. 5. Annual average of salinity at surface (SS) and near the bottom (SF) for each station.

The discriminant function analysis kept as variables of primary interest the tide coefficient, the average of debits in the Coimbra bridge-dam and the water salinity near the bottom, to build the model to discriminate the groups previously established. The Fig. 6 shows the projection of the five groups obtained by hierarchical classification on the plan of the first and second discriminant functions, at which are associated 89 % of discriminatory power, mainly based respectively in tide coefficient and salinity near the bottom. The first function discriminated the group of marine stations (Gala and Porto Comercial – G 4:4) from that of brackish stations (Pranto – G 3:3 – and Braço Sul – G 5:5) and the second discriminated the freshwater station of Vila Verde (G 2:2) from those of marine and from Braço Sul stations based mainly in the bottom salinity. The third function with a weaker discriminatory power differentiated the group of Vila Verde, in freshwater conditions, from the group that include all stations in the months that occurs greater discharges in Coimbra dam and great pluviosity. It can be noted on this picture some affinity between Pranto and Braço Sul stations, being the difference between them only suggested by the trend of Pranto points to fall above the central line (0).

Summarising it can be said that the freshwater discharges at Coimbra dam and atmospheric precipitation are two covariables whose effects, in the rainy periods, led to attenuate the differences among the several regions of the system. The persistence of the affinity among abiotic factors from Gala and Porto Commercial and the identity of remainder stations were observed, being noted also some approach among the

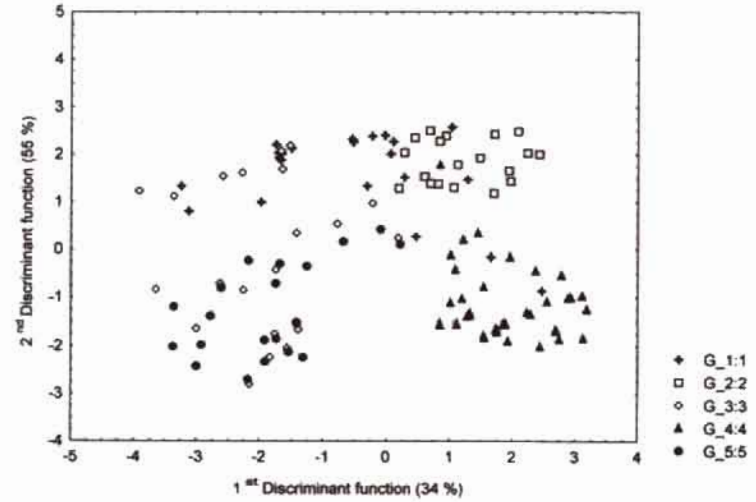

Fig. 6. Plot of discriminant function analysis showing how the two first functions discriminate the five groups of stations identified by hierarchycal classification (G_1:1 stations in overflow months; G_2:2 Vila Verde; G_3:3 Pranto; G_4:4 Gala e Porto Comercial; G_5:5 Braço Sul).

characteristics of Pranto and Braço Sul stations. Therefore, three space units can be considered corresponding to a freshwater habitat in the upstream region of north arm (Vila Verde), a marine one in Gala/Porto Comercial zone and a brackish area on the inner part of south arm which continues by the downstream section of Pranto river up to the Bizorreiro sluice. In consequence for fish community analysis: Gala and Porto Comercial are marine habitats (M), Pranto and Braço Sul are brackish habitats (S) and Vila Verde is freshwater habitat (D).

Fish fauna composition and situation

The simplest way to study the fish fauna is to obtain a list, which indicates the species of fish that use estuaries. In this study were identified 62 species distributed by 27 families according to the criterions of Whitehead et al. (1986).

a) List of species

A number of classifications scheme for estuarine fish species have been referred by several authors (Claridge et al. 1986, Costa 1982, Monteiro 1989, Paris and Quignard 1971, Bach 1986, Rebelo 1993). Based on the occurrence of sampling species and in the characteristics of their life cycles, there were considered the following community status group, represented in Table I:

Table 1. Fish fauna list of each community group (code of each species in brackets)

Sedentary	Occas. Freshwater	Occas. Marine	Migrants (MIG.)		
(SED.)	(O.D.)	(O.M.)	Anadromous (Mig. an.)	Catadromous (Mig. ca.)	Cyclic (Mig. cy.)
Atherina boyeri (64)	*Barbus bocagei* (7)	*Ammodytes tobianus* (45)	*Alosa alosa* (3)	*Anguilla anguilla* (15)	*Chelon labrosus* (62)
Atherina presbyter (66)	*Carassius auratus* (8)	*Aphia minuta* (48)	*Alosa fallax* (4)		*Dicentrarchus labrax* (32)
Gobius niger (50)	*Carassius carassius* (9)	*Belone belone* (17)			*Diplodus sargus* (35)
Pomatoschistus minutus (54)	*Chondrostoma polylepis* (10)	*Boops boops* (40)			*Diplodus vulgaris* (36)
Pomatoschistus microps (53)	*Cyprinus carpio* (11)	*Callionymus lyra* (57)			*Engraulis encrasicolus* (6)
Syngnathus abaster (24)	*Gambusia affinis* (16)	*Ciliata mustela* (29)			*Liza aurata* (63)
Syngnathus acus (25)	*Gasterosteus aculeatus* (27)	*Echiichthys vipera* (46)			*Liza ramada* (64)
	Gobio gobio (13)	*Entelurus aequoreus* (18)			*Mugil cephalus* (65)
	Micropterus salmoides (14)	*Gobius cobitis* (49)			*Mullus surmuletus* (34)
	Rutilus macrolepidotus (12)	*Hyppocampus ramulosus* (21)			*Platichthys flesus* (70)
		Labrus merula (41)			*Sardina pilchardus* (5)
		Labrus viridis (42)			*Scophthalmus rhombus* (69)
		Nerophis lumbriciformis (22)			*Solea senegalensis* (72)
		Nerophis ophidion (23)			*Solea vulgaris* (73)
		Pagrus pagrus (38)			*Sparus aurata* (37)
		Parablennius gattorugine (58)			*Spondyliosoma cantharus* (39)
		Pomatoschistus kneri (56)			*Trigla lucerna* (68)
		Pomatoschistus lozanoi (52)			
		Pomatoschistus pictus (56)			
		Scomber japonicus (47)			
		Spinachia spinachia (28)			
		Symphodus bailloni (43)			
		Symphodus melops (44)			
		Syngnathus typhle (26)			
		Trachurus trachurus (33)			

Sedentary species (Sed): have a great tolerance to salinity changes and can complete their entire life cycles within the estuary, and are represented by seven species.

Migrants which includes: the anadromous species (M. an.) migrating from the sea to fresh water for breeding (*Alosa alosa* and *Alosa fallax*); the catadromous species (M. ca.) – migrating from freshwater to the sea to breed (*Anguilla anguilla*); the cycling migrants (M. cy.) with a marine origin, occupies temporarily the estuarine waters for feeding, protection or spawning, are represented by 17 species generally juvenile forms. This important group dominated in biomass and abundance during all the year in marine, freshwater and brackish habitats.

Occasional visitors from sea-water (O. m.): intolerant to the salinity changes, appear irregularly in the estuary when the environmental conditions are favourable. They correspond to 25 species with a strong preference for marine environment and the less rainy years.

Occasional visitors with a freshwater origin (O. d.) have, consequently, a freshwater affinity occurring mainly in freshwater conditions in rainy periods and with freshwater discharges in Coimbra bridge-dam and Bizorreiro sluice. They are represented by 10 species.

Based on the evolution of length frequency distributions and age readings, an attempt was made to represent migration flows for the main marine cyclic migrants (Fig. 7). For the most of considered species (*Trigla lucerna, Plathichthys flesus, Sardina pilchardus, Dicentrarchus labrax, Sparus aurata, Solea vulgaris, Chelon labrosus, Liza ramada* and *Scophthalmus rhombus*) the recruitment starts in spring which means that their spawning periods occur in winter. A second recruitment takes place in summer with *Diplodus sargus* and *Diplodus vulgaris* in the beginning of the season and later, in August, *Mullus surmuletus* and *Engraulis encrasicolus*. The youngs of *L. aurata* begins to appear in the third recruitment in autumn.

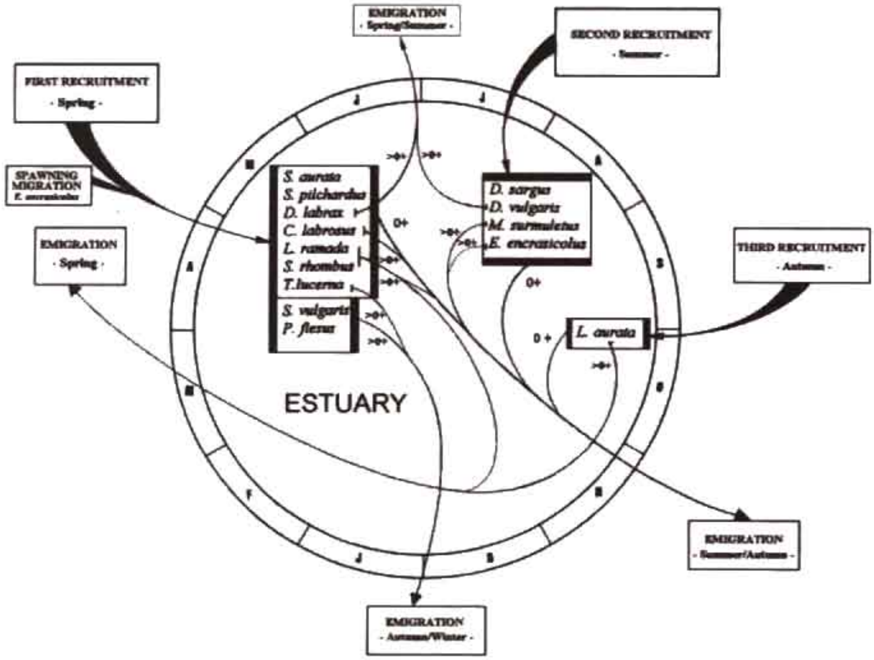

Fig. 7. Scheme indicating the beginning of migrations for the main marine species which use the estuary as a nursery ground.

Sparus aurata, Diplodus sargus, Mullus surmuletus, Engraulis encrasicolus, and *Sardina pilchardus* seems to stay in the estuary for few months and *Plathichthys flesus, Liza ramada*, and *Chelon labrosus* for more than three years, generally at about four years. Another species have a intermediate period of colonisation, normally one year, like *Dicentrarchus labrax, Trigla lucerna, Diplodus vulgaris, Scophthalmus rhombus*, or less than three years as *Solea vulgaris* and *Liza aurata*. In fact it was the calendar of the disponibility of the young fish to the estuary.

b) Comparison of species list of Mondego estuary with those of other european systems

The fish fauna composition was compared with those of Severn estuary in Southwest coast of Great Britain (Claridge et al. 1986), Étang de Thau in Mediterranean coast (Paris and Quignard 1971, Bach 1986), Ria de Aveiro (Rebelo 1993) and Tejo estuary in the western coast of Portugal (Costa 1982), and Ria Formosa in the southern portuguese coast) (Monteiro 1989) (Fig. 8).

One can verify that the sedentary proportion of species from portuguese systems are in intermediate position between the Étang de Thau (18%) and the Severn estuary (3%).

Excepting for Tejo estuary, the percentage of occasional marine species are greater in the atlantic systems and the marine migrants that use estuaries or lagoons as nursery grounds have more importance in southern systems – Tejo estuary, Ria

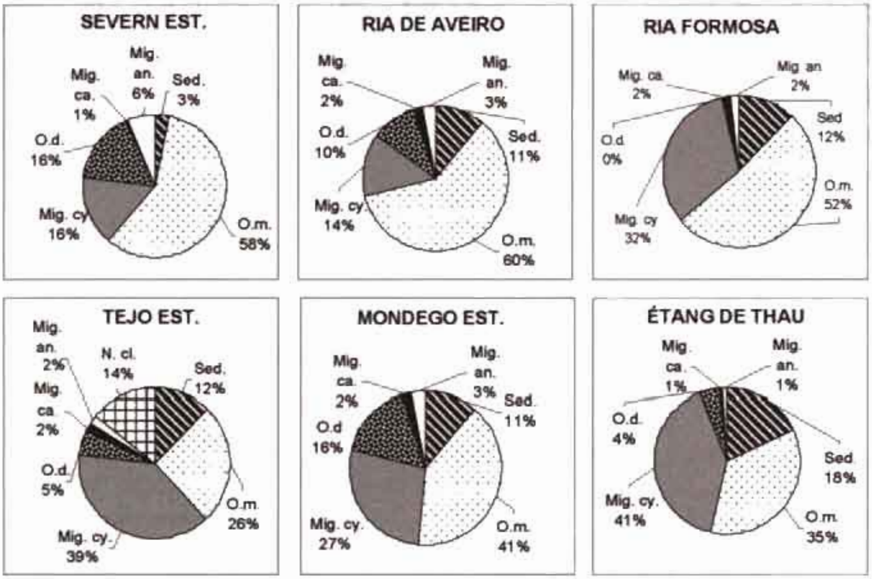

Fig. 8. Comparison of community components of fish fauna from different european coastal systems: sedentary (Sed.), marine occasional (O.m.), marine cyclic migrant (M.cy.), occasional freshwater (O.d.), migrant catadromous (Mig. ca.) migrant anadromous (Mig. an.), non classified (N.cl.).

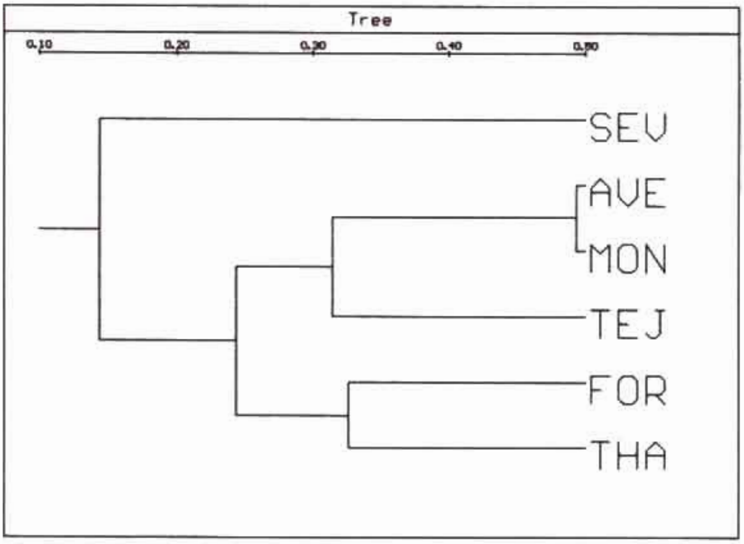

Fig. 9. Dendrogram obtained by comparison of fish fauna lists from different european coastal systems: Severn estuary (SEV), Ria de Aveiro (AVE), Ria Formosa (FOR), Tejo estuary (TEJ), Mondego estuary (MON), Étang de Thau (THA).

Formosa, and Étang de Thau – perhaps related with most favourable and stable conditions that lasts for a long period.

The anadromous and catadromous species had the lowest values in all cases and the freshwater species had the highest in the northern ones, especially in Severn and Mondego estuaries, probably due to strong freshwater discharges.

The classification analysis (Fig. 9) separate the estuary of Severn from the others which are distributed in two groups, one with Ria Formosa and Étang de Thau and another with remainder systems showing a strong association between Mondego estuary and Ria de Aveiro. The resembling lists of Ria Formosa and Étang de Thau, reflects the influence of climatic characteristics of Mediterranean and southern coast of Portugal, which are different from those of west coast.

The assemblages of the remainder portuguese systems, with a greater homogeneity of Mondego and Aveiro lists seems to be determined by its proximity and the size of sea entrance which is lesser (up to a 1 km) than that of the Tejo estuary (about 4 km). The difference of list from Severn estuary is probably due to its situation in a different biogeography region with large river and its vast sea entrance. Thau and Ria Formosa being lagoons with no permanent river they are different from Ria de Aveiro and are not estuaries like Mondego and Tejo.

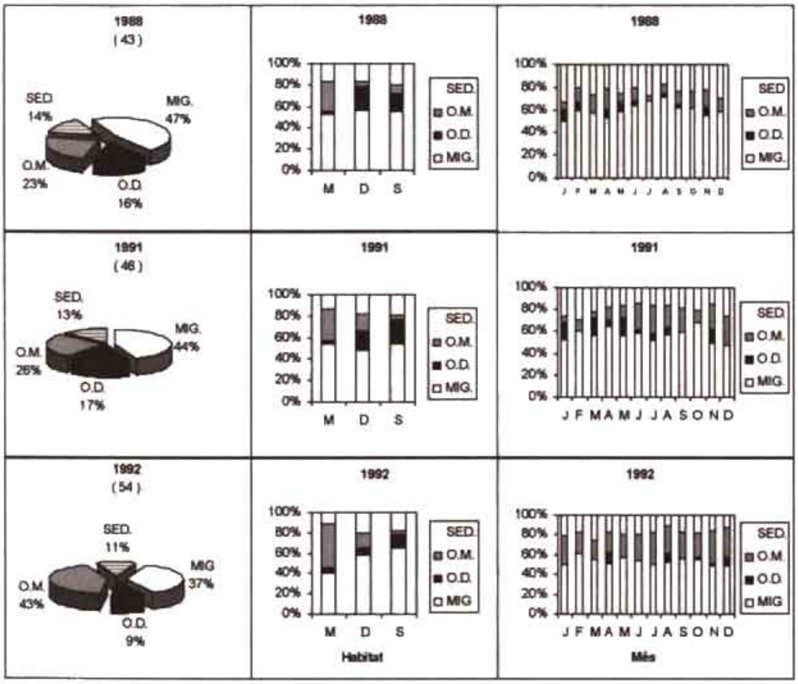

Fig. 10. Variation of species number (%) of each community group: sedentary (SED.), occasional marine (O.M.), occasional freshwater (O.D.) and marine migrant (MIG.). A - in the estuary as a whole; B - by habitat: marine (M), freshwater (D) and brackish (S); C - by month.

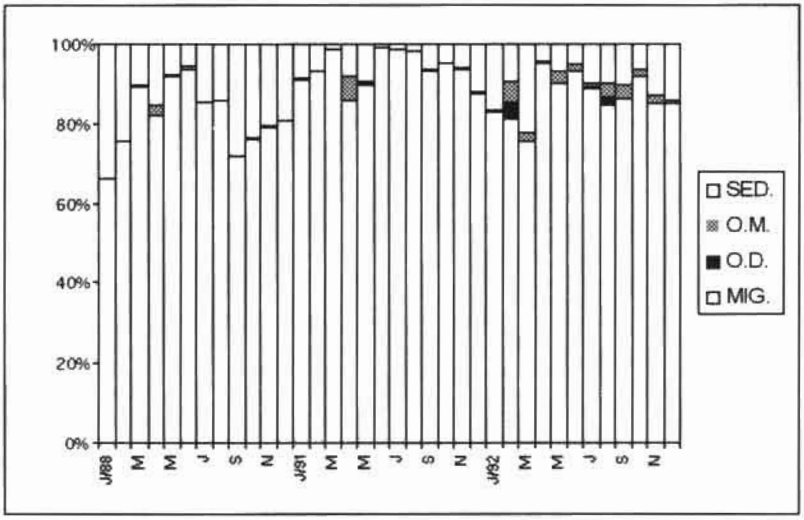

Fig. 11. Variation of abundance (%) of each community group: sedentary (SED.), occasional marine (O.M.), occasional freshwater (O.D.) and marine migrant (MIG.).

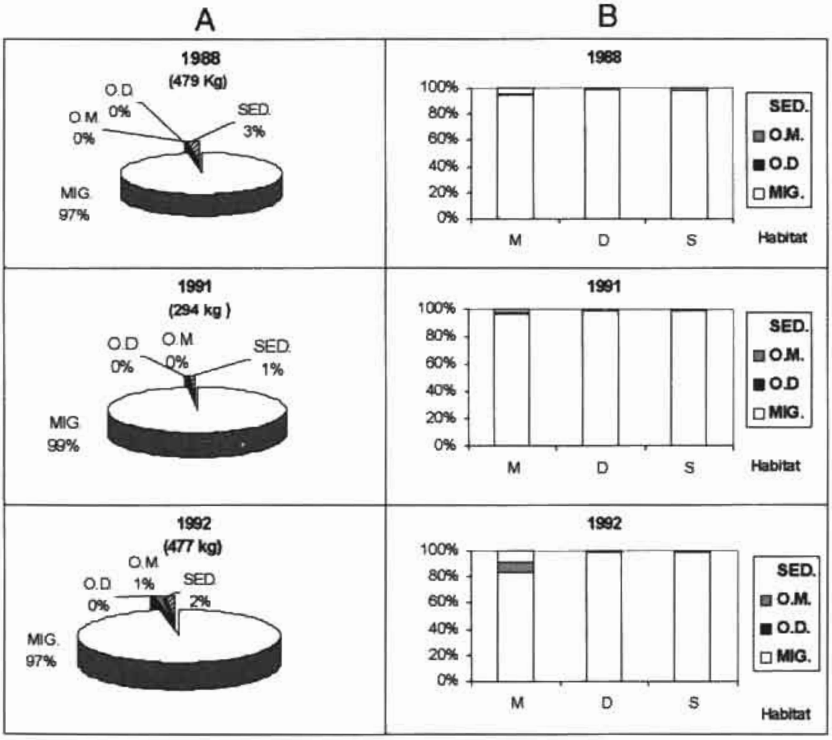

Fig. 12. Variation of biomass (%) of each community group: sedentary (SED.), occasional marine (O.M.), occasional freshwater (O.D.) and marine migrant (MIG.). A - in the estuary; B - in each habitat: marine (M), freshwater (D) and brackish (S).

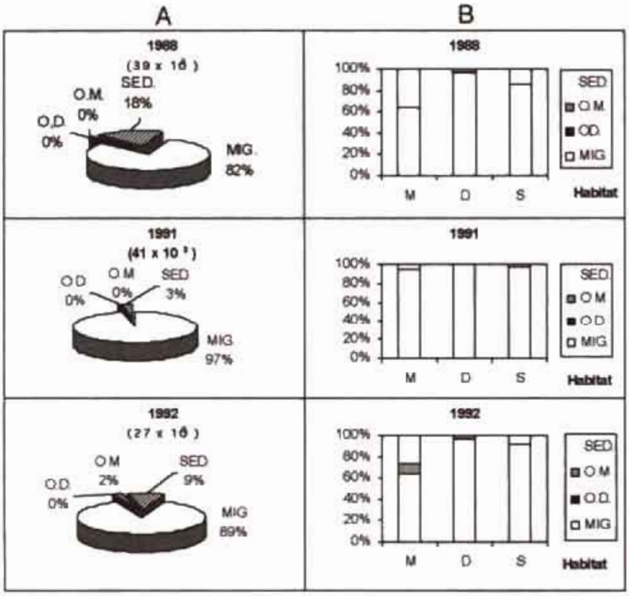

Fig. 13. Variation of abundance (%) of each community group: sedentary (SED.), occasional marine (O.M.), occasional freshwater (O.D.) and marine migrant (MIG.). A - in the estuary; B - in each habitat: marine (M), freshwater (D), and brackish (S).

Space-temporal variation of fish communities with the status

To detect annual differences between the community components of fish fauna from the three habitats, its proportions were analysed by year, zone, and month and for the system as a whole.

The fish fauna of Mondego estuary is dominated by the migrants status species which represent a percentage of 37% – 47% of species number, 82% – 97% of individuals and 97% – 99% of biomass (Fig. 10 – 13), occurring lowest abundance in autumn, winter and spring.

Sedentary presented a number of species similar from year to year and its importance in number of individuals or in biomass was greater in marine region, occupying the second place after migrants.

Occasional freshwater species were present mainly in periods of greater freshwater discharges and their species numbers were higher in the brackish and freshwater habitats.

Occasional marine visitors were mainly represented in marine zone. In 1992, considering the three areas as a whole, their species numbers were greater than that observed for migrants and, like the freshwater group, had little number of individuals and biomass.

The occurrence and importance of these groups in marine, brackish, and freshwater zones seem be related with seasonal evolution of parameters and migrants behaviour. Thus, the lowest values of migrants abundance in autumn, winter, and beginning of spring, (Fig. 11) can be determined by migration impulses (Fig. 7) in connection with decreases of salinity and temperature as well as trophic needs of individuals above certain size. In 1992 the more regular presence of occasional marine visitors from April till September is certainly in relation with the dry climate that characterised this year.

Space-temporal variations of fish communities diversity

The structure of a community is one of its essential characteristics and can be represented by the species number or by the abundance or biomass of each species and may be, in more or less degree, influenced by environmental changes. A diversity index is a way to express the community structure that is closely related with the diverse solutions adopted by the different community groups when they colonise a ecosystem (Vieira da Silva 1979). The evenness indicates if a community is near or far from the steady state, which is reached when evenness is maximum, equal to 1. At this point there is an adjustment of the species in a progressive way to the environmental constraints (Amanieu and Lasserre 1982).

The evolution of specific richness, the diversity index of Shannon-Winner and evenness, based on biomass, were determined by month and habitat studied, to point out their space-temporal variations. The time variability of all indexes is lower in marine habitat then in freshwater and brackish habitat (Fig. 14). The specific richness was higher in marine habitat (Fig. 15) and lower in the freshwater one. The most important values were observed, in general, in summer and the lowest in winter or at the end of autumn. The diversity index and evenness had a parallel evolution in all zones, with bigger values in Gala/Porto Comercial region. In all habitats studied the maxima values occurred in general from April until the end of summer and the smallest took place in winter or at the end of autumn. In spite of the different methodologies used in the community indexes determination, the results of Mondego estuary have shown a trend similar to those recorded for other fish nursery areas in portuguese coast (Costa 1986 in Tejo estuary, Rebelo 1993 in Ria de Aveiro and Monteiro 1989 in Ria Formosa): the highest values in spring or summer and the lowest in autumn/winter. However, exceptions to these general trends were sometimes found in Mondego estuary, related with the instability that characterises estuaries: high diversity or evenness indexes at the end of autumn or winter when biomass were equi-distributed by the species with more resilience (in freshwater habitat in December of 1988; in marine and freshwater habitats on January 1991 and in brackish one in March of 1991; in January and December of 1992 in freshwater zone); low values of those indices in summer when a species is strongly dominant (brackish zone in August of 1991), what may happen when the salinity drops due to a freshwater discharge, abundant rain or a sluice opening (brackish zone in August of 1992).

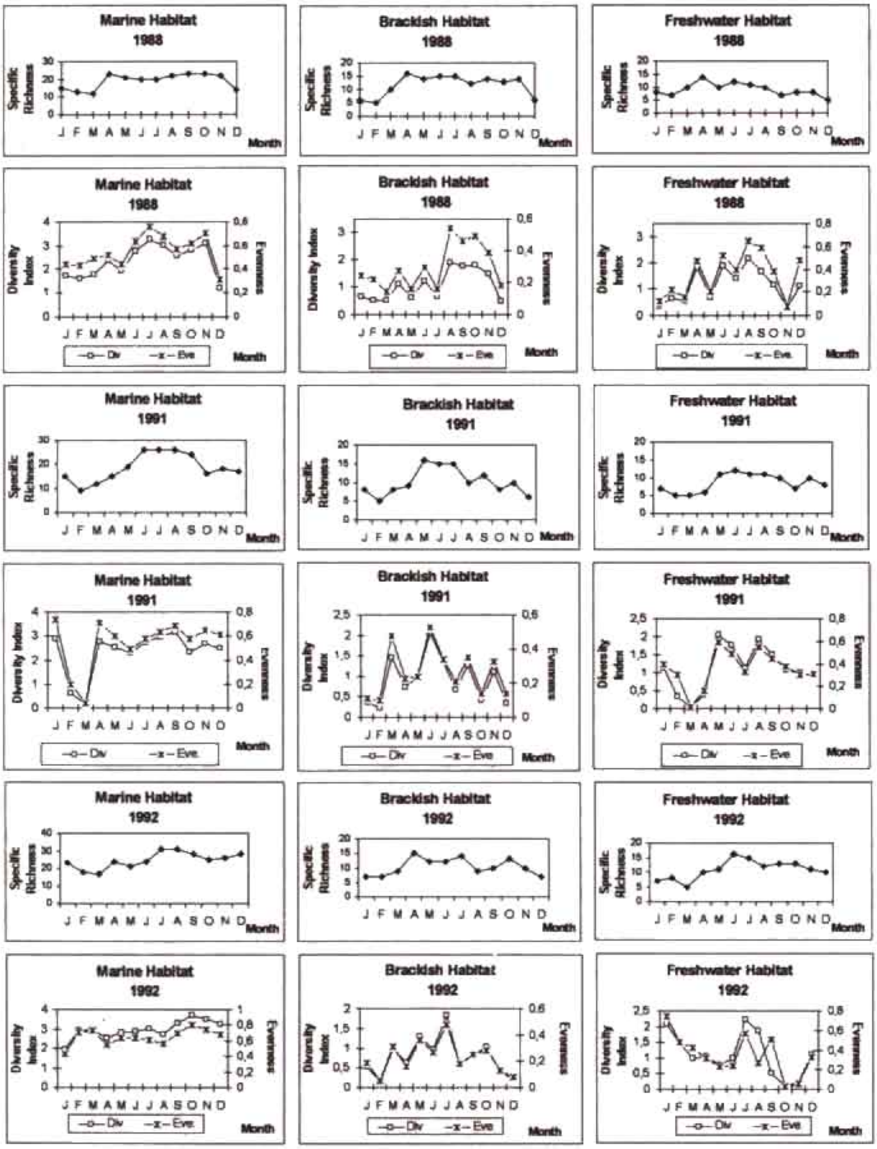

Fig.14. Monthly evolution in each habitat and year of community indexes: specific richness, diversity and evenness.

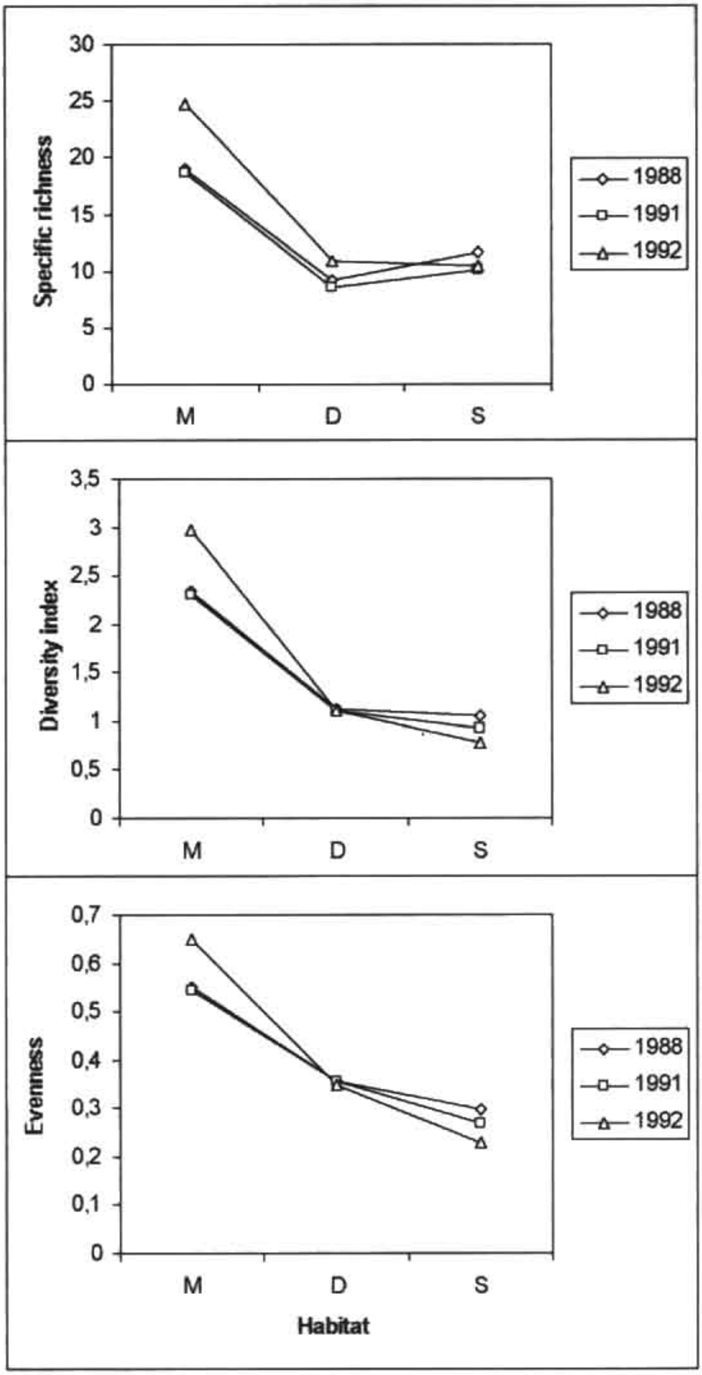

Fig. 15. Yearly values of community indexes by habitat

Multidimensional analysis of fish communities assemblages

The results of correspondence factorial analysis applied to fish fauna biomass matrix are represented in Fig. 16-18. The graphics show the projections of species and sampling habitats on the plan of two first axes that explain for 1988, 1991 and 1992 respectively, 44%, 40% and 42% of variance in the total data set.

In 1988 (Fig. 16) there are three groups relating species and stations: one concerning marine sector in summer/autumn with stenohaline marine migrants – *Trigla lucerna* (68), *Mullus surmuletus* (34), *Diplodus sargus* (35) and occasional marine species – *Symphodus bailloni* (43); other with Pranto station in summer/autumn and the migrant species *Engraulis encrasicolus* (6) and *Diplodus vulgaris* (36); another in spring with Pranto and Vila Verde stations, associated to eurialine species *Liza ramada* (64) and *Dicentrarchus labrax* (32).

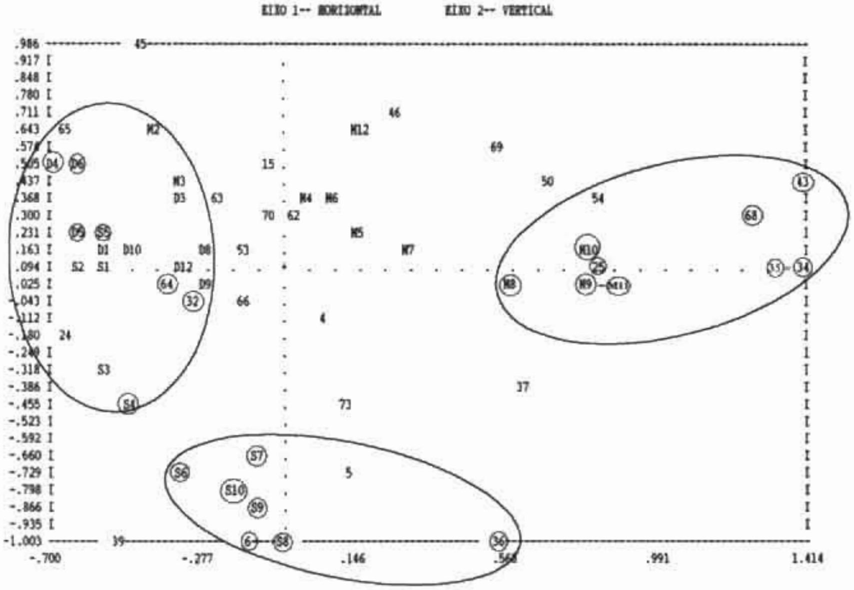

Fig 16. Correspondence factor analysis of biomass matrix from 1988.

In 1991 (Fig. 17) there is one group corresponding to a temporal association of the three habitats in winter, including the freshwater region during spring and December. The remainder two groups comprise the marine and the brackish habitats. The first habitat corresponds to Gala/Porto Comercial station in summer/autumn, with marine migrants – *Trigla lucerna* (68) and *Scophthalmus rhombus* (69), the sedentary *Gobius niger* (50) and the marine occasional *Callionymus lyra* (57). The second group comprising Pranto station in summer is related with *Sardina pilchardus* (5), *Engraulis encrasicolus* (6) *Diplodus vulgaris* (36) and *Sparus aurata* (37).

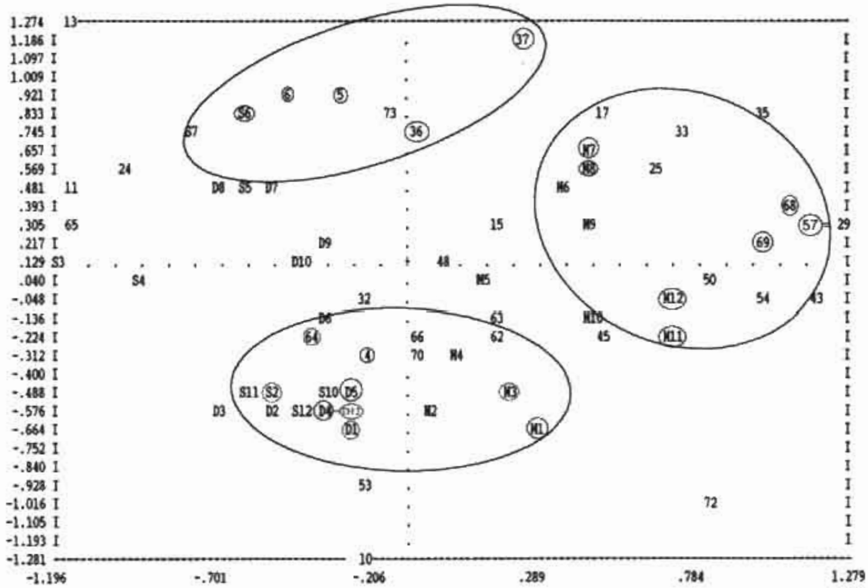

Fig. 17. Correspondence factor analysis of biomass matrix from 1991.

In 1992 (Fig. 18) were identified four groups: one with marine habitat in summer/autumn associated to migrants species – *Trigla lucerna* (68), *Scophthalmus rhombus* (69), *Diplodus vulgaris* (36) – occasional marine species – *Ciliata mustela* (29), *Callionymus lyra* (57) and *Symphodus bailloni* (43) – and sedentary species like *Gobius niger* (50) and *Pomatoschistus minutus* (54). Other group concerning brackish habitat in summer is associated with *Engraulis encrasicolus* (6) and *Sparus aurata* (37). The remainder two groups correspond to the freshwater sector in winter associated with *Platichtys flesus* (70) and the brackish area in spring/autumn related with *Liza ramada* (64) and *Dicentrarchus labrax* (32).

Conclusion and perspectives

The space-temporal variability of abiotic factors allowed us to characterise three habitats: marine, brackish and freshwater.

Each year two space temporal units were systematically found: (i) one concerning marine stations (M) in summer/autumn with an assemblage of fish species composed by stenohaline migrants (*Trigla lucerna, Scophthalmus rhombus, Mullus surmuletus, Diplodus sargus*), occasional marine species (*Ciliata mustela, Callionymus lyra, Symphodus bailloni*) and sedentary species (*Gobius niger* and *Pomatoschistus minutus*); (ii) other with brackish habitat in summer (or summer/autumn in 1988), in connection with *Engraulis encrasicolus, Sardina pilchardus, Sparus aurata* and *Diplodus vulgaris*. Less consistent in time of occurrence were found temporal units related with low salinity due to stronger

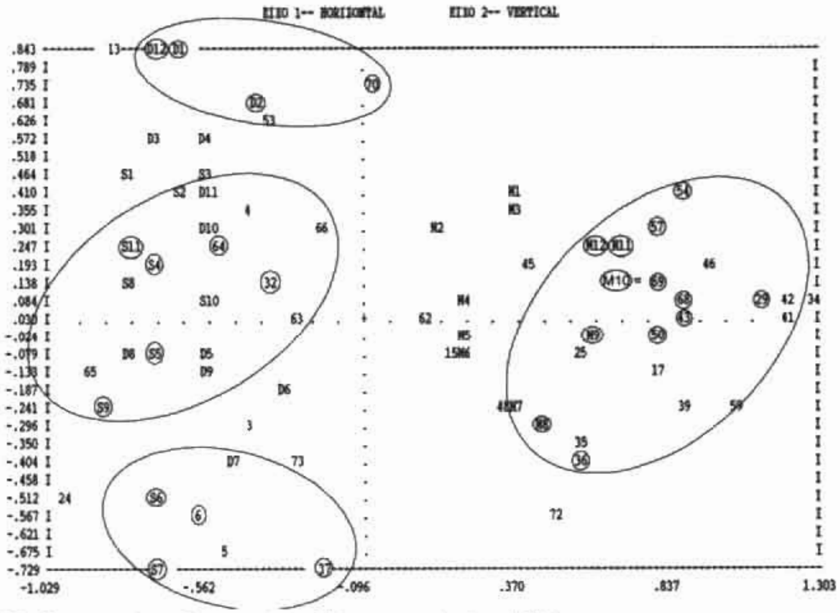

Fig. 18. Correspondence factor analysis of biomass matrix from 1992.

freshwater discharges associated with the presence of eurialine species assemblages (*Liza ramada, Dicentrarchus labrax, Platichtys flesus* and *Alosa spp.*).

The marine habitat, Gala/Porto Comercial, is a zone submitted to a daily marine influence where there are the lowest yearly amplitudes of temperature and salinity and consequently, with a greater capacity for receive stenohaline species and those sedentary which prefer the places where the abiotic parameters are more stable.

The freshwater habitat Vila Verde located in upstream of north arm is the station most exposed to the freshwater discharge of Coimbra bridge-dam.

The brackish habitat of Pranto station is plenty influenced by the tide currents when the dam of Bizorreiro is closed. In summer the sunstroke is frequently strong and can induce to higher temperature and salinity. These conditions in calm waters leads some migrants like *Engraulis encrasicolus, S. pilchardus, Sparus aurata* and *Diplodus vulgaris* to frequent this habitat, despite temporary disturbances caused by the overture of Pranto sluice. The result is a very high variability of community indexes like diversity with its lowest level.

Besides the spatial and seasonal variations of assemblages there are interannual fluctuations, and as an estuary is a dynamic system, it functioning can change due to natural causes or impacts of human activities, that can interfere for example in the volume or quality of freshwater flow. If marine influence will decrease we can assist to a diminution of salinity and the assemblages that characterised the previous marine and brackish habitats will be replaced by associations with species more euryhaline or freshwater species because the capacity to receive the first will decrease. *Liza ramada, Plathichthys flesus, Dicentrarchus labrax* are good indicators of this situation.

On the contrary, if marine influence increases due to a deficit of pluviosity, or artificial reduction of freshwater discharges in dams, the assemblages of species evolve towards to a marinization and some of the preceding occasional species may behave migrants because the capacity of the system to receive the marine species will increase. In a small scale it was what happened in the 1992 dry year, when the number of marine occasional species enhanced in estuary, staying some of them (*Ciliata mustela, Belone belone, Symphodus bailloni, Labrus merula, Callionymus lyra* and *Ammodytes tobianus*) during more time.

The perspectives are according to the principle of biological indicator to the functioning and ecological classification of ecosystem:

1. select the most indicative migrant species of each habitat;
2. select the date of their disponibility to the estuary;
3. quantify their exact tolerance to the environment factors (salinity, temperature, oxygen, chemical pollution…).

The objective is monitoring the estuarine ecosystem quality in relation to its traditional socio economic activity and with agriculture and industrial activity in river basin.

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STATUS AND HABITAT USE OF WADERS IN THE MONDEGO ESTUARY

Abstract

This paper describes the status and habitat use of the wading birds community at the Mondego estuary. Special emphasis is given to trends of population size and the importance of major habitats for waders, intertidal mudflats and salinas. Dunlin *Calidris alpina* is the most important species in terms of numbers but more 29 other species also occur. Numbers of some important species have been decreasing during last years (e.g. Avocet *Recurvirostra avosetta*) while for other species an increase was detected (e.g. Black-winged Stilt *Himantopus himantopus*). The Mondego wader assemblage is relatively important in the whole Portuguese wintering community (between 1.1 and 2.4% of the national totals during 1994-97). The main threat for wader conservation in the estuary is habitat loss, especially due to destruction or degradation of the preferred supratidal habitat.

Introduction

Estuaries are well known ecosystems with diversified and important bird communities (McLusky 1989). Since the order Charadriiformes (including waders, gulls and terns) and in particular the suborder Charadrii (waders) has a very important role on most of the estuarine trophic chains (Moreira 1997), this determined the focus of ornithological investigation during last decade in the Mondego estuary. The Mondego estuary (40°08' N 8°50' W, fig. 1) along with other portuguese estuaries (Tagus, Sado and Minho) and rias (Aveiro and Formosa) supports a large number of aquatic birds, due to the existence of suitable supratidal habitats and an important intertidal benthic macroinvertebrate community (Marques et al. 1993, Flindt et al. 1997, Múrias et al. 1997).

While the estuary has been recognised as one of the major wintering sites in Portugal for Lesser Black-backed Gull (*Larus fuscus*), for other species its importance

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was more difficult to assess. Exploratory wader mid-winter counts in the Mondego estuary were first performed in 1977 and 1978 (Rufino 1979) to evaluate the major habitats for coastal waders in Portugal. Since these census did not reveal important numbers of waders, the Mondego estuary was excluded from the national mid-winter wader census in the following years. In 1986 the estuary was again included, but less than 300 birds were counted (Rufino and Neves 1986). Between 1986 and 1993, several mid–winter census were performed (Múrias, unpublished work; Rufino 1989,1990) but the number of birds counted never reached the threshold of 1000 birds, due mainly to incomplete coverage of the estuary and misrepresentation of several key species. Therefore, an accurate determination of wader abundance was still unavailable by 1993, and the quality of previously collected data prevented any further interpretation.

The main objective of this work was to assess the current knowledge about bird communities in the estuary, to clarify the importance of Mondego estuary for waders and to present data on seasonal and annual variation in wader numbers and use of main estuarine habitats (intertidal areas and salinas). This will provide a comprehensive framework for research and conservation of waders at Mondego Estuary.

Methods

Bird community

Studies on the whole bird community of Mondego estuary began in 1993, when during the whole year a monthly census was performed, comprising transects throughout the most common habitats (Pacheco, unpublished work). This study recorded the presence of species but no density estimates were performed at that time. Apart from this census, during the last decade several species were also added from observation records.

Wader populations

From October 1993 to May 1995, fortnight low-tide and high-tide counts were performed by Múrias (1997), providing the first accurate census of wader abundance. From January 1996 onwards, Lopes (1999, unpublished work) implemented a fortnightly (1996) and monthly (1997-1999) low-water census, with the main objective of providing information about trends on the abundance of the major species of waders.

The census carried by Múrias (1997) covered the south arm and most of the salinas of Murraceira island (fig. 1). The mudflats were surveyed during spring tides from three fixed points while a transect was used to survey the supratidal habitats (salinas, fish farms and saltmarshes). Most of the censuses were performed in one day. After 1996 the coverage was even more extended with the inclusion of the salinas of the south-arm and the mudflats in the north arm by Lopes (1999).

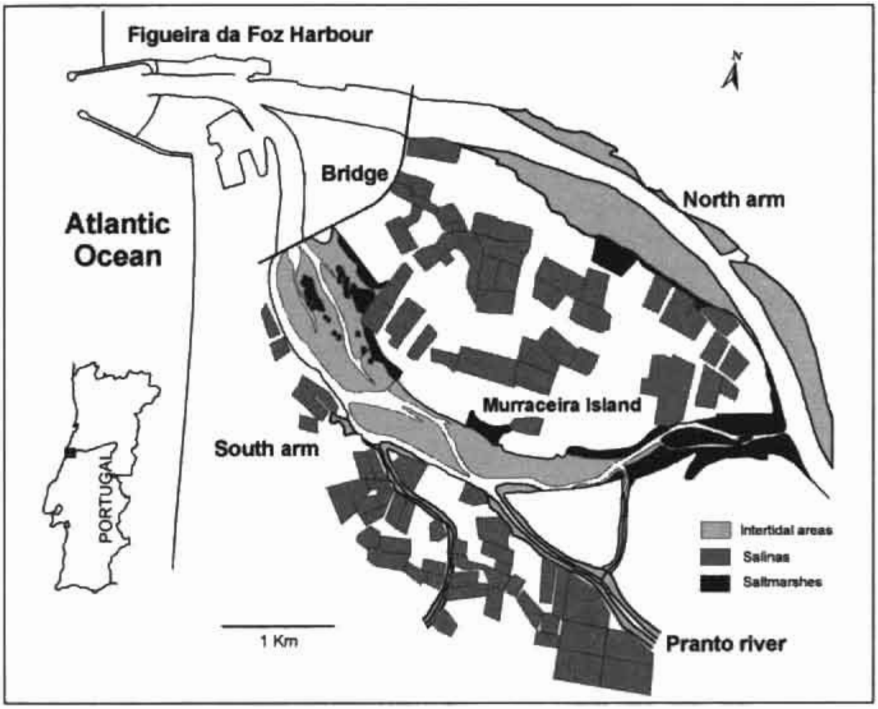

Fig. 1. The Mondego estuary with the two main habitats for waders, intertidal areas (light grey) and salinas (medium grey). The converted or abandoned salinas where the degradation is still reversible are also represented.

In order to allow for comparisons of results, the fortnight census were averaged by month for each species. The few missing censuses were interpolated by calculating the average of the two nearest census made before and after the missing data (Wolff and Smit 1990). Unfortunately, a gap of six months occurred between the census carried by Múrias (1997) and the beginning of the present census program (Lopes 1999). However both census were analysed together in order to provide a longer time-series for interpretation, but no missing values and trend analysis were performed at this stage.

Trends in use of habitat

The census performed by Múrias (1997) and Lopes (1999) discriminated the number of birds in these two types of habitat and the proportion of birds in each type of habitat was calculated. In fortnight counts the average monthly proportion of birds in salinas was used. However, since the number of salinas covered by the first census (1993-1995) was lower, not including south arm salinas, the pattern of use may be biased so it wasn't included in the analyses, which used only data from 1996-1999.

Results and discussion

Bird community

The results confirmed the high bird diversity of Mondego estuary, with 137 bird species recorded in the estuary during last decade. The bird community in Mondego estuary was dominated by two main orders (Passeriformes and Charadriiformes) with 35 and 31% of the species (fig. 2). However, most of the species used the estuary as migratory and wintering ground and only 18 species were confirmed breeders. Other 35 species still need to be confirmed as breeding in the estuary.

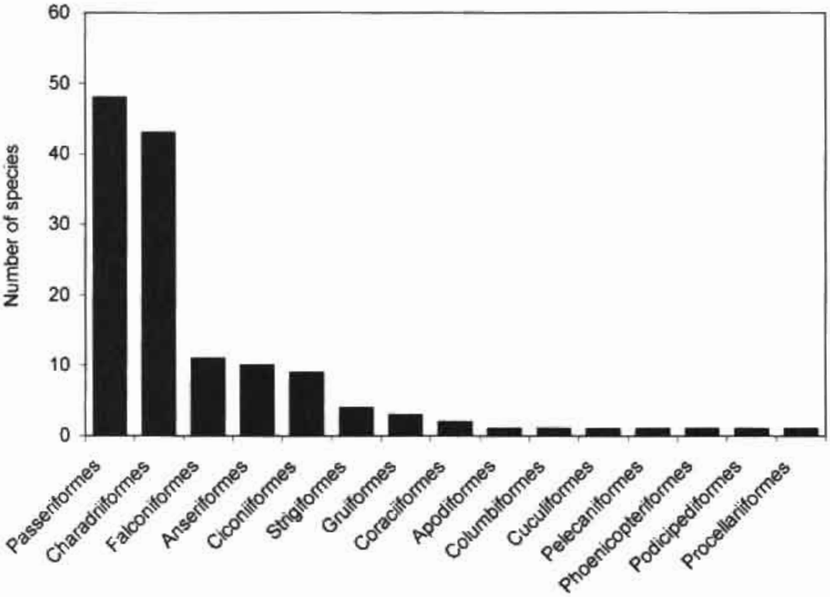

Fig. 2. Number of bird species grouped by order, recorded during the 1990's in the Mondego estuary.

Wader species composition and relative abundance

Total number of species and birds

From 1993 to 2000 were recorded 30 species of waders (appendix 1). The number of species recorded monthly seemed to increase from 1993-1995 to the period 1996-1999, probably due more to inter-observer variability than to a real trend. The maximum of birds occurred during winter months, especially in December and January (fig. 3). A second peak was observed in April-May (pre-nuptial migration) while another peak occurred from August to October (post-nuptial migration). During summer a maximum of few birds were present in the estuary (mainly breeding birds).

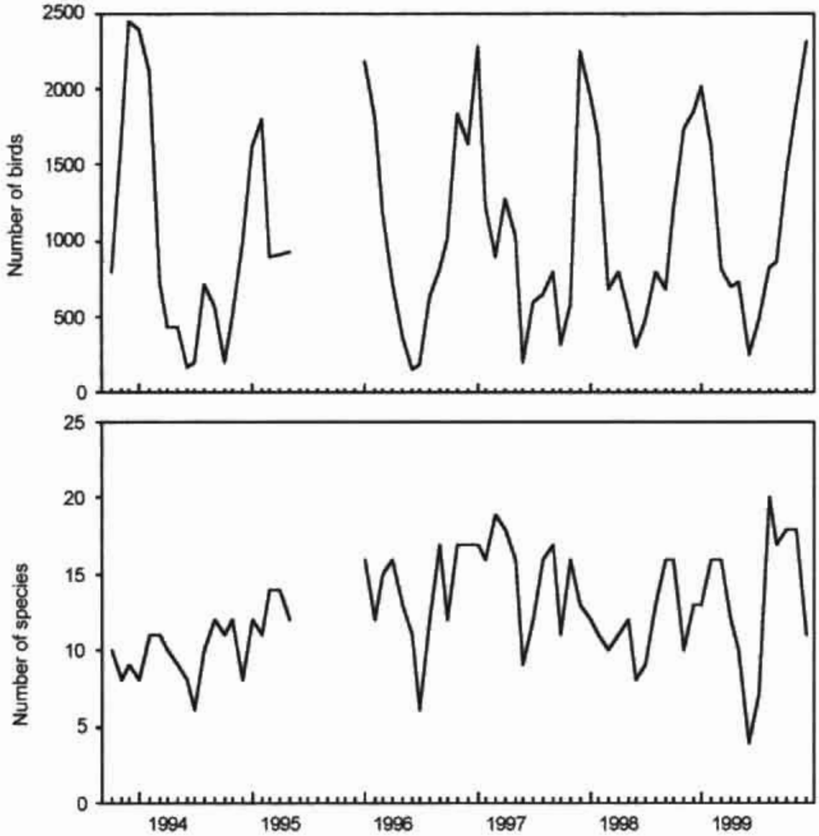

Fig. 3. Number of waders and species that were observed in low tide census from October 1993 to December 1999. (Data from Múnas 1977, Múrias et al. 1997; Lopes 1999, unpublished data).

Six species (fig. 4) represented a large proportion of the wader assemblage (mean monthly percentage = 89%). The community was dominated by Dunlin *Calidris alpina* and Avocet *Recurvirostra avosetta* during winter (November-February). In summer (June-July) the Black-winged Stilt *Himantopus himantopus* and Kentish Plover *Charadrius alexandrinus* were the most important species. During both migration periods Dunlin was the dominant species but high number of Kentish Plovers and Ringed Plover *Charadrius hiaticula* were also present.

The wader assemblage of the Mondego estuary did not differ much from other estuaries in Portugal (Múrias 1997). Dunlin is also the main species for most of the sites along southwest European coast (Cramp and Simmons 1983, Smit and Piersma 1989). Portugal is one of the countries with highest number of wintering avocets (Smit and Piersma 1989) and they were also one of the most important species in the estuary.

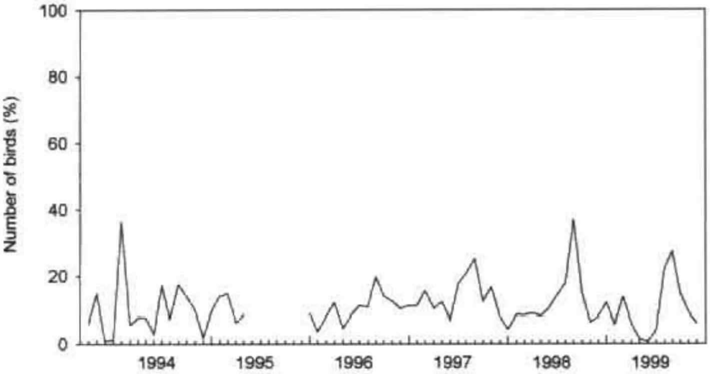

Fig. 4. Proportion of the six most abundant important species in the estuary (Dunlin, Avocet and Grey Plover, Kentish Plover, Ringed Plover and Black-winged Stilt) that were observed in low tide census from October 1993 to December 1999.

Important species

Dunlin was the most abundant wader species in the estuary in most of the counts (fig. 5). It was usually more abundant during winter with a smaller peak count in spring due to the passage of migrating birds. During autumn migration (August-October) an increase was observed, corresponding to a more continuous passage of birds than in spring migration. Ringing data during autumn migration suggest the occurrence of differential migration between adults and juveniles, with a very low percentage of adults in September (Lopes 1999, unpublished data).

The Avocet was the second most abundant species (fig. 5). It was a typical wintering species, found from October to March, but the number of avocets wintering in the estuary decreased during last years. Most of the colour-ringed birds observed in the estuary came from Germany and Holland, although a ring recovery was also found from Denmark. Since the majority of these birds were ringed as pulli or breeding, we can be confident that most of them breed in those countries.

Grey Plover *Pluvialis squatarola* was another important wintering species with a very typical seasonal pattern (fig. 5). It differed from the avocet since some migrating birds were also present during spring and autumn passage. There was an increase in the number of wintering birds in recent years while no trend was observed for migrating birds.

The Kentish Plover was present throughout the year (fig. 5) and was also one of the three species that bred in the estuary, along with Black-winged Stilt and Redshank *Tringa totanus*. Its seasonal pattern is very difficult to interpret due to the existence of several distinct populations (breeding, wintering and migrating birds).

Another species with a typical pattern was the Ringed Plover (fig. 5). The highest numbers occurred during autumn migration with a continuous decrease during the

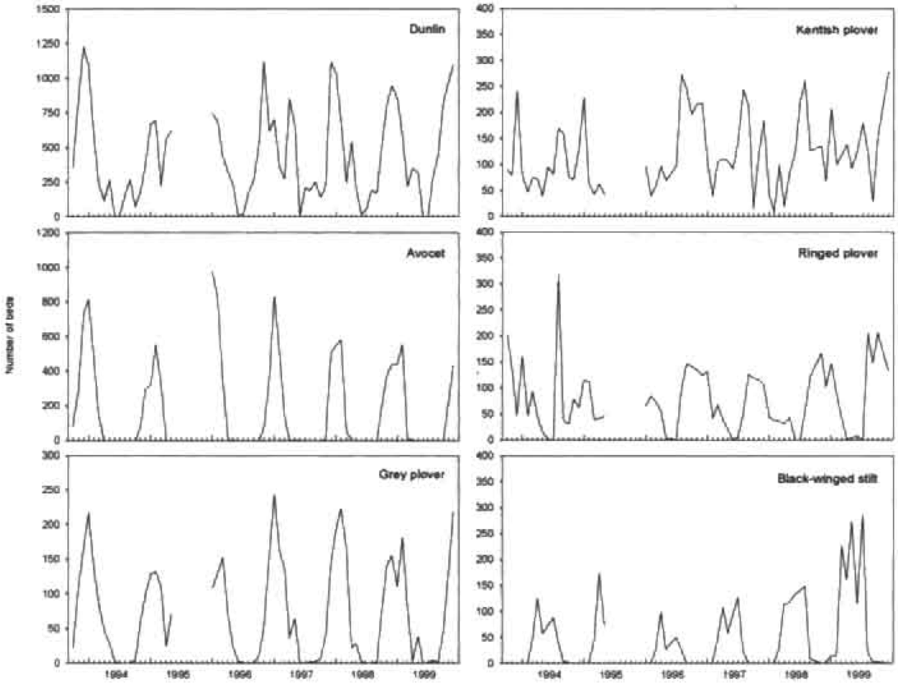

Fig. 5. The number of birds for the six most abundant species that were observed in low tide census from October 1993 to December 1999.

winter and a smaller peak during spring migration. The absence of this pattern during 1993-1995 was probably due to inter-observer variability than to a real trend. In 1998-1999 the number of Ringed Plovers that use the estuary during autumn migration increased while during spring their number decreased substantially.

The black-winged Stilt has been increasing in the Mondego estuary (fig. 5). During April a peak occurred due to the presence of a large number of birds including the local breeding population and migrating birds. The observation of colour-ringed birds showed that the estuary was used as a stopover for migratory birds breeding in France and Spain. In the last years some birds were present during some part of the winter. These observed seasonal patterns did not differ from those observed in other Portuguese estuaries (Batty 1992, Encarnação 1993).

Trends in wintering populations of waders

The mid-winter counts have been adopted to estimate population sizes of waders along the east-Atlantic flyway, assuming that inter-site movements are minimal (Prater 1981). In Portugal these censuses have been performed in the major estuarine areas, including Mondego. The January census performed by Múrias (1999) and Lopes (1999, unpublished work) have been incorporated in the national mid-winter wader

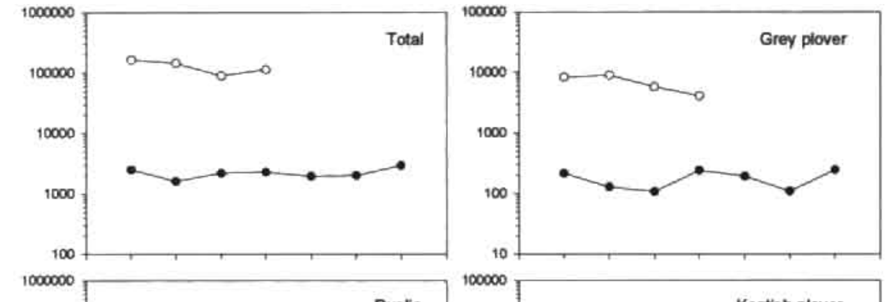

Fig. 6. Number of birds observed in the mid-winter national census and in the Mondego estuary from 1994 to 2000. Shown are total number of birds for all species and for the six most abundant species in the estuary (Dunlin, Avocet ,Grey Plover Kentish Plover, Ringed Plover and Black-winged Stilt).

census since 1994 (Costa and Rufino 1994, 1996a, 1996b, 1997), providing accurate information about the trends of wintering bird populations in the Mondego estuary and their national importance.

For same species there has been a trend of increase of birds during the last years (small in Dunlin and higher in Kentish Plover). This also occurred slightly for the total number of birds (fig. 6). Grey Plover and Ringed Plover exhibited a fluctuation between years with no discernible trend while avocets showed a negative trend in the last years with a recent stabilisation in numbers.

Unfortunately at this time no national data is available for the last three years. According to Farinha and Trindade (1994) the Mondego estuary supported less than 0,5% of wintering waders in Portugal during 1989-92. We think that this was due more to a deficient coverage of the area than to real estimates. The total numbers in the Mondego estuary represented between 1.1 and 2.4% of the national totals during 1994-97, which seems a more representative figure of the importance of the estuary as a wintering site. For some species this proportion is much higher. In national terms the wintering population of avocets in the Mondego estuary was for most of the years, an important proportion of the total number of avocets in Portugal (3.4 to 8.2%). Grey Plover (1.4 to 5.9%), Kentish Plover (1.9 to 12.9%) and Ringed Plover (1.9 to 3.6%) were also well represented in the Mondego estuary. This relative importance was probably the reason for the existence of correspondence in the fluctuations on the total numbers of Avocet, Kentish plover and Ringed plover between Mondego and Portugal as a whole (fig. 6). For the total values, Dunlin and Grey plover no correspondence was found.

Trends in use of habitat

Waders use several kinds of habitat in the Mondego estuary, including intertidal mudflats, salinas, aquaculture ponds and rice fields (Múrias 1997, Lopes 1999). However, during low-tide periods, waders use two main types of habitats (intertidal areas and salinas). Intertidal mudflats and sandflats (227 ha) occur in both arms of the estuary and during last decade the area of intertidal habitat increased with the formation of new mudflats and sandflats in the north arm (fig. 1). Seasonal intertidal macroalgal blooms (mainly *Enteromorpha* spp.) have occurred in both arms during the last decade (Marques et al. 1993, Flindt et al. 1997, Marques et al. 1997, Martins et al. 1997, Pardal 1998, Lillebø et al. 1999), probably due to an increase of nutrients in the estuary, which supports industrial activities and aquaculture and receives nutrients from 15000 ha of cultivated land (mainly rice fields). This problem is described further in other articles of this book. The salinas (251 ha in 1995) are located in the Murraceira island and in the left margin of the south arm and a considerable number has been abandoned or converted in the last decades. This conversion can be irreversible, with the construction of fish farms or reversible when they are used as fishponds without the destruction of the pans.

The results indicated a seasonal variation in the use of intertidal areas and salinas during low-water periods (fig. 7). During winter the proportion of birds that used salinas was very low. This was mainly due to the presence of two wintering species (Avocet and Grey Plover) that didn't use the salinas very often during low-tide. However, this proportion increased during recent years. During summer, because most species breed in the salinas (Black-winged Stilt, Redshank and Kentish Plover) the proportion of birds using salinas during low-tide was very high. The importance of salinas during migrations has changed throughout years but most of the times it showed an intermediate importance between summer and winter.

It seems that during low-tide salinas are used as complementary feeding areas during winter and migration periods, which also occurs in other estuaries (e.g. Ria Formosa, Cadiz bay) with salinas (Rufino et al. 1984, Batty 1992, Perez-Hurtado and Hortas 1993). However, during winter most of these sites support a higher proportion of birds in salinas (Batty 1992, Perez-Hurtado and Hortas 1993). Since these areas also support a higher number of waders, the competitive pressure on mudflats may be higher than in Mondego estuary, forcing birds to move to alternative supratidal habitats. The salinas of Mondego are very small (average size = 4 ha),which may contribute for

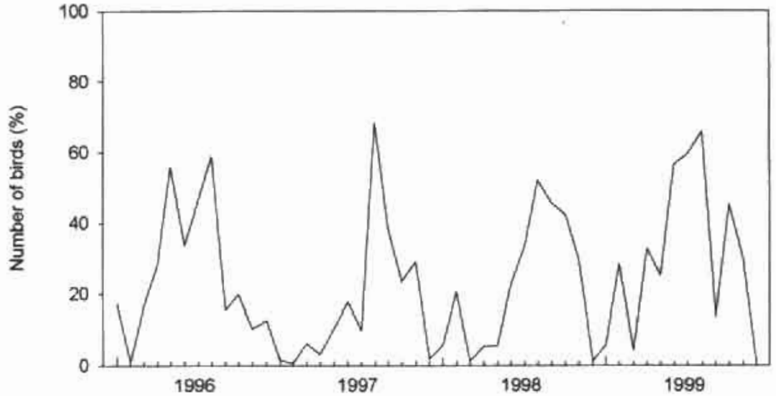

Fig. 7. Proportion of birds observed in salinas during low tide census from January 1996 to December 1999.

a larger variation in physical factors such as water depth and presence of vegetation. This variation is usually higher during winter decreasing the area of salinas available for feeding by waders. During summer salinas are very important because they constitute the preferred waders' habitat for breeding. Despite the ongoing conversion of salinas, some species such as the Black-winged Stilt have increased, presumably because they may prefer salinas with both abandoned and active sections, providing not only food but also camouflage and shelter from predators.

Conclusions

This review managed to compile most of the available data on wader bird communities in the Mondego estuary. Information is provided on seasonal and annual patterns of variation of bird numbers. These results are very important to understand the dynamics of wader populations in Mondego, Portugal as a whole and for the conservation of Mondego estuary. Despite the small area and human pressure, the Mondego estuary still holds an important and diversified wader community. This situation may change in the future if the degradation or destruction of the main habitats is not arrested.

Acknowledgements

We would like to thank all the people that helped in the collection of the data throughout these years.

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Appendix I

List of wader species recorded in the estuary during this work.

Species	Common name
Haematopus ostralegus	Oystercatcher
Himantopus himantopus	Black-winged Stilt
Recurvirostra avosetta	Avocet
Charadrius hiaticula	Ringed Plover
Charadrius alexandrinus	Kentish Plover
Charadrius dubius	Little Ringed Plover
Pluvialis apricaria	Golden Plover
Pluvialis squatarola	Grey Plover
Vanellus vanellus	Lapwing
Calidris canutus	Knot
Calidris alba	Sanderling
Calidris minuta	Little Stint
Calidris ferruginea	Curlew Sandpiper
Calidris maritima	Purple Sandpiper
Calidris alpina	Dunlin
Philomachus pugnax	Ruff
Gallinago gallinago	Snipe
Limosa limosa	Black-tailed Godwit
Limosa lapponica	Bar-tailed Godwit
Numenius phaeopus	Whimbrel
Numenius arquata	Curlew
Tringa ochropus	Green Sandpiper
Tringa glareola	Wood Sandpiper
Tringa erythropus	Spotted Redshank
Tringa totanus	Redshank
Tringa nebularia	Greenshank
Tringa flavipes	Lesser Yellowlegs
Actitis hypoleucos	Common Sandpiper
Arenaria interpres	Turnstone
Phalaropus fulicarius	Grey Phalarope

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SOURCES OF ENERGY IN LOW-ORDER STREAMS

Abstract

To quantify and characterise the sources of energy in low-order streams of the Mondego River Basin, data on the amounts and dynamics of litterfall, retention, decomposition and accumulation of allochthonous organic matter and on the standing stock of epilithic biofilm was collected on several years and streams. Our results showed that the deciduous forests of the Mondego River Basin produce abundant litter inputs, mainly in the form of leaves, and especially during late autumn. Leaves entering low-order streams tend to be rapidly retained especially by substrate structures such as debris dams. Therefore, at any given time, the organic matter transported by the streams is low when compared to benthic organic matter. Retained leaves decompose at different rates, depending on the stream and on leaf species. The different processing rates of several leaf species within a stream may provide a continuum of conditioned leaf litter available for the benthic invertebrate community throughout the year. Standing stock of benthic organic matter was 29-88 times higher in the deciduous and 5 times higher in the mixed forest stream than standing stock of epilithic biofilm. Allochthonous organic matter constitutes thus the main energy source to low-order streams of the Mondego River Basin.

Introduction

Organic matter in lotic systems comes from two main sources: (a) autochthonous matter from photosynthetic production within the stream and (b) allochthonous matter of terrestrial origin from the surrounding forest (Bunn 1986). Autochthonous sources of organic matter are considered to make only a minor contribution to the total energy pool of forest streams (Bunn 1986). Fisher and Likens (1973) found that less than 1% of the total energy inputs to a forested stream in New Hampshire (U.S.A.) was derived from photosynthesis within the stream.

Autotrophy may play an important role in the functioning in some streams, however, namely those that do not flow through dense canopy. Moreover, the relative importance of autochthonous sources of organic matter is also predicted to increase

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with stream order (Vannote et al. 1980). The wider channels of larger streams and rivers diminish the effect of shading by riparian vegetation and primary productivity is enhanced due to the increased solar radiation.

Allochthonous organic material entering streams from riparian vegetation depends on the amount of litter produced in the adjacent forest and on morphologic characteristics of the streams. The amount of litter produced in forests varies considerably and depend on factors such as climate, vegetation, type of soil, and age of the trees (Hernandez et al. 1992). For almost all kinds of wood, the massive litterfall occurs yearly during certain periods, depending on the phenology of the dominant species (Hernandez et al. 1992).

The continuous unidirectional flow through lotic ecosystems tends to transport matter to downstream reaches. After reaching a stream, allochthonous inputs will flow with the water being of little use to local biota, unless they are somehow retained. Retention removes matter from transport and makes it available for utilization by stream biota, providing a critical link between input and storage (Speaker et al. 1984). The process of retention includes both the immediate trapping of matter in transport and the sub-sequent longer-term storage of this material (Speaker et al. 1984).

Benthic detritus is an integral component of the functioning of headwater streams in forested areas; the knowledge of detritus storage is thus necessary to understand the structure and functioning of streams (Smock 1990). Accumulation of benthic organic matter depends on several factors, including litter inputs, discharge patterns and morphologic characteristics of the streams. After reaching a stream, CPOM is transformed to dissolved organic matter (DOM) through leaching (e.g. Petersen and Cummins 1974) or converted to fine particulate organic matter (FPOM) by physical abrasion or biological breakdown (Wallace et al. 1995). Litterfall, retention and breakdown are thus key processes in the energetics of low-order streams.

The objectives of this paper were to quantify and characterise the inputs and dynamics of detritus in several low-order streams of the Mondego River Basin, with special reference to litterfall, retention, decomposition and accumulation of allochthonous organic matter and to the standing stock of epilithic biofilm. The present paper is a synthesis of results included in the publications: Abelho 1994, Abelho 1999, Abelho and Graça 1996, Abelho and Graça 1998, Canhoto and Graça 1996, Canhoto and Graça 1998.

Material and methods

Study sites

The study was conducted in 6 streams of the Mondego River Basin, located at the Açor, Caramulo and Lousã Mountains. The sites were classified according to the surrounding vegetation into deciduous (streams flowing through mixed deciduous forests), "mixed" (streams flowing through eucalyptus plantations with a riparian corridor of deciduous trees: *Salix* spp., *Quercus* spp., etc.) and "agricultural" (streams draining agriculture fields and bordered by mixed deciduous riparian vegetation).

Table 1. Selected descriptive characteristics of the six study streams.

	Margaraça 1	Margaraça 2	Fraga da Pena	S. João	Laceiras	Sobral Cid
Location	Açor Mountain	Açor Mountain	Açor Mountain	Lousã Mountain	Caramulo	Coimbra
Riparian vegetation	Deciduous	Deciduous	Deciduous	Deciduous	Mountain Mixed	Agricultural
Stream order	1	3	3	5	3	1
Catchment area (ha)	29	182	559	1800	705	-
Discharge ($m^3 s^{-1}$)						
Mean	0.020 (N=15)	0.005 (N=3)	0.040 (N=3)	0.811 (N=18)	0.089 (N=3)	-
Range	0.005-0.068	0.003-0.009	0.021-0.071	0.140-1.526	0.067-0.131	-
Dissolved Oxygen (%)						
Mean	103 (N=15)	100 (N=3)	100 (N=3)	106 (N=17)	100 (N=5)	100
Range	97-113	97-104	94-104	100-112	93-107	-
mg O_2 L^{-1}						
Mean	11 (N=15)	11 (N=3)	11 (N=2)	12 (N=17)	12 (N=4)	-
Range	9-13	10-13	10-11	10-13	11-13	-
Temperature (°C)						
Mean	12 (N=16)	11 (N=4)	12 (N=4)	10 (N=17)	11 (N=5)	11
Range	9-17	9-14	9-16	7-12	9-13	-
Conductivity (µS cm^{-1})						
Mean	66 (N=12)	33 (N=3)	25 (N=3)	42 (N=18)	35 (N=2)	-
Range	55-74	10-58	10-45	38-47	30-40	-
pH						
Mean	6.8 (N=15)	6.4 (N=3)	6.7 (N=3)	6.4 (N=13)	5.8 (N=3)	7.0
Range	6.3-7.7	6.1-6.5	6.2-7.2	5.6-6.9	5.6-6.1	-
Alkalinity (mg $CaCO_3$ L^{-1})						
Mean	19 (N=15)	21 (N=3)	10 (N=3)	-	5 (N=3)	290
Range	15-24	17-23	9-11	-	4-6	-

Three deciduous streams were located at the Açor Mountain, two at Margaraça Forest (Margaraça 1 and Margaraça 2) and one at Fraga da Pena (Fraga da Pena). The other deciduous stream (S. João) was located at the Lousã Mountain. The "mixed" stream (Laceiras) was located at the Caramulo Mountain. The "agricultural" stream (Sobral Cid) was located near Sobral Cid Hospital, Coimbra. The study was conducted in riffle areas of the streams. The bed substrate was composed of boulders, pebbles and gravel. The streams were well oxygenated, circumneutral, with low conductivity and alkalinity values (Table 1). Discharge patterns were highly seasonal in all streams, with lowest flow during summer and highest flow during autumn and spring (Fig. 1).

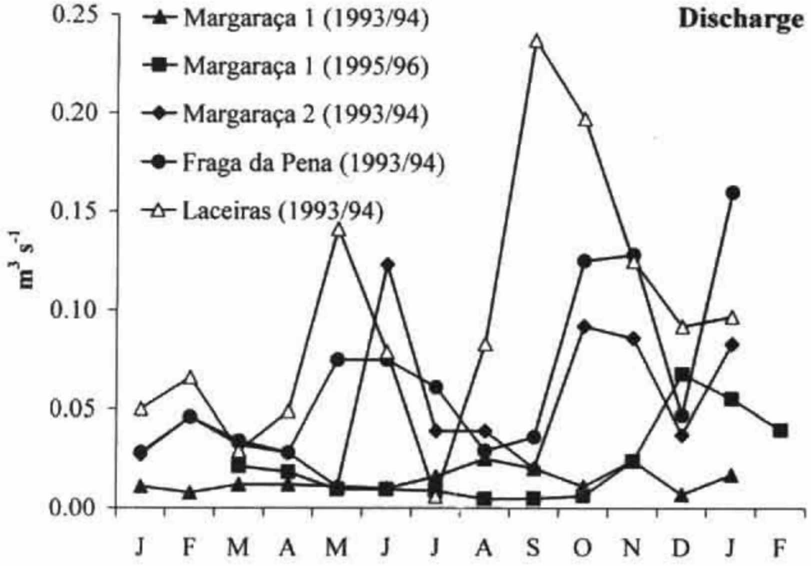

Figure 1. Temporal variation in discharge of three deciduous (Margaraça 1, Margaraça 2 and Fraga da Pena) and one mixed (Laceiras) forest streams during two non-consecutive years.

Litterfall

Litterfall was quantified during two non-consecutive years, in the riparian corridors of several streams. In the first year (February 1993 to February 1994), litterfall was determined at streams flowing through deciduous and mixed forests (Abelho 1994, Abelho and Graça 1996). The deciduous streams were Margaraça 1, Margaraça 2 and Fraga da Pena, and the mixed stream was Laceiras. In the second year (March 1995 to March 1996), litterfall was determined only at the site Margaraça 1 (Abelho and Graça 1998, Abelho 1999).

At each site, vertical litterfall was determined from 0.152 m^2 traps randomly placed at ground level in the riparian forest (four replicates during 1993/94 and 10 replicates during 1995/96). Each trap consisted of a circular plastic collector, pierced to allow rainwater to drain. Samples were collected every month during the study periods. Litter was sorted into three categories: leaves, twigs/branches, and fruit/flowers. Each category was oven-dried at 60°C for 48 hours, weighed, and ashed at 500°C for 5 hours to determine ash free dry mass (AFDM). A mean monthly value expressed in g AFDM m^{-2} was calculated for each fraction per stream.

Short-term retention experiment

The experiment was carried out during the summer of 1995 in S. João stream, using leaves of *Alnus glutinosa, Castanea sativa, Quercus faginea* and *Eucalyptus globulus* as the experimental units (Canhoto & Graça 1998). Four to five hundred of each leaf species were released at one point of the stream and collected in 1-mm mesh nets stretched across the stream and placed at fixed distances from the releasing point (maximum 90-m). The leaves collected in each net were immediately counted and released. Leaves were considered retained when not arriving to the next net for longer than 3.5 h (Canhoto and Graça 1998).

Transported organic matter

Transported organic matter was determined during 1993/94, in the same streams described for the litterfall inputs (Abelho 1994). Every month, organic matter was collected in three drift nets (aperture 0.33 m x 0.33 m, 0.5 mm mesh size) during a period of 45 minutes. The water volume flowing through the nets during the exposure period was calculated from the width of the nets (m) and from measurements of current velocity (m s^{-1}) and depth (m) at the nets. Total trapped organic matter was dried and ashed as described above and final values were expressed in g AFDM m^{-3} per stream.

Benthic organic matter

Benthic organic matter was determined in the same streams and years described for the litterfall inputs (Abelho 1994, Abelho and Graça 1996, Abelho and Graça 1998, Abelho 1999). In each stream, six Surber samples (area 0.09 m^2, mesh 0.5 mm) were randomly taken from the stream bottom every month during the study periods. AFDM of litter was determined as described for the litterfall samples. A mean monthly value expressed in g AFDM m^{-2} was calculated for each fraction per stream.

Decomposition

Four independent decomposition experiments were carried out in several streams using different leaves and methodology (Abelho 1994, Abelho and Graça 1996, Canhoto and Graça 1996, Abelho 1999). One experiment was carried out during 84 days (February-April 1993) in Sobral Cid stream using oven-dried (50°C, 48 h) leaves of *Alnus glutinosa, Castanea sativa, Eucalyptus globulus* and *Quercus faginea* (Canhoto and Graça 1996). A second experiment was carried during 1993

(September-November) in the same streams described for the litterfall inputs, using oven-dried (50°C, 24 h) leaves of *Castanea sativa* and *Eucalyptus globulus* (Abelho 1994, Abelho and Graça 1996). The other two experiments were carried out during January 1997 and January-March 1998 in S. João stream using air-dried leaves of *Castanea sativa* (Abelho 1999).

Leaves (approximately 3 g) were enclosed in fine-mesh bags (0.5-1.0 mm), and exposed in the streams. In each sampling occasion, 3-7 replicate bags were randomly retrieved. In the laboratory, the leaves were gently washed to remove attached sediments and oven-dried (50-60°C, 24-72 h). Weight loss was expressed as percentage of initial weight.

Autochthonous standing stock

Autochthonous standing stock was estimated from the epilithic biofilm of the upper surface of stones randomly collected from the streambed in the same streams described for the litterfall inputs (Abelho 1994, Abelho and Graça 1998, Abelho 1999). Samples were taken twice (July/August and October) during 1993 (five stones) and monthly from February 1995 to February 1996 (six stones) by scraping the stone surface with a scalpel in two areas of 0.0013 m^2. The collected material was oven-dried (60°C, 48 h), weighed and ashed (500°C, 5 h) to determine ash free dry mass (AFDM). The mean value of the two areas scraped was converted to g AFDM m^{-2}.

Results

Litterfall

Annual litterfall was 250-765 g m^{-2} yr^{-1} in the deciduous streams and 463 g m^{-2} yr^{-1} in the mixed stream (Table 2). Leaves were the most abundant component, comprising 64-79% of total litterfall in all streams (Table 2). Temporal patterns of litter inputs were seasonal in the deciduous streams where litterfall peaked during the autumn-winter period (Fig. 2). In the mixed streams, litterfall showed no clear seasonal pattern (Fig. 2).

Table 2. Litter production and percent composition at the deciduous and mixed forest sites during the years 1993/94 and 1995/95.

	Annual total organic matter (g AFDM m^{-2} yr^{-1})	% of total		
		Fruit and flowers	Twigs and branches	Leaves
Deciduous				
Margaraça 1 (1993/94)	693.4	12.4	19.9	67.7
Margaraça 1 (1995/96)	715.3	16.0	20.4	63.5
Margaraça 2 (1993/94)	765.0	9.1	11.7	79.2
Fraga da Pena (1993/94)	249.7	17.2	11.0	71.8
Mixed				
Laceiras (1993/94)	463.2	5.5	18.5	76.0

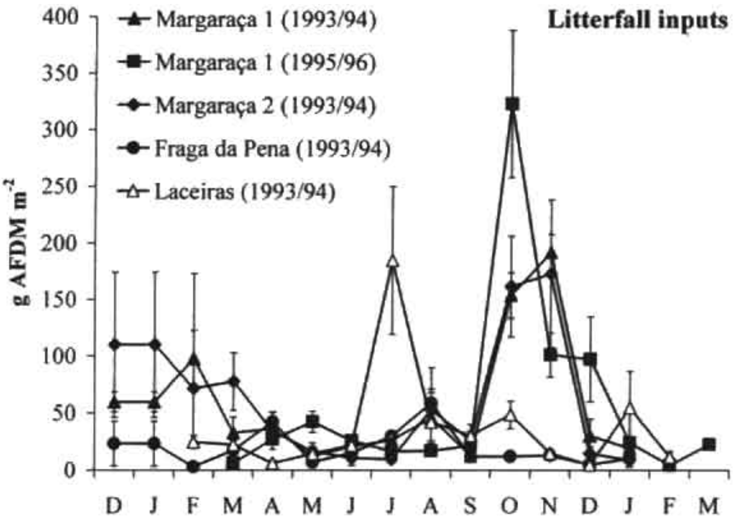

Figure 2. Temporal variation in litterfall at three deciduous (Margaraça 1, Margaraça 2 and Fraga da Pena) and one mixed (Laceiras) forest sites (mean ± 1 SE) during two non-consecutive years.

Short-term retention experiment

The stream was highly retentive trapping 90% of all leaves at short distances (mean 67 m) from the releasing point (Fig. 3). Leaves were more efficiently retained on substratum (i.e. debris dams) than on hydrological (i.e. margins and riffles) features of the stream (Canhoto and Graça 1998). Although no differences were found in the retention patterns of the four leaf species (ANCOVA, P>0.05), the substratum features retained

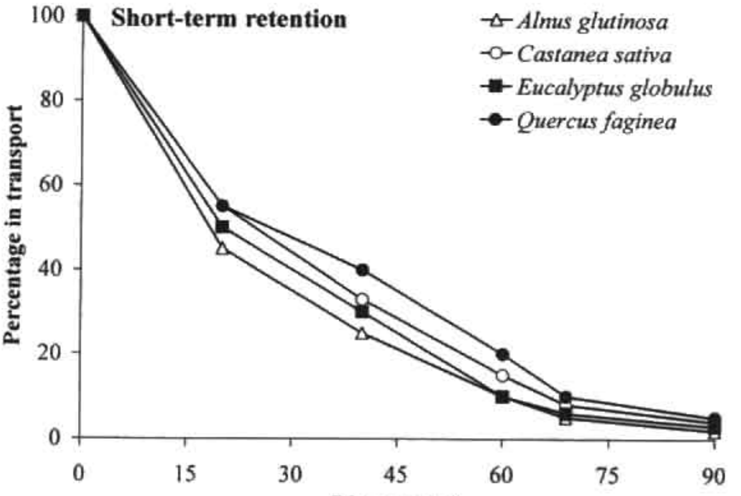

Figure 3. Percentage of released leaves remaining in transport along the experimental reach in S. João stream.

more efficiently the more flexible leaves of *Alnus glutinosa* and *Castanea sativa* than the harder leaves of *Quercus faginea* and *Eucalyptus globulus* (Canhoto and Graça 1998).

Transported organic matter

Transported organic matter was 0.9-1.8 g AFDM m^{-3} yr^{-1} in the deciduous and 0.3 g AFDM m^{-3} yr^{-1} in the mixed forest stream (Fig. 4). Transported organic matter was seasonal in the deciduous forest streams but showed no clear seasonal pattern in the mixed forest stream (Abelho 1994). In the deciduous forest streams, transported organic matter was negatively correlated with discharge ($r = -0.41$, DF = 29, $P<0.05$), but no correlation was found between transported organic matter and discharge in the mixed stream (Abelho 1994).

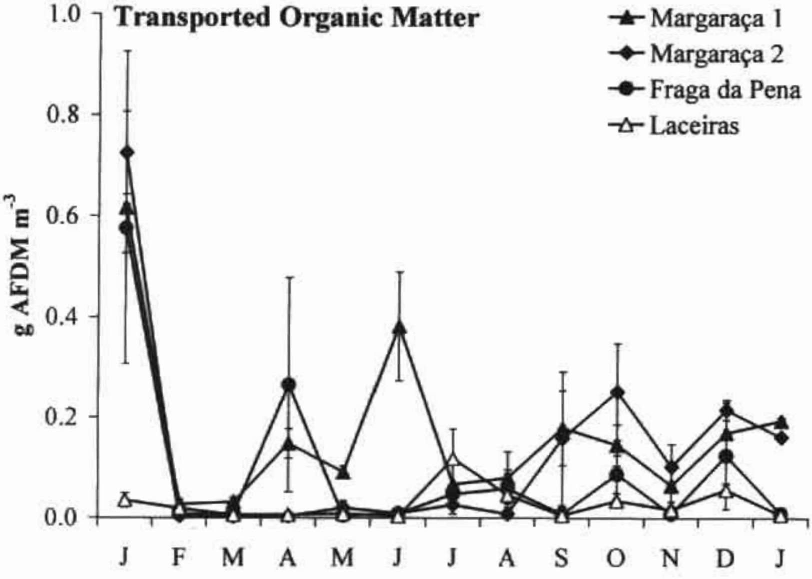

Figure 4. Temporal variation in transported organic matter of three deciduous (Margaraça 1, Margaraça 2 and Fraga da Pena) and one mixed (Laceiras) forest streams (mean ± 1 SE) during the year 1993/94.

Benthic organic matter and epilithic biofilm

Mean monthly benthic organic matter standing stock was 43-157 g m^{-2} in the deciduous and 24 g m^{-2} in the mixed stream (Fig. 5). The temporal patterns of benthic organic matter tended to be seasonal in all streams (Abelho and Graça 1996, Abelho and Graça 1998), with higher standing stock during spring and autumn (Fig. 5).

Standing stock of epilithic biofilm was 0.3-6.8 g AFDM m^{-2} in the deciduous and 1.3-5.5 g AFDM m^{-2} in the mixed forest stream (Fig. 6).

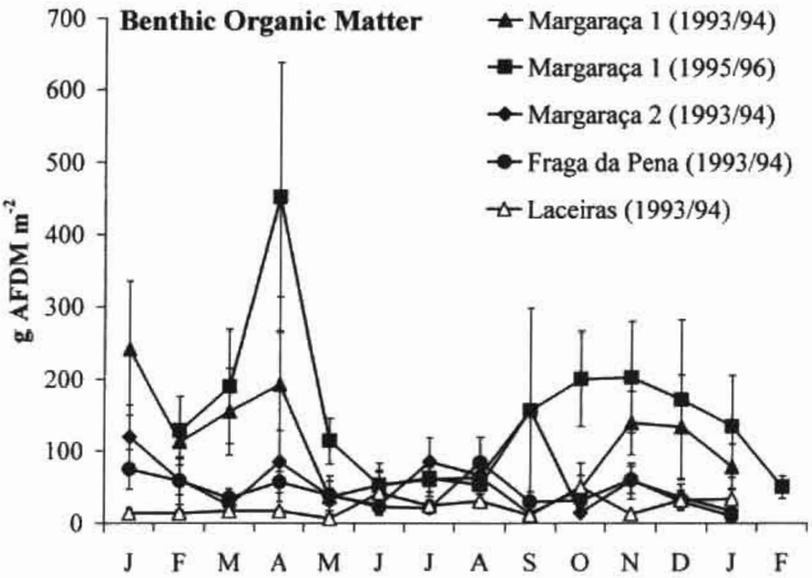

Figure 5. Temporal variation in standing stock of benthic organic matter in three deciduous (Margaraça 1, Margaraça 2 and Fraga da Pena) and one mixed (Laceiras) forest streams (mean ± 1 SE) during two non-consecutive years.

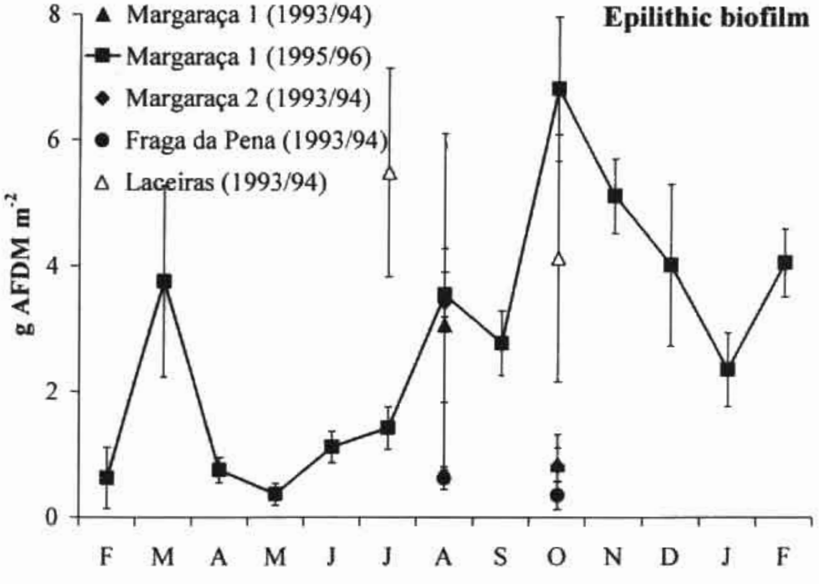

Figure 6. Temporal variation in standing stock of epilithic biofilm in three deciduous (Margaraça 1, Margaraça 2 and Fraga da Pena) and one mixed (Laceiras) forest streams (mean ± 1 SE) during two non-consecutive years.

Decomposition experiments

In Sobral Cid stream (Table 3), breakdown rates were fastest for the leaves of Alnus glutinosa (k = - 0.0161) and slowest for the leaves of *Quercus faginea* (k = - 0.0037). Decomposition rates of leaves of *Castanea sativa* (Table 3) varied between - 0.0066 and - 0.0079 day^{-1}. Decomposition in terms of mass remaining (Table 3) was faster for leaves of *Castanea sativa* (37-46%) than for leaves of *Eucalyptus globulus* (23-62%).

Table 3. Dry mass remaining at the end of the experimental periods and processing rates of different leaf species *(Alnus glutinosa, Castanea sativa, Eucalyptus globulus and Quercus faginea)* in one "agricultural" (Sobral Cid) and four deciduous streams (Margaraça 1, Margaraça 2, Fraga da Pena and S. João) of the Mondego River Basin.

Leaf species	Stream	Beginning	Experimental period (days)	Dry mass remaining (%)	Breakdown rate (*k* day^{-1})
Alnus glutinosa	Sobral Cid	February 1993	84	25	-0.0161
Castanea sativa	Sobral Cid	February 1993	84	50	-0.0079
Castanea sativa	Margaraça 1	September 1993	60	37	
Castanea sativa	Margaraça 2	September 1993	60	46	
Castanea sativa	Fraga da Pena	September 1993	60	42	
Castanea sativa	S. João	January 1997	31	78	-0.0066
Castanea sativa	S. João	January 1998	102	53	-0.0073
Eucalyptus globulus	Sobral Cid	February 1993	84	50	-0.0068
Eucalyptus globulus	Margaraça 1	September 1993	60	53	
Eucalyptus globulus	Margaraça 2	September 1993	60	62	
Eucalyptus globulus	Fraga da Pena	September 1993	60	23	
Quercus faginea	Sobral Cid	February 1993	84	70	-0.0037

Discussion

The litterfall inputs in the study sites (250-765 g AFDM m^{-2} yr^{-1}) are within the reported range for other European streams (Weigelhofer and Waringer 1994, Pozo et al. 1997). Litterfall had a seasonal distribution in the deciduous forests, with maximum inputs in autumn/winter primarily due to leaf abscission.

Due to their mixed nature, biofilms may have both heterotrophic and autotrophic activities. In our study, the standing stock of epilithic biofilm was very low. The source of energy it provides seems thus unimportant when compared with the allochthonous inputs and the standing stock of benthic organic matter, suggesting that the study streams are basically heterotrophic. The biofilm seems, in this case, to act mainly as an organic matter trap, removing dissolved organic matter from the water column (Abelho and Graça 1998, Abelho 1999).

It has been argued that, due to the highly seasonal patterns of litterfall, the availability of organic matter for the benthic community of deciduous forest streams is limited in winter (Campbell and Fuchshuber 1994). In the deciduous study streams, the rapid trapping of the falling leaf litter, the different decomposition rates of different leaf species and the retention structures of the streams provide a high standing stock of benthic organic matter throughout the year (Abelho and Graça 1998, Abelho 1999). Moreover, the seasonal quality of litter is highly variable. The spring inputs, although quantitatively small, may be very important as an energy source on a time when leaf inputs are minimal, due to the high nutritive content of flowers (Fittkau 1964, Winterbourn 1976).

In temperate regions, the highest CPOM standing stock occurs in late autumn following the period of highest annual litterfall (Bärlocher 1983). In the study streams, the annual input of litter is reflected by the dynamics of benthic organic matter under changing discharge conditions (Abelho and Graça 1996). In the deciduous forest streams, the autumn peak of litterfall resulted in accumulation of organic matter in the streambed, but the highest peak of standing stock was observed in spring, coinciding with a smaller increase in litterfall. Thus, only when relatively high inputs coincide with a period of low discharge, an increase of these materials on the streambed is expected (Abelho and Graça 1996, Abelho and Graça 19989.

In summary, this study showed that the deciduous forests of the Mondego River Basin produce abundant litter inputs, mainly in the form of leaves, and especially during late autumn. Leaves entering low-order streams tend to be rapidly retained especially by substrate structures such as debris dams. Therefore, at any given time, the organic matter transported by the streams is low when compared to benthic organic matter.

Retained leaves decompose at different rates, depending on the stream and on leaf species. The different processing rates of several leaf species within a stream may provide a continuum of conditioned leaf litter available for the benthic invertebrate community throughout the year.

Standing stock of benthic organic matter was 29-88 times higher in the deciduous and 5 times higher in the mixed forest stream than standing stock of epilithic biofilm. Allochthonous organic matter constitutes thus the main energy source to low-order streams of the Mondego River Basin.

Acknowledgements

The work synthesised on this paper was financed by JNICT (projects PEAM/C/CNT/31/91 and PBIC/C/CEN 1131/92) and by the European Community (contract CI1*-CT94-0100). Manuela Abelho was financed by JNICT (BM/2265/91-RN), and by FCT (PRAXIS XXI/BD/2952/94). We wish to thank all colleagues who helped in fieldwork.

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NUTRIENT DYNAMICS IN THE INTERTIDAL POOLS OF THE MONDEGO ESTUARY.

I – NUTRIENTS SOURCES, SEDIMENT PROFILES, MINERALISATION AND ADSORPTION DYNAMICS

Abstract

During ebb situations in tidal estuaries with high tidal amplitude most of the system is out of water. In this period intertidal pool formation takes place in very extended areas. These pools differ with respect to internal nutrient loading, hydraulic conditions, duration, plant coverage, sediment properties such as organic content in the sediment, C/N/P-ratios of the sediment nutrient sources and porewater concentrations of nutrients.

Therefore, the objective was to study and compare the nutrient sources and dynamics in different types of intertidal pools of the Mondego estuary. In each intertidal pool the nutrient dynamics were followed on a seasonal scale by measuring oxygen dynamic, phosphate, ammonia, nitrate besides physical parameters as salinity, temperature, sediment organic matter, sediment total phosphorus, potential phosphate adsorption, total sediment nitrogen, sediment water content, pool volume and pool area.

The results showed that the nutrient dynamics in shallow intertidal pools are much faster than in subtidal areas. This is most probably due to the shallowness of the systems giving anabolic as well as catabolic processes optimal conditions with respect to light climate, oxygen concentrations, higher concentrations of dissolved inorganic nutrient and higher mean temperature during the growth season. Altogether, this study indicate that in the *Spartina maritima* covered areas the plants are able to control the phosphorus dynamic due to *Spartina maritima* take up phosphate for growth purposes, that without plant coverage would have returned to the watercolumn and that *Spartina* do also facilitates the increased phosphate adsorption. On the system level most pools are situated on bare bottom without connection to rooted vegetation, and these leaks with substantial amounts of phosphate. So the intertidal pool system of the Mondego estuary altogether are exporting significant amounts of phosphorus in these years.

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Introduction

Estuaries represent gradient in both physical, chemical and biological processes and their function as filters for dissolved and particulate nutrients from land drainage is determined by these gradients (Shubel and Kennedy 1984, Nixon 1995). The longitudinal gradients of nutrients created by dilution, dispersion and loss processes over short stretches make estuaries ideal for studies of nutrient dynamics (Borum 1997). From a physical point of view the deposition of fluvial material depends on the gravitational circulation pattern, sediment stability and the basin bathymetry, but also processes like flocculation are important (Pejrup et al. 1997, Amos et al. 1998). The physical-geochemical deposition is leaving dissolved nutrients for biological uptake in the watercolumn followed by sedimentation. Or alternatively by nutrient uptake for anabolic purposes at the sediment surface. The study of the close benthic-pelagic coupling of nutrient dynamics and the net losses of nutrients in estuaries therefore requires description of both transport-dispersion and biogeochemical processes. And only proper mass balances can verify whether an area inside the estuary acts as a source or a sink for nutrients.

Earlier, mass balances for the Mondego estuary were calculated (Flindt et al. 1997, Pardal 1998), where about 14 tons of phosphorus where missing. The question was where to search for this deficit? Was it caused by incorrect land based discharge or mixing at the outer boundary? Not with an order of magnitude! Being out in the field area, we recognised that although most of the area is submerged during high tide, when the water level drops during the ebb most of the system is out of the water-phase. In this situation tidal pools formation takes place in very extended areas. The pool size can vary from cm^2 only holding ml's of water up to several m^2 with volumes reaching m^3. Almost nothing is presented in the literature about the nutrient dynamics in small intertidal pools. Moreover, depending on the topographic conditions, these shallow tidal pools will remain either for a short period or during the whole ebb phase. The long existing pools have a duration of about eight to ten hours, before the next flood reach them and the water become mixed with the estuarine channel water. During this long period the water temperature increases dramatically facilitating high mineralization rates of organic matter, organic nitrogen and phosphorus (Lillebø et al. 2002b). Furthermore, during night ebbs these pools may become anoxic, when microphytobentos, macroalgae and rooted macrophytes are unable to compensate the heterotrophic oxygen consumption. Under such conditions, the adsorbed phosphate releases from the adsorption sites and diffuses to the porewater resulting in increased phosphate efflux from the sediment to the water column.

Altogether, the enhanced nutrient dynamics in the pools, the differences in the sediment properties, and the topography may support macroalgae growth rates that differs from the rates recognised during high tide. Only the fact that the macroalgae never become light limited in the shallow pools changes the growth potential completely. These changes together with the fast nutrient turnover in the intertidal pools may be of significant importance for the nutrient dynamics of the whole estuary and influence the seasonal export balance of nutrients from the system.

In this suite of four papers we share a major objective by studying if different intertidal pools function as a source or a sink for nutrients in the Mondego estuary. The work is separated into the following topics:

I. Nutrient pools, nutrient sediment profiles, nutrient mineralisation and phosphorus adsorption dynamics.
II. Seasonal efflux of PO_4-P and NH_4-N in bare bottom and vegetated pools.
III. The importance of nutrient effluxes to macroalgal growth (*Enteromorpha* sp.).
IV. Pools contribution to the dissolved inorganic phosphate loading.

The selected pool types were considered as representative for the salt marsh area of the estuary. Inside this area all kinds of dominating sediment types was represented. The chosen pools were *Spartina maritima* covered muddy pools, sandy and muddy bare bottom pools with different amount of organic matter, and nitrogen and phosphorus content. By repeating the studies during day and night periods and over the season net efflux calculations could be made and the export/import balance for the nutrients from the different pool areas will appear.

Study site

General information and more detailed description of the Mondego estuary can be found in Marques et al. (2002) . Figure 1 shows the placement of the different sites and the pool that was used in the present study. The sandy pools had the shortest duration of about 3 hours, while the *Spartina* and the muddy pools lasted for 8-10 hours depending on the tidal phase. So the intertidal pools differed with respect to hydraulic conditions, residence time, plant coverage and organic content in the sediment, porewater profiles of phosphate, ammonia and nitrate.

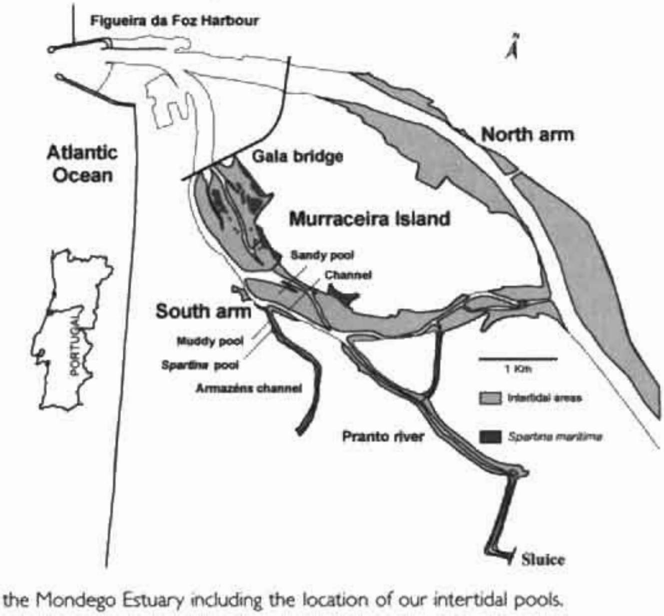


Fig. 1. Map of the Mondego Estuary including the location of our intertidal pools.

Material and Methods

From the formation of each pool when the high tide faded the nutrient dynamics were followed in the watercolumn of each type pool. This was realised by measuring concentrations of oxygen, phosphate and ammonia with a temporal resolution of 1 hour. In addition physical parameters as salinity, temperature, pool volume and pool area was measured.

Three sediment cores were harvested from each pool in July 1997 and brought to the laboratory where they were sectioned into 1 cm subcores. All subcores from the same depth interval were initially mixed to improve the homogenity among the samples. Triplicate subsamples were analysed for sediment water content, dry matter (105 °C in 24 hours), loss of ignition (550 °C in 6 hours), total phosphorus, potential phosphate adsorption capacity and total nitrogen.

Phosphate adsorption measurements were done as a saturation kinetic experiment on the same subcores used for measurements of the other sediment parameters. All analysis were performed by methods appropriate for the ambient salinity according to Standard methods (1996). The adsorption of phosphate was measured over a broad range of phosphate concentrations. Eight subsamples from each sediment layer (about 1.0 g wwt. sediment) were exposed to increasing concentrations of phosphate ranging from: 0.0-5 mg PO_4-P l^{-1}. The experiments were done in 250 ml open medical glass bottles where the sediment was suspended with 200 ml pre-filtered estuarine water. Afterward, the bottles were placed in a shaking bath for 36 hours and the adsorption or desorption of phosphate were measured following the method used by Jensen et al. (1992) The measured adsorption (µg PO_4-P g^{-1} dwt. sediment) was afterward statistical fitted to the non-linear saturation Langmuir expression:

$$SP = (PSC*Ce)/(Ce + K) - NAP \qquad (1)$$

where SP is the adsorption of phosphate in mg g^{-1} dwt. sediment, Ce is the final phosphate concentration, K is the half saturation constant, PSC is the Phosphate Sorption Capacity (maximal adsorption capacity) and NAP is the Native Adsorbed Phosphate (the adsorption at concentration 0.0 µg PO_4-P l^{-1}). The SAS non-linear regression model was used to find PSC, K and NAP.

Experiments of the quality of the organic matter in the sediment were performed for each inter tidal pool. The sediment cores were harvested in July 1997. These experiments were performed as mineralization studies, where 2 × 10g wwt of sediment from the depth range of 0.0 – 5.0 cm represented each pool. At time zero, the particulate organic nitrogen (PON) were measured in all samples representing the initial 100% of PON in each intertidal pool. The well-mixed sediment was transferred into 250 ml glass bottles, and afterwards added 200 ml pre-filtered estuarine water. All bottles were incubated in darkness at 18 °C ± 1 °C. At each time interval a subsample of the sediment were taken and measured at a Carlo Alba CHN-analyser. It was decided that the experiments terminated when the mineralization of the sediment particulate organic matter had entered a slowly and stable mineralization pattern without further nutrient dynamics. Subsamples were taken at day 0, 5, 10, 20, 30, 40, 50, 60 and 70 when this mineralization experiment was finished.

All dissolved inorganic nutrient analyses were measured on a rapid flow auto-analyzer (RFA 300 Alpkem) and performed by methods appropriate for the ambient salinity according to Alpkem methodologies (Alpkem 1990).

Results

Sediment properties

The sediment water content was measured in the upper 15 cm in all the different pools. The water content in the *Spartina* pools was about 75 % at the sediment watercolumn interface, which decreases to about 55 % in the lower part of the core. In the bare muddy sediment the water content was 95 % in the top decreasing to 80 % in 12.5 cm depth. The sandy pools had the driest sediment with a water content of 50 % in the interface decreasing to about 25 % in the deepest part.

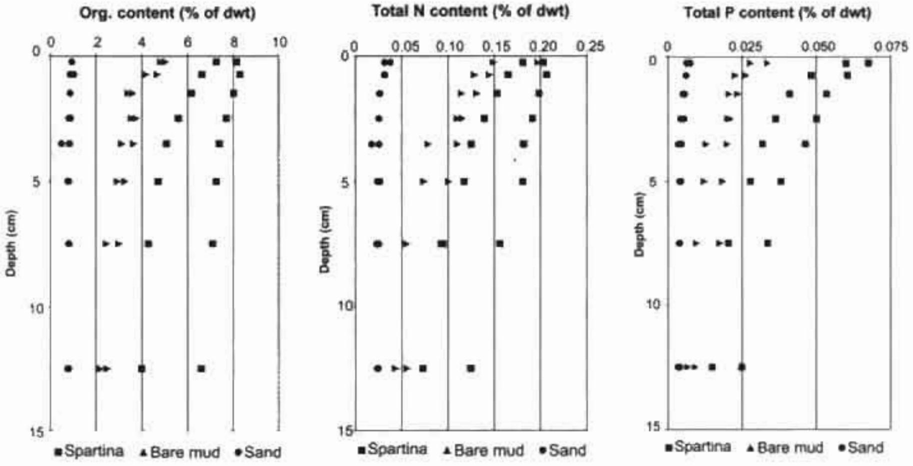


Fig. 2. Sediment organic matter content (a), nitrogen (b) and phosphorus content (c) measured as % of the sediment dry weight.

The organic content of the different pools is shown in Figure 2a. It was about 8 % in the upper part of the *Spartina* sediment decreasing continuously to about 4-6 % in 12.5 cm depth. The muddy bare bottom sediments had an organic content of about 5 % in the top decreasing to about 2 % in the bottom, while the sandy bare bottom sediments had the lowest organic content with 0.95 % in the surface without any depth variations. The total nitrogen content was again the highest in the *Spartina* covered pools where the values were about 0.2 % total nitrogen in the top part decreasing to about 0.1 % in the deepest layer (Fig. 2b). The muddy bare bottom sediment had about 0.15-0.2 % in the sediment surface, which continuously decreased to 0.5 % in 7.5 cm depth without any further variation downward. The total nitrogen content in the sandy bare bottom pools was about 0.04 % without any consistent changes with depth (Fig. 2.b). The total phosphorus content was also highest in the *Spartina* pools with about 0.06 % in the sediment surface reaching 0.02 in 12.5 cm depth with a well-defined gradient (Fig. 2.c). The muddy bare bottom content of total phosphorus was only 0.03 % in the surface decreasing to about 0.01 % in the bottom. The sandy bare bottom sediment was again the lowest with only 0.01 % of total phosphorus in the surface decreasing to about 0.005 % in the bottom (Fig.2.c).

Nitrogen mineralisation experiments.

It is assumed that this type of mineralisation experiments carried out in the laboratory verify how much of the nitrogen pools that becomes mineralised (Nixon 1981, Klump and Martens 1983) and thereby is exchangeable between the sediment and the watercolumn (Kamp-Nielsen 1992). The non-mineralised fraction after these 70 days are assumed to be less labile to microbial degradable and therefor has a very long turnover time. The results are shown in Figure 3. The mineralization experiment shows the general tendency with highest initial mineralization rates that decreases over time. Major differences existed between the amount of mineralised nitrogen in the different pools. Within the first 10 days of the experiment 20% of the PON were mineralised in the *Spartina* pool while only 10% were mineralised in the muddy pools and 5% in the sandy pools. In the end of the experiment about 50 % of the sediment PON from the *Spartina* pools was mineralised while about 30% of the PON from the muddy pools and only 20% of the PON from the sandy pools was mineralised.

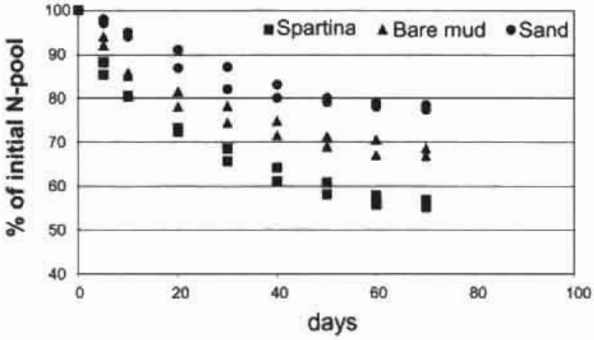


Fig. 3. Sediment particulate nitrogen (PON) mineralisation at the three study sites. The initial amount of PON was set to 100 %.

Based on the above experimental data, mineralisation rate constants were calculated for the PON in the respective pool types. The results are shown in Fig. 4. The rate constants obtained in the *Spartina* pool indicate that three nitrogen sources with different lability became mineralised. The first fast source was mineralised within the first 10 days with a rate constant of about 0.02 d^{-1}, while the lesser labile source with an average rate constant of about 0.01 d^{-1} was only used at day 40. The less labile nitrogen source with a rate constant of 0.005 d^{-1} continued throughout the experimental period until day 70. The initial mineralization rate constant in the bare muddy pools was about the half of the *Spartina* value, and became used until day 20, while the rate constant for the last nitrogen sources only was a little lesser than the final *Spartina* source. It seems likely that there is only one particulate nitrogen source in the sandy pools, due to the fact that there were no changes in the mineralisation rate constant throughout the experiment. The rate stayed low throughout the experiment ranging between 0.004-0.006 d^{-1}.

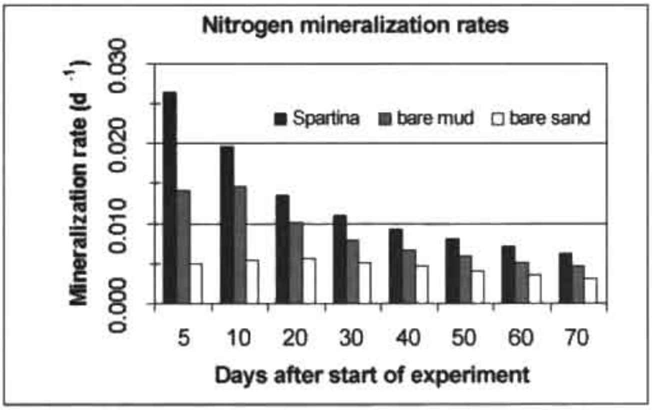


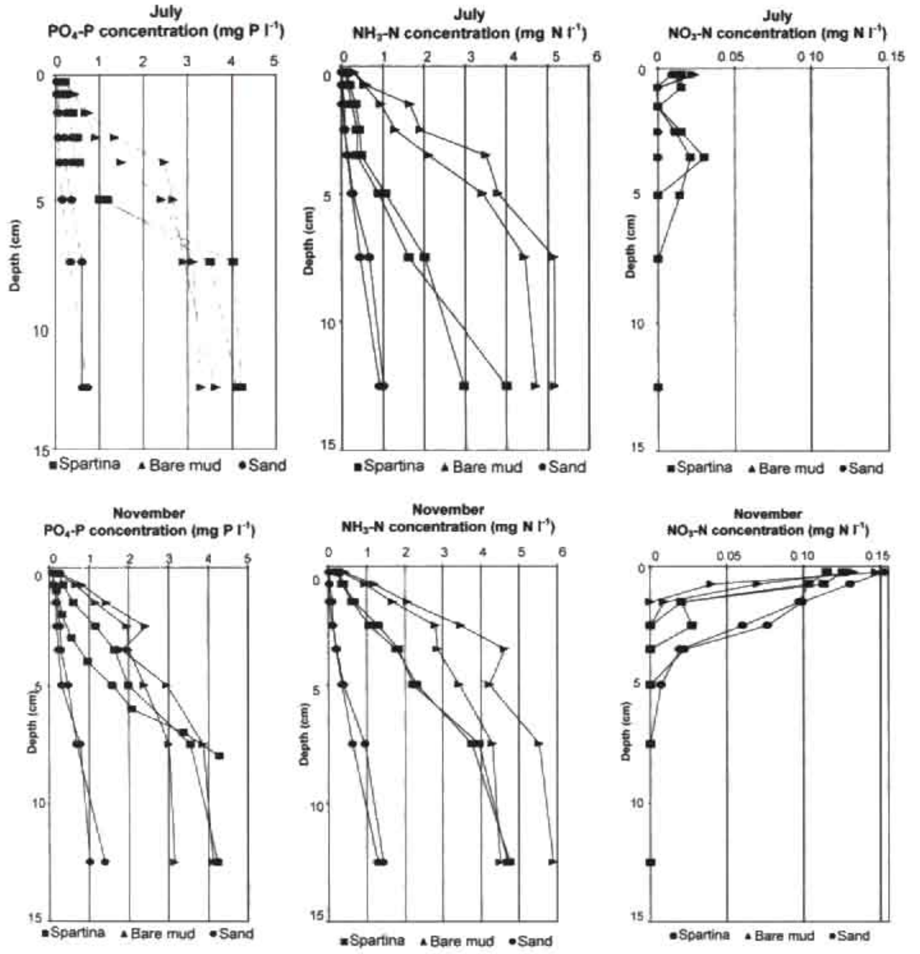
Fig. 4. Dynamics in the sediment mineralisation rate constant during experiments.

Porewater profiles of dissolved inorganic nutrients.

There were very pronounced differences in porewater concentrations of dissolved inorganic nutrients between the locations and from summer to fall/winter.

Phosphate porewater profiles.

Comparing bare sandy and muddy pools in the summer, the porewater concentrations of phosphate was 0.02-0.04 mg PO_4-P l^{-1} in the sandy sediment water interface while the concentration in the muddy pools reached about 0.2 mg PO_4-P l^{-1}. At the sandy pool the concentration never increased above 0.6 mg PO_4-P l^{-1} in the depth, while it reached a concentration of more than 4 mg PO_4-P l^{-1} in the deepest part of the muddy porewater (Fig. 5.a). In the *Spartina* covered muddy sediments phosphate was very depressed in the upper part of the rhizosphere (0-8 cm depth) with a concentration range of 0.2-0.4 mg PO_4-P l^{-1} compared to the bare muddy pools where the concentration was 1.5-2.8 mg PO_4-P l^{-1} at the same depth. Deeper in the sediment (8-15 cm depth) the concentrations equalised. The fall/winter profiles of the sandy area was slightly higher (Fig 5.d). In the deeper parts of the sediment, the concentration was about 1 mg PO_4-P l^{-1}. In the muddy area the PO_4-P l^{-1} profile



July
PO$_4$-P concentration (mg P l^{-1})

July
NH$_3$-N concentration (mg N l^{-1})

July
NO$_3$-N concentration (mg N l^{-1})

November
PO$_4$-P concentration (mg P l^{-1})

November
NH$_3$-N concentration (mg N l^{-1})

November
NO$_3$-N concentration (mg N l^{-1})

Fig. 5. Sediment porewater concentration of PO$_4$-P summer (a) and winter (d), NH$_3$-N summer (b) and winter (e), NO$_3$-N at summer (c) and winter (f) in the Spartina, the bare muddy and the bare sandy pools.

increased continuously from just above detection limits to 3-4 mg PO$_4$-P l^{-1} in the deeper part of the sediment. The *Spartina* PO$_4$-P profile did not show the same rhizosphere depression in fall/winter period as in the summer, and was more comparable to profile in the bare muddy location.

Ammonia porewater profiles.

The same differences existed between the *Spartina* covered area and the bare muddy area with respect to porewater ammonia concentrations – although more pronounced (Fig. 5.b). From an ammonia concentration of about 0.2 mg NH_3-N l^{-1} in the upper part of the bare muddy pools the concentration level increased continuously to 4-5 mg NH_3-N l^{-1} in the deeper part during summer sampling. In the *Spartina* covered area the porewater profile increased from about 0.150 mg NH_3-N l^{-1} in the sediment water interface to 1.5 mg NH_3-N l^{-1} in 7.5 cm depth. This was followed by a further increase below the rhizosphere in the depth range of 10-15 cm where the concentration peaked with about 3 mg NH_3-N l^{-1}. In the sandy station the profile increased continuously from about 0.1 mg NH_3-N l^{-1} at the sediment surface to a little less than 1 mg NH_3-N l^{-1} in the deeper part. The winter profiles of ammonia showed the same tendency (Fig. 5.e). The bare muddy station had again the highest porewater concentrations with about 0.6 mg NH_3-N l^{-1} in the sediment water interface steeply increasing to about 4 mg NH_3-N l^{-1} at 5 cm depth with a further increase to 5 mg NH_3-N l^{-1} in the deeper part. The *Spartina* profiles were again depressed in the rhizosphere where the concentration reached 2.5 mg NH_3-N l^{-1} and increased to about 5 mg NH_3-N l^{-1} in the bottom. In the bare sandy area the ammonia concentration was close to zero at the sediment water interface slowly increasing to about 1 mg NH_3-N l^{-1} in the bottom.

Nitrate porewater profiles

Both the bare sandy and bare muddy pools had very low concentrations of nitrate in the top part of the sediment during the summer (Fig 5.c). At both stations the concentration was around 0.020-0.025 mg NO_3-N l^{-1} in the upper part of the sediment and depleted completely at about 1cm depth. The *Spartina* covered bottom followed the same pattern, but increased again down in the rhizosphere, where the concentration peaked with about 0.030 mg NO_3-N l^{-1} at 3.0-5.0 cm depth. Further down the concentration decreased again and was depleted in a depth of 7.5 cm. In the winter period all profiles extended deeper into the sediment (Fig. 5.f). All station had about the same concentration of about 0.150 mg NO_3-N l^{-1} in the sediment water interface. The bare mud concentration profile decreased fastest and depleted at a depth of 1.5 cm, where the concentration profile in the plant covered pools depleted at the depth of about 3 cm, while the bare sand concentration profile first depleted at about 7.5 cm depth.

Phosphorus adsorption

A non-linear regression model (SAS, Nline procedure, no weighting, DUD fitting procedure) the Langmuir adsorption isotherm was introduced and afterward given the measured values of Ce and SP as input data. The statistical model then predicted values of NAP, K and PSC inside the phosphate concentration range of 0.0 – 5.0 mg PO_4-P l^{-1}. The graph is shown in Figure 6. while the parameter values are presented in Table 1. The *Spartina* covered pool showed the highest PSC of 140 µg PO_4-P g cm^{-3} sediment and the lowest K of 400 mg PO_4-P l^{-1}. The bare muddy pools also had a high PSC of 80 µg PO_4-P g cm^{-3}, but a much higher K of 2000 400 µg PO_4-P l^{-1}. With respect to the sandy bare bottom pools they had the lowest PSC of 30 µg PO_4-P g cm^{-3} while the K was equal to that obtained for the muddy pools. The NAP value did not change essentially between the stations. By traditional statistics (SAS, ANOVA, T-test) the three types of pools differed significantly ($P< 0.01$).

Table 1. Phosphate adsorption characteristics at the three study sites.

	Spartina pools	Bare mud pools	Bare sand pools
NAP	0	5	10
PSC	140	80	30
K	400	2000	2003

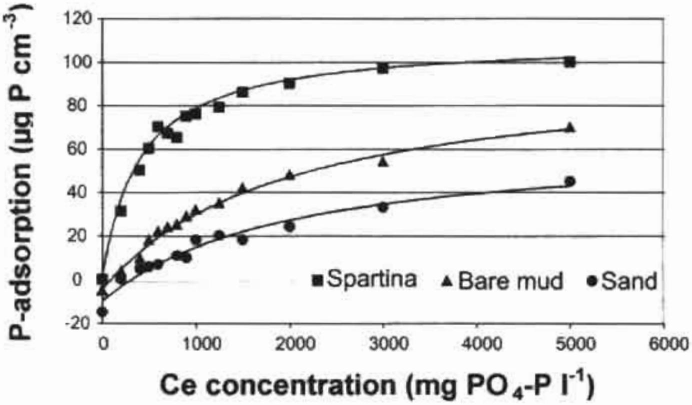


Fig. 6. Phosphate adsorption capacity at the different sites.

Discussion

The organic content of the different pools are results of local autothropic production, sedimentary processes and the internal nutrient turnover in each pool. The low nutrient content of the sandy pools may be explained by the fact that these pools are located near to the channel where the mass flow of the estuarine water is highest. The current velocities peak with values of about 2 m s^{-1} during spring tide periods (Martins et al. 2002). This means that only small amount of organic matter remains in the area, while the majority becomes resuspended and exported (Flindt et al. 1997, Salomonsen et al. 1997, Flindt et al. 1999, Salomensen 1999). The high nutrient content in the *Spartina* pools is the results of vice versa processes. The high plant biomass act as a sediment trap for both the local *Spartina maritima* production as wel as for external produced detritus and macroalgae (Pusceddu et al. 1999). The sediment nutrient content in the different pools showed the same tendency, with much higher nitrogen and phosphorus masses in the *Spartina* pools compared to the muddy and sandy pools. In all three pool types the nutrient content continuously descresed towards the depth. Nutrients did not decresed until a certain level and stayed constant at the last cm. This indicate that the sediment is reworked by biomixing and that the nutrient pools are bioavailable until 15 cm depth. This is also supported by visual observations where *Scrobicularia plana* and *Carcinus maenas* often bring organic matter like *Ulva* sp. and *Enteromorpha* sp. sheets down into the sediment.

The knowledge about macrophyte production is well established, but less is known about the fate of the production. Although, some birds are reported to graze on rooted macrophytes (Tubbs and Tubbs 1983), the grazing is seldom extensive in temperate areas (Mann 1975, Odum et al. 1967). Instead leafs are sloughed with aging or at the end of the growth season, or they are broken or dislodged during periods with violent wave and wind motion. Thus, most of the production enters detrital food webs. Therefore a mineralisation study was initiated to obtain information about the quality of the organic matter in the sediment. Not much qualified information can be harvested from total measurements of nutrients in the sediment if the lability of the particulate organic matter is not quantified (Harrison 1989, Lillebø et al. 1999). Evaluating the particulate organic nitrogen (PON) mineralization in the different pool types, it becomes evident that besides the lowest organic content at the sandy pool, the PON content is also lesser degradable than at the other sites. Together this gives the low porewater concentration of ammonia measured in the present study, which also results in lower flux rates between the sediment porewater and the water column in the sandy pools (Lillebø et al. 2002b). A higher labile fraction of PON was found at both the muddy pool and the *Spartina* pool with the fastest mineralisation going on in the *Spartina* pool that also had the highest organic content. Altogether this gives more than double PON turnover in the *Spartina* pools compared with the muddy bare bottom pools.

Several experiments have been made on the decomposition of *Zostera marina* and they agree on very low mineralization rates (Mann 1972, Mann 1975, Harrison and Mann 1975, Fenchel 1977, Mann 1982, Harrison 1989, Enriquez et al. 1993, Flindt et al. 1998), which ranges between 0.002 and 0.007 d^{-1}. In comparison, the particulate organic nitrogen from the *Spartina* pools are decomposed much faster than these averages from *Zostera marina* degradation studies, and within the same temporal range as newly sloughed leaves from *Spartina maritima* (Lillebø et al. 1999). This indicates that most of the particulate matter in the *Spartina* pools are based on local production. Although no experiment were performed on the particulate organic phosphorus fraction, it is well-known that phosphorus mineralization is even faster than the nitrogen mineralisation, while the carbon decomposes slowest of the elements (Flindt et al. 1998).

Evaluating the nutrient porewater concentration of the different pools it is evident that the significant differences exist among them. The pools located on the sand bottom are topographic placed lower than the other pools, which give these pools a mean residence time of only 3 hours per tidal cycle. The muddy pools and the *Spartina* pools are located further up so the mean residence time for these pools is about 6-8 hours. Alone, this fact has impact on the nutrient profiles and efflux from the different pools. Due to the differences in the nutrient turnover that was found in the mineralisation study it would have been expected that the lowest porewater concentration of nutrient would be measured in the sandy pools as we did. Further it was expected that the highest nutrient porewater concentrations would be measured in the *Spartina* sediment, which was not the case. The highest concentration of ammonia and phosphate was found in the muddy pools. This difference in concentration between the bare muddy pools and the Spartina covered pool may most reasonable be explained by plant uptake, indicating that *Spartina maritima* affects the efflux rates of dissolved inorganic nitrogen. Comparing the measured porewater concentration from the *Spartina* pools and the muddy pools from the Mondego estuary with nutrient porewater profiles from other shallow estuaries, the ammonia concentrations are within the same range as measured in the eutrofied broad of Roskilde Fjord, Denmark (Kamp-Nielsen & Flindt 1993, Flindt 1994, Flindt & Kamp-Nielsen 1998). The phosphate concentrations in the Spartina and the muddy pools are much higher than these found in the Roskilde Fjord indicating that the locations in the Mondego estuary have a higher specific phosphorus loading and a higher P-turnover than the Danish Roskilde Fjord.

Due to the adsorption isotherm the *Spartina* rhizosphere had the highest adsorption capacity with about 140 µg PO_4-P cm^{-3} sediment, while the bare muddy area in the same depth had a capacity of 80 µg PO_4-P cm^{-3} sediment. The sandy sediment showed a capacity of only 30 µg PO_4-P g^{-1} cm^{-3} sediment. No essential differences existed with respect to NAP between the three sediment types. The major difference in adsorption capacity between the muddy and the *Spartina* covered pools were due to differences in half saturation level, where the *Spartina* sediment showed much lesser K values than the bare muddy sediment. This may be explained by differences in sediment iron content giving the *Spartina* pool a higher adsorption capacity at lower phosphate concentration than these in the muddy pool. The result is that the realised adsorption inside the actual phosphate concentration range of the upper sediment becomes about 3-4 times higher in the *Spartina* pools than in the bare muddy pools. This fact together with an enhanced phosphate uptake due to plant growth is most properly the reason for the measured difference in the phosphate porewater profiles between the muddy pools and the *Spartina* covered pools. In the summer period, the *Spartina* phosphate profile are depressed in the rhizosphere compared to the muddy pools, while it is less expressed in the winter profiles.

Although we only found very small internal peaks of nitrate in the porewater profiles of the *Spartina* covered pool, it indicates that these plant are able to stimulate nitrification. This may only occur if oxygen is present, so most properly the plants realise oxygen or other oxidants from the roots to the porewater. This has also been found for *Zostera marina* (Flindt 1994), where the plants indirectly were able to stimulate the denitrification inside the plant covered areas. Comparing these to species potential for realising oxygen the root biomass per area of *Spartina maritima* is much higher than these inside *Zostera marina* beds (Lillebø 2000).

Altogether, this study indicates that *Spartina* also is able to control the nutrient dynamic in the intertidal sediment due to two processes: 1) *Spartina* takes up phosphate for growth purposes that without plant coverage would have returned to the watercolumn and 2) the plant does also facilitate increased phosphate adsorption.

Both process results in a closer coupling of the phosphorus cycle between the plant and the sediment to the benefit for the plant dynamic.

At the system level most pools are situated on bare bottom without connection to rooted vegetation, and these leaks with huge amounts of phosphate, so the intertidal pool system of the Mondego estuary altogether is exporting essential amounts of phosphorus in these years. Lillebø et al. (2002c) have tried to evaluate the internal loading from the different pools over a full discharge year.

The next of this suite of papers concerning the nutrient dynamics in the Mondego estuary will 1) compare the nutrient fluxes and dynamics in the different pools (Lillebø et al. 2002b), 2) demonstrate how macroalgae growt potential are affected by the nutrient fluxes in the different types of pools (Martins et al. 2002) and 3) discuss and perspect the role of the nutrient dynamics in the different pool types on the system level for the Mondego Estuary (Lillebø et al. 2002c).

Acknowledgements

The present work was supported by the WET-project, (Wetland Ecology and Technology), funded by the European Scientific TMR program (TMR) and the F-ECTS project founded by the European Scientific MAST 3 program inside the ELOISE frame. The authors would also like to thank the freshwater Biological Laboratory, University of Copenhagen, for the analytical support.

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NUTRIENT DYNAMICS IN THE INTERTIDAL POOLS OF THE MONDEGO ESTUARY.
II – SEASONAL EFFLUX OF PO_4-P AND NH_4-N IN BARE BOTTOM AND VEGETATED POOLS

Abstract

In intertidal estuaries, such as the Mondego estuary, not much is known about the importance of the low water intertidal pools, with regard to sediment/water exchanges of ammonia and phosphate, as a result of anabolic/catabolic balances. Therefore, the main topic of this work was a comparative study of the role and the importance of the nutrient efflux in intertidal bare bottom (sandy and muddy) and in *Spartina maritima* covered pools, during day and night periods. Seasonal variation and daily variations were studied in July and November 1997.

Although, the availability of nutrients is reflected by the organic content in sediments, *S. maritima* dynamics clearly influence ammonia and phosphate efflux rates, especially in summer and during the day. Considering the fact that sediments constitute a major source of nutrients for rooted macrophytes, during the day there might be a certain competition between two processes: *Spartina maritima* facilitated adsorption capacity and the efflux from the sediment.

Ammonia and phosphate fluxes were higher in bare bottom muddy pools, followed by *Spartina maritima* covered pool and sandy bare bottom pools. It was also possible to observe the importance of plant dynamics. Ammonia efflux rates where higher during fall, due to *Spartina* was less productive, while the *Spartina* kept the efflux low during summer (flowering season). On the system level, the results express the importance of intertidal low water pools to the internal nitrogen loading, particularly in the bare bottom muddy pools.

This study also shows that in July, high temperatures and anoxic conditions, especially during the night, promoted the phosphate efflux to the overlaying water column. Moreover, *S. maritima* also controlled phosphate efflux from the sediment during the day, but in absence of enough light for primary production to take place, during the night, phosphate efflux increases, especially in July. Although in a seasonal scale, the daily mean

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(day/night) efflux rates were similar. On the system level, results also evidences the importance of these intertidal pools to the internal dissolved inorganic phosphate loading, especially the muddy bare bottom pools, which showed a clear seasonal variation.

Introduction

Many studies have focused on the importance of freshwater runoff from land and the exchange of water with adjacent open sea regarding biogeochemical processes in estuaries (e.g. Valiela and Teal 1979, Hinga 1990, Staver et al. 1996, Pardal 1998, Flind et al. 1999).

It is know, that in estuaries vertical distribution and relative abundance of inorganic nitrogen compounds (NH_4^+, NO_2^-, and NO_3^-) in the sediment are controlled by the redox state (Valiela 1995). In anaerobic sediments ammonium is far more abundant and it has been shown that ammonium diffuses upward to the overlying water (Valiela 1995). Rocha (1998) described a mechanism where, in warm climates intertidal estuaries and during the day, temperature gradient between the exposed sediment and water at the time of flooding causes a free convection of ammonia to the water column. On the other hand, if the water is warmer than the sediment surface, which is the intertidal pool situation, ammonia efflux only occurs by diffusion (Rocha 1998).

The movement of particulate organic matter transports most of the phosphorous to the sediment. In an intertidal system, sediments get exposed to air and subsequent drying conditions during ebb tide, and it has been show (Baldwin 1996 *in* Mitchell and Baldwin 1998) that under this desiccated/oxidation conditions, sediments have a substantially higher phosphate sorption capacity than submerged sediments. Nevertheless, in the intertidal pools sediments are always submersed, and may represent 30-40% of the system area (Flindt et al. 2002, Lillebø et al. 2002). Phosphorus dynamics is also influenced by sediment resuspension and macrophyte coverage (Flindt and Kamp-Nilsen 1998) and bacteria might directly or indirectly mediate phosphorous release from sediments (Mitchell and Baldwin 1998). On the other hand, temperature seems to be of crucial importance to the efflux of phosphorus from the sediment (e.g. Asmus et al. 2000), with a resulting increased of phosphorus internal loading (e.g. Perttilä et al 1995, Valiela 1995, Pardal 1998, Sfriso 1988).

The importance of benthic mineralisation to the trophic chain increase as the water column gets shallower (e.g. Nixon and Pilson 1983 *in* Rocha 1998), and particularly in these ephemeral pools system, physic and chemical conditions change in few hours, reaching high water temperature and low oxygen concentrations, especially during summer night ebbs tides. Moreover, submersed rooted macrophytes link the nutrients in sediments with the overlying water, which may have important implications for nutrient cycling within such systems (e.g. Valiela 1995, Kamp-Nilsen and Flindt 1993, Flindt et al. 1999). But the relative contribution from the sediment pore-water and the overlying water column to N /P supply is not very well documented (Flindt et al. 1999). Therefore, the main topic of this work was a comparative study of the seasonal nutrient efflux in intertidal bare bottom (sandy and muddy) and macrophyte (*Spartina maritima*) covered pools during 24-hours tidal cycles.

Material and methods

Field procedure

In the present study *Spartina maritima* salt marsh area was chosen for being the most representative regarding vegetated tidal pools in the Mondego estuary (Marques et al. 2002, Pardal 1998, Lillebø et al. 1999) (Fig 1). A tidal pool in this area was selected to represent an average pool size in respect to surface area and depth. Simultaneously, the same study was performed in two bare bottom areas characterised respectively by muddy and sandy sediments. Pools were studied during 24-hours tidal cycles in July 97 and November 97, representing respectively summer and fall situations. From the pool formation until the pool was submerged again, water temperature, salinity and dissolved oxygen were measured, and water samples (10 ml each) were taken for dissolved reactive phosphate and ammonia analysis. The sampling frequency was one hour. All the water samples were filtered in the field through a Whatman GF/F glass-fibber filter and stored in ice. In the laboratory they were kept frozen at – 18 °C until analysis. Each pool was measured for surface area and depth at each sampling date. Flux chambers were placed in the main channel (under day and night situations, July 2001) for comparison of the efflux rates in the low water pools.

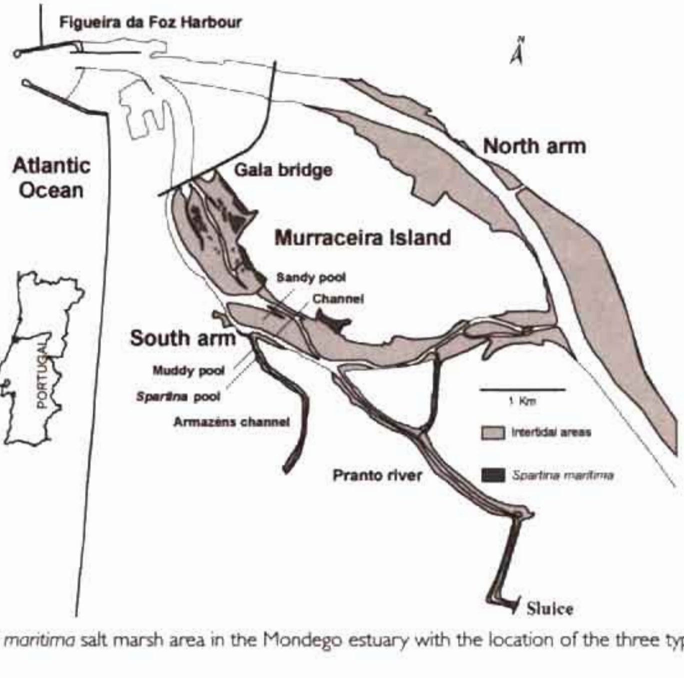

Fig 1. *Spartina maritima* salt marsh area in the Mondego estuary with the location of the three types of tidal pools.

Analytical procedure

Analysis of dissolved reactive phosphate and ammonia were carried out using a rapid flow autoanalyser (RFA 300 Alpkem) and performed according to Alpkem methodologies (Alpkem, 1990).

Calculations

Phosphate, ammonia and oxygen fluxes were calculated taking into account the initial concentrations in the pool, which corresponded to the very first measurement just after the formation of the tidal pool (t_0). So, for each consecutive sample (t_1, t_2...t_n) the calculated rates were given by the difference between concentration in time (t_n) and the initial concentration, an then divided by the number of hours between the two samples.

$$\text{Rate} = \left[\frac{Cf - Ci}{tn - to}\right]$$

All data were previously standardised (µmol l^{-1} m^{-2}) to avoid bias caused by differences in the pools size. For the same reason, all data were also standardised (mg m^{-2}) to calculate the total amount of nutrients per hour.

Calculations of the mean efflux rates (mg. m^{-2} d^{-1}) were performed considering: a) the mean daily efflux rates (day and night) from each type of pool (Lillebø et al. 2002); b) a visual estimation of the contribution of each type of pool to the estuarine area (1.9 km^2: 17% *Spartina*, 50% mud and 33% sand); c) a visual estimation of the area covered by pools during low tide (40%); d) the mean efflux rates in July could represent a mean value for spring and summer, and that the mean efflux rates in November could represent a mean value for fall and winter. In the main channel it was assumed that there is no significant seasonal variation.

It is known that nutrient fluxes are strongly dependent on temperature dynamics, and several studies have demonstrated this promoting effect of rising temperatures (e.g. Nixon et al. 1976, Fisher et al. 1982 *all in* Asmus et al. 2000). As a preliminary approach, we therefore used a simple dynamic temperature model to simulate the yearly variation in the ammonia fluxes. The model equations are:

$$NH_4\_flux_{actual} = NH_4\_fluxt_n{}^{*}K(n\text{-}temp_{actual})$$

$$\text{Accumulated } NH_4\_flux = NH_4\_flux_{t+1} + NH_4\_flux_t$$

Where the $NH_4\_flux_n$ is the measured phosphate flux at a certain temperature (n°C), K is temperature dependency constant, while $temp_{actual}$ is the simulated ammonia fluxes. K varied between 1.02-1.12 indicating that some pools are more temperature dependent than others. The only forcing function was the temperature. Afterward the simulated fluxes becomes numerical integrated so the yearly internal loading, due to the flux dynamics, for the pools becomes simulated.

Calculations of the ammonia mass efflux (kg N) were also performed assuming that the mean efflux rates in July could represent a mean value for spring and summer, and that the mean efflux rates in November could represent a mean value for fall and winter. In the main channel it was assumed that there is no significant seasonal variation.

Results

During the life span of the pools temperature increased dramatically during the day (Fig. 2) and, as we should expect, mean water temperature increased seasonally, reaching a maximum value of 32 °C, in July and a minimum of 10 °C in November. Oxygen concentration decreased during the night, (always less than 5 mg O_2 l^{-1}), when primary production was unable to compensate for heterotrophic oxygen consumption. This situation was more accentuated in July when temperature was about to 20 °C, (1 mg O_2 l^{-1}). So, pools become anoxic, for short periods. Salinity varied seasonally as a function of the freshwater discharges from the Pranto River (Fig. 1) (Marques et al. 2002). The lowest salinities were recorded in November, always less than 5 and the highest in July, reaching 36.

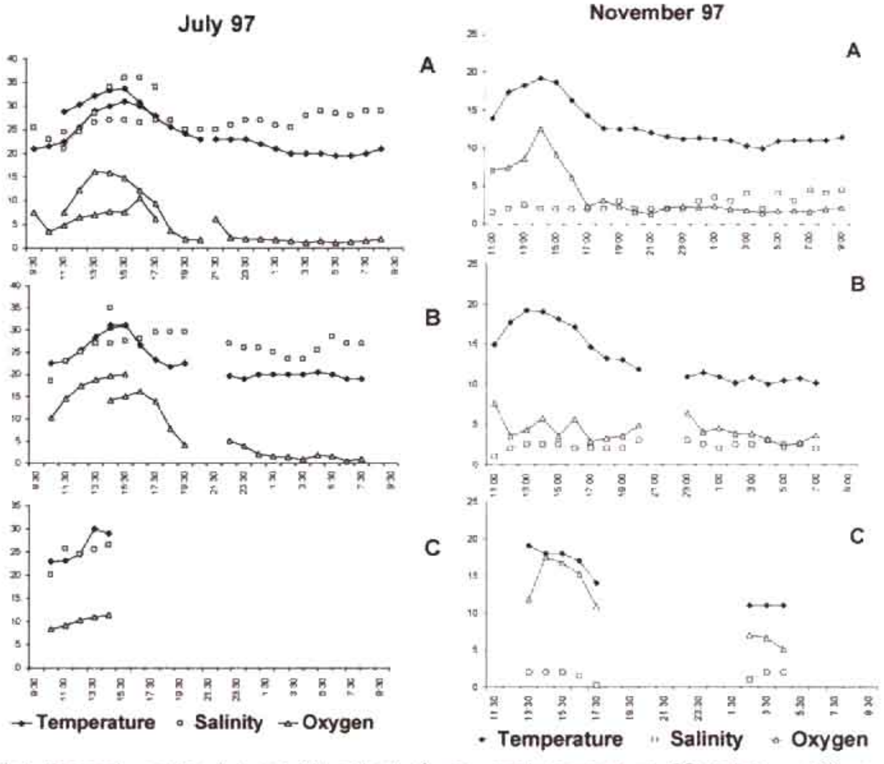

Fig 2. Seasonal variation (July and November) of water pools temperature (°C), Salinity and Oxygen (mg O_2 l^{-1}): a) *Spartina maritima* covered pool. b) Muddy bare bottom pool. c) Sandy bare bottom pool.

Ammonia dynamics

Seasonal differences were observed concerning the initial total amount of ammonium in the pools (Fig. 3). In July 97 the initial values varied between 8 and 12 mg m^{-2} (579 and 724 μmol m^{-2}) in the *Spartina* covered pool, 8 and 12 mg m^{-2} (547 and 742 μmol m^{-2}) in the muddy bare bottom pool and 0.7 mg m^{-2} (49 μmol m^{-2}) in the sandy bare bottom pool (Fig 3). In November, we quantified 46 mg m^{-2} (3275 μmol m^{-2}) in the *Spartina* covered pool, 14 and 21 mg m^{-2} (1009 and 1513 μmol m^{-2}) in the muddy bare bottom pool, and 8 and 11 mg m^{-2} (566 and 812 μmol m^{-2}) in the sandy bare bottom pool (fig 3). The total amount of ammonia was therefore much higher in November. Moreover, the total amount of ammonia in *Spartina* covered pool was much more dynamic in November, which may be a reflection of plants annual dynamics.

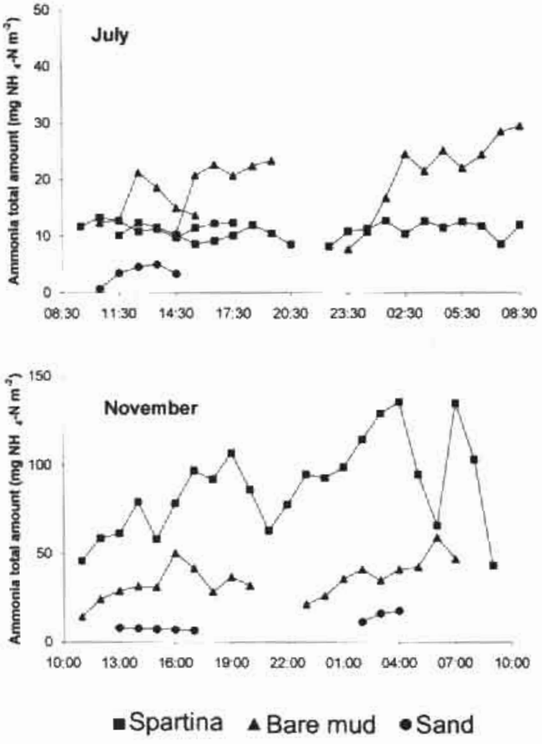

Fig 3 Seasonal variation of ammonia total amount (mg NH_4-N m_{-2}) during the life span of the low water pools.

Ammonia formation is close related to mineralisation, and therefore plotted against oxygen consumption.

Results show that in all three pools, higher ammonia efflux rates occurred during the night period when heterotrophic respiration was not compensated by primary production (Fig 4). Considering separately each type of pool, results showed that the

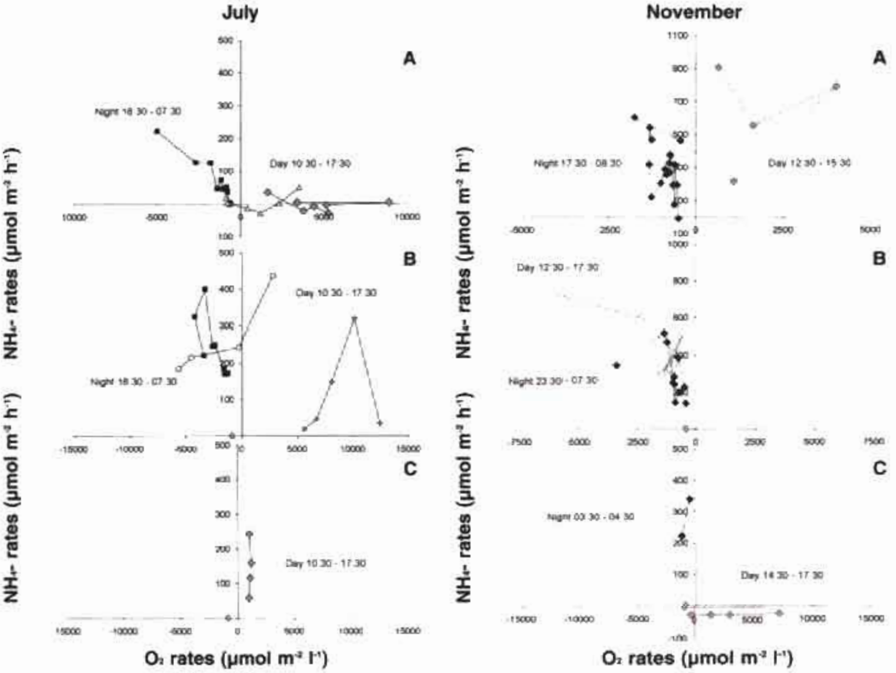

Fig 4. Seasonal variation of ammonia efflux and uptake rates vs. oxygen efflux and uptake rates; a) *Spartina maritima* covered pool, b) Muddy bare bottom pool, c) Sandy bare bottom pool.

efflux rates of ammonia in the *Spartina maritima* covered pool was very dependent on plant dynamics. In July there was ammonia influx during the day and an efflux during the night (Fig 4.1A). Furthermore, although oxygen depletion during night was higher in July, ammonia efflux was much higher in November and occurred even during the day (Fig 4.2A). In the muddy bare bottom pool, there were always effluxes of ammonia (day and night), and oxygen concentration decreased along the life span of the pool during day and night periods (Fig 4.1B, 4.2B). Oxygen depletion was similar in July and November. In the sandy bare bottom pool, although, efflux rates during the day were lower in November, it also seemed to increase during night (Fig 4.1C, 4.2C).

Considering the mean daily efflux rates (day and night) from each type of pool there was a clear seasonal variation of the efflux rates, with higher N-effluxes during fall and lower during summer (Fig. 5). This seasonal variation is particularly clear in the *Spartina* covered pool. The efflux rates measured in the main channel are comparatively very low (Fig. 5).

As a preliminary approach, we simulate the internal loading of ammonia based on a temperature-dependent model (Fig. 6). Despite cautions in interpreting this simulation, due to the lack of points for the calibration of the model, it express a decrease of ammonia efflux during the warmer periods (spring and summer) and an increase during fall and winter, especially in the *Spartina* covered pools.

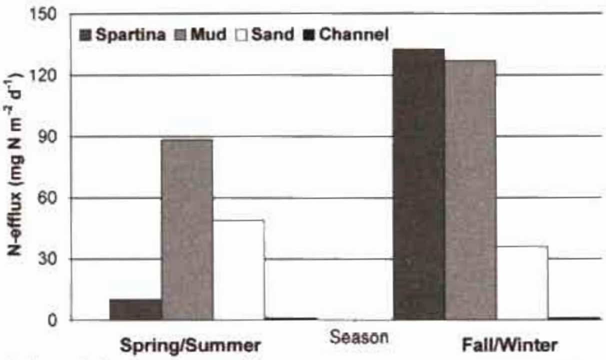

Fig 5. Seasonal variation of phosphate mean efflux rates (mg NH_4-N m^{-2} h^{-1}) at each pool type and the main channel.

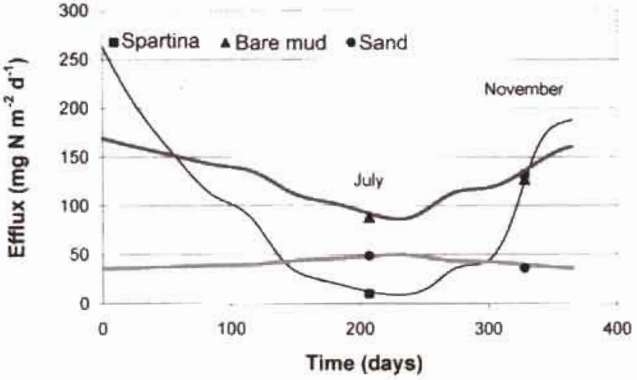

Fig 6. Simulation of the yearly variation in ammonia fluxes, in pools located in the bare bottom muddy areas, sandy areas and in *Spartina maritima* covered areas of the estuary.

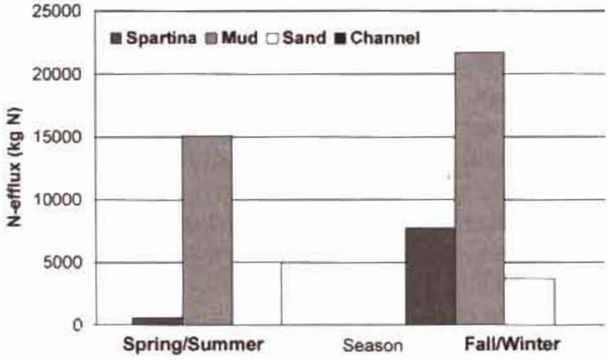

Fig 7. Yearly net efflux of ammonia (kg N) for each type of pool and the main channel.

Calculations of the ammonia net effluxes (kg N), based on the simulated values, suggest a strong contribution of the bare bottom muddy pool to the whole system internal ammonia loading (Fig 7), and especially during fall and winter. The *Spartina* covered pool and the sandy covered pool had a similar yearly contribution to the system internal ammonia loading, but higher than the channel (Fig. 7). Although, sandy pools did not show a clear seasonal variation.

Phosphate dynamics

The initial total amount of phosphate in the pools decreased from July to November in *Spartina* covered pool and in the bare bottom muddy pool, but increased slightly in the bare bottom sandy pool (Fig. 8). In July 97, the initial values varied between 2 and 3 mg m^{-2} (115 and 126 µmol m^{-2}) in the *Spartina maritima* covered pool, 1 and 4 mg m^{-2} (52 and 310 µmol m^{-2}) in the muddy bare bottom pool and 0.8 mg m^{-2} (55 µmol m^{-2}) in the sandy bare bottom pool (Fig 8). In November, we quantified 4 mg m^{-2} (268 µmol m^{-2}) in the *Spartina maritima* covered pool, 1 mg m^{-2} (75 µmol m^{-2}) in the muddy bare bottom pool, and 1 and 1.5 mg m^{-2} (85 and 107 µmol m^{-2}) in the sandy bare bottom pool (fig 8). The phosphate total amount was therefore higher in July. Moreover, the total amount of phosphate in *Spartina* covered pool was much more dynamic in November, which may also reflect the plants annual dynamics.

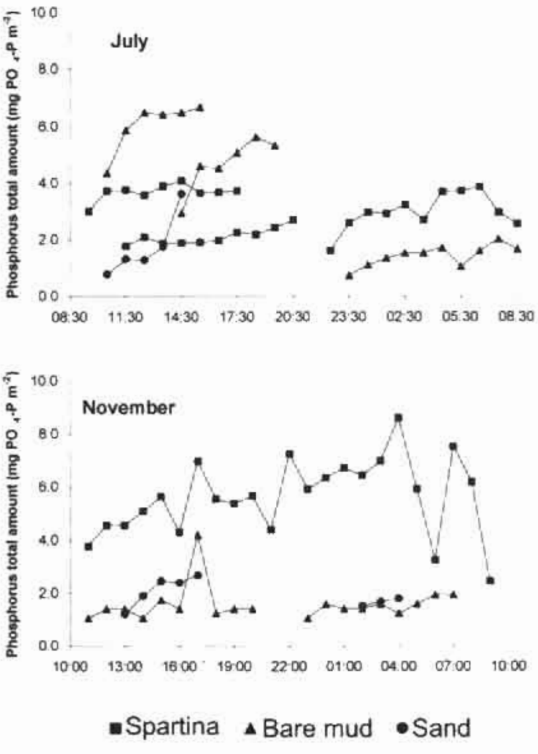

Fig 8. Seasonal variation of phosphorus total amount (mg PO_4-P m^{-2}) during the life span of the low water pools.

The comparison of phosphate and oxygen efflux rates (µmol m^{-2} h^{-1}) in July and November shows that for the same type of pool, the process varies seasonally (Fig. 9), with lower efflux rates in November. Efflux rates were always higher within the first hour after pool formation. Considering each type of pool, *Spartina* covered pool phosphate dynamics seems much dependent on plant dynamics. In fact efflux rates are much lower during the day and increase during the night, in July (Fig. 9.1A). On the other hand, in November, although efflux rates are lower (Fig. 9.2A), higher total amount of phosphate occurred during the day and under oxic conditions (Fig. 8). In the muddy bare bottom pool there was always efflux of phosphate (Fig. 9.1B, 9.2B). In the sandy bare bottom pools, values found for the phosphate efflux were lower than in the other two types of pools, but showing the same tendency as in the bare bottom muddy pool (Fig. 9.1C, 9.2C).

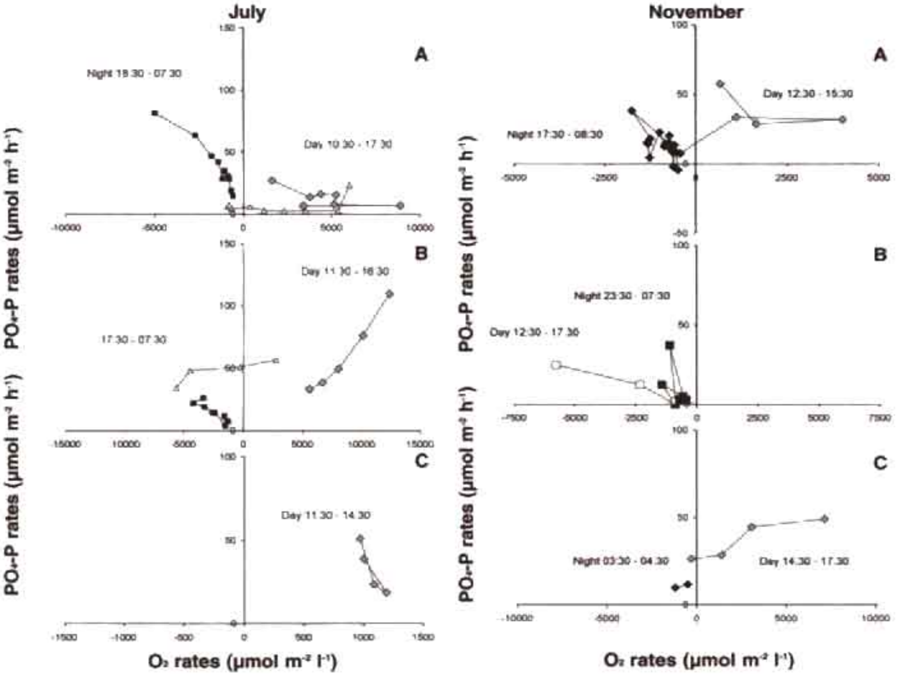

Fig 9. Seasonal variation of phosphate efflux and uptake rates vs. oxygen efflux and uptake rates: a) *Spartina maritima* covered pool, b) Muddy bare bottom pool, c) Sandy bare bottom pool.

Considering the mean daily efflux rates (day and night) from the muddy bare bottom pool there was a clear seasonal variation of the efflux rates, with higher P-effluxes during Spring/Summer and lower during Fall/Winter (Fig. 10). This seasonal variation is not visible in the *Spartina* covered pool, or in the sandy pools. The efflux rates from the main channel are comparatively low (Fig. 10).

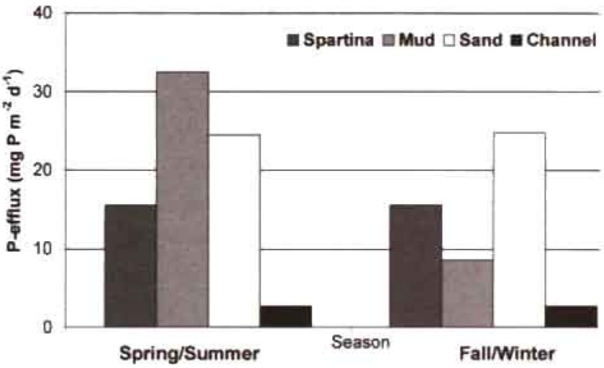

Fig 10. Seasonal variation of phosphate mean efflux rates (mg PO_4-P m^{-2} d^{-1}) at each pool type and the main channel.

Discussion

Ammonia dynamics

Results clearly show the influence of plant coverage, especially with higher temperatures and during the day. In fact, rooted macrophytes keep nutrients cycling close coupled to the rhizosphere, and it is known that rooted vegetation potentially enhances denitrification (Valiela 1995, Flindt et al. 1999). Moreover, ammonia can be removed from water by plants uptake, algae and bacteria, which may also explain the depressed fluxes, meaning uptake of ammonia from the water column in the *Spartina maritima* covered pool. Therefore, we might expect a different nutrient dynamics between *Spartina maritima* covered intertidal pools and bare bottom pools (muddy and sandy sediments). Furthermore, the organic content in sediments also reflects the availability of nutrients, and results from a previous study (Flindt et al. 2002) showed that in *S. maritima* covered pools the organic content was about 8 % in the sediment upper layer, decreasing to about 5% at 12.5 cm's depth. Comparatively, muddy sediments presented an organic content of about 5 % in the upper layer, decreasing to about 2 % while sandy bare bottom sediments exhibited the lowest organic content with approximately 0.95 % without any variations as a function of depth. All these facts may explain the higher effluxes of ammonia in bare bottom muddy pools, followed by *Spartina maritima* covered pools and sandy bare bottom pools. Comparatively with a study performed in the River Colne estuary, efflux rates of ammonia are in the same range (µmol NH_4 -N m^{-2} h^{-1}) respectively for sandy sediments and muddy sediments, probably as a function of organic matter content and higher ammonification rates in the sediment (Ogilvie et al. 1997). On the other hand, if we consider a day/night cycle, ammonia efflux rates are higher during the night, which is in agreement with an experimental study performed by Kristensen (1993). Furthermore, anoxic conditions occurred during the night, especially in July, so it may also be responsible for the higher ammonia efflux (Pettine 1983). On a seasonal scale, ammonia effluxes were always higher during winter in the muddy sediment pools (bare bottom and *Spartina* covered). These results are in agreement with results from Ogilvie and others (1997), which could just find this seasonal variation in a muddy sediment site with higher organic matter content, although Kristensen (1993) could not find a significant seasonal pattern. In the sandy sediment there was no clear seasonal variation in ammonia efflux, which is in agreement with results from Ria Formosa (Portugal), (Falcão and Vale, 1998), were the NH_4^+ flux in sandy sediments was production-rate controlled, and the flushing from intertidal sediments was similar in February in June.

Calculations of the ammonia net effluxes (kg N), express not only the N-efflux seasonal variation in *Spartina* and muddy pools, but evidences the importance of intertidal low water pools to the internal nitrogen loading, as well the clear contribution of the bare bottom covered pools to the whole system internal nitrogen loading.

Most probably differences in ammonia fluxes between the muddy bare bottom pool and the *Spartina* pool is due to plant associated process. Based on this difference in the temporally integrated ammonia mass, we were able to calculate the plant production in nitrogen units. Taking into account, field data from Lillebø (2000) of aboveground mean C/N-ratio (35.5) for the *Spartina maritima*, we calculated the local plant production to a mean yearly net primary production of 503 g C m^{-2} y^{-1}, which corresponds to a mean aboveground biomass of 1200 g m^{-2}. This result is in agreement with literature values for *Spartina maritima* production: 670 g C m^{-2} y^{-1} for 200-2500 g m^{-2} aboveground biomass, in the Tagus estuary (Catarino 1981), and 296 g C m^{-2} y^{-1} for a mean aboveground biomass of 628 g m^{-2}, in Bay of Biscay (Benito and Onaindia 1991).

Phosphate dynamics

In the Mondego estuary, the highest phosphate effluxes occurred in summer, which is in agreement with previous studies (Sfriso 1988, Valiela 1995, Falcão and Vale 1998, Pardal 1998, Asmus et al. 2000) showing that phosphate regeneration, into the dissolved form is dependent on temperature and redox-state of the sediment. This is also evident if we compare day and night ebb tides. These effluxes are more evident in muddy bare bottom pools, followed by *Spartina maritima* covered pools, and by the sandy bare bottom pools. In July 97 *Spartina maritima* covered pool was the only one where night efflux is greater than day. This is most probably related to sediment properties such as, sediment organic content, sediment pore-water profiles of phosphate, and phosphorus adsorption capacity of the sediment (Valiela 1995, Flindt et al. 1999, Flindt et al. 2002, Martins et al. 2002). Although, as described before, the organic matter content in the sediment was higher in *Spartina maritima* covered pools, muddy *Spartina* rhizosphere had also the highest adsorption capacity, 4.5 µmol PO_4-P g^{-1} dwt. sediment. While the bare muddy area in the same sediment depth had a capacity of 2.6 µmol PO_4-P g^{-1} dwt. sediment and the sandy sediment showed a capacity of only 0.97 µmol PO_4-P g^{-1} dwt. sediment. Furthermore, several authors (Carignan and Kalff 1980, Smart and Barko 1985, Chambers et al. 1989) have shown that rooted submersed macrophytes are able to uptake almost all needed phosphorus from the sediments. So, most probably, during the night ebb, when primary production does not occur and oxygen concentration in the pool decreases to anoxic levels, phosphate efflux takes place. In fact, other studies have focussed this close relation between phosphorous and oxygen concentration suggesting that under anoxic conditions sediments may turn from sinks to sources of phosphorous (Sfriso 1988, Perttilä et al. 1995).

Considering the fact that sediments constitute a major source of phosphorous supply for submersed macrophytes, during the day there might be a certain competition between two processes: *Spartina maritima* facilitated adsorption capacity and the efflux from the sediment, expressing the efficiency of the plants in oxidizing reduced compounds of the sediment (Caçador and Vale 2000). On the other hand this situation did not occur in November, probably as a result of decrease temperature and decrease plant activity. So, mean efflux rates were closer among the muddy pools at this time of the year. The efflux rates in the sandy pool were in the same range as recorded by Asmus et al. (2000) for Ria Formosa lagoon (Portugal) sandy sediments (63 – 8.8 μmol m^{-2} h^{-1}), with less than 1m water depths and similar temperature range. The efflux rates in the muddy bare bottom pool were lower but in the same range as recorded by Forja et al. (1994 *in* Asmus et al. 2000) for muddy sediments in the Bay of Cadiz (Spain) (20.8 – 379.2 μmol m^{-2} h^{-1}).

This study shows that macrophytes such as *Spartina maritima* controls phosphate efflux from the sediment, but in absence of enough light for primary production to take place, during night, high temperatures and anoxic conditions promote phosphate efflux to the overlaying water column. On a seasonal scale, results suggest that phosphate efflux is temperature dependent and that in *Spartina maritima* covered pools it is also dependent on plant dynamics. Moreover, the highest mean phosphate effluxes occurred in summer with a clear contribution of the bare bottom muddy areas, which may be explained by the organic matter content, and the P-sorption capacity of the plants (Flindt et al. 2002).

Taking into account these results, we hypothesised that the efflux of phosphorus in these intertidal pools could constitute an important contribution to the system internal loading of phosphorus (See Lillebø et al. 2002).

Acknowledgements

The present work was supported by the Programme MAST II (MAS 2 CT 92 0036), by the WET-project, (Wetland Ecology and Technology), funded by the European scientific TMR program, (TMR) (ERB 4061 PL 95-0832), by the F-ECTS project founded by the European Scientific MAST 3 program inside the ELOISE frame, and by the Praxis XXI program (Portugal) through a PhD grant (BD/ 9290/ 96). The authors would like to thank the Freshwater Biological Laboratory, University of Copenhagen, Denmark, for the support to conduct this study.

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NUTRIENT DYNAMICS IN THE INTERTIDAL POOLS OF THE MONDEGO ESTUARY.

III – THE IMPORTANCE OF NUTRIENT EFFLUXES TO MACROALGAL GROWTH (*Enteromorpha* sp.)

Abstract

The aim of the present work was to evaluate the importance of nutrient fluxes across the sediment-water interface to the growth of opportunistic macroalgae (*Enteromorpha* sp.) within tidal pools of an intertidal estuary. We hypothesised that different tidal pools, in relation with organic matter content in the sediment and the presence/absence of rooted-macrophytes, promote distinct growth responses of *Enteromorpha* sp. Nutrient dynamics (PO_4-P, NH_4-N and NO_3-N) and *Enteromorpha intestinalis* growth were assessed during low-tide period in 4 different places: a sandy bare bottom pool, muddy pools (a bare bottom pool and a *Spartina maritima* covered pool) and the low-depth subtidal channel. Macroalgal growth in the subtidal area was estimated to compare with results from the tidal pools. Results showed that the growth rate of *E. intestinalis* was higher in the muddy bare bottom pool and statistically different from growth in the other places. No significant differences were found between macroalgal growth in the *S. maritima* covered pool, the sandy pool and the channel. The differences are partially explained by different nutrient dynamics between the muddy bare bottom pool and the other places. The availability of NH_4-N was higher in the muddy bare bottom pool, followed by the *S. maritima* covered pool, the channel and the sandy bare bottom pool. N:P atomic ratios of the water suggested that, with the exception of the muddy bare bottom pool, all the other places showed a potential N-limitation for primary production. Results also suggested that *Spartina maritima* affect the nutrient dynamics in tidal pools. However in this type of pools, the growth of *E. intestinalis* may also be affected by shading caused by the rooted-macrophyte presence. In the channel, other factors (e.g. hydrodynamics) affect macroalgal growth, which determines differences in relation with tidal pools. At the system level, these results suggest that in terms of nutrient availability, the occurrence of macroalgal blooms may be independent from external inputs.

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Introduction

Algal abundance in coastal eutrophic systems is largely related with physical factors such as exposure to wind, waves, water exchange (Morand and Briand 1996, Pihl et al. 1999) and topography (Hernández et al. 1997). However in intertidal shallow estuaries, where the coupling between benthic and pelagic processes is very significant (*in* Falcão and Vale 1990, Flindt et al. 1999), macroalgal growth may at a certain extent be determined by the exchange of nutrients across the sediment-water interface. For example, in Narragansett Bay (USA), regeneration from the sediments can provide enough phosphate to support 50 % of the primary production in the water column (*in* Valiela 1995), while in the archipelago of southern Fyn (Denmark), during spring (June), 20 % of inorganic nitrogen comes from the sediment (*in* Morand and Briand, 1996). In the same way, in the Venice Lagoon, the water-sediment nutrient fluxes were considered as a major factor controlling algal growth (Sfriso 1995).

The south arm of the Mondego estuary is a shallow intertidal system characterised by an extensive tidal pool formation during low-tide (Flindt et al. 2002). Intertidal pools differ in relation with organic matter content in the sediment, rooted-macrophytes presence and mean residence time of nutrients (Flindt et al. 2002). These characteristics may determine different nutrient availability in different intertidal pools (Flindt et al. 2002, Lillebø et al. 2002a). Throughout the last decade, opportunistic green macroalgae mostly *Enteromorpha* spp., were the dominant primary producers in the south arm of the Mondego estuary (Pardal 1998, Martins 2000, Pardal et al. 2000, Martins et al. 2001) and *E. intestinalis* (L.) Link and *E. compressa* (L.) Greville constituted the most abundant species (Martins 2000, Martins et al. 2001). During the spring of 1993, the biomass of green macroalgae reached a maximum value of 500 g AFDW.m^{-2} (Pardal et al. 2000). On a yearly basis, at the beginning of the growing season (late winter/early spring), new individuals of *Enteromorpha* proliferate in the muddy areas, while later in spring, larger macroalgal accumulations tend to occur in the inner sandy areas of the south arm of the estuary.

The aim of this work was to assess for the contribution of nutrient fluxes between sediment and water-interface to macroalgal growth in different intertidal pools of the Mondego estuary. Besides verifying the importance of benthic-pelagic interactions on ongoing processes in shallow intertidal systems, the present work may also contribute to understand the annual pattern of macroalgal coverage in the Mondego estuary.

Material and methods

Experimental procedure

The work was carried out on the 17th of July 1999 from 9am until 5pm (low-tide period) in the south arm of the Mondego estuary (Fig. 1). Three different experimental places located in the intertidal area were chosen in relation to sediment composition and the presence or absence of rooted-macrophytes (for a better characterisation see Flindt et al. 2001): a organic-poor sandy pool (0.95 %), a organic-rich unvegetated muddy pool (5 %) and a organic-rich *Spartina maritima* pool (8 %). For comparison with the tidal pools, a fourth experimental place located in the subtidal channel (average depth during low-tide 0.5-1 m) was chosen to assess for macroalgal growth in the subtidal areas where the nutrient dynamics is not so dependent on the sediment-water interface.

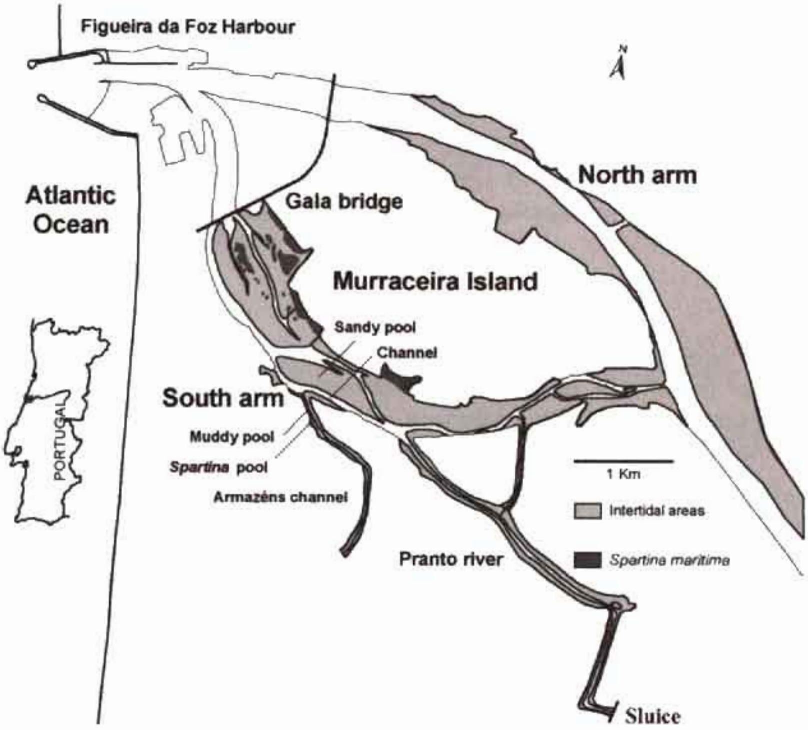

Figure 1. The Mondego estuary with location of the field stations (Picture by R. Lopes)

At that time of the year, *Enteromorpha* was not very abundant in the south arm of the estuary (personal observation) nevertheless, healthy individuals of *Enteromorpha intestinalis* were harvested and taken to the experimental areas inside cages containing estuarine water locally collected. All *E. intestinalis* individuals were cut to the same initial length (approximately) (Table 1) and they were placed inside the tidal pools in such a way that they could not shade themselves. For growth estimation in the channel, *Enteromorpha* replicates were placed inside experimental plexiglass cages surrounded laterally and in the bottom by plastic net with a mesh size of 2-3 mm, which allowed water circulation inside. Experimental cages were always kept immersed by ropes fixed to sticks buried in the sediment (for a more detailed description see Martins 2000).

Table 1. *Enteromorpha intestinalis* individuals used in the experiment: number of replicates (N), initial length of replicates (cm), exposure time of replicates (initial time-final time) and average specific growth rate (h^{-1}).

	Sandy pool	**Channel**	**Muddy pool**	**Spartina pool**
N	12	15	8	11
Initial length (cm)	20	20	23	20
Experimental hours (with macroalgae)	11.40am- 4.10pm	12.25am- 4.10pm	12.45am- 5pm	11.45am- 5.15pm
Average growth (h^{-1})	0.0193	0.0212	0.0329	0.0214

The initial and final length (cm) of all macroalgal replicates was registered and macroalgal growth was estimated by a mathematical deduced formula considering some assumptions. *Enteromorpha* thallus is considered as a cylinder with a wall one cell in thickness and its growth is two-dimensional (*in* Poole and Raven 1997). In order to infer growth rate in weight from length variation, *Enteromorpha* is considered as a volume which, increases proportionally in length (a) and width (b). No thickness (c) or density (γ) variations were considered and it was assumed that macroalgal growth in weight increases exponential by the formula (e.g. Brown and Rothery 1994):

$$W_t = W_0 \times e^{k_w t} \tag{1}$$

W_0 – initial weight, W_t – final weight, t – experimental time, k_w – specific growth rate (time unit^{-1}).

Density (γ) is defined as:

$$y = \frac{W}{V} \tag{2}$$

W-weight, V-volume

Equation 2 may be written in function of the weight (W), considering the volume of a cylinder ($V = \pi \times \frac{1}{2} a \times \frac{1}{2} b \times c$) and the assumptions made above:

$$W_t = y_t \times \pi \times \frac{1}{2} a_t \times \frac{1}{2} b_t \times c_t \tag{3}$$

γ_t- macroalgal density in time t (weight/volume) and the other factors are the volume of the thallus also at time (t). In the same way as W_t, an identical formula may be written for W_0.

Substituting in equation 1, we obtain:

$$y_t \times \pi \times \frac{1}{2} a_t \times \frac{1}{2} b_t \times c_t = y_0 \times \frac{1}{2} a_0 \times \frac{1}{2} b_0 \times c_0 \times e^{kw.t} \tag{4}$$

Assuming that

$$a_t = a_0 \times e^{ka\,t} \quad \text{and} \quad b_t = b_0 \times e^{kb.t} \tag{5}$$

and substituting in equation 4, we obtain

$$y_t \times \pi \times \frac{1}{2} a_0 \times e^{ka.t} \times \frac{1}{2} b_0 \times e^{kb.t} \times c_t = y_0 \times \frac{1}{2} a_0 \times \frac{1}{2} b_0 \times c_0 \times e^{kw.t} \tag{6}$$

Considering, as previously mentioned, that $\gamma_0 = \gamma_t$, $c_0 = c_t$, $k_a = k_b = k$, equation 6 may be simplified to:

$$2k = k_w \tag{7}$$

k_w– growth rate based on weight variation and k is the analogous rate for length variation.

From equation 7 it is possible to calculate macroalgal growth rate in weight from the growth rate in length (or width) variation.

In all experimental places, initial and final measurements of water temperature, salinity and dissolved oxygen were carried out and water samples (10 ml volume) were taken hourly for posterior quantification of dissolved reactive phosphate (PO_4-P), ammonia (NH_4-N) and nitrate (NO_3-N). All water samples were filtered in the field through Whatman GF/F filters and stored in ice. In the laboratory, samples were kept at −18°C until analysis in a rapid flow autoanalyser (RFA 300 Alpkem) (see also Flindt et al. 2002, Lillebø et al. 2002a). PFD was measured using a quantum sensor LICOR LI-1000.

Significant differences between macroalgal growth rates in different experimental places were assessed by one-single factor ANOVA with unequal n (Sokal and Rolf 1995) after tests of normality (Kolmorogov's test) and homogeneity (Bartlett's test) had been carried out. A multiple comparison test (Tukey's test) for unequal n was performed to discriminate for differences between samples (Zar 1999).

Results

Temperature, salinity, dissolved oxygen variation and PFD

Over the experimental period and in all experimental places, temperature didn't show important variations. As expected, dissolved oxygen increased from morning to the middle of the day and from 4 p.m. on, it started to decrease again (Figs. 2a and b).

The initial and final salinity measurements suggest that through the experimental period and in all places, salinity was high (ranging between 26 and 28) and showed no variations. Although it was summer, the weather was very cloudy and a maximal PFD of 400 μmol.ph.m^{-2}.s^{-1} was registered.

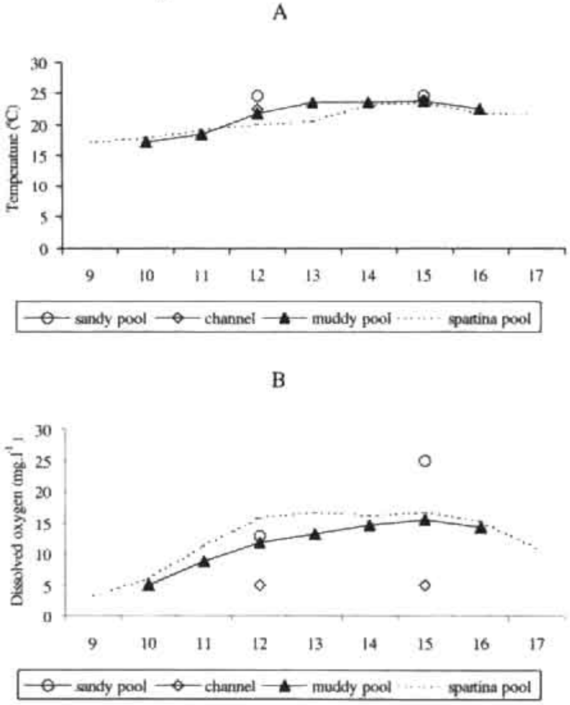

Figure 2. Variation of temperature (°C) (A) and dissolved oxygen (mg.l^{-1}) (B) throughout experimental period in the different places. In the channel and in the sandy pool, only initial and final measurements were done.

PO_4-P, NH_4-N and NO_3-N variation

In the *Spartina maritima* covered pool, PO_4-P concentration showed an important increase from 9 to 10 am but then it was kept more or less constant. Furthermore, throughout the experimental period, *S. maritima* pool showed the highest PO_4-P concentrations. Also in the muddy bare bottom pool, PO_4-P concentration didn't show important variations and the same tendency was observed in the sandy bare bottom pool, where PO_4-P concentrations were similar to the ones of the muddy bare bottom pool (Fig. 3a)

Initially, both the pattern of NH_4-N variation and the concentration values were very similar in the muddy bare bottom pool and in the *Spartina maritima* pool, but from 11 to 12 am this situation changed. While in the muddy bare bottom pool, NH_4-N concentration kept increasing gradually until the end of the experiment, in the *Spartina* pool, from 11 to 12 am, NH_4-N decreased significantly and then it didn't show important variations. In the sandy pool, NH_4-N concentration was very low comparatively to the other places and it decreased more significantly in the first experimental hour (Fig. 3b).

The concentration of NO_3-N in the channel was considerably higher than in the other places and it didn't show important variations. In the *Spartina* pool, NO_3-N concentration decreased exponentially from 9 to 11am. and then it decreased smoothly until the end of the experiment. In the muddy pool, NO_3-N concentration also showed a significant decrease, although more gradual than in the *Spartina* pool. The sandy pool didn't show important variations, although the final value was lower than the initial one (Fig. 3c).

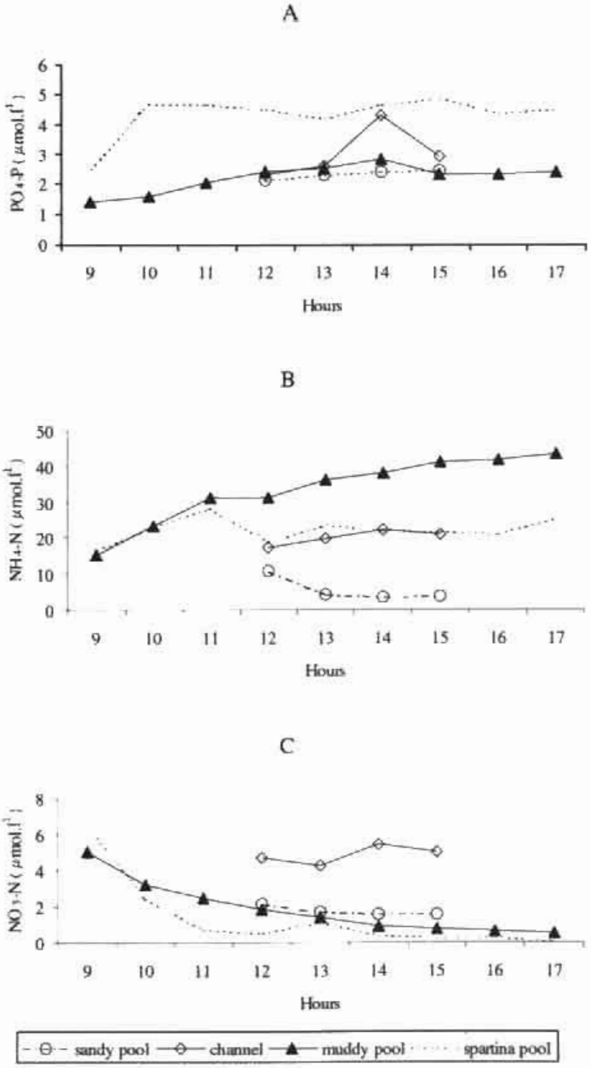

Figure 3. Nutrient dynamics throughout the study period in experimental areas. A - PO_4-P variation, B - NH_4-N variation. C - NO_3-N variation.

The muddy area showed higher N:P ratios of the water, close to the Redfield ratio, while the other places showed very low N:P ratios, especially, the sandy pool (Fig. 4).

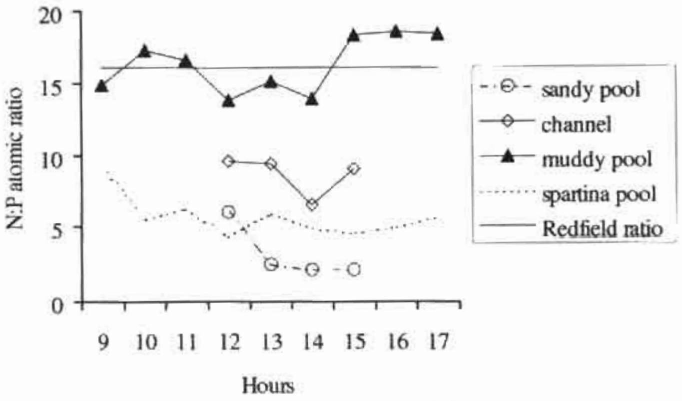

Figure 4. Variation of N:P atomic ratio of the water in all experimental places.

Macroalgal growth rate

The average *Enteromorpha* growth rate was higher in the muddy bare bottom pool (0.0329 h^{-1}), followed by the subtidal channel (0.0212 h^{-1}), the *Spartina maritima* covered pool (0.0200 h^{-1}) and the sandy bare bottom pool (0.0193 h^{-1}) (Fig. 5). These results corresponded to significant growth differences between experimental areas (ANOVA, $p<0.05$). Macroalgal growth in the muddy bare bottom pool was significantly different from growth in the other places (Tukey test, $p<0.05$) but *E. intestinalis* growth was not statistically different in the *Spartina maritima* covered pool, the sandy pool and the channel (Tukey test, $p>0.05$).

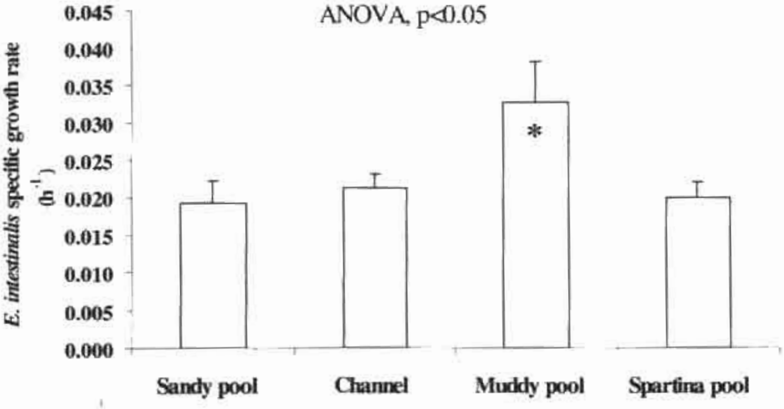

Figure 5. Average *Enteromorpha intestinalis* specific growth rate + 1 standard error in experimental areas. Macroalgal growth was statistically different between experimental areas (ANOVA, $p<0.05$): *Enteromorpha intestinalis* growth rate in the muddy pool was significantly different from growth in the other places (Tukey test, $p<0.05$).

Discussion

The previous results suggest that *E. intestinalis* growth is favoured in muddy bare bottom pools in relation with *Spartina maritima* covered pools, sandy pools and the low-depth channel. Results also suggest that these differences be partially explained by different nutrient dynamics within the tidal pools related with nutrient effluxes from the sediment to the water column.

In the case of NH_4-N, the variation of concentration in the muddy bare bottom pool was in agreement with the result of Lillebø et al. (2002a), which found out that in this type of intertidal pools, ammonia efflux occurs both during night and day. In fact, the observed increase of NH_4-N concentration in the muddy bare bottom pool throughout the experiment suggests that ammonia efflux took place during the day and that, the efflux rate was higher than the rate of NH_4-N uptake by macroalgae. The low NH_4-N concentrations in the sandy pool and the decrease throughout the experimental period are explained by the low organic matter content of the sediments (0.95 %, Flindt et al. 2002, Lillebø et al. 2002a) and by macroalgal uptake. Additionally, Flindt et al. (2002) found that, in the sandy pools, the particulate organic nitrogen (PON) is less degradable than in the other experimental pools, which results in low porewater nutrient concentrations and low efflux rates from the sediment to the water column.

The variation of NH_4-N in the *Spartina maritima* covered pool is also in agreement with the result of Lillebø et al. (2002a), who measured a lower NH_4-N efflux, during the day, in *S. maritima* covered pools compared to the muddy bare bottom pools. Apparently, in the presence of light due to plant photosynthesis and O_2 translocation, *Spartina maritima* roots contribute to oxidise the surrounding sediment, which promotes lower ammonia efflux rates (Lillebø et al. 2002a). The decrease in NH_4-N concentration that was observed from 11 to 12 a.m. in the *Spartina maritima* covered pool must reflect both the uptake by macroalgae and the lower NH_4-N efflux.

Thus, it seems to exist a parallelism between NH_4-N efflux rates and *E. intestinalis* growth, which in summer are higher in muddy bare bottom pools, followed by *Spartina maritima* covered pools and finally by sandy bare bottom pools. This result is corroborated by the values for potential macroalgal growth (g $WW.m^{-2}.d^{-1}$) estimated for the different pools, based on daily (including night and day values) N-availabilty calculated by Lillebø et al. (2002a) (Table 2). Assuming a C:N ratio of 10 and that carbon constitutes 40 % of macroalgal dry weight, which corresponds to experimentally obtained values for Chlorophyta (e.g. Viaroli et al. 1993, Thompson and Valiela 1999), in summer, the N-availability in the mud pools may potentially contribute for a macroalgal daily increase of 38 g $WW.m^{-2}$, while in the *Spartina* pools, there may be an increase of 4.3 g $WW.m^{-2}.d^{-1}$. This difference may be related with high N-uptake rates by the plant at this time of the year (Lillebø et al. 2002a). On the contrary, in autumn, the potential for macroalgae growth based on N-availability is very similar in the *Spartina* and in muddy pools and even slightly higher in the *Spartina* pool (Table 2). This may be related with the higher organic matter content of these pools (see above,

Table 2. Potential macroalgal weight increase (wet weight) in different intertidal pools in summer and autumn estimated from N-availabilty calculated by Lillebø et al. 2001a and considering a C:N=10 and that C constitutes 40 % of macroalgae dry weight. $N_{availability}$-N concentration in mg $N.m^{-2}.d^{-1}$, N_{req}-N internal requirement for maximum growth (7.4 mg N $(gDW)^{-1}.d^{-1}$, Pedersen and Borum 1996). $P_{availability}$-P concentration in $\mu mol.m^{-2}.d^{-1}$ calculated by Lillebø et al. 2001a,b. Vmax-rate of uptake at phosphate saturation (96 μmol P $(gDW)^{-1}.d^{-1}$, in Poole and Raven, 1997)

Season	**Summer**		**Autumn**		
Type of intertidal pool	Spartina pool	Mud pool	Spartina pool	Mud pool	Sand pool
Potential macroalgal growth (g $WW.m^{-2}.d^{-1}$)	4.3	38	65	59	11
$N_{availability}/N_{req}$	2.7	24	41	37	7
$P_{availability}/V_{max}$	10	22	13	6	18

Flindt et al. 2002) and with the possible lower uptake rates by the plant at this time of the year (Lillebø et al. 2002a).

Assuming a N-internal requirement of 7.4 mg N (g DW)$^{-1}$ d^{-1} for the maximum growth of opportunistic macroalgae (*Ulva* sp., Pedersen and Borum 1996), the N-availability per square meter in the Mondego intertidal pools range between 3-40 times this value (Table 2), which indicates the high potential for macroalgae growth within these pools.

According to Lillebø et al. (2002a), in summer, phosphate efflux is higher in muddy bare bottom pools followed by *Spartina maritima* covered pools and the sandy bare bottom pools, however such pattern is not clear in the present results. This difference could have been partially determined by temperature. In July 1997, Lillebø et al. (2002a) registered a maximum temperature of 32°C, while in the present study, the maximum temperature was 25°C. Actually, phosphate efflux is promoted by higher temperatures and by increased redox-state of the sediment (Valiela 1995, Lillebø et al. 2002a). This may explain the difference between the present results and those of Lillebø et al. (2002a). Nevertheless, if we consider the initial concentration (1.35 μmol PO_4-P.l^{-1}) at 9 a.m. in the muddy bare bottom pool and its concentration at 2 p.m. (2.82 μmol PO_4-P.l^{-1}), it corresponds to a two-fold increase. This also agrees with the occurrence of PO_4-P efflux during the day in this type of pools as measured by Lillebø et al. (2002a).

The increase in PO_4-P concentration verified in *Spartina maritima* pool from 9 to 10 a.m. could have resulted from the occurrence of strong anoxic conditions during the night associated with the presence of the rooted-macrophyte. In this situation, reduced conditions may persist until the next morning (until the point when O_2 consumption is balanced by O_2 production). Under anoxic conditions, sediments may turn from sinks to sources of phosphorus (Sfriso et al. 1988, Perttilä et al. 1995, Flindt et al. 2002, Lillebø et al. 2002a,b).

Although in summer, PO_4-P effluxes are higher in the muddy pools (Lillebø et al. 2002a,b), *Spartina maritima* covered pools present the highest initial and average PO_4-P concentrations. This may reflect the fastest mineralisation rates promoted by the highest organic matter content of the sediments in the *Spartina* covered pools (Flindt et al. 2002).

The ratio of total (day and night) PO_4-P availability in each type of intertidal pools (Lillebø et al. 2002a) by the maximum rate of phosphate uptake (V_{max}=96 µmol P $(gDW)^{-1}.d^{-1}$ in Poole and Raven 1997) suggests PO_4-P sufficiency in all pools (Table 2). In autumn, there is a slight increase of this ratio in the *Spartina* pool compared to summer, which again such as in the case of nitrogen may reflect seasonal plant growth variations. The significant decrease verified in the mud pool in autumn compared with the summer value may be related with temperature variation. The highest value of $P_{availability}/V_{max}$ in autumn was measured in the sand pool, which is apparently unexplained.

The low NO_3-N concentrations verified in the water of tidal pools are in accordance with other values measured during summer in the Mondego estuary (Pardal 1998, Martins 2000, Martins et al. 2001, Flindt et al. 2002). However, the observed decrease in NO_3-N concentrations especially in the muddy bare bottom and in the *Spartina maritima* covered pool is not completely explained by macroalgal uptake. In fact, most marine algae seem to preferably take up NH_4-N over NO_3-N (Valiela 1995), particularly if NH_4-N concentration exceeds that of NO_3-N (Riccardi and Solidoro 1996) as was the case. It is possible that, denitrification processes be related with NO_3-N depletion in tidal pools during summer, particularly in the presence of rooted vegetation (Valiela 1995, Flindt et al. 1999).

Nutrient dynamics in the subtidal channel depends on other factors (e.g. hydrodynamics), which may not be significant to nutrient dynamics in the tidal pools. In the present results, the difference between nutrient dynamics in intertidal pools and the subtidal channel areas was more evidenced by NO_3-N, which was always higher and less variable in the channel compared with the intertidal pools. This is in agreement with previous results (Pardal 1998, Martins 2000, Martins et al. 2001), which suggested that the NO_3-N enrichment of the system depends mainly on the input from external sources (namely from the Pranto River, see Fig. 1). Several factors related with hydrodynamics (e.g. higher light extinction coefficients) may contribute to a lower macroalgal growth in the channel relatively to the muddy bare bottom pools. However, the suitability of the subtidal channel to macroalgal growth, at this time of the year, is very similar to that of *Spartina maritima* covered pools and sandy pools. It is also possible that in the *Spartina maritima* (average plant height = 50 cm) pools, macroalgae suffer from light-limitation, especially in early morning and late afternoon, when shading by the rooted plant is higher.

The variation in N:P ratios of the water suggest a strong potential of N-limitation in all places except the muddy bare bottom pool and particularly in the sandy pool. Nevertheless, *E. intestinalis* grew in all places, which may be related with macroalgal nutrient storing capacity (Björnsäter and Wheeler 1990, Fong et al. 1998). Although macroalgae-storing capacity is lower than that of seagrasses, macroalgal growth is considered to depend on internal nutrient concentrations (Björnsäter and Wheeler 1990, Bendoricchio et al. 1994, Solidoro et al. 1997), while their uptake rates depend on the difference between internal and external nutrient concentrations. Nevertheless, the present results suggest that the differences in *E. intestinalis* growth between places might have been related with different N:P ratios in the different experimental places. In fact, the rate of dependency on external conditions is also determined by macroalgal growth rate (Poole and Raven 1997). If macroalgae growth is low, it can rely for a longer period in internal storage than if growth is higher, as was the case. However, the significant N-limitation of the water in the sandy pool, *Spartina* pool and the channel did not necessarily cause N-limitation of *E. intestinalis* but it might have contributed to slow down their growth in relation with *E. intestinalis* growth in the muddy pool.

In conclusion, the present results show that different nutrient availability causes distinct growth responses of green macroalgae in different tidal pools. Nevertheless, between muddy bare bottom and muddy vegetated pools, nutrients may not be the only factor contributing for the different growth of green macroalgae. In fact, although the presence of rooted vegetation affects nutrient dynamics within intertidal pools, light-limitation of macroalgal growth due to shading by high rooted-macrophytes is likely to occur.

Furthermore, this work highlights the importance of nutrient effluxes from the sediment to the growth of opportunistic macroalgae. Although seasonal differences take place, the nutrients provided by the sediment-water effluxes provide enough nutrients to allow maximum macroalgal growth, suggesting that in terms of nutrients, the occurrence of macroalgal blooms in the south of the estuary may be independent from external input. On the other hand, the effective growth of macroalgae will depend on the variation of other factors (e.g. light conditions, salinity, current speeds, N:P ratio see Martins et al. 2001) that will control altogether the productivity of this primary producer.

At the system level, these results may at a certain extent explain the higher abundance of new macroalgal individuals in muddy areas during late winter/early spring. At this time of the year, when external conditions are still unstable (e.g. high tidal currents due to high input of freshwater, high light extinction coefficients, low salinity), the places with a higher nutrient availability may be comparably more suitable to macroalgal growth.

Acknowledgements

This work was supported by the Portuguese Foundation for Science through a PhD grant attributed to I. Martins (PRAXIS BD/3744/94) and by the EU project "WET – Wetland Ecology and Technology (FMRX –CT96 – 0051. Inside this frame IMAR and Freshwater Biological Laboratory, University of Copenhagen, Denmark, made the joint scientific study in the Mondego Estuary. The study was also supported by the European Scientific MAST 3 program (the F-ECTS project) inside the ELOISE frame.

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NUTRIENT DYNAMICS IN THE INTERTIDAL POOLS OF THE MONDEGO ESTUARY.
IV – POSSIBLE CONTRIBUTION TO DISSOLVED INORGANIC PHOSPHORUS LOADING

Abstract

In the Mondego estuary, the freshwater from Pranto River represents an important input of phosphorus into the south arm, and the estuary is exporting inorganic dissolved nutrients to the adjoining coastal zone. In fact, a previous study concerning the loading of dissolved inorganic phosphorus showed that the system had a net production of 14 tons (PO_4-P) y^{-1}.

Results concerning the phosphate efflux rates from intertidal pools level to the water column, evidence the importance of these pools to the internal dissolved inorganic phosphorus loading, especially the muddy bare bottom pools, which showed a clear seasonal variation, with higher efflux in summer. This seasonal variation of phosphate effluxes is also in agreement with previous data concerning the inorganic dissolved phosphate concentrations in the south arm.

The aim of this work was to evaluate the contribution of the efflux of inorganic phosphate from the intertidal pools to the system, and relate it with the export into coastal waters.

At the system level, we considered the seasonal contribution of each type of pool (formed in muddy sediment with and without *Spartina* cover, and sandy sediment) to the whole system. These preliminary approach shows that the contribution of intertidal pools, and especially in the muddy bare bottom areas which are also the most representative of the system, appears to be very important to the increased concentration of dissolved inorganic phosphorus during summer. Although, this evaluation is quite limited in interpretation, the phosphate efflux from the intertidal pools level seems to have an important contribution to the export of dissolved inorganic phosphorous from the Mondego estuary.

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Introduction

Rivers may supply much of the nitrogen and phosphorus into estuaries, but within the estuary, large fractions may be internally recycled through biogeochemical processes. In the south arm of the estuary (Fig. 1) water circulation is mainly dependent on tides, and on the freshwater input of a tributary, the Pranto River (Marques et al. 1997, 2002, Pardal et al. 2000, Martins et al. 2001). A sluice controls the Pranto river outflow, and its opening frequency is crucial to the nutrient loading into the estuary (Pardal 1998).

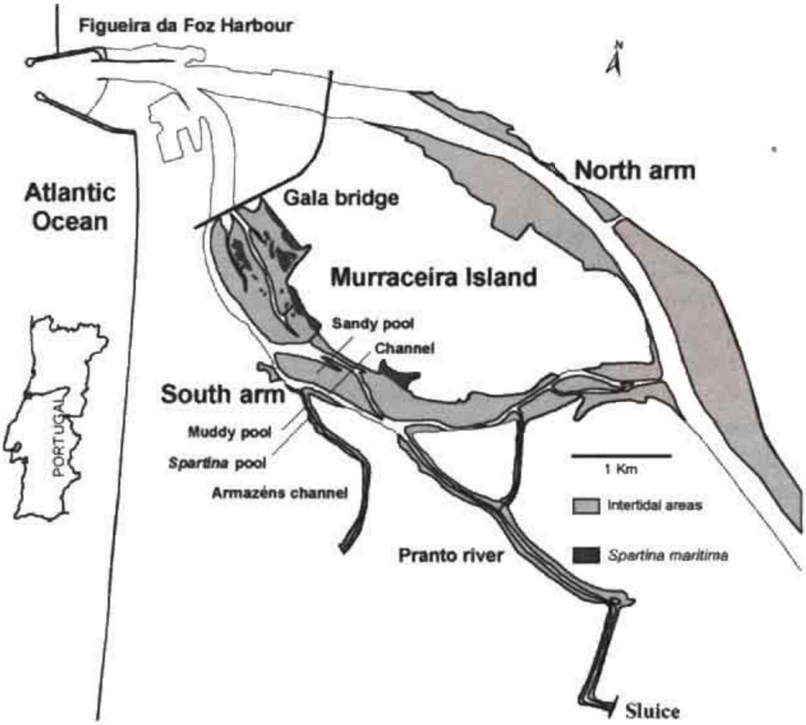

Fig. 1. The Mondego estuary with the location in the south arm of the Pranto River sluice, and the Gala bridge outer boundary.

The study of the close benthic-pelagic coupling of nutrient dynamics and the net losses of nutrients in estuaries requires the description of both transport-dispersion and biogeochemical process, and only proper mass balance calculations can verify whether an area inside the estuary acts as a source or as a sink for nutrients (Flindt et al. 1999, 2002).

An estimate of the nutrient loading into the south arm of the Mondego estuary, carried out between May 1993 and June 1994, showed that dissolved inorganic nutrients were exported from the system (Fig. 2) (Flindt et al. 1997, Pardal 1998). For

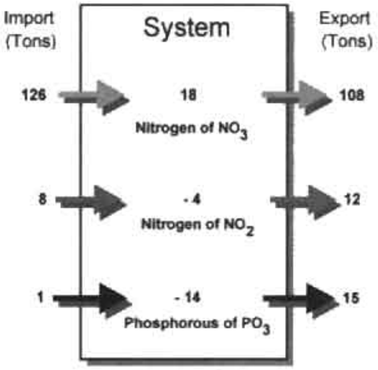

Fig. 2. Nitrogen and Phosphorus annual balance, from June 93 to June 94, in the south arm of the Mondego estuary.

dissolved inorganic phosphorus, 1 ton was imported to the system, while 15 tons were exported to the sea (Fig. 2), which means that 14 tons were net released from the south arm of the estuary.

Discharges from the Pranto River sluice are usually higher during winter and early spring, following the seasonal variation of precipitation (Pardal 1998, Martins et al. 2001). During this period the system presents low phosphate concentrations in the water column, and increases during late spring and summer, showing a clear inter-annual seasonal variation. So, most probably phosphorous input from the Pranto River is transported adsorped to particulate matter, and dissolved phosphate in the main water-body results from desorption and mineralisation of phosphate from particulate organic matter (e.g. Pettine et al. 1983, Hinga 1990, Sundby et al. 1992, Valiela 1995, Falcão and Vale 1998, Mitchell and Baldwin 1998).

In the estuarine area, although most of the system is submerged during high tide, when the water level drops in the ebb, intertidal pools formation takes place. A previous study (Lillebø 2002a) showed that in July high temperatures and anoxic conditions, especially during the night, promoted the phosphate efflux to the overlaying water column. Moreover, results also suggested that these intertidal pools could play a major role to the internal dissolved inorganic phosphorus loading. Therefore we considered necessary to evaluate the contribution of the efflux of phosphate from the intertidal pools level to the main water-body and relate it with phosphorous export into coastal waters.

Material and methods for the existing data

Loading estimates

In summer the south arm Mondego estuary can be seen almost as a coastal lagoon, with inflow and outflow regimes depending essentially on tides, and on another inflow of a small tributary, the Pranto River (See also Marques et al. 2002) (Fig. 1). The discharge of the Pranto River changes seasonally as a function of precipitation and the needs for water in the rice fields from the Pranto valley (Martins et al. 2001). A sluice consisting of three sections with 3m widths each controls this discharge. The water column height, during discharges, varies between 0.5 and 2.5 m (Pardal 1998). To avoid salt-water intrusion into the rice fields, discharges take place always during ebb tide (Pardal 1998).

An almost natural downstream boundary of the south arm, where marine water and estuarine water exchange, was considered at the Gala bridge cross-section (Fig. 1). This cross-section is 200 m width and 2 to 4 m deep, in high tide situation, with an average depth varying between 2 and 3 m, meaning that during spring ebb tides part of this cross-section may be emerged for short periods (Pardal 1998). The Pranto River sluice was the only upstream input considered, and the downstream cross-section at the Gala bridge was the exchange (outer) boundary. No other inputs were taken into account because they were considered too small to be relevant (Pardal 1998).

The Pranto River discharges and exchanges in the downstream outer boundary were monitored from May 93 to June 94, to quantify the dissolved inorganic phosphorus loading in the south arm of the estuary (Flindt et al. 1997, Pardal 1998). During this period there were 144 discharges from the Pranto River and the monitoring program covered 50% of them. At Gala bridge outer boundary the monitoring program took place every month, with greater effort when the Pranto sluice was open, covering 18 complete tidal cycles. At both sites water samples were collected with one-hour intervals. Simultaneously, current velocity was measured and salinity and temperature were determined in situ. Water samples were kept frozen until analysis, and dissolved reactive phosphate was quantified according to Strickland and Parson (1968) methodology.

Estimates of total dissolved phosphate inflow were carried out taking into consideration the volume of water discharged during the period the sluice was open, at the Pranto station, and also the tidal regime and wave components at the Gala station. During the discharges, at the Pranto River sluice, current velocity and water height were measure, and the concentration of nutrients quantified. The estimation by simple integration through time of the flow, allowed the calculation of the total water discharged into the estuary, as well as the loading of nutrients discharge at each time the sluice was open (Pardal 1998).

The outer boundary at the Gala bridge is mainly subjected to the physical action of the tidal wave, which is constituted by the overlap of elementary waves. The harmonic components of these elementary waves could be calculated based on continuous measurement during one annual cycle at a sampling station in the same area (Table 1), (Almeida and Seabra Santos 1993 *in* Pardal 1998). In the referred study (Pardal 1998) it was just considered the waves with amplitude equal or bigger than 4% of the fundamental harmonic M^2:

Table 1. The harmonic compounds of elementary waves, equal or bigger than 4% of the fundamental harmonic M_2 (from Pardal 1998).

Component	Period	Amplitude		Phase (h)
	(h)	(m)	(%M_2)	
M_2	12.421	0.910	100.0	9.785
S_2	12.000	0.333	36.6	3.620
N_2	12.658	0.200	22.0	4.127
O_1	25.819	0.067	7.4	13.216
K_1	23.934	0.065	7.2	4.009
L_2	12.192	0.039	4.3	7.718
MS_f	354.367	0.036	4.0	201.876

The evolution of the water surface *(t), in the considered section was given by the following equation.

$$n(t) = \sum_{i=l}^{7} A_i \cos\left[\varpi_i (t - \phi_i)\right]$$

Where Ai, *i e *i represent the amplitude, the angular frequency respectively (=2*/T) and the phase correspondent of each harmonic.

Taking into consideration the small distance of the whole system the wave can be considered a stationary wave whose velocity at time t, is given by the equation:

$$U(t) = \sum_{i=l}^{7} A_i \sqrt{8/_{h_0}} \sin\left[\varpi_i (t - \phi_i)\right] \sin\left[\varpi_i \frac{x_\beta - x_0}{\sqrt{g h_0}}\right]$$

Where $x_\beta - x_0$ represent the distance between Gala bridge and the upstream section (≈ 4800 m), h_0 represent the wave height at the outer boundary, creating the forcing, and g the gravitational acceleration (Pardal 1998).

The instantaneous flow was calculated multiplying the signal with the transversal section area, correspondent to the water height in the considered instant. The loading of nutrients was estimated by multiplying the flow with the instantaneous concentration measured. Which means that, the difference between tides conditioning the water column height at the Gala cross-section was taken into account (Pardal 1998). This methodology allowed to estimate the loading of dissolved inorganic phosphorous (phosphate) in the south arm of Mondego estuary (Flindt et al. 1997, Pardal 1998).

Long-term field monitoring program

The variation of nutrient concentration in the water column in the south arm of the estuary has been monitored for several years in order to understand the eutrophication process (e.g. Pardal 1998, Martins 2000). This database allowed us to evaluate the seasonal variation of dissolved inorganic nutrients in the system, as well as salinity and temperature. Part of the water analysis was performed according to Stickland and Parson (1968) and others according to Standard Methods (1995).

Inorganic phosphate dynamics in intertidal pools

Within the downstream section of the south arm of the Mondego estuary, three study sites were chosen, one located inside a muddy *Spartina maritima* bed while the others where located on bare muddy sediment and bare sandy sediment (See Lillebø et al. 2002a). These pools were studied during 24-hours tidal cycles in July 97 and November 97, respectively in summer and fall, and representing different average temperature, hydraulic conditions, emersion time, plant coverage, and organic content in the sediment (Flindt et al. 2001, Lillebø et al. 2002a, Martins et al. 2002). Nutrient dynamics was followed in the water of each pool, with 1-hour periodicity, from its formation until it was submerged again. Flux chambers were placed in the main channel (under day and night situations, July 2001) for comparison of the efflux rates in the low water pools.

In July 97, sediment cores were sampled from each pool and analysed for potential phosphate adsorption capacity (μmol PO_4-P g^{-1} dwt. sediment), (See Flindt et al. 1999, 2002). Calculations of the mean phosphate efflux rates (mg. m^{-2} d^{-1}) were performed considering: a) the mean daily efflux rates (day and night) from each type of pool (Lillebø et al. 2002a); b) a visual estimation of the contribution of each type of pool to the estuarine area (1.9 km^2: 17% *Spartina*, 50% mud and 33% sand); c) a visual estimation of the area covered by pools during low tide (40%); d) the mean efflux rates in July could represent a mean value for spring and summer, and that the mean efflux rates in November could represent a mean value for fall and winter. In the main channel it was assumed that there is no significant seasonal variation.

It is well known that nutrient fluxes are strongly dependent on temperature dynamics, and higher phosphate effluxes in temperate regions are found at elevated temperatures (Asmus et al. 2000). We therefore used, as a preliminary approach, a simple dynamic temperature model to simulate the yearly variation in the phosphate fluxes. The model equations are:

$$PO_4\_flux_{actual} = PO_4\_fluxt_n * K(temp_{actual}^{-n})$$

$$Accumulated\ PO_4\_flux = PO_4\_fluxt_{+1} + PO_4\_flux_t$$

Where the $PO_4\_flux_n$ is the measured phosphate flux at a certain temperature (n°C), K is temperature dependency constant, while $temp_{actual}$ is the simulated phosphate fluxes. The only forcing function was the temperature. Afterward the simulated fluxes becomes numerical integrated so the yearly internal loading, due to the flux dynamics, for the pools becomes simulated.

Calculations of the phosphate mass efflux (kg P) were also performed assuming that the mean efflux rates in July could represent a mean value for spring and summer, and that the mean efflux rates in November could represent a mean value for fall and winter. In the main channel it was assumed that there is no significant seasonal variation.

Results

Loading

Between June 93 and June 94, discharges of dissolved inorganic phosphorus proceeding from the Pranto River occurred mainly between October and April (Fig. 3). This variation is related to precipitation and the needs to balance the water level in rice fields located in the Pranto River valley. The maximum input of dissolved inorganic phosphorous occurred in November (50.73 Kg). (Pardal 1998).

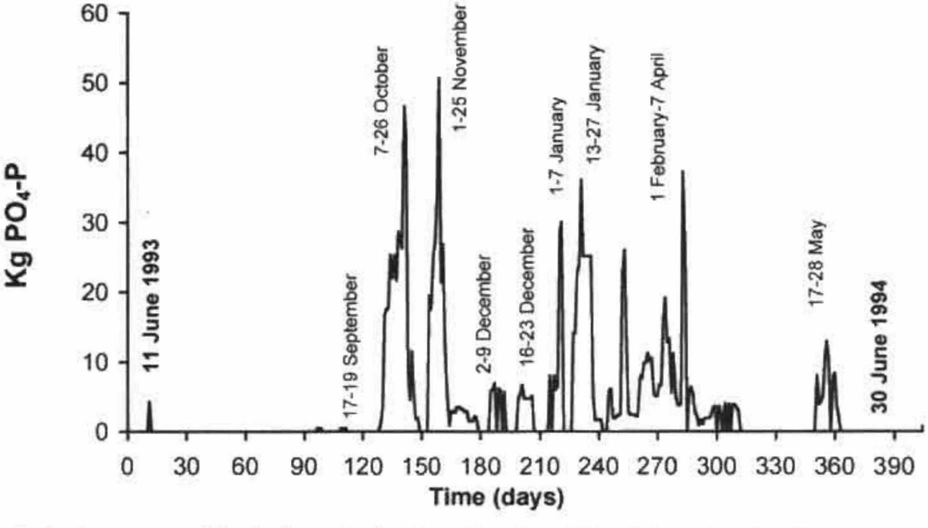

Fig. 3. Monthly input of dissolved inorganic phosphorus from Pranto River discharge, from June 93 to June 94.

The loading estimates for dissolved inorganic phosphorous (Fig. 4) showed that between June 93 and June 94, the estuary was exporting phosphorous almost all year around except for a short period, from late winter (February) to early spring (April), when phosphorous uptake was observed in the system (Pardal 1998).

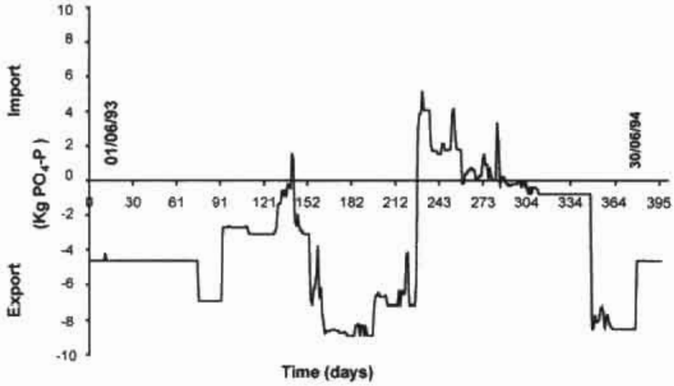

Fig. 4. Monthly mass balance of inorganic dissolved phosphate in the south arm, from June 93 to June 94. Positive values mean consumption within the system and the negative values represent release from the system.

Field monitoring program

Results show a clear seasonal variation of dissolved inorganic phosphorus in the south arm of the estuary (Fig. 5), with higher concentrations recorded always in August/September (values varied between 0.08 and 0.14 mg l^{-1}) and lowest during winter (always close to 0.016 mg l^{-1}). Moreover, if we consider only data between June 93 and June 94, it is possible to see that dissolved inorganic phosphorous proceeding from the Pranto River discharge did not increase the concentration at the system level.

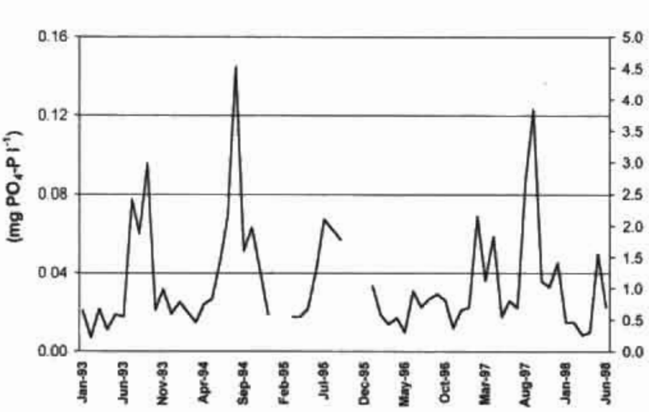

Fig. 5. Monthly concentration of dissolved inorganic phosphorus in the south arm, from January 93 to June 98.

Data from January 93 to June 98 showed seasonal variations of temperature and salinity, and some inter-annual variation especially regarding salinity (Fig. 6). During summer, water temperature varied between 23 °C and 28 °C, and during winter it was always close to 10 °C, during the day. Salinity seasonal variation was basically a function of precipitation and the fresh water input from the Pranto River. Values were usually higher than 25 in the summer and lower than 15 in the winter (Fig. 6). Moreover, during a dry hot summer salinity could reach values of 35 (e.g. September 97), and during rainy winter periods salinity values could be lower than 5 (e.g. January 96 and December 97).

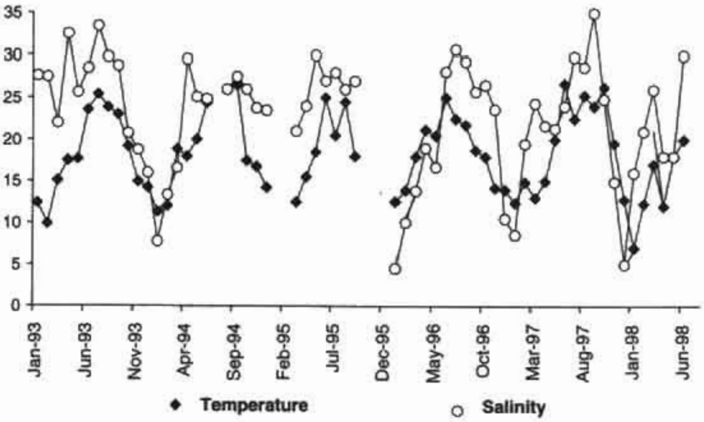

Fig. 6. Monthly measurements of temperature and salinity in the south arm, from January 93 to June 98.

Intertidal pools study

Considering the mean daily efflux rates (day and night) from the muddy bare bottom pool, there was a clear seasonal variation of the efflux rates, with higher P-effluxes during summer and lower during fall (Fig. 7). In the *Spartina* covered pool and in the sandy pools, this seasonal variation is not visible, and in the main channel the efflux rates are comparatively low, and we assumed that there is no seasonal variation (Fig. 7).

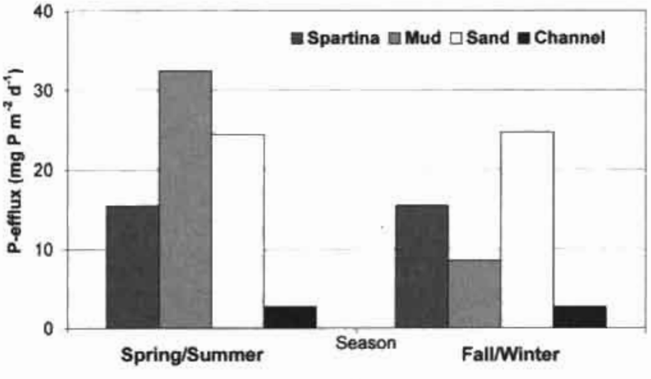

Fig. 7. Seasonal variation of phosphate mean efflux rates (mg PO_4-P m^{-2} d^{-1}) at each pool type and the main channel.

This seasonal variation is closely related with temperature and redox state of the sediment/water column inter-phase, and differences among pools appeared to be related to pool plant coverage and organic matter content in the sediment. Adsorption/desorption isotherms were also studied in July 97 showing that the muddy *Spartina* rhizosphere had highest adsorption capacity (Fig. 8) with 4.5 μmol PO_4-P mol^{-1} dwt. sediment, while in bare muddy areas, at the same sediment depth, the adsorption capacity was only 2.6 μmol PO_4-P mol^{-1} dwt. sediment (Flindt et al. 1999, 2002). In bare sandy sediment the adsorption capacity was clearly lower than in muddy sediments, reaching 0.97 μmol PO_4-P mol^{-1} dwt. sediment (Flindt et al. 1999, 2002).

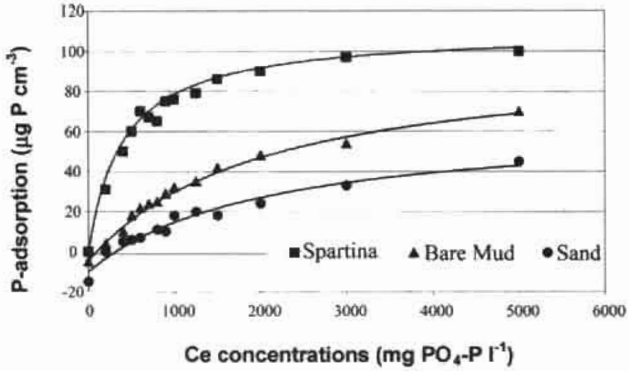

Fig. 8. Sediment phosphate adsorption capacity (P-sorption fitted to Langmuir exp.) at the three study sites.

As the P-efflux seasonal variation was only clear for the muddy bare bottom pools, we simulate the internal loading of phosphate based on a temperature-dependent model, as a preliminary approach (Fig. 9). Despite cautions in interpreting this simulation, due to the lack of points for the calibration of the model, it express an increase of phosphate efflux during the warmer periods (spring and summer) and a decrease during fall and winter.

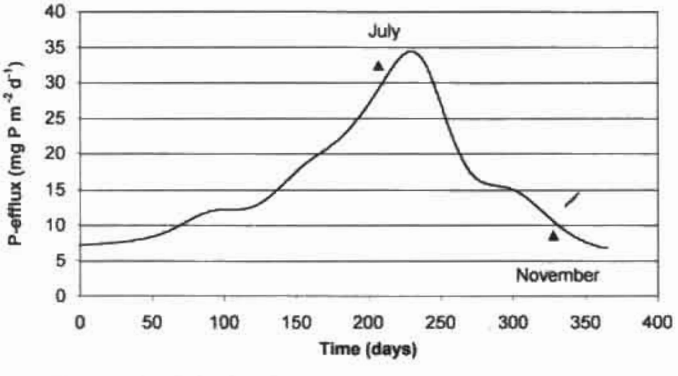

Fig 9. Simulation of the yearly variation in phosphate fluxes, in pools located in the bare bottom muddy areas of the estuary.

Phosphate dynamics at the system level

Calculations of the phosphate net effluxes (kg P), assuming that July data could represent summer and spring and that November data could represent fall and winter, suggest a strong contribution of the bare bottom muddy pool to the whole system internal phosphate loading (Fig 10), and especially during the warmer periods. The *Spartina* covered pool had comparatively a lower effect on the internal phosphate loading than the sandy covered pool, but higher than the channel (Fig. 10).

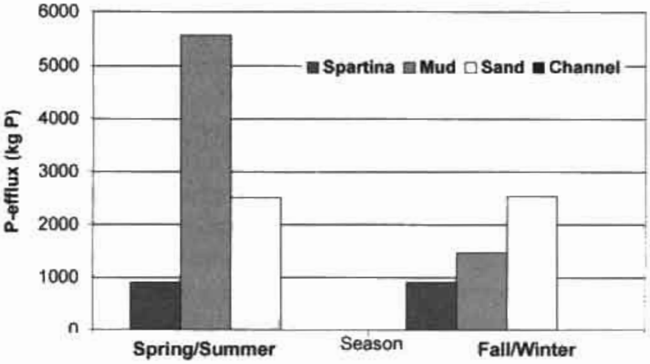

Fig 10. Yearly net efflux of phosphate (kg P) for each type of pool and the main channel.

This preliminary approach show that the phosphate efflux from the pool system level can contribute with several tons (13.8 t, by these calculations) of dissolved inorganic phosphorus to the net internal loading, which corresponds to the previous loading calculations for the estuary.

Discussion and conclusions

The annual mass balance of dissolved inorganic phosphorous shows that the estuary is exporting 14 ton y^{-1}, but also that the system is retaining most of the dissolved inorganic phosphorus during late winter and early spring (Pardal 1998). Such retention probably results from an enhancement of primary producers growth during this period, namely from phytoplankton, macroalgae (e.g. *Enteromorpha* sp.) and rooted macrophytes (e.g. *Zostera noltii, Spartina maritima* and *Scirpus maritimus*) (e.g Pardal 1998, Lillebø 2000, Martins 2000). On the other hand, the Pranto River discharges were more significant between October and April (Pardal 1998), but during this period there was not an increase in the concentration of dissolved inorganic phosphorous at the system level. Therefore, the south arm of the Mondego estuary may be functioning as a sink of phosphorous during winter and the growing season, but also as a source of phosphorous to the estuarine plume zone or even to open coastal waters during summer.

Values of dissolved inorganic phosphorus concentration in the south arm of the estuary were higher in summer, and other authors have also noticed the phenomena of increase phosphorus concentration during this period (Hinga 1990, Tian et al. 1993, Valiela 1995) and under anoxic conditions (Sfriso 1988, Perttilä et al. 1995). Moreover, the temperature and the oxic state of the sediment may be very important to the efflux of phosphorus due to an intensification of anaerobic decomposition pathways of organic matter (Forja et al. 1994 *in* Falcão and Vale, 1998).

Results obtained may express the inorganic dissolved phosphorus adsorption and consumption, but the question remains: How does the south arm of the Mondego estuary supply the increase of inorganic dissolved phosphorus concentration during late spring and summer, and consequently its export from the system?

In intertidal systems, sediments get exposed to air and subsequent drying conditions during ebb tide, and it has been shown that under this desiccated/oxidation conditions sediments have a lower affinity for phosphorus than submerged sediments (Baldwin 1996 *in* Mitchell and Baldwin 1998). Phosphorus dynamics in sediments is strongly influenced by cycles of iron and sulphur (Sundby et al. 1992), and the lower phosphorus release under desiccated conditions may be explained by three main factors: drying and ageing of minerals with which phosphorus is associated, (particularly iron hydroxides), carbon limitation as a result of drying, and by the shift in bacterial community structure, specially the loss of viable sulphate-reducting bacteria (Mitchell and Baldwin 1998). However, it has been showed that these elements (iron and sulphur) can be very reactive when flooding water spreads over the sediment (Caetano 1977 *in* Falcão and Vale 1998). Moreover, as a result of the reduction of the insoluble ferric hydroxides compounds to more soluble ferrous hydroxides compounds, under flooded conditions sediments have many more sorption sites than under drained conditions (Patrik 1992). So, in the presence of bio-reducible iron, the redox state of the sediment and the amount of phosphorus in pore-water or water column determine whether phosphorus is released or absorbed. Furthermore, in intertidal estuaries, such as the Mondego estuary, the low water intertidal pools may cover 30-40% of the total estuarine area. Within these pools, physic and chemical conditions usually change quite fast (Flindt et al. 2002, Lillebø et al. 2002a, Martins et al. 2002), in comparison to the main water-body, reaching very high water temperature and low oxygen concentrations, especially during spring night ebb tides. During this period, and in consequence of respiration by microfauna, meiofauna and macrofauna, these pools may become anoxic, when microphytobentos, macroalgae and rooted macrophytes are unable to compensate the oxygen consumption. Under such conditions, the adsorbed phosphate can be released to the pore-water and an increased efflux from the sediment to the water column may occur (Patrik 1992), depending on whether the phosphate concentration in the bottom water is lower or higher than the equilibrium concentration in the pore water (Sundby 1992).

Considering the fact that sediments constitute a major source of phosphorous supply for macrophytes, during the day, there might be a certain competition between two processes: *Spartina maritima* increased adsorption capacity and the efflux from the sediment. In fact, under more oxygenated conditions, like the ones surrounding plants rhizosphere, the ferrous iron precipitates as ferric hydroxides, removes dissolved phosphate by readsorption to oxidized iron compounds (Berner and Berner 1996, Anderson and Ring 1999).

The highest mean phosphate effluxes occurred in summer with a clear contribution of the bare bottom muddy areas, which may be explained by the organic matter content, and the P-sorption capacity of the plants (Flindt et al. 2002, Lillebø et al. 2002a).

Taking into account these preliminary approach, we may consider that phosphorus release in intertidal pools during low-water periods appears to represent an important contribution to the increase concentration of dissolved inorganic phosphorus in the main water-body, and that this process can be particularly significant during the warm periods. In fact, our results suggest that, in absence of external sources, the daily phosphate efflux from the pools level is able to support the main water-body phosphate concentration. Although, we are still not able to quantify exactly the relatively contribution of each type of pool, it is clear that the intertidal pools system, by providing a dramatically increase of temperature conditions, stimulates P-mineralisation and P-desorption and thereby the efflux of phosphate. Nevertheless, these results should be regarded as a preliminary approach for two main reasons: the lack of sampling points to calibrate the simulated fluxes, as well as the yearly net efflux of phosphorus; and due to the fact that *Scirpus maritimus*, annual dynamics has not been taken into consideration. Although from July until December only bellow-ground part of the plants persists (Lillebø 2000, Lillebø et al. 2002b). So, further research is being provided in order to accurate these results.

Acknowledgements

The present work was supported by the Programme MAST II (MAS 2 CT 92 0036), by the WET-project, (Wetland Ecology and Technology), funded by the European scientific TMR program, (TMR) (ERB 4061 PL 95-0832), by the F-ECTS project founded by the European Scientific MAST 3 program inside the ELOISE frame, and by the Praxis XXI program (Portugal) through a PhD grant (BD/ 9290/ 96). The authors would like to thank the Freshwater Biological Laboratory, University of Copenhagen, Denmark, for the support to conduct this study.

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Enteromorpha spp. (ULVALES: CHLOROPHYTA) GROWTH IN THE SOUTH ARM OF THE MONDEGO ESTUARY: FIELD GROWTH RATES WITH AND WITHOUT MACROFAUNAL GRAZER EFFECTS

Abstract

The aim of this study was to quantify field growth rates of *Enteromorpha* spp. and to compare macroalgal growth with and without macrofaunal grazer effects in the south arm of the Mondego estuary. From January 1996 to January 1997, *Enteromorpha* growth was characterised by null or very low values in winter and late autumn, which fitted to negative exponential models. In spring, macroalgal growth enhancement took place according to exponential models. During summer, although lower than in spring, *Enteromorpha* growth rates were positive with the exception of July. In spite of the growth enhancement observed in spring, average *Enteromorpha* growth rates were low for this time of the year. This was attributed to the significant input of freshwater to the south arm during winter and spring 1996. According to the results, *Enteromorpha* growth rates are positively correlated with salinity and negatively correlated with precipitation, which partially results from the dependency of salinity on precipitation. However, this dependency is only valid for certain periods of the year. The present results are inconclusive relatively to grazer effects on *Enteromorpha* growth, since macroinvertebrates were found inside both types of experimental devices.

Introduction

It is widely known that eutrophic conditions stimulate the growth of opportunistic primary producers. Higher surface to volume ratios (SA:V) of phytoplankton and ephemeral macroalgae (e.g. *Enteromorpha* spp.) determine high maximal uptake rates of nutrients, high initial slopes of photosynthesis versus irradiance and lower half-

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saturation constants for the uptake of nutrients (Hein et al. 1995, Valiela et al. 1997), which in turn determine high growth rates especially under nutrient enriched conditions.

On the other hand, it has been suggested that even under eutrophic conditions, grazing control may be an important factor regulating biomass accumulation of free-floating macroalgae, such as *Ulva* sp. and *Enteromorpha* sp. (Geertz-Hansen et al. 1993). Ultimately, the abundance and success of a given alga in a certain environment results from the balance between nutrient uptake, nutrient requirements, cell growth and loss rates due to grazing and physical processes (Hein et al. 1995).

In the Mondego estuary, eutrophication is characterised by significant accumulations of green macroalgae mainly *Enteromorpha* spp. (Marques et al. 1993, Pardal 1998, Lillebø et al. 1999, Martins et al. 1999). *Enteromorpha compressa* (L.) Greville and *Enteromorpha intestinalis* (L.) Link were identified as the most abundant species (Martins et al. 1999, Martins 2000). In fact, throughout the last decades *Enteromorpha* spp. became the dominant primary producer in the intertidal areas of south arm of the estuary. At the same time, there was a decrease in the area occupied by rooted macrophytes (especially *Zostera noltii*) (Pardal 1998, Martins 2000). The two processes may be related and may have resulted in the occurrence of less structured and more impoverished macrofaunal benthic communities in the south arm of the estuary (Lillebø et al. 1999, Pardal 1998, Pardal et al. 2000).

The general aim of the present work was to follow *Enteromorpha* spp. growth in the south arm of the Mondego estuary, throughout one year in order to detect the main external factors controlling it. In particularly, we tried to assess for macrofaunal grazer effects on *Enteromorpha* spp. growth.

Material and methods

Preparation of macroalgae for growth experiments

From January 1996 to January 1997, one experiment was run, every month, in an inner area of the south arm of the Mondego estuary (Fig. 1). Field work was always carried out during low-tide. One day before the beginning of the experiment, *Enteromorpha* spp. individuals were collected randomly on the intertidal muddy flats of the south arm of the estuary, placed in recipients containing estuarine water and carried to the lab. Forty healthy individuals were chosen, carefully washed with estuarine water and placed on kitchen paper inside a temperature-controlled room at 20°C, which allowed to remove the excess of water. After this procedure, macroalgal individuals were weighted for initial wet weight adjustment corresponding to 4-5 g of algal tissue. Each macroalgae portion was then placed inside identified experimental devices, consisting of semi-cylinder cages built in plexiglass (Fig. 2). The sides and bottom of 20 devices were surrounded by 0.5 mm mesh-size net to prevent grazers from enter, while sides and bottom of the other 20 devices were surrounded by 4 mm mesh-size net, which allowed grazers to enter (Fig. 2). The experimental devices

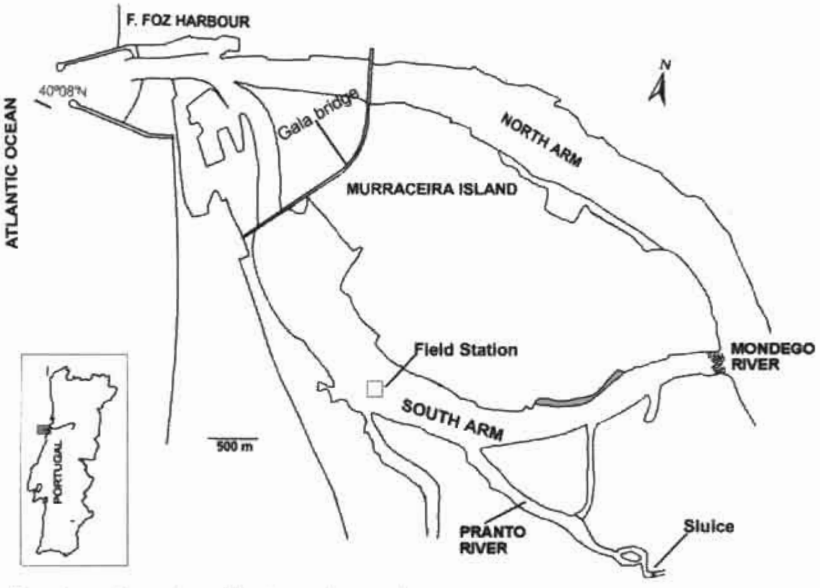


Figure 1. Experimental area located in the south arm of the Mondego estuary.

allowed sufficient water circulation and light penetration inside. Nevertheless under field conditions, the amount of light decreased with exposure time due to sediment deposition on device surfaces.

All experimental devices were carried out to the field and fixed by thin ropes to wood sticks buried in the sediment. Groups of five replicates of devices from both types were removed from the field after 5, 10, 15 and 20 days. In the laboratory, macroalgal individuals were carefully washed with estuarine water and weighted to final wet weight, following the water removal procedure already described.

For each sampling date, temperature (°C), salinity, dissolved oxygen (mg.l^{-1} and %) and pH data were measured *in situ* and water samples (approximately 250 ml) were collected to estimate dissolved inorganic nutrients (PO_4-P, NO_3-N, NO_2-N and NH_4-N). In the laboratory, water samples were filtered and analysed following Standard Methods (1992) procedures for orthophospate and N-compounds. Data on precipitation were obtained from the Geophysics Institute of the University of Coimbra and concern precipitation values measured at the city Coimbra.

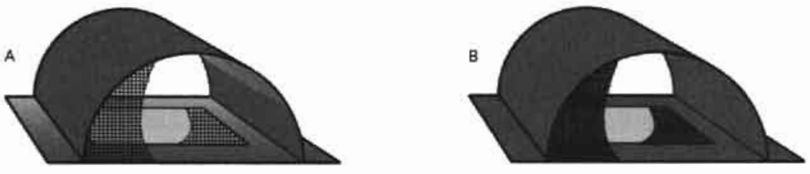


Figure 2. The two types of experimental "plexiglass" cages used to estimate *Enteromorpha* spp. growth: A) surrounded by 4 mm mesh size net to allow grazers entrance; B) surrounded by 0.5 mm mesh size net to prevent grazers entrance

Data analysis

Monthly growth rates of *Enteromorpha* spp. were calculated according with the exponential growth model:

$$W_t = W_0 \times e^{kt} \quad (1)$$

W_t – *Enteromorpha* weight (g wet weight) at time t, W_0 – *Enteromorpha* initial weight (g wet weight), k – coefficient of specific growth (d^{-1}).

Enteromorpha weight data were ln transformed, which allowed the exponential curve to become a straight line. The slope of that line is k, i.e., *Enteromorpha* growth rate (Sokal and Rolf 1995; Zar 1999). After calculating *Enteromorpha* growth rates as the slopes of fitted regressions, the significance of regressions was tested by analysis of variance (ANOVA). Analysis of covariance (ANCOVA) was used to compare slopes (i.e. growth rates) and the Tukey test was used to detect significant differences between them.

The t-test was used to compare *Enteromorpha* growth rates with and without grazer effects, after checking for normality (Kolmogorov test) and for homogeneity of variances (Bartlett test). Correlation between growth rates and physicochemical parameters was assessed by Pearsons' correlation coefficient and the significance of the correlation was assessed by an F-test (Zar 1999). MICROSOFT EXCEL 97 and STATGRAPHICS software packages were used to perform all statistical analysis.

Results

In winter and late autumn, *Enteromorpha* weight variation inside both types of devices was well fitted to negative exponential models (Figs 3 and 4). In spring and August, *Enteromorpha* weight variation without grazer effects was well described by positive exponential models (Fig. 3), which was not the case of *Enteromorpha* spp. weight variation with grazers (Fig. 4). In both situations (with and without grazers), the weight variation of *Enteromorpha* in July was poorly fitted to exponential models (Fig. 3 and Fig. 4).

Enteromorpha spp. specific growth rates without grazers ranged from –0.33 to 0.063 d^{-1} in January 1996 and June/August, respectively. Macroalgal growth in the presence of grazers ranged from –0.38 to 0.13 d^{-1} in January and May, respectively (Fig. 5). Higher growth rates occurred in spring and also in August, while in July *Enteromorpha* had a negative growth rate (Fig. 5).

Monthly growth rates were significantly different when compared to each other (ANCOVA, $P<0.05$). *Enteromorpha* growth in January and February 1996 was significantly different from growth in any other month (Tukey test, $P<0.001$) (Tables 1 and 2).

No significant differences were found between *Enteromorpha* spp. growth rates with and without grazer effects (t test, $P>0.05$). Additionally, macroinvertebrates were found inside both type of experimental devices. Table 3 shows the most representative taxa and their relative abundance inside experimental devices.

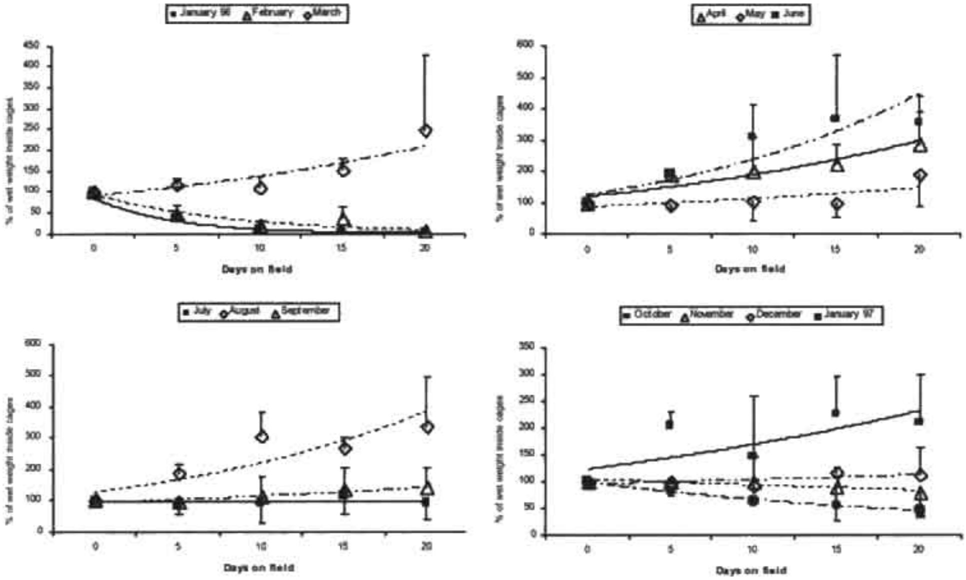


Figure 3. Variation of *Enteromorpha* spp. wet weight (%) inside experimental cages without grazers. R^2 between data and exponential models in March, April, May and June were 0.80, 0.85, 0.52 and 0.83, respectively.

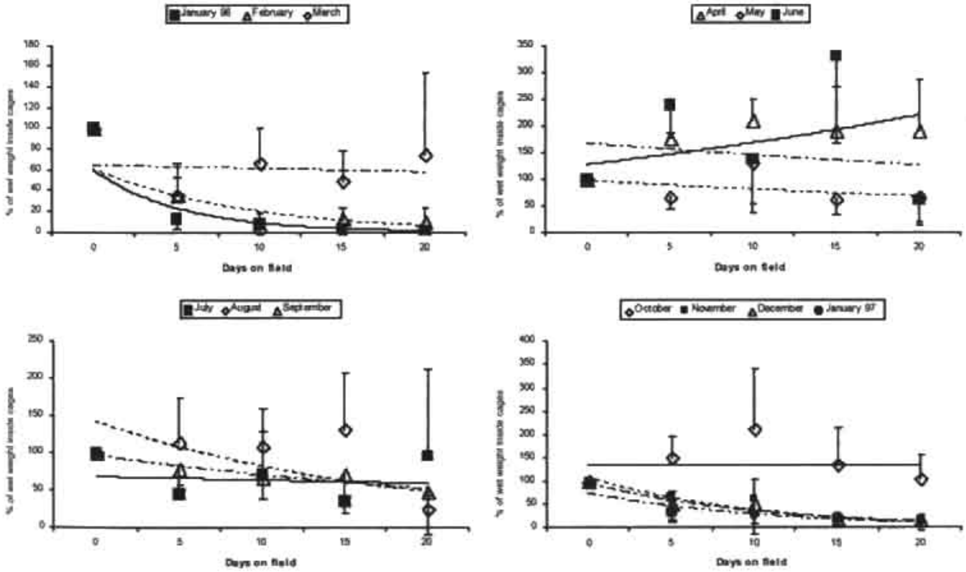


Figure 4. Variation of *Enteromorpha* spp. wet weight (%) inside experimental cages with grazers. R^2 between data and exponential models in March, April, May and June were 0.01, 0.54, 0.19 and 0.033, respectively

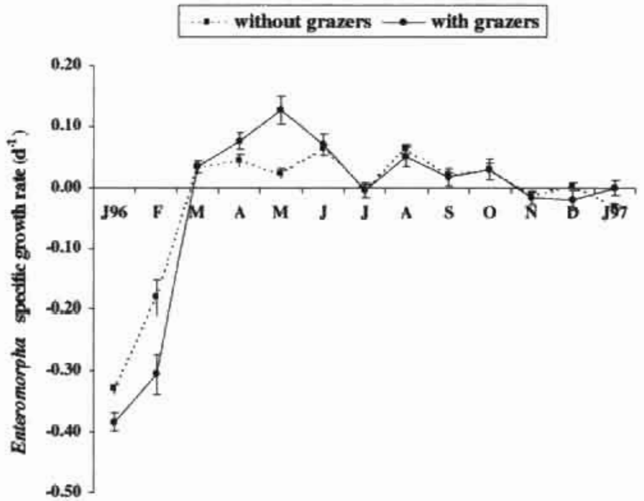


Figure 5. Variation of *Enteromorpha* spp. growth rate (d^{-1}) without and with grazer effects ± standard error

		Jan-96	Feb-96	Mar-96	Apr-96	May-96	Jun-96	Jul-96	Aug-96	Sep-96	Oct-96	Nov-96	Dec-96	Jan-97
	slopes	-0.332	-0.182	0.033	0.044	0.023	0.063	-0.005	0.063	0.020	0.029	-0.014	0.002	-0.031
Jan-96	-0.332	-												
Feb-96	-0.182	S^{***}	-											
Mar-96	0.033	S^{***}	S^{***}	-										
Apr-96	0.044	S^{***}	S^{***}	NS	-									
May-96	0.023	S^{***}	S^{***}	NS	NS	-								
Jun-96	0.063	S^{***}	S^{***}	NS	NS	NS	-							
Jul-96	-0.005	S^{***}	S^{***}	NS	S^{*}	NS	S^{***}	-						
Aug-96	0.063	S^{***}	S^{***}	NS	NS	NS	NS	S^{***}	-					
Sep-96	0.020	S^{***}	S^{***}	NS	NS	NS	NS	NS	NS	-				
Oct-96	0.029	S^{***}	S^{***}	NS	NS	NS	NS	NS	NS	NS	-			
Nov-96	-0.014	S^{***}	S^{***}	NS	S^{**}	NS	S^{***}	NS	S^{***}	NS	NS	-		
Dec-96	0.002	S^{***}	S^{***}	NS	NS	NS	S^{***}	NS	S^{***}	NS	NS	NS	-	
Jan-97	-0.031	S^{***}	S^{***}	S^{***}	S^{***}	S^{***}	S^{***}	NS	S^{***}	S^{*}	S^{***}	NS	NS	-

Table 1. Tukey test results from monthly *Enteromorpha* spp. growth without grazers. NS-not significant, S-significant (*p<0.05, **p<0.01, ***p<0.001)

		Jan-96	Feb-96	Mar-96	Apr-96	May-96	Jun-96	Jul-96	Aug-96	Sep-96	Oct-96	Nov-96	Dec-96	Jan-97
	slopes	-0.384	-0.305	0.034	0.076	0.127	0.070	-0.004	0.051	0.017	0.030	-0.017	-0.021	-0.001
Jan-96	-0.384	-												
Feb-96	-0.305	NS	-											
Mar-96	0.034	S^{***}	S^{***}	-										
Apr-96	0.076	S^{***}	S^{***}	NS	-									
May-96	0.127	S^{***}	S^{***}	S^{**}	NS	-								
Jun-96	0.070	S^{***}	S^{***}	NS	NS	NS	-							
Jul-96	-0.004	S^{***}	S^{***}	NS	NS	S^{***}	NS	-						
Aug-96	0.051	S^{***}	S^{***}	NS	NS	NS	NS	NS	-					
Sep-96	0.017	S^{***}	S^{***}	NS	NS	S^{***}	NS	NS	NS	-				
Oct-96	0.030	S^{***}	S^{***}	NS	NS	S^{**}	NS	NS	NS	NS	-			
Nov-96	-0.017	S^{***}	S^{***}	NS	S^{**}	S^{***}	S^{*}	NS	NS	NS	NS	-		
Dec-96	-0.021	S^{***}	S^{***}	NS	S^{***}	S^{***}	S^{***}	NS	NS	NS	NS	NS	-	
Jan-97	-0.001	S^{***}	S^{***}	NS	S^{*}	S^{***}	S^{*}	NS	NS	NS	NS	NS	NS	-

Table 2. Tukey test results from monthly *Enteromorpha* spp. growth with grazers. NS-not significant, S-significant (*p<0.05, **p<0.01, ***p<0.001)

Table 3. Relative percentage of macrofaunal species inside the two types of experimental cages

Taxa	Genus and species
Molusca: Gastropoda, Bivalvia	*Hydrobia ulvae*-45 % *Cerastoderma edule*-1.7 %, *Scrobicularia plana*-3 %
Arthropoda: Crustacea Isopoda Amphipoda Decapoda	*Cyathura carinata*-0.5 %, *Sphaeroma* spp.-0.11 %, *Idotea* spp.-0.19 % *Melita palmata*-34.5 %, *Amphitoe* spp.-0.2 %, *Gammarus* spp.-0.1 % *Carcinus maenas*-10.3 %, *Crangon crangon*-3 %, *Palaemon* spp.-1.3 %

High precipitation occurred in winter and spring of 1996, which was accompanied by low salinity values, particularly, from January 1996 to May and again in December 1996 and January 1997 (Fig. 6). In fact, throughout 1996, salinity was strongly dependent on precipitation (Fig. 7).

Enteromorpha growth was positively correlated with salinity (F 0.05(2),11,11, r=0.61, P<0.05) (Fig. 8a) and negatively correlated with precipitation (F 0.05(2),11,11, r= -0.65, P<0.05) (Fig. 8b).

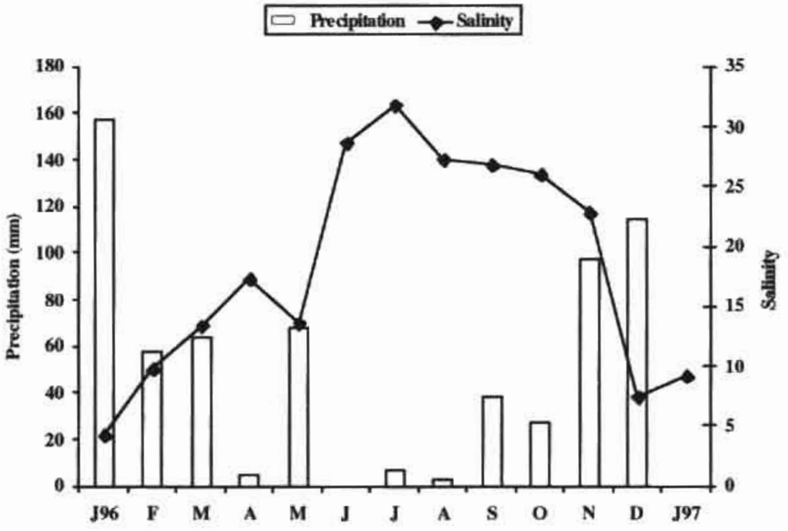


Figure 6. Variation of precipitation (mm) and salinity throughout the study period

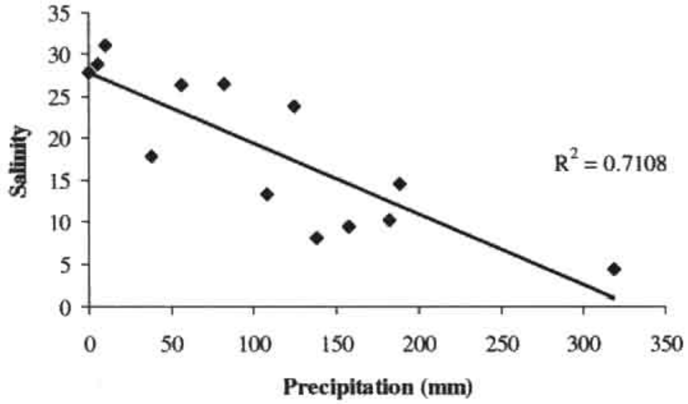
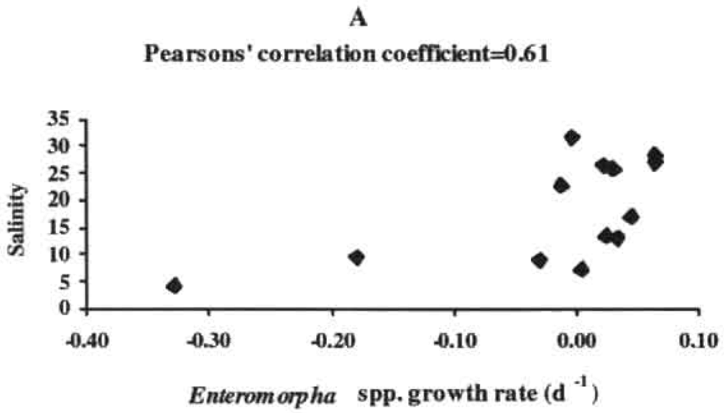


Figure 7. Relationship between salinity and precipitation (mm) in the Mondego estuary during 1996



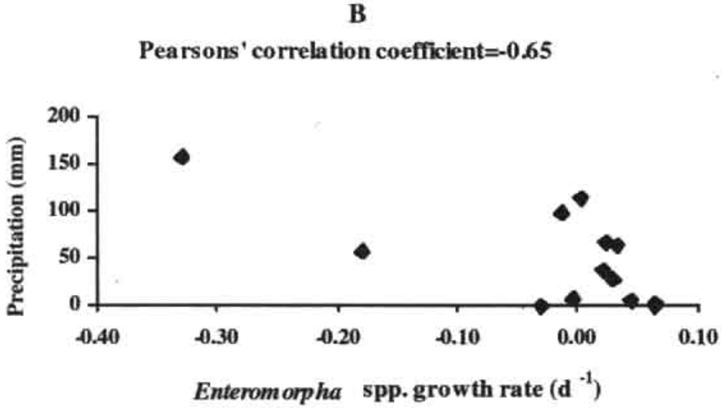


Figure 8. *Enteromorpha* spp. growth rate (d^{-1}) *versus* salinity (A) and *versus* precipitation (mm) (B).

Discussion

The yearly growth variation of *Enteromorpha* spp., over the study period, followed the typical variation of Ulvaceae populations in the Northern Hemisphere (e.g. Hull 1987, Sfriso 1995, Schories 1995, Hernández et al. 1997). During winter, Ulvales vanish or become reduced to few adult individuals in more sheltered areas, whereas in spring and summer macroalgal growth is enhanced, usually originating the development of significant amounts of biomass (Pregnall and Rudy 1985, Everett 1994, Schories 1995). However, while macroalgal populations from northern Europe usually start to grow in May (Schories and Reise 1993, Kolbe et al. 1995), in the Mondego estuary, *Enteromorpha* spp. started to grow in March. The earlier growth enhancement of *Enteromorpha* in the Mondego estuary probably reflects differences in light and temperature conditions between northern and southern Europe.

However, the quantified *Enteromorpha* growth rates (maximum values of 6 % and 13 % d^{-1} without and with grazers, respectively) are low when compared with other populations of Ulvales, some of them located further north. For example, in the Venice Lagoon, *Ulva* sp. presents growth rates of 23 % d^{-1} (Sfriso 1995), while in the Roskilde Fjord (Denmark), *Ulva* sp. growth may range between 4-20 % d^{-1} (Geertz-Hansen et al. 1993). In spite of an earlier improvement of temperature and light conditions, *Enteromorpha* growth in 1996 at the south arm of the Mondego estuary was comparatively low. On the other hand, the winter and spring of 1996 were quite rainy.

This situation is in agreement with the finding that, the amount of freshwater flowing through the south arm of the estuary is an important factor controlling macroalgal growth (Martins et al. 2001). The amount of freshwater in the south arm depends on precipitation and on river management practices. High freshwater discharge to the system causes significant decreases in salinity values and increases in the light extinction coefficient and in the water currents (Martins et al. 2001). Furthermore, because the freshwater is highly enriched in inorganic nitrogen, mostly nitrate (Pardal 1998, Martins 2000, Martins et al. 2001), it may also contribute for a potential P-limitation of primary producers. The combined effect of all these factors seems to determine *Enteromorpha* growth and standing crop in a given year (Martins et al. 1999, Martins et al. 2001).

In the winter and spring of 1996 precipitation was quite high, about 811 mm. On the other hand, the low salinity values quantified between January and May 1996 suggest that, the upstream sluices remained opened for long periods, which determined significant freshwater flow to the south arm of the estuary. This also agrees with the observed positive correlation between *Enteromorpha* growth and salinity and with the negative correlation between macroalgal growth and precipitation. In 1996, since it was a very rainy year and the sluices remained opened for long periods, salinity was strongly dependent on precipitation. Nevertheless, this is not always the case because it may rain and still the sluices may be kept closed (Martins et al. 2001).

The decrease in *Enteromorpha* spp. growth observed in July may be related with environmental conditions at that time of the year. Frequently, summer is reported as a nutrient limiting period of the year, which consequently may restrict macroalgal growth (e.g. Rivers and Peckol 1995, Pedersen 1995, Pedersen and Borum 1996). On the other hand, in lower latitudes, the limitation of macroalgal growth during summer has also been attributed to temperature and photon flux density (PFD), which may act synergistically to suppress the photosynthetic capacity of emersed macroalgae. (Pregnall and Rudy 1985, Rivers and Peckol 1995). Actually, desiccation stress has been suggested as the main cause of the summer decline of southern European populations of Ulvales (Hernández et al. 1997, Aníbal 1998). In the Mondego estuary, temperatures easily increase to 25°C during summer, while photon flux densities can reach 2000 µmol photon $m^2.s^{-1}$ (Martins 2000). Considering that photosynthetic saturation of *Enteromorpha* spp. takes place between 500 and 1000 µmol photon $m^2.s^{-1}$ (Shellem and Josselyn 1982, Beer and Shragge 1987), *Enteromorpha* photoinhibition may be a common process in the Mondego estuary during summer, especially during diurnal low tides. However, this process requires further investigation, since August growth rates were relatively high considering the obtained results.

In spring, *Enteromorpha* weight variation inside devices with grazers was more irregular than growth without grazers and, it seldom fitted a positive exponential model. This situation may be the result of some disturbance caused by the animals. On the other hand, the highest average growth rate of *Enteromorpha* spp. occurred within cages with grazers. Moreover, some of the animals (e.g. *Crangon crangon*, *Carcinus maenas* and Palaemonidae) found inside experimental devices are known to macerate and ingest *Enteromorpha* directly (Warwick et al. 1982). However, since such macroinvertebrates were found inside both types of experimental devices, it is not possible to draw conclusions relatively to the effects of grazers on *Enteromorpha* spp. growth.

Other studies, carried out in the south arm of the Mondego estuary (Pardal 1998, Pardal et al. 2000), have established clear relationships between the biomass of macroalgae and the abundance of some macroinvertebrate (e.g. *Amphitoe* spp., *Melita palmata*). The highest densitiy and biomass of such species was observed in the place where green macroalgae biomass was higher, which may reflect some kind of feeding dependency (Pardal 1998, Pardal et al. 2000). According with this and if indeed these anfipods graze on *Enteromorpha* spp. then, they can not ultimately prevent spring macroalgal blooms to take place. Perhaps that, as suggested by Valiela et al. (1997), the potential control of macroalgae by grazers is likely only in estuaries subject to low rates of N loading, which is not the case of the Mondego estuary.

Acknowledgements

To the Portuguese Foundation for Science and Technology (FCT) which supported this work through a PhD grant attributed to I. Martins (PRAXIS XXI/BD/3744/94), EU projects "MUST – Marine Universal Structural Model" (MAS2-CT92-0036) and "WET – Wetland Ecology and Technology (FMRX –CT96 – 0051).

The authors are indebted to all colleagues that assisted in field and laboratory work, particularly, to Maria Gabriel Fontes.

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NITROGEN DYNAMICS IN THE MONDEGO ESTUARY: LEAF SENESCENCE AND N MOBILISATION IN *Spartina maritima*

Abstract

Spartina maritima (Curtis) Fernald is a dominant species in the Mondego salt marsh playing a significant role in estuarine productivity and energy cycling. A study conducted between February 1996 and January 1997 revealed that nitrate was mainly transported from roots and rhizomes to the leaves where it was accumulated. Nitrate concentration increased during summer in all types of studied leaves, probably playing an important role in osmotic regulation. Ammonium, on the contrary, was incorporated into organic compounds in the root rather than transported into the leaves. Total nitrogen quantified in the leaves, showed an obvious pattern, decreasing greatly during summer and early autumn. From March to June, nitrogen content in green leaves decreased nearly 75 %. This variation of nitrogen content was highly correlated with its concentration in the sediment. Leaf ageing in *Spartina maritima* was associated to chlorophyll losses, to nitrate and total nitrogen remobilization, and also to ammonium accumulation.

Introduction

In salt marshes, nitrogen supply is mediated by redox of the sediments and limits plant growth (Valiela 1995). Nitrate and ammonium are the major sources of inorganic nitrogen taken up by roots of *Spartina alterniflora* as well as in general higher plants (Wirén et al. 1997). Nitrate is readily mobilised in xylem and can be stored in vacuoles of roots, shoots, and storage organs (Martinoia et al. 1981). Its accumulation in vacuoles is of considerable importance for cation-anion balance and osmoregulation (Smirnoff and Stewart 1985). Ammonium is incorporated into organic compounds and generally not stored in cells (Schortemeyer et al. 1997).

Spartina maritima is a dominant species in the Mondego salt marsh (Portugal) and plays an important role in nutrient and energy dynamics in European salt marshes similar to that played by *Spartina alterniflora* in North American marshes. Like most

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coastal producers, throughout the world *Spartina maritima* is subject to eutrophication, which elevates nitrogen levels in the sediment (Valiela et al. 1992). Increased nitrogen supply increases growth and biomass, and affects plant morphology, nutrient content, density and reproduction (Rogers et al. 1998).

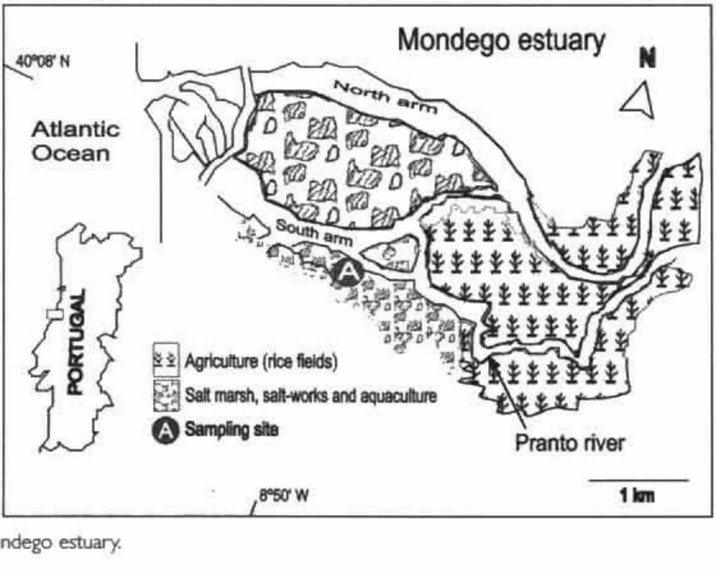
In general, plant leaves undergo a series of developmental changes. Initially, leaves are predominantly heterotrophic organs, maturing to become a net exporter of photosynthates. After reaching maturity, the leaf exhibits a reduced capacity to act as a source of photosynthetically fixed carbon and enters a senescent phase (Turgeon 1989). Leaf senescence represents a key developmental phase in plant life, which is as ordered and complex as any other phase of development. During leaf senescence, N, C and minerals are mobilised from the mature leaf to other parts of the plant. This process involves a series of events like cessation of photosynthesis, disintegration of chloroplasts, breakdown of proteins, loss of chlorophyll and removal of amino acids (Buchanan-Wollaston 1997).

The main objective of this work was to estimate seagrass contribution to the nitrogen dynamics in a Portuguese saltmarsh. This paper reports the seasonal variation of inorganic and total nitrogen concentrations in the Mondego saltmarsh and *Spartina maritima* contribution to this process. Nitrogen mobilisation during leaf senescence and its importance for plant survival in this system was also studied.

Material and methods

Study site

This work was conducted at the Mondego estuary located on the western coast of Portugal (40°08' N / 8°50' W) (Fig. 1). The Mondego estuary consists of two arms



1. The Mondego estuary.

with different hydrographic characteristics. The northern arm is deeper, while the southern arm is largely silted up, routing most of the freshwater input through the northern arm. Water circulation in the southern arm is mainly dependent on tidal cycles, receiving also small freshwater inputs of the Pranto river, a tributary with large amounts of nitrogen substances, frequently used as fertilisers by rice farmers in upstream areas (Marques 1989, Flindt et al. 1997). Sampling site was located on the south arm, in a salt marsh dominated by *Spartina maritima* (Fig. 1A).

Field program and laboratory procedures

Spartina maritima plants were sampled monthly from February 1996 to January 1997. In the laboratory, biomass samples were separated into roots, rhizomes, and leaves. Leaves were separated in 3 leaf age groups: green leaves (GL) – live tissue with green colour; senescent leaves (SL1 and SL2) – senescent tissue with at least 1/3 of area yellow (SL1), leaves with almost 2/3 of their area with yellow-brown color (SL2) and standing-decaying leaves (SDL) – yellow-brown dead material ready to fall. Leaves detached via decay were collected using 20 nylon mesh litter traps 100 cm long x 40 cm wide, opened at the top for monthly collections (Castro and Freitas 2000), designated in this paper as litter trap leaves (LTL). Samples were weighed and dried at 60°C until constant weight. Dry tissues were ground and analysed for NO_3 by the acid salicylic method (Cataldo et al. 1975), for NH_4^+ by the indophenol blue method (Solorzano 1969), and for total N (only in leaf samples) by the Kjeldhal method (Bremner and Mulvney 1982).

Immediately after collection, the chlorophyll (a + b) content was measured spectrophotometrically on triplicate 300 mg (fresh weight) of leaf samples (GL, SL1, SL2 and SDL), after extraction with 80 % acetone (Henry and Price 1993).

Sediment samples were collected each month in parallel to plant samples. Sediments were oven-dried at 60°C for 3 days and stored in sterilised plastic bags. Total N was determined in 5 g of sediment following the method in Bonneau and Souchier (1979).

Statistical analysis

One Way Analysis o Variance (ANOVA) was used to test for differences between mean nitrogen concentrations in plant samples, chlorophyll content in leaves, and seasonal variation of these variables. Non-parametric analysis of variance (Kruskal-Wallis) was used whenever parametric assumptions were not verified (Zar 1996).

Results

Water content

Water content (percentage of fresh weight) was higher in belowground tissues than in leaves (Fig. 2). Water content was relatively constant in roots and rhizomes, with higher values in root tissues. Rhizomes showed a medium annual value of 76 % of water and roots of 84 %. Young leaves were more succulent than senescent and dead leaves. All types of leaves revealed a decrease in water content from February to April, increasing the month after. Green and senescent leaves showed a slight decrease during summer. Water content in standing dead leaves increased from May to October, remaining high in winter.

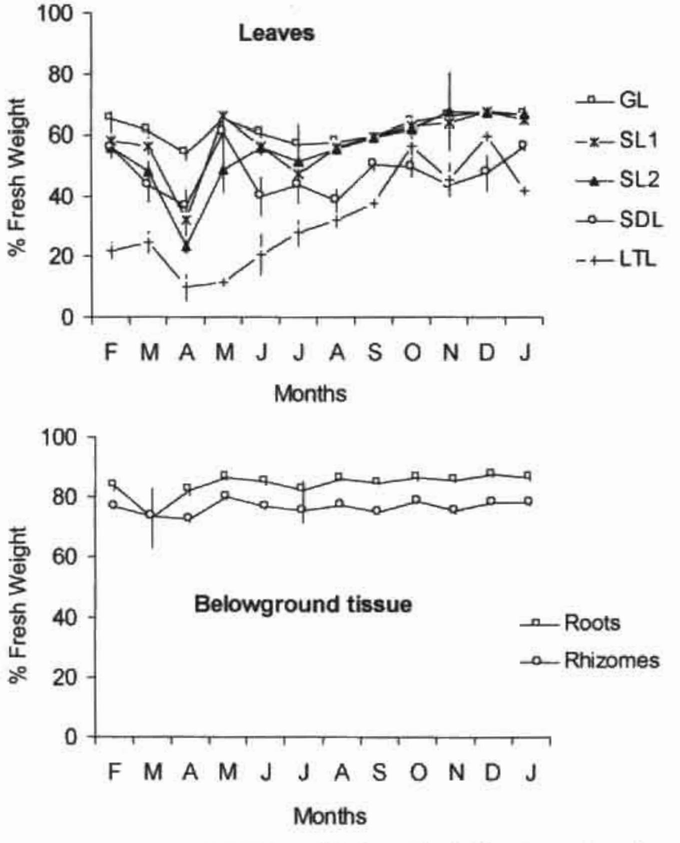


Fig. 2. Water content, expressed as percentage of fresh weight, in *Spartina maritima* leaves, roots and rhizomes, collected from February 1996 to January 1997.

Chlorophyll

Chlorophyll levels were different between the four types of leaves ($H_{3,N=144}=123$; $P<0.001$; Kruskal-Wallis ANOVA), decreasing with leaf senescence (Fig. 3). Overall, mean chlorophyll concentration for GL was 24.5 ± 3.1 mg L^{-1}. Mean losses of 17 %, 39 % and 58 % were measured for SL1, SL2 and SDL, respectively. Although each type of leaf followed its own seasonal change, a late-summer decrease and autumn increase were common features of the annual pattern.

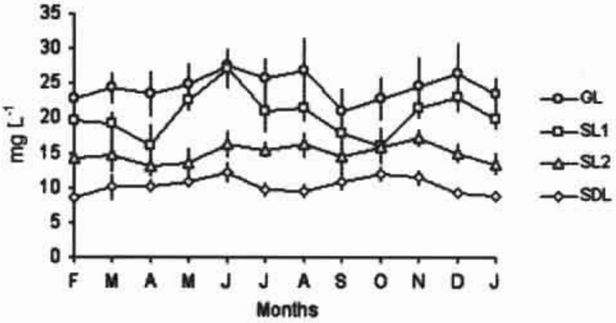


Fig. 3. Chlorophyll content (a + b) in leaves of *Spartina maritima*, monthly collected from February 1996 to January 1997.

Nitrogen dynamics

Nitrate was clearly transported and accumulated in young leaves (Fig. 4). During the study period, belowground roots and rhizomes showed levels of NO_3^- 4-5 times lower than GL. Seasonal variations were observed in all types of leaves, with higher

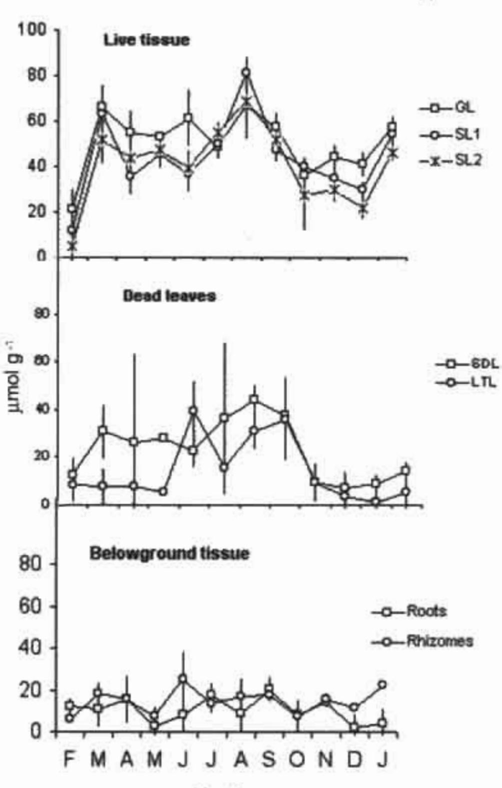


Fig. 4. Nitrate concentrations determined in plant samples between February 1996 and January 1997.

values in summer months. Differences between root and rhizome NO_3^- concentrations were verified ($F_{1,219}$=7.46; P<0,01). Seasonal variations for belowground tissues were also observed with slight increase in dry months. NO_3^- mean concentrations in rhizomes ranged from 6.4 ± 1.7 μmol g^{-1} dry wt (February) to 25.0 ± 12.8 μmol g^{-1} dry wt (June). Nitrate leaf content changed greatly with leaf age ($F_{4,374}$=49.78; P<0,001). Higher concentrations consistently occurred in the youngest age class, with large decrement from mature to senescent leaves. Leaves detached via decay (LTL) revealed about 70 % less nitrate than GL.

Mean monthly concentrations of NH_4^+ increased with leaf senescence ($F_{4,175}$ = 14.35; P<0,05). Like NO_3^-, all types of leaves showed NH_4^+ seasonal changes (Fig. 5). A slight decrease in NH_4^+ content was observed in leaves collected during summer. Standing-decaying leaves (SDL) showed ammonium concentrations 2-3 times higher than GL. Similar concentrations were found in belowground tissues, and roots and rhizomes did not show statistical differences in their values ($F_{1,70}$ = 0.52; P=0.47).

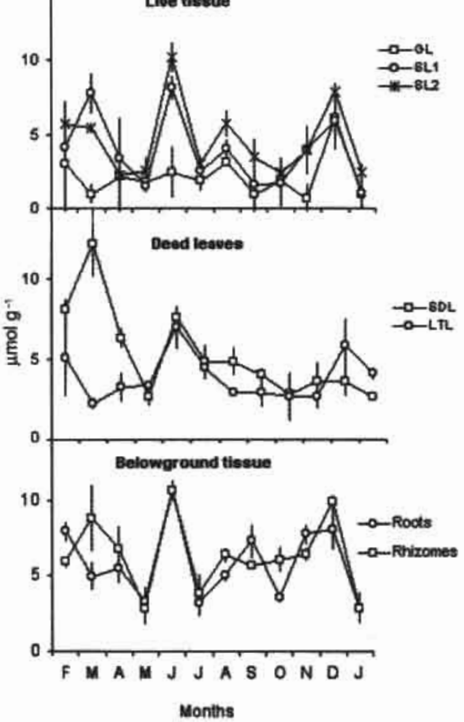


Fig. 5. Ammonium concentrations determined in plant samples between February 1996 and January 1997.

All leaves revealed a strong seasonal variation, with leaves produced in early spring and winter showing higher N levels (Fig. 6). Total nitrogen content (TN) was significantly different between the types of leaves analysed ($F_{4,155} = 6.94$; $P<0.05$). Mean N concentrations in GL and SL1 were generally higher from that observed in older leaves from February to June. This difference decreased and was even inverted during summer and autumn, recovering in the last month.

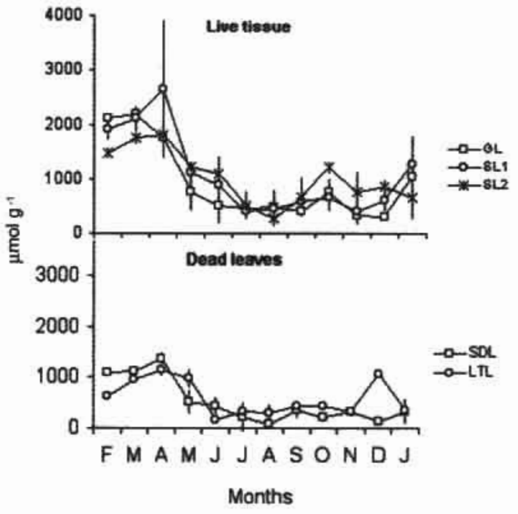


Fig. 6. Total nitrogen content measured in leaves of *Spartina maritima* between February 1996 and January 1997.

Total nitrogen (TN) concentrations in the sediment were much higher in spring and winter (Fig. 7). Maximum value of 20 ± 0.5 mmol g^{-1} dry wt was measured in April. In June, total nitrogen concentrations decreased to 5 ± 1.1 mmol g^{-1} dry wt.

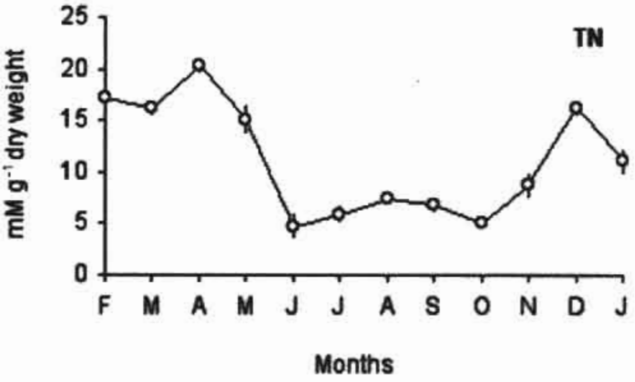


Fig. 7. Seasonal changes in nitrogen concentrations in sediment samples, collected in the Mondego salt marsh from February 1996 to January 1997.

Discussion

Nitrate translocation from roots to leaves (Fig. 4) can be explained by the need of tissue osmotic adjustment, particularly important during summer, when soil salinity increases (Flowers et al. 1986). The accumulation of salts participate in this function and is the least energetic costly mechanism of osmolarity generation (Raven 1985). Water content in *Spartina maritima* leaves did not change greatly along the year in living leaves, suggesting that succulence is not an important strategy for tissue osmoregulation (Fig. 2). Considering that, contrarily to dicotiledone plants (Flowers et al. 1986), grasses do not use succulence as a major adaptation to soil salinity, nitrate can thus be considered to play an important role in this process for this species. Our results, particularly the NO_3^- accumulation in summer leaves, showed that NO_3^- can also work as an important mechanism to lower the energy costs of the plant for nitrate reduction and assimilation in these tissues, since the energy can be directly given by the photosynthesis process (Abrol et al. 1983), and also decreasing the production of toxic oxygen radicals. Several studies demonstrate that light increases nitrate metabolism rates (Delhon et al. 1995, Keller et al. 1995, Cárdenas-Navarro et al. 1998).

Toxic ammonium effects are well known in terrestrial plants (Gerendás et al. 1997, Pearson and Stewart 1993), but for marine environments we can expect to have higher NH_4^+ concentrations in plant tissues, since salt concentration in sediments is much higher, and plants are probably more tolerant to this nutrient. However, this was not observed. Whereas NO_3^- was stored in leaves without detrimental effect, NH_4^+ content was quite low in both aerial and subterranean organs. This showed that, despite the high NH_4^+ concentrations in the soil (Fig. 5), *Spartina maritima* has several physiological mechanisms to prevent the increase of this nutrient in its tissues. Van Katwijk et al (1997) showed that the presence of concentrations as low as 25 µM of NH_4^+ in sediments were toxic to Zostera marina plants. Fan et al. (1997) also suggested that the use of NO_3^- instead of NH_4^+ as an N source is important for rice plants to tolerate anaerobiosis conditions of the soil, particularly for seed germination, because it gives a more efficient economy of energy and minimises lower pH effects in cells cytoplasm.

The lower N concentrations observed in summer were expected because the flowering period of this species occurs between May and July, and nutrients become thus necessary for plant reproduction (Marschner 1995). What is interesting is that the N seasonal variation in the soil corresponds almost exactly to the same variation in the sediment (Fig. 7) reflecting an important strategy that *Spartina maritima* plants use to improve nutritional efficiency during low external N availability (Thornton et al. 1994). During summer a decline of N absorption at root level is expected (Correia et al. 1992), mainly due to the reduction of carbohydrate levels in root cells (Marschner 1995).

Ammonium concentrations have been shown to increase during leaf senescence for several species (e.g. Thomas 1978, Peters and Van Laere 1992, Chen et al. 1997). This study showed that it also happens in *Spartina maritima* (Fig. 5). A decrease in glutamine synthetase activity, breakdown of macromolecules (e.g., proteins and nucleic acids) and an increase in nitrate reduction during leaf ageing, are some of the factors controlling ammonium accumulation (Storey and Beevers 1978, Platt and Anthon

1981, Kar and Feierabend 1984, Chen and Kao 1998). Chen et al. (1997) suggested that NH_4^+ could participate in senescence regulation of rice leaves, with the argument that increases in ammonium concentrations could make foliar tissues more sensitive to the action of ethylene, thus promoting senescence.

An opposite pattern was observed for nitrate and total N; senescent leaves clearly showed lower nitrate concentrations than green leaves. Castro and Freitas (2000) showed that higher leaf fall rates of *Spartina maritima* occur during summer, peaking in July and that these results are clearly related to the decrease of N and NO_3^- in leaves before they fall, contributing to nutrient and energy saving of the plant (Marschner 1995, Hayati et al. 1995).

Conclusions

Leaf senescence and decomposition are important processes for the cycling of nutrients in the salt marsh system (Cornelissen 1996, Rutigliano et al. 1996, Aerts and Caluwe 1997, Mugendi and Nair 1997). Nutrient regulation, particularly nitrogen, is a very important process during plant development (Marschner et al. 1997). This work showed that *Spartina maritima* was capable of adjusting N distribution when growing in low N conditions and also before leaf fall in order to avoid the waste of N and energy, indispensable for plant survival in salt marsh systems. Nitrate and total N content decreased greatly with leaf senescing and before major leaf fall occurred. NH_4^+ was not accumulated in plant cells, and it's concentration increased with leaf ageing, resulting from the normal process of senescence, but most likely to have an important role in controlling such process.

Acknowledgements

The Portuguese Foundation for Science and Technology supported this study (grant: PRAXIS XXI/BM/8091/96). We thank colleagues of IMAR for laboratory assistance, and soil analysis. We also thank Prof. Ivan Valiela (Boston University) for is comments on the manuscript. Thanks are extended to the anonymous reviewer for his comments and suggestions.

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THE ROLE OF *Spartina maritima* AND *Scirpus maritimus* TO SEDIMENT PORE-WATER PROFILES, AND POSSIBLE IMPLICATIONS TO THE MONDEGO ESTUARY NUTRIENT DYNAMICS

Abstract

Previous studies concerning the concentrations of inorganic nutrients in the south arm of the Mondego estuary showed a consistent yearly pattern, with an increase of nitrogen (nitrite, nitrate and ammonia) from summer until early winter, and higher phosphorus concentrations from late spring until summer. In *Spartina* and *Scirpus* salt marshes the nutrients concentrations in low water pools were monitored, and results suggested that *Scirpus* primarily contributes with the nitrate form of nitrogen to the sediment/water phase dynamics, and that *Spartina* had a stronger contribution of ammonia to the nitrogen dynamics. The aim of the present study was to compare the pore-water profiles of the two emergent macrophytes in the south arm of the Mondego estuary, and relate it to the nutrient concentrations in the two salt marshes low water pools. Our results showed statistically significance differences between *Spartina* and *Scirpus* sediment nutrient profiles (nitrate, ammonia and phosphate) (Mann-Whitney test, $p<0.05$), during day and night situations, suggesting that coupling between plant and sediment are species-specific processes. Although the ability to transport oxygen to the belowground parts has been described for both species, where it is used for root respiration and oxidation of the rhizosphere, it seems that the *Scirpus* rhizosphere had higher phosphorus adsorption capacity. Moreover, when comparing the two nutrient profiles *Scirpus* rhizosphere presented higher concentrations of the nitrate form of nitrogen, while *Spartina* rhizosphere presented higher concentrations of the ammonia form of nitrogen. In addition, the specificity of these processes will have repercussions on the sediment/water interface nutrient dynamics, which may be reflected in the entire system nutrient dynamics.

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Introduction

In the south arm of the Mondego estuary (western Portugal) the *Scirpus maritimus* population occupies the inner areas, while *Spartina maritima* occurs in the downstream areas (Fig. 1) following the salinity gradient (Lillebø 2000). These two species differ in their annual dynamics: *Spartina maritima* is a rhizomatous grass, forming extensive monotypic stands (Castellanos et al. 1994, Sanchéz et al. 1997), with a continuous but very slow growth (Pierce 1983 *in* Adams and Bate 1995); *Scirpus maritimus* is a stoutly rhizomatous perennial sedge (Karagatzides and Hutchinson 1991) which usually forms similar dense monospecific stands in shallow brackish marshes (Lieffers and Shay 1982 *in* Charpentier and Stuefer 1999). Moreover, in this species, ramets produce photosynthetic shoots that are active for a single growing season, and only belowground parts persist into the next year (Charpentier and Stuefer 1999, Lillebø 2000). In the Mondego estuary mature phase, with maximum aboveground biomass, occurs in April/May (Lillebø 2000).

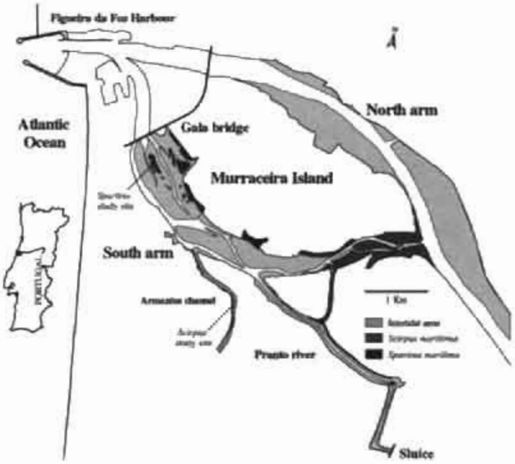

Fig. 1. The location of the *Spartina maritima* and *Scirpus maritimus* salt marshes in the Mondego estuary.

During the last decade the south arm of the Mondego estuary has been almost continuously monitored with respect to inorganic nutrients in the water column (Flindt et al. 1997, Marques et al. 1997, Pardal 1998, Martins 2000, Martins et al. 2001). Throughout a four years study period, from January 93 to January 97, the variation of nitrite and nitrate in the water column followed a similar yearly pattern, usually with higher concentrations in winter and lower concentrations in spring and summer (Fig. 2). Moreover, ammonia concentration represented an important contribution to the total inorganic nitrogen concentration in the system, and tended to increase from summer until autumn and early winter (Fig. 2). Furthermore, during the same study period, the highest concentration of dissolved inorganic phosphorus occurred mostly during summer (Fig. 2). So, these may be considered as the general pattern for inorganic nutrients concentrations in the south arm of the Mondego estuary (Martins et al. 2000).

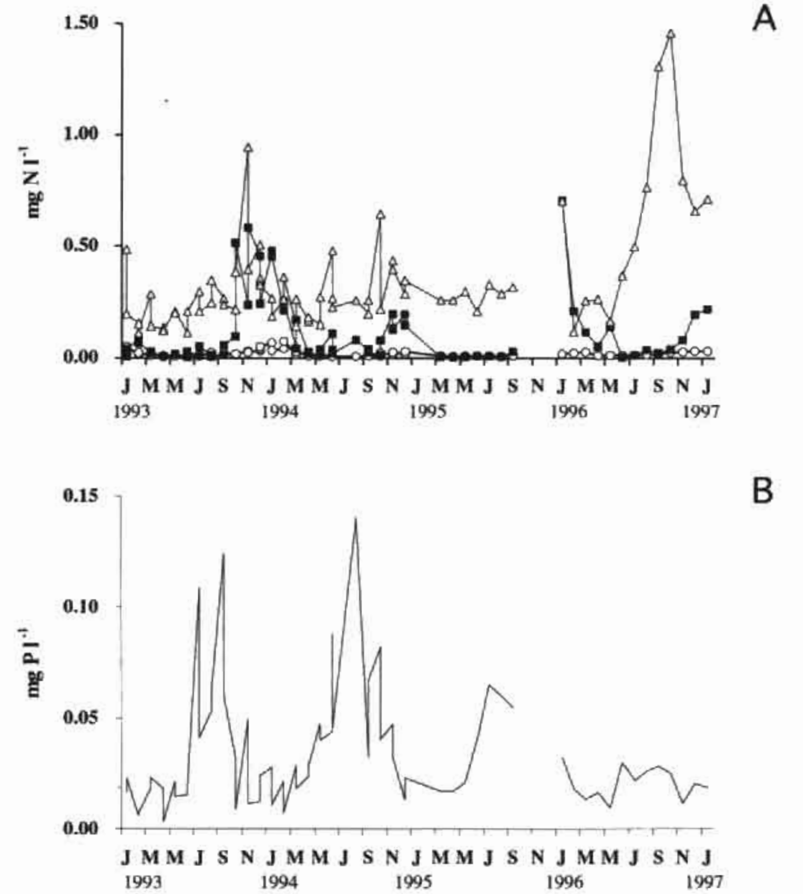

Fig. 2. Variation of dissolved inorganic nutrients in the water column between January 93 and January 97. A: nitrogen (circles: nitrite; squares: nitrate; triangles: ammonia), B: phosphorus.

Salt marshes have been characterised as efficient sinks for nutrients, and buffering of the effects of nutrients inputs (e.g. Anderson et al. 1997, Andersen and Rins 1999). The interaction of roots with the surrounding sediments is exceedingly complex, covering a wide range of biogeochemical processes (Caçador et al. 1996, Wigand et al. 1997). Vegetation may act as sediment traps playing an important role in the settling of suspended matter, and also, plant roots interact with the surrounding sediment (Caçador et al. 1996, 2000, Andersen and Rins 1999, Flindt et al. 1999). Several papers have reported chemical changes in the rhizosphere of several salt marsh plants, including the redox potential (Eh), organic matter, metal availability and also the nutrient profiles (e.g. Kamp-Nielsen and Flindt 1993, Caçador et al. 1996, 2000, Anderson et al. 1997, Wigand et al. 1997, Cartaxana and Lloyd 1999, Flindt et al. 1999). Moreover, it is known, that submersed rooted macrophytes link the nutrients in sediments with the overlying water which may have important implications for nutrient cycling within such systems (e.g. Kamp-Nilsen and Flindt 1993, Valiela 1995, Anderson et al. 1997, Flindt et al. 1999).

The first objective of the present study was to compare the dissolved inorganic nutrients in the low water pools formed in the two salt marsh areas (*Spartina maritima* and *Scirpus maritimus*), with the general yearly pattern described for the system. Furthermore, we also compared the importance of the two salt marsh species to the sediment nutrient profiles, and relate it with the concentrations in the low water polls, in a typical temperate intertidal estuary (Mondego estuary).

Material and methods

In the scope of a field-monitoring program focusing on the nutrient dynamics in the Mondego estuary salt marshes (Fig 1), water and sediment samples were collected during thirteen months, from March 97 to June 98, at monthly intervals (Lillebø 2000). Water samples for nutrient analyses (dissolved inorganic phosphate, ammonia, nitrate and nitrite) were collected in low water pools. All water samples were filtered (GF/F) and kept frozen until analysis. Analyses of dissolved reactive phosphate, ammonia, nitrate and nitrite were also performed according to Standard Methods (1995). Sediment triplicate sub-samples were analysed for dry weight, loss on ignition (8 h, 450 °C), total nitrogen and phosphorus. The granulometry was analysed seasonally and classified according to the nomenclature proposed by Brown and McLachlan (1990). Analyses of sediment total nitrogen and phosphorous were performed according to Standard Methods (1995).

Sediment profiles were studied by placing vertically dialysis chambers in the plants rhizosphere in May because this corresponds to the time where *Scirpus maritimus* reaches the mature phase in the Mondego estuary (for a more detailed description of the technique see Kamp-Nielsen and Flindt 1993). In our case, the water samples were collected with a syringe (2,5 ml, corresponding to each chamber volume), and simultaneously the chamber was filled up with distilled water. To evaluate the effect of plants, samples were taken after a 12 hours exposure under dark conditions (night period) and 12 hours under light conditions (day). Water samples were analysed for dissolved inorganic phosphate, ammonia and nitrate in a rapid flow autoanalyser (RFA 300 Alpkem) and performed according to Alpkem methodologies (Alpkem 1990). A non-parametric Mann-Whitney test was applied to determine the significance of differences (95% confidence level) between the sediment nutrient profiles (Zar 1996).

Results

Sediment properties

In these two salt marshes, sediments consisted mainly of fine particles between 63µm and 125µm in size, corresponding to silt and clay, and do not show a significant seasonal variation (Table I). Sediment water content, during low tide, did not show a clear seasonal variation although it slightly increased during the winter period, probably as a result of higher precipitation, and also lower evaporation due to lower temperatures. Moreover, results do not evidence a spatial variation. The mean percentage of sediment water content (± std) during low tide, at the *Spartina* salt marsh was 60.68 ± 3.5% and 61.12 ± 3.0% at *Scirpus* salt marsh (Lillebø 2000).

Table I. Relative frequency of sediment granulometry classes determined seasonally in *Spartina* and *Scirpus* salt marshes (April, July and October 97, and in January and April, 1998).

Classes (mm)	>0.500 <1.0	>0.250 <0.500	>0.125 <0.250	>0.063 <0.125	>0.063	
Spartina	A-97	1.2	8.1	30.1	**57.2**	3.5
	J-97	0.1	14.1	24.9	**54.9**	5.9
	O-97	2.0	39.5	28.1	**28.6**	1.7
	J-98	0.2	4.5	16.0	**67.7**	11.5
	A-98	0.7	5.1	19.2	**69.5**	5.5
Scirpus	A-97	0.9	8.5	37.0	**50.4**	3.2
	J-97	2.0	9.6	27.4	**54.9**	6.1
	O-97	2.2	27.9	25.1	**41.4**	3.4
	J-98	1.3	4.8	26.7	**60.3**	6.9
	A-98	2.0	24.3	21.7	**49.0**	3.0

The sediment organic matter content in the *Spartina* and *Scirpus* salt marshes was statistically different (Mann-Whitney test, p=0.0047), with lower organic matter content in the *Spartina* salt marsh (Mann-Whitney test, p=0.0023). The *Scirpus* salt marsh did not show a clear seasonal variation (Fig. 3). In the *Spartina* salt marsh there was a slight increase during summer, probably due to the decomposition of green macroalgae (*Enteromorpha* spp. and *Ulva* sp.) that usually occurs in the downstream areas of the estuary (Pardal 1998, Martins et al. 2001).

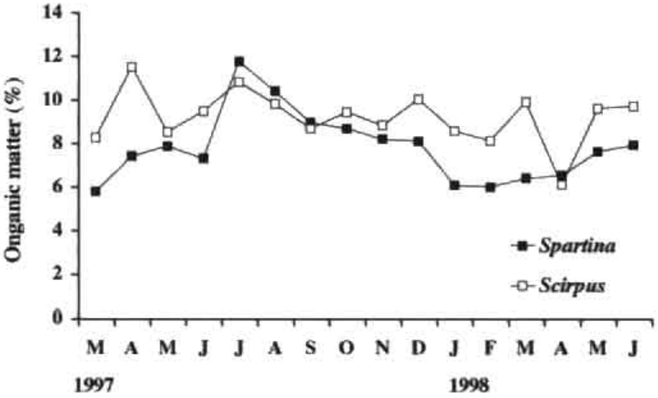

Fig. 3. Seasonal variation of sediment organic matter content in *Spartina* and *Scirpus* salt marshes.

Water nutrient dynamics

As general pattern of dissolved inorganic nutrients in the water column (Martins et al. 2001), phosphorus concentration in the salt marshes low water pools showed an inverse seasonal pattern of nitrogen. In the low water pools nitrate concentrations were much higher than the nitrite concentrations (Fig. 4A). Both study sites showed a clear seasonal variation with an increase on nitrate and nitrite concentrations during winter, following the general pattern for dissolved inorganic nitrogen in the Mondego estuary. Nevertheless, the low water pools formed in *Scirpus* salt marshes presented higher nitrate concentrations than the pools at *Spartina* salt marshes (Fig. 4A), which may reflect the importance of plant coverage to the efflux of nutrients from the sediment to overlaying water.

Ammonia concentrations in the low water pools were plotted together with the total nitrogen in the sediment (mg.g^{-1} sediment dWt) (Fig 4B) in order to compare the dynamics between these two compartments. In this comparison we did not use the total nitrogen in the water (ammonia, nitrate and nitrite), because ammonia is the most representative form of nitrogen in the water column (Fig. 2). Results show that the total nitrogen in the sediment is quite stable all over the study period. *Spartina* salt marsh presented slightly higher nitrogen concentrations in the sediment, but generally lower ammonia concentration in the water (Fig. 4B). Furthermore, the seasonal variation of ammonia concentration in the water is much more evident in the *Spartina* salt marsh, with a higher increase during fall and winter, while the total nitrogen in the sediment increases during late winter and early spring. In the *Scirpus* salt marsh, the ammonia concentration in the water is much more constant, which is not in agreement with the general pattern for ammonia concentrations in the south arm of the Mondego estuary. The total nitrogen in the sediment decreased in November and in March, and was probably related to the plant annual dynamics. New shoots started to emerge in December/January and the plant reaches the mature phase in April/May (Lillebø 2000).

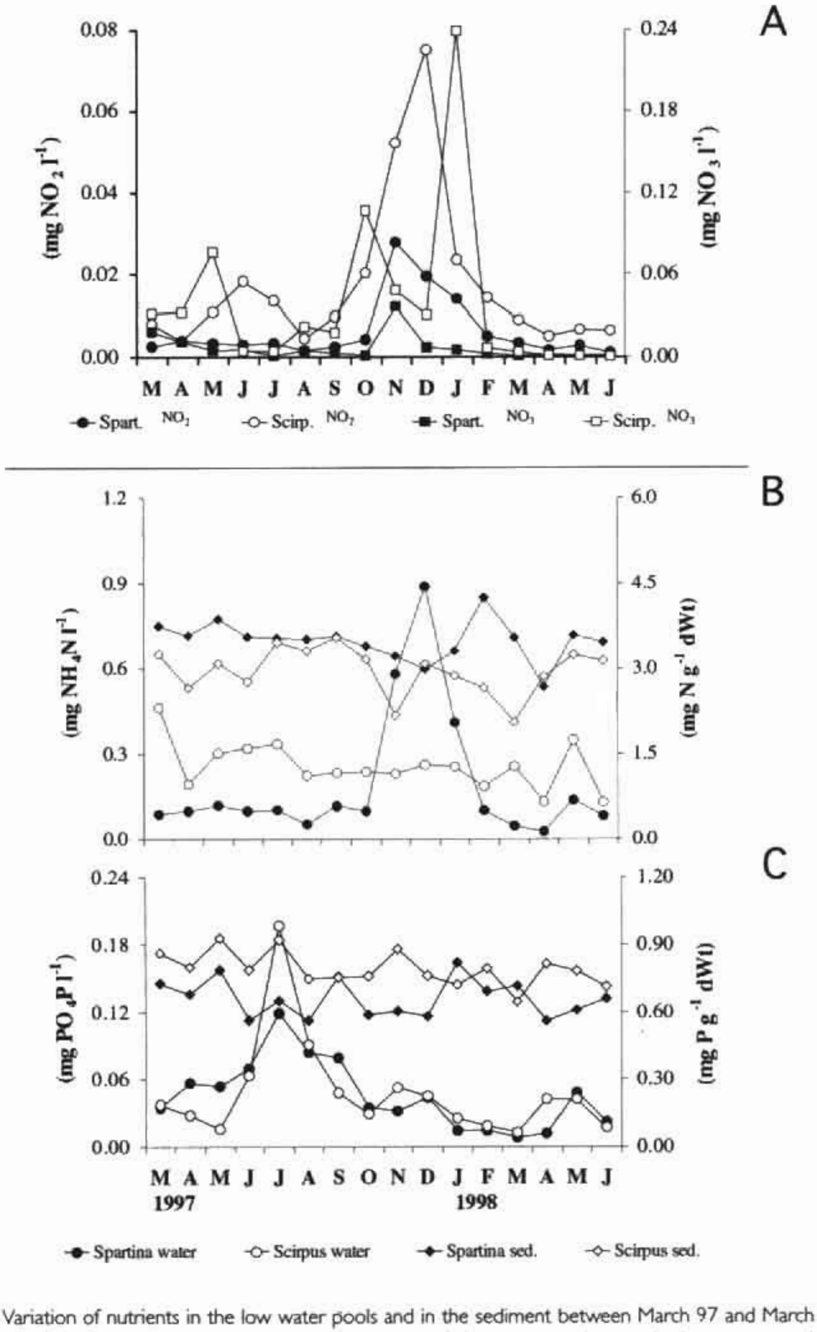

Fig. 4. Variation of nutrients in the low water pools and in the sediment between March 97 and March 98 in *Spartina* and *Scirpus* salt marshes. A: nitrate and nitrite concentrations in the low water pools; B: Nitrogen dynamics in sediment (total N) and in the low water pools (ammonia); C: Phosphorus dynamics in sediment (total P) and in the low water pools (phosphate).

The concentration of dissolved inorganic phosphate concentration in the low water pools was plotted together with the total phosphorus in the sediment ($mg.gl^{-1}$ sediment dWt) in order to compare the dynamics between the plant rhizosphere and the low water pools (Fig. 4C). The downstream areas of the estuary show lower phosphorus content in the sediment, which was quite stable during the study period. In the low water pools phosphate concentration showed a clear seasonal variation, with higher concentrations during summer, which is in agreement with the general pattern for dissolved inorganic phosphate in the south arm of the Mondego estuary. During the summer period, July and August, the low water pools in *Scirpus* salt marsh showed higher phosphate concentration when compared with *Spartina* salt marsh. This increase is probably related to *Scirpus* annual dynamics, due the plants enter the senescent phase in June/July (Lillebø 2000), corresponding to the mineralisation period of the above ground part of the plants.

Sediment nutrient profiles

As hypothesised, the sediment pore-water profiles showed that plant coverage influences differently the nutrient profiles. The non-parametric Mann-Whitney test show statistically significance differences between *Spartina* and *Scirpus* sediment nutrient profiles, namely concerning the NO_3-N profile during the day ($p=0.0000$) and night period ($p=0.0000$), the NH_4-N profiles during day ($p=0.0007$) and night period ($p=0.0016$), and PO_4-P profile during day ($p=0.0000$) and night period ($p=0.0000$).

The nitrate concentration in pore-water is much higher in the *Scirpus* salt marsh, and it is particularly higher during the day and in the first 6 cm of sediment (Fig. 5), below this depth nitrate concentration decreases especially during the night period. This difference between day and night period is statistically significant ($p=0.0017$). In *Spartina* sediments there were no evident differences between day and night situations ($p=0.5591$), and the higher concentration corresponded to the first millimetres during the day period. Moreover, in the *Scirpus* rhizosphere, the nitrate concentration in the first 0.3 millimetres in depth (which correspond to the sediment/water interface) was in the same range as the concentrations recorded in the low water pools. On the other hand, in *Spartina* rhizosphere the nitrate concentration was ten times lower.

The sediment pore-water profiles for ammonia concentration in *Spartina* and *Scirpus* marshes showed a different dynamics, which is evident in the first 4-5 cm in depth (Fig. 6). *Spartina* rhizosphere had much higher ammonia concentration in the first 10 cm. From the 10 cm to 20 cm, *Scirpus* rhizosphere showed an increase of ammonia concentration, although *Spartina* rhizosphere seems more stable (Fig. 6). Furthermore, in *Spartina* rhizosphere, ammonia concentration in the first 0.3 millimetres in depth was much higher than in the low water pools, while in *Scirpus* rhizosphere ammonia concentration is much lower. There were no statistically differences between the ammonia profiles in day and night situations in *Spartina* rhizosphere ($p=0.5591$) or in *Scirpus* rhizosphere ($p=0.6785$).

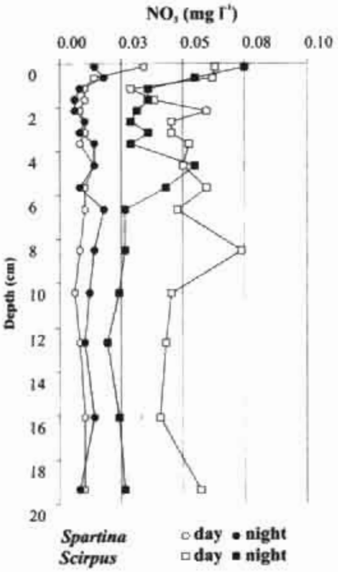

Fig. 5. Nitrate concentration in sediment pore-water profiles in *Spartina* and *Scirpus* salt marshes.

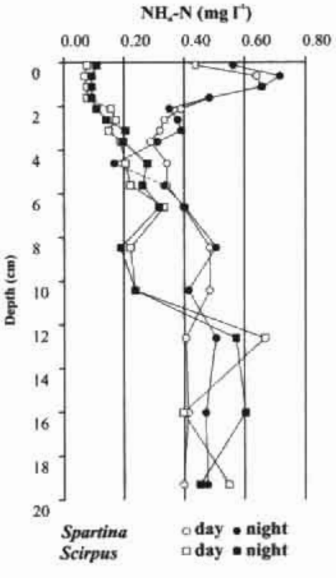

Fig. 6. Ammonia concentration in sediment pore-water profiles in *Spartina* and *Scirpus* salt marshes.

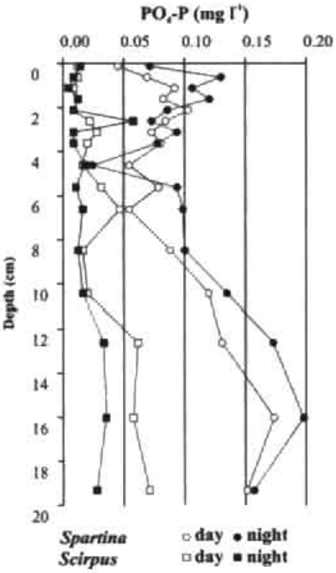

Fig 7. Phosphate concentration in sediment pore-water profiles in *Spartina* and *Scirpus* salt marshes.

Phosphate concentration in *Scirpus* pore-water rhizosphere was lower than the *Spartina*, and very constant during the night period (Fig. 7). During the day, phosphate concentrations in the *Scirpus* pore-water increases, especially between 10 and 20 cm in depth, although day and night situations are not statistically different ($p=0.1092$), and were always much lower than in *Spartina* rhizosphere. The *Spartina* rhizosphere showed a more dynamic phosphate profile, with an increase concentration with depth, especially after the first 10 cm. Phosphate concentrations were always higher during the night period, although day and night situations were not statistically different ($p=0.1689$). Moreover, in the *Spartina* rhizosphere, phosphate concentrations in the first 0.3 millimetres in depth was in the same range as concentrations in the low water pools (Fig. 4), while in *Scirpus* rhizosphere phosphate concentration was much lower.

Discussion

Results concerning the field-monitoring program in the Mondego estuary showed that *Scirpus maritimus* salt marsh presented higher organic matter content in the sediment when compared to the *Spartina maritima* salt marsh. This may result from differences in particulate organic matter settling rates within the estuary, because water residence time is higher in the inner areas of the estuary (Duarte et al. in press). The

Spartina salt marshes occur in the downstream areas while *Scirpus* in the inner areas of the estuary. These may also explain why the total phosphorus concentration in the sediment is higher in the *Scirpus* salt marsh (Day et al. 1989). The sediment total nitrogen is lower in *Scirpus* salt marsh, and especially during *Scirpus* growing season, which corresponds to the period from December/January until April/May (Lillebø 2000). In salt marshes plants rhizosphere bacteria fix part of the total nitrogen, but the nitrogenase activity tends to be suppressed by the presence of exogenic nitrates and oxygen (Carter 1988), like in the *Scirpus* rhizosphere.

Differences in nutrient concentrations in the pore-water profiles seem to be related to the plants dynamics, and generally the interaction between plants and the surrounding sediment increases during the growing season, and decreases during winter (Ernst 1990, Otte 1991 *all in* Caçador et al. 2000). Due to that, the uptake of nutrients is coupled to the photosynthethic rate, and follows the same pattern as photosynthesis at different light intensities (Valiela 1995).

Spartina is an alternative type (C4) of photosynthetic mechanism (Carter 1988), or just a (C4) type (Benito and Onaindia 1991), while *Scirpus* is a C3 type (Boschker et al. 1999). It has been discussed that plants with C4 photosynthetic pathway have, at least theoretically, a number of competitive advantages over C3 species (Adam 1990), namely a higher potential productivity, higher water-use efficiency and also a more efficient use of available nitrogen (Adam 1990). The form of inorganic nitrogen will be dependent upon the oxic state of the sediment (e.g. Nixon and Pilson 1983, Valiela 1995, Cowan et al. 1996). According to Adam's review (1990), although there is a likely predominance of ammonium over much of the marshes, measurements of nitrate reductase in several species indicated that nitrate was the major available nitrogen source. The same author identified two possibilities, either the heterogeneity of the environment provided aerobic micro sites with available nitrate, or rhizosphere oxidation mitigated the prevailing ammonium. Moreover, it was found a declining gradient from seaward to landward in both nitrogen redutase and tissue nitrogen concentrations in *Spartina*, as well as in other species (Adam 1990). These may explain the fact that *Scirpus* rhizosphere presented higher concentrations of the nitrate form of nitrogen, while *Spartina* rhizosphere presented higher concentrations of the ammonia form of nitrogen. Furthermore, in the *Scirpus* rhizosphere, nitrate concentrations in the first millimetres in depth, was in the same range as the concentrations recorded in the low water pools, while in the *Spartina* rhizosphere it was ten times lower. In addition, the concentration of ammonia was much higher in the *Spartina* pore-water profiles and was in the same range as the concentrations recorded in the low water pools.

The yearly pattern of dissolved inorganic nutrients in the water column, in the south arm of the Mondego estuary, showed an increased concentration of nitrogen (nitrite, nitrate and ammonia) from summer until early winter, in which ammonia was the dominant form of nitrogen (Martins et al. 2001), which is also the pattern frequently found in temperate estuaries (Nixon and Pilson 1993). Our results concerning the nutrients concentrations in the *Spartina* and *Scirpus* salt marshes low water pools showed differences in nitrate and ammonia dynamics, which may represent a different contribution from each type of salt marsh sediment/water phase to the overlaying water. In fact these results suggest that the *Spartina* salt marsh may contribute to the nitrogen pool in the water column by ammonia efflux process, while *Scirpus* salt marshes may contribute by nitrate efflux process.

Both species have the ability to transport oxygen to the belowground parts, where it is used for root respiration and oxidation of the rhizosphere (Teal and Kanwisher 1966, Bertness 1991 *all in* Adams and Bate 1995, Armstrong and Armstrong 1988, Laan and Blom 1990 *all in* Cleavering et al. 1995). This process may lead to the precipitation of iron oxides around the roots (Vale et al. 1990), and eventually increase the adsorption capacity of the sediment. In our results, although, *Scirpus* sediment rhizosphere had higher total phosphorus concentration, the pore-water profiles show that in May the phosphate concentration was much lower than in *Spartina* salt marsh. Moreover, in *Spartina* rhizosphere, phosphate concentration in the first millimetres in depth was in the same range of concentrations found in the low water pools, while in the *Scirpus* rhizosphere phosphate concentration was much lower. These results suggest that the *Scirpus* rhizosphere may have a stronger adsorption capacity, at least during the mature phase.

Vale et al. (1990) described the formation of metal-enriched concretions around the roots of *Spartina maritima* in the Tagus estuary, and suggests that this process is probably related to the increased oxygen in the rhizosphere. In the Mondego estuary salt marshes, we observe a thin layer of iron oxides surrounding only the *Scirpus maritimus* rhizomes (personal observation). Besides, under anoxic conditions, Fe^{3+} hydroxides are reduced to Fe^{2+} ions and phosphate is released to solution, while under more oxygenated conditions, like the ones surrounding plants rhizosphere, the ferrous iron is precipitated again as ferric hydroxide, which removes dissolved phosphate by readsorption (Berner and Berner 1996). This chemical reaction may explain the differences between phosphate concentrations in *Spartina* and *Scirpus* rhizosphere. Additionally, in May, *Scirpus* was still in the growing season, and so phosphate uptake from the sediment pore-water could also be more intense. Therefore, this may also be the result of modifying the chemistry of the rhizosphere during *Scirpus* growing season. Moreover, during the summer period, which corresponds to the senescent phase of the plant, the efflux of phosphorus from the sediment seems to be an important contribution to the concentrations in the low water pools. Also, a study performed in Chesapeak Bay (USA) (Wigand et al. 1997), showed that macrophyte species composition influence the pore-water phosphate and solid phase phosphorus and metal levels. One of the possible explanations, pointed out by these authors, were the differences in root oxygenation capabilities.

Our results reinforce the general idea that salt marsh plants regulates the fluxes of dissolved inorganic nutrients between the sediment surface and overlying water, suggesting that coupling between plant and sediment is a species-specific process.

Results obtained from this study can conduct to a more comprehensive and environmentally correct understand of estuarine systems functioning. The knowledge acquired may be of particularly importance on management programs due to excess of nutrient inputs into estuarine systems.

Acknowledgements

This study was carried out in the scope of the Praxis XXI program (Portugal) through a PhD grant (BD/ 9290/ 96).

The authors would like to thank the Freshwater Biological Laboratory, University of Copenhagen, Denmark, for the support to this study.

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SHREDDERS IN LOW ORDER STREAMS

Abstract

In low order streams the riparian vegetation canopy generally restricts light reaching the river bed and therefore primary production. Riparian vegetation also provides streams with organic matter that is decomposed by the joint action of fungi and invertebrates. The trichoptera *Sericostoma vittatum* Rambur and the diptera *Tipulla lateralis* Meig. are two common shredders present in Ribeira de S. João, a 4th order river, and at other local streams. In this chapter we integrate results of several publications (Canhoto and Graça 1995, Feio and Graça 2000, Graça et al. 2001) aiming to know important aspects of their trophic ecology: consumption rates, preferences among leaf types, importance of leaf conditioning and activity patterns.

Introduction

In low order streams the riparian vegetation canopy generally restricts light reaching the river bed and therefore primary production. On the other hand, riparian vegetation provides the stream with organic matter such as leaves, branches and berries, depending on the season (Triska et al. 1982). Consequently allochtonous organic matter is the main energy source for low order streams running through forests.

After falling into the water, leaves are leached out of soluble compounds, colonised and decomposed by microorganisms and eaten by aquatic invertebrates (Gessner et al. 1999). Animals feeding on this organic matter are known as "detritivores" and can be classified in sub-groups according to the feeding mechanism: shredders, collectors, filters and gatherers (Cummins and Klug 1979). Shredders actively feed on large particles of organic matter such as entire leaves, incorporating organic matter into secondary production and accelerating the

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decomposition (Webster and Benfield 1986, Gessner et al. 1999, Graça 2001). They are particularly abundant in low order streams and provide a trophic link between the head waters and lower stream sections by the transformation of large particles into fine particulate organic matter which can be used as food by collector-gathers and filter-feeders further downstream (Vannote et al. 1980, Heard and Richardson 1995).

Forests in the Centre of Portugal have been recently subjected to major changes with the replacement of pine and deciduous species by *Eucalyptus globlus* Labill.. This affects the dynamic of organic matter and consequently has the potential to influence the structure of aquatic communities (Abelho and Graça 1996, 1998, Pozo et al. 1997).

Feeding activities of shredders may be affected by the nutritional value of leaves (Irons III et al. 1988) and microbial colonisation (conditioning) (Golladay et al. 1983; Rounick and Winterbourn 1983, Bärlocher 1985, Graça et al. 1993, Graça 1993). Given the importance of leaf stream invertebrates changes in the riparian vegetation have the potential to affect performances of shredders and the dynamic of organic matter (Canhoto and Graça 1995).

In Mondego basin catchment area, studies on the ecology of shredders have been carried out at Ribeira de S. João, Serra da Lousã (4th order stream, catchment area 18 km^2, mean bank full width in the lower section 6.8 m, mean depth 0.35m; mean flow 8.9×10^{-2} m^3s^{-1}, pH 7.3, temperature 12.4 °C, O_2 96% saturated; Canhoto and Graça 1995).

Two common shredder species have been selected for experimental work at the Dep. of Zoology, University of Coimbra: the cadisfly *Sericostoma vittatum* (Thrichoptera: Sericostomatidae) and the cranefly *Tipula lateralis* (Diptera: Tipulidae). In this chapter we describe experiments aimed to get information on their growth, feeding rates and food preferences. This chapter summarises information published by Canhoto and Graça 1995, Feio and Graça 2000 and Graça et al. 2001.

Material and Methods

Experimental conditions

Experimental animals were captured in the location of "Piscinas", Lousã. In the laboratory the animals were kept in plastic boxes containing filtered aerated stream water (GF/C Whatman) or APW (Ca: 80 mg l^{-1}, Cl: 145 mg l^{-1}, Mg: 12 mg l^{-1}, Na: 18 mg l^{-1}, K: 3 mg l^{-1}, pH 7.9) and ignited stream sand (<1mm; 500°C for 1h). A photoperiod was set to 12:12 hours and temperature to 15 °C. Senescent leaves from tree species were collected in autumn and air dried. For conditioning leaves were placed inside bags (10x14 cm; 0.5 mesh size) and submerged in the river for 3 weeks. When unconditioned leaves were offered as food they were previously leached under tap running water for 24 hours.

Consumption and selectivity

To quantify food consumption, disks were cut from leaves with a cork borer (9 mm ø for *Sericostoma vittatum* and 14 mm ø for *Tipula lateralis*). Consumption was measured as the difference between the initial and final mass of disks. In experiments with *Tippula lateralis* initial mass was obtained by weighting the leaf disks previously dried at 50 °C for 48h; the disks were then re-hydrated and offered to the shredders. In the case of experiments with *Sericostoma vittatum* pairs of disks were obtained from contiguous areas of the leaves. The disks were assumed to have identical mass. One disc was exposed to the invertebrate whereas the other disk was hanged inside the same cup but protected from the invertebrate by a bag (0.5 mesh size). Invertebrates were allowed to fed until ≈ 2/3 of the offered food was consumed. Consumption was expressed as the difference between the unexposed and exposed discs, divided by the elapsed time in days and the initial mass of the experimental animals. When more than one leaf type was exposed to the invertebrates they were marked with a coloured pin. For more details of the experimental designs see Canhoto and Graça 1995, Feio and Graça 2000.

This design was used to determine: (a) Feeding rates of *Sericostoma vittatum* (0.81-0.19 mg initial dry mass; n=20) and *Tipula lateralis* (9.27-10.81 mg initial dry mass; n=30). (b) Selection among conditioned and unconditioned *Castanea sativa* Mill. leaves by *Sericostoma vittatum*. c) Selection among leaves of 4 tree species differing in their quality: *Alnus glutinosa* L., *Castanea sativa*, *Quercus faginea* Lam. and *Eucalyptus globulus*.

Influence of food quality on growth

Eighty individuals of *Tipula lateralis* (0.65 – 0.93 mg dry mass) were individually allocated to rearing cups (with 200 ml of water) containing leaves of alder, oak, chestnut or eucalyptus. The leaves were previously conditioned for 2 weeks. The experiment lasted for 126 days. Food, water and sand was changed weekly when animals were weighted. Dry mass (Dma) of larvae was estimated from their wet mass (Wma) by the expression (r^2=0.985):

$\log_e \mathrm{Dma} = [(\log_e \mathrm{Wma} - 0.0596) / 1.0837]$ (Canhoto and Graça 1995)

Patterns of activity and metabolism

Larvae of the species *Sericostoma personattum* are known for their higher nocturnal than diurnal activity (Wagner 1990). *Sericostoma vittatum* is an Iberian endemic species and no equivalent data was available.

A total of 23 *Sericostoma vittatum* individuals were allocated into 3 plastic containers (27 × 15 × 10 cm; containing 1.5 L of water) in the laboratory. The number of active animals in each container was registered during darkness and during light periods with intervals of 10 minutes (n=7 for each night and day periods).

Assuming that respiratory rates are indicators of activity, ten *Sericostoma vittatum* larvae were selected to measure oxygen consumption during dark (n=5) and light periods (n=5). The animals were individually placed in 8 ml chambers (glass syringes) to which oxygen saturated water was pumped (through-flow: 5-8 ml/h). The difference in oxygen between incoming (O_2i) and outcoming (O_2o) water from the syringes was attributed to the invertebrate respiration (see Graça 1990). The dry mass (Dma) of each experimental animal was determined and a mean respiratory rate (RR) was calculated for each animal for light and dark using the expression:

$$RR = [\ (O_2i) - (O_2i) \times F] / Dma$$ (Feio and Graça 2000)

Results

Consumption rates of conditioned leaves of *Castanea sativa* by *Sericostoma vittatum* were 0.47 ± 0.42 mg mg animal^{-1} day^{-1}, while the consumption registered for *Tipulla lateralis* was 0.02 ± 0.006 mg mg animal^{-1} day^{-1}. When given the choice, larvae of *Sericostoma vittatum* feed preferentially upon conditioned leaf disks, almost ignoring unconditioned disks (t = 2.464, d.f = 30; $p<0.05$) (Fig. 1).

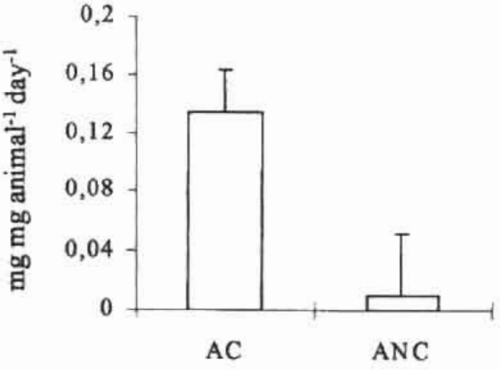

Figure 1. Consumption of *Alnus glutinosa* leaves by *Sericostoma vittatum* when exposed to conditioned (AC) and unconditioned (ANC) leaves (mean+s.e.).

In the multiple selection tests, *Tipula lateralis*, larvae prefered leaves of *Alnus glutinosa*. Consumption on leaves of *Castanea sativa*, *Quercus robur* and *Eucalyptus globulus* was significantly lower (F= 70.06, $p<0.001$; Tuckey test: $p<0.05$; Fig.2).

Differences in consumption rates were consistent with growth when only one leaf type was available. Growth followed a logistic curve when larvae were fed with alder ($y = -12.99+15.85 \log x$; $r^2=0.93$) and presented the fastest mean specific growth rate (7.23% day; Fig. 3). Individuals fed with chestnut grew at a slower rate ($y = -9.83+8.71$; r2=0.78; specific growth rate = 3.01% day^{-1}; Fig.3). Differences in growth were significant (t=9.02; $p<0.001$). No growth occurred in larvae fed with oak or eucalyptus and all specimens died after 63 and 91 days, respectively. For individuals fed with alder,

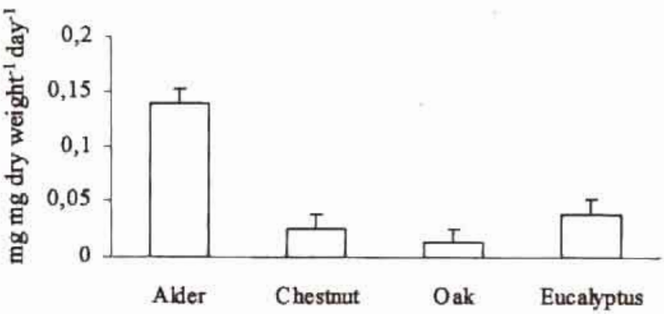

Figure 2. Food preferences of *Tipula lateralis* when leaves of *Alnus glutinosa*, *Castanea sativa*, *Quercus faginea* and *Eucalyptus globulus* were offered (mean+95%CL).

the survival was 90% and for chestnut 80% (logrank statistic > 10.83; p>0.05). There was therefore a tendency for *Tipula lateralis* to select leaves promoting high growth.

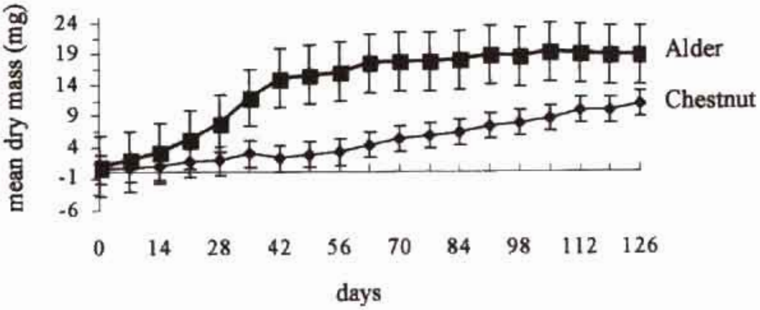

Figure 3. Growth of *Tipula lateralis* larvae fed with *Alnus glutinosa* or with *Castanea sativa* leaves during 126 days (mean±95%CL).

Active *Sericostoma vittatum* larvae were significantly more frequent during the light than during the dark periods (t=8.18; df=12; p<0.001; Fig.4). Consistently respiratory rates increased from 0.46-2.91 μg O_2 h^{-1} mg animal^{-1} during the day to 0.95-4.49 μg O_2 h^{-1} mg animal^{-1} during the night (paired t-test: t=5.56; df=6; p<0.001; Fig.5).

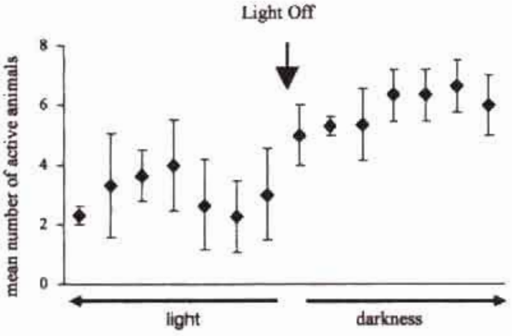

Figure 4. Number of active animals during day and night measured at 10 minutes intervals (mean±s.e.).

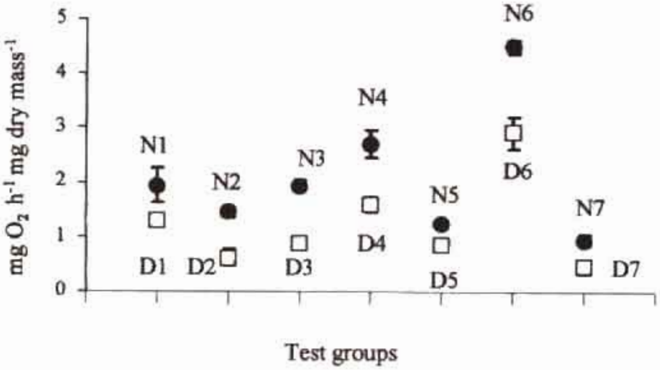

Figure 5. Nocturnal (N1-N7) and diurnal respiratory rates (D1-D7) for each specimens of *Sericostoma vittatum* (mean±s.e.).

Discussion

The experiments here reported using stream species from Mondego river Basin reinforce some known facts about the detritivores in general, reported for other geographic areas: (1) Some stream invertebrates feed on decomposing allochtonous organic matter. (2) Stream invertebrates of the same species and similar sizes can feed at different rates. (3) They prefer conditioned to unconditioned leaves. (4) They discriminate among leaf types. (5) Selected leaves are the ones promoting high growth and survivorship. (6) Some detritivores are more active during the night.

Larvae of *Sericostoma vittatum* are strong food processors when compared with the larger *Tipulla lateralis*, a diptera larvae that reaches greater sizes. Considering the high densities of the trichoptera (annual mean of 25 individuals m^{-2} for Ribeira de S. João) and the presented values for leaf consumption, we consider this species as having a key role on the fragmentation of allochtonous organic matter in Ribeira de S. João.

The importance of alder leaves as a food resource for shredders is also here reinforced (see also Kaushik and Hynes 1971, Iversen 1974, Irons et al. 1988). This riparian species produce leaves that are rapidly colonised and consumed by aquatic shredder detritivores. This is probably due the higher intrinsic content of nitrogen of the alder leaves along with the low levels of refractory compounds, absence of leaf protecting structures and reduced hardness (Nykvist 1962, Mellilo 1982, Cortes et al. 1994, Pereira 1998). Other authors have reported that alder leaves become rapidly conditioned and are probably more palate and easy to digest by larvae (Martin et al. 1980, Bärlocher 1985). This information underlines the link between stream systems and terrestrial systems and has important implications for the management of riparian zone.

Microbial colonisation enriches the nutrient content of leaves and may result in a decreased hardness (Suberkropp 1992, Graça et al. 1993). Once more this was verified in our tests with the obvious preference of *Sericostoma vittatum* individuals for conditioned leaves when faced with the possibility to eat unconditioned material. The implication is that the river health has to take in consideration factors affecting decomposing microbes, a subject normally underestimated in the rivers management.

Nocturnal behaviour of detritivores is understand as a predator avoidance mechanism (Elliot 1969, Wagner 1990) and it was also suggested that could also be a way of avoiding the competition for food since when the temperatures become higher (day) search for food also increases (Wagner 1990). These are probable explanations for the clear nocturnal behaviour of *Sericostoma vittatum*. The low mobility of these animals may force them to do their food search and the observed relative long distance displacements during the night, period when most of the predators (other insects, fishes or ducks) rest or can not easily detect them.

Leaves are a primordial food source for stream invertebrates. Therefore the knowledge of the stream ecology is strongly dependent on the knowledge of the interactions of the trilogy: leaves, microbes and invertebrates. This is still a wide and complex study field.

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CRAYFISH *Procambarus clarkii* (GIRARD) IN THE LOWER MONDEGO RIVER VALLEY

Abstract

Crayfish *Procambarus clarkii* populations in the Lower Mondego River valley, were studied. The main recruitment period occurs from the second half of October to the end of November, but juveniles are found throughout the year. Average growth rate is 1.16 mm of total length per week. Production was estimated at 27.22 $g.m^{-2}$ per year, average biomass 5.4 $g.m^{-2}$ and the $P/\overline{B}$ ratio 5.03. Females outnumber males throughout the year. Reproductively active (Form I) males are abundant from May to October. Fulton's condition factor was determined for individual crayfish. Condition is dependent on sex and reproductive state, but the seasonal pattern of variation is similar for females, for all males, for reproductive males and for non-reproductive males (Form II). From December to May there is a gradual decrease in condition, ending as a steep decrease. This may be explained by a mass molting to reproductive forms. A post-reproductive decrease in condition is observed in September and October. Our data corroborate the idea that there is a relation between male condition and reproductive activity.

Introduction

The introduction of alien freshwater crayfishes in new habitats, mainly as food value, has been practiced since 1746 (Hobbs et al. 1989), and the case of *Procambarus clarkii* (Girard), a species from northeastern Mexico and the southern U.S.A., is undoubtedly a good example to illustrate the problems arising from an uncontrolled introduction of alien species (Holdich 1988). In general, this species revealed a high adaptive capacity to new available habitats, showing good tolerance to a wide range of environmental conditions (Hobbs et al. 1989). A high growth rate (Huner 1978) associated with a successful reproductive strategy is responsible for the development

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of extremely large populations, which can have a severe negative impact on agriculture areas in wetlands (e. g. rice) (Ocete and Gallego 1985). To prevent damage to rice production, farmers have repeatedly tried to eradicate crayfish populations by means of xenobiotic chemicals. Such methods proved to be ineffective and had a devastating impact on useful species (Velez 1980, Roqueplo and Hureaux 1989). In any case, once introduced into favourable habitats, *Procambarus clarkii* is rather difficult to eliminate (Holdich 1988).

The reproductive period of *Procambarus clarkii* is dependent on both environmental (e. g. climate) and endogenous physiological factors (Sommer 1984), and therefore, due to the combined effects of these factors, it may change after the species is introduced into different regions. These geographical variations are apparently a function of the hydrological cycle and temperature. For instance, a short arid period interchanging with a prolonged rainy season will increase the active period of the species, causing the development of dense populations with high growth rates, while the opposite situation will decrease the reproductive period. Nevertheless, alternating rainy and dry seasons do not fit precisely with reproductive and sexually inactive periods (Sommer 1984). On the other hand, the influence of water temperature is obvious and, at decreasing latitudes, *Procambarus clarkii* populations tend to change from univoltin to multivoltin life cycles (Huner 1981).

The growth process of *Procambarus clarkii* (Girard 1852) leads mostly to an increase in size, while sexual maturation causes allometric modifications (Romaire et al. 1977, Huner et al. 1988, Correia 1990). These morphological changes coexist with internal changes, which may be helpful to assess the condition of the population. The hepatopancreas is the main energy storage organ in crayfish (Armitage et al. 1972) and energy, moisture content, and the weight of this organ change during crayfish biological cycles (Armitage et al. 1972, Huner et al. 1985, Lahti 1988, Huner 1995, Viikinkoski et al. 1995). Thus, hepatopancreas analysis may be useful to assess crayfish condition, but alternative processes include measures of relative muscle weight and condition indices. These indices provide no information on the state of internal organs or body composition and can also be biased by the molt stage (Lindqvist and Lahti 1983, Musgrove and Geddes 1995). Nevertheless, the main advantages of a condition factor are its simplicity, and the fact that no animals are sacrificed during the process. The effects of season, sex or reproductive state can thus be identified with a small effort. Moreover, the possibility of the use of a condition factor by crayfish farmers should not be excluded. It may provide them a simple tool to diagnose crayfish condition.

The purpose of this synthesis of results included in the publications Anastácio 1993 and Anastácio et al. 1995 is to describe and understand the bioecology of *P. clarkii* populations in the lower Mondego river valley. This type of data will be useful for the management of crayfish populations.

Materials and methods

Study sites:

Three sampling sites were chosen in typical freshwater habitats for crayfish, a rice field, a swamp drainage channel and an agriculture drainage channel, respectively (Fig 1). The rice field was subjected to manipulation of water level and sampling was not possible during a considerable part of the year due to very low water levels. Water was always present in the swamp drainage channel although levels varied throughout the year. Reeds *(Phragmites australis)* were quite abundant on the banks. In the agriculture drainage channel, located in the perimeter of an agricultural area, there was a slight water flow. As a result this channel was similar to a lotic system.

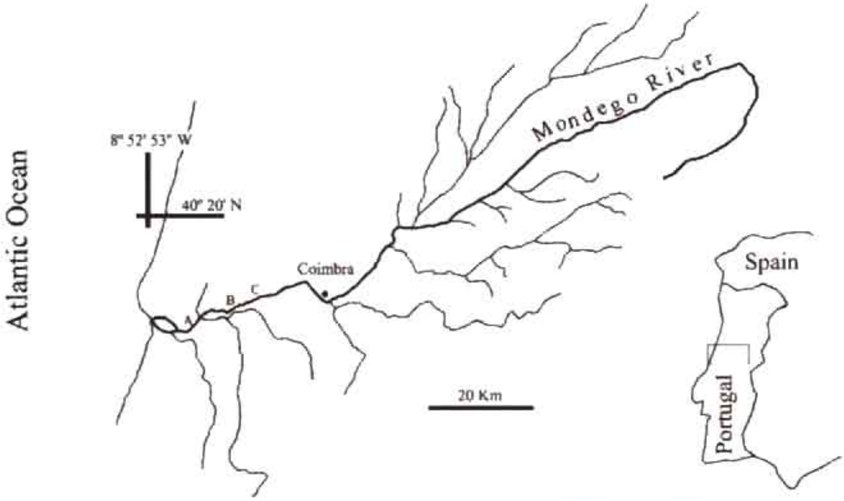

Fig. 1. Location of the sampling areas on the Lower Mondego river valley. A. rice field; B. swamp drainage chanel; C. agriculture drainage chanel.

Sampling

There were three sampling programs, and the first was carried out in all three sampling sites from October 1991 to December 1992. A fortnightly periodicity was used during the most important recruitment period and a monthly periodicity whenever recruitment was negligible. Crayfish were collected with a 1mm-mesh handnet, samples being taken from areas delimited with nets. The sampled areas corresponded to 9 m^2 in the rice field and ranged from 6 to 12 m^2 in the other two sites. In all cases sampling was carried out until no more crayfish could be caught. At each site three replicates were taken randomly using this technique. For each date and at each sampling site, temperature, dissolved oxygen (percentage of saturation) and pH were measured.

The second sampling program was carried out in the agriculture drainage channel and covered the period from June 1992 to February 1993, corresponding to one reproduction and recruitment period. Twenty-five baited Swedish type traps (20 mm mesh) were set overnight on a fortnightly basis and checked in the morning. The traps were spaced 5 m apart and baited with sardines (*Sardina pilchardus* Walbaum, 1792). Crayfish were sexed, weighed and post orbital carapace length (POCL) was measured. All crayfish were released.

The third sampling program was also carried out in the agriculture drainage channel and covered the period from May 1993 to September 1994. Due to the low number of adult crayfish obtained in the 2nd sampling program the sampling strategy was modified. Samples were collected on a monthly basis and traps were set for 3 consecutive nights and checked every morning. The same procedures as those of the previous sampling program were followed but in addition the reproductive state of males was determined. Form I males (reproductive) were identified based on the presence of copulatory hooks (Suko 1953, Taketomi et al. 1990).

Laboratory procedures (first sampling program)

Samples obtained in the first sampling period were preserved in neutralised 4% formalin and organisms were separated in the laboratory after washing with water.

Postorbital carapace length (POCL), dry weight and wet weight were determined for each animal. Crayfish smaller than 1cm (POCL) were measured using a dissecting microscope with a calibrated micrometer. The other animals were measured with a vernier caliper. Measurements were made to the nearest 0.005cm and 0.015cm, respectively. POCL proved to be better correlated with total length than full carapace length (CL). In order to compare our results with other works, which often use CL, the following regression model can be used to convert POCL into full carapace length (CL): $CL = 0.149355 + 1.20916\ POCL$ ($n=941$; $r^2=0.943624$), (Adão 1991)

Wet weight was determined following the Ulomski method, described by Winberg (1971) and the dry weight was determined after drying in an oven at 60°C for 7 days. Ash free dry weight was determined after reducing dried crayfish to powder, which was dried again, weighed, burned in a muffle furnace at 450°C for 9 hours and weighed again. Weight determinations were carried out with a 10^{-5}g precision.

Animals were sexed by the presence (in males) or absence (in females) of developed gonopodia, which was usually applicable for individuals larger than 1cm (POCL). Form I males (reproductive) were identified based on the presence of copulatory hooks (Taketomi et al. 1990) and on the hardness and colour of the gonopodia.

Growth and mortality rates (first sampling program)

Growth and mortality rates were estimated by tracking recognisable cohorts along size-frequency distributions (2 mm length classes) at successive sample dates. Size-frequency analysis was executed by using the probability paper method (Harding 1949) as performed by Cassie (1954, 1963). Reliability was tested employing both X^2 and G tests ($P<0.05$) (Fisher 1950, Sokal and Rohlf 1981). Computations were performed using ANAMOD software (Nogueira 1992). Normally, growth rates are not constant through the year, therefore, seasonal variations were taken into consideration using a model proposed by Gaschütz et al. (1980, see Anastácio and Marques 1995 for details).

Production and Average Biomass estimates (first sampling program)

Production was estimated using Allen curves (Peer 1970, Wildish and Peer 1981). The production estimate for each cohort was achieved by determining the average wet weight of the animals of a given cohort. Density was then plotted against time and the same was done with regard to the weight. A new data set was then obtained by the adjustment of curves to both plots. The final step was the plot of density against average wet weight for each sampling date, where cohort production was given by the integral of the curve adjusted to this plot.

The average biomass was determined by the ratio of the biomass integral within a given time interval and the time interval (Allen 1971). The biomass of a cohort in a given time is the product of individual average weight and the density of the cohort. Total biomass was calculated by the sum of the biomasses of all cohorts tracked in a given instant. Average biomass was determined after fitting a 4th order polynomial function to data.

Condition factor (second and third sampling programs)

For each crayfish captured Fulton's condition factor (Ricker 1975) was calculated by the formula: Weight/Length^3. Wet weight and POCL were used in this formula. Average condition factors were calculated for each sampling date.

Data were tested for correlations of POCL vs. condition. The analysis was performed using individual crayfish values, and also daily averages. Correlation was also determined for the relative abundance of Form I males vs. the average condition factor of the males. Pearson's product-moment correlation coefficient (Zar 1984) was used for all the correlations. A one-way ANOVA (Zar 1984) was performed with data from the third sampling program. The objective was to determine if there were differences in the average condition of the following population sub-groups: females, Form I males, Form II males, and all males.

Results

Physicochemical data

With the exception of the temperature, a pattern of variation could not be recognised throughout the year. The main difference between temperatures at the three sites was the higher annual variation at the rice field (R.F.). Temperatures ranged from 5°C to over 30°C. Median oxygen saturation were higher and more variable at the agriculture drainage channel (A.D.C.), usually ranging from 40% to 180%. The highest values of pH were found at A.D.C. with a median value of approximately 7.5. pH values ranged between 6.3 and 8.5. In general terms physicochemical characteristics were not very different at the three sampling sites.

1st sampling program

Population structure

Seven cohorts were tracked for variable periods of time (Fig. 2). Cohorts 1, 2 and 3 were recognised on 26 October, cohort 4 was identified on 18 January, cohort 5 on 19 August, cohort 6 on 25 September and cohort 7 on 6 November. Cohort 3 exhibited the longest recruitment period, which lasted for approximately one month. Cohorts recognised in August and September did not consist of newly born individuals, and therefore recruitment must have taken place previously, although it was not detected. Recruitment dates of these two cohorts were estimated after knowing the approximate growth rate.

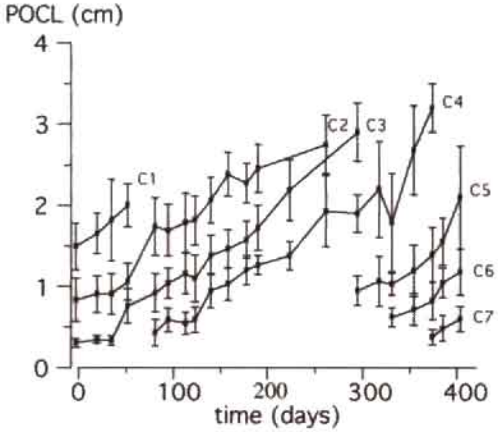

Fig. 2. Increase on the average POCL (Post Orbital Carapace Length) of each cohort throughout the study period. Standard deviations are also represented. C1, C2... C7 are the cohorts 1 to 7.

Recruitment was higher from the second half of October to the beginning of December. Other recruitment periods were also noticed in May and August. Animals with a POCL smaller than 1cm, which were considered to be juveniles, could be found throughout the year, except from May to July.

The average sex ratio was 0.24, ranging from 0.143 to 0.5 and it was not possible to recognise any regular pattern of variation. A total of 40 adult males was recognisable from samples, the smallest one (a form I male) 2.52 cm of POCL. Of these, 26 (67%) were form I. Only one ovigerous female was observed and due to the small number of adults collected it was difficult to determine the type of variations in reproductive stage or condition that occurred throughout the year.

Size-weight relationships

Taking into consideration animals equal or larger than 0.25 cm POCL, POCL-dry weight relationships may be expressed by the following regression model: $Y=0.08025091 6x^{3.07156}$ ($r^2=0.939$; $n=1278$; $P<0.001$). For animals smaller than 0.25 cm POCL no significant correlation was found ($P>0.05$; $r^2=0.0006$; $n=122$), and therefore a median value of 0.00096g was considered for their individual weight.

Also, POCL and wet weight were found to be significantly correlated according to the following regression model: $Y=0.49675243x^{2.879349}$ ($r^2=0.922$; $n=758$; $P<0.001$). In this case only animals larger than 0.4cm POCL were considered. The correlation for animals smaller than 0.4cm was not significant ($P>0.05$; $r^2=0.0077$; $n=106$) and, like in the previous case, an average individual weight of 0.0012g was considered.

Growth

Size-frequency analysis provided the average POCL size of each cohort and the correspondent standard deviation at each sampling date (Fig. 2). Seven cohorts were tracked for periods variable from 30 (cohort 7) to 237 days (cohort 4). Data on each cohort were used to adjust growth curves, and the fit was carefully compared based on r^2 values for a given number of data points. The curve with the best fit was then selected.

Cohort 3 provided the most consistent data on field growth rates, ($r^2=0.98757$ for 12 data points). The first two points were not taken into consideration since they seemed not to make sense. Therefore data on cohort 3 were merged with some data on cohort 2 in order to calibrate a growth model for winter cohorts of *Procambarus clarkii* in the Lower Mondego River valley.

The estimated Gauschutz growth model parameters are as follows: $L_\infty = 5.6$, $t_0 = -0.077838123$, $K = 0.68220933$, $t_s = 0.57738118$, $C = 0.44322345$ and $D = 1$. The value of "D" was assumed to be 1, since information on metabolic rate of this species was not obtained. From the model, average growth rate was 1.16mm per week for total length, corresponding to 0.61mm per week for carapace length and to 0.5mm per week for postorbital carapace length.

Density and mortality

Size frequency analysis also allowed an estimate of the density of each cohort at each sampling date, although some sampling constraints related to the capture technique might have caused some bias on the data. Actually, newly released individuals and especially adults have most likely been underestimated. This problem was partially solved by the adjustment of a negative exponential curve to the densities of the cohorts: $y=a.10^{-bx}$ where x and y are time in days and density ($ind.m^{-2}$), respectively and "a" and "b" are parameters to be determined. All the curves with more than three data points presented a good fit ($P<0.05$). Cohort 7 was adjusted to a curve with a value of "b" equal to the average of that parameter for all the other curves.

For these calculations, mortality (m) was assumed to be constant for each cohort, and can be determined from:

$$m = \left| \frac{(a \cdot 10^{-bx})'}{a \cdot 10^{-bx}} \right| = \left| \frac{-b \cdot \ln \cdot a \cdot 10^{-bx}}{a \cdot 10^{-bx}} \right| = \left| -b \cdot \ln 10 \right|$$

where "m" is the daily mortality rate, "a" and "b" are parameters of the density equation for each cohort, and "x" is the time in days.

Cohort production estimates

The Allen curve method as originally described takes into account a limited number of data points. However, a previous determination of equations for density and wet weight, made possible the use of more data points. The method used to estimate production was based on the plot of the daily relationship between the density and the average wet weight of each cohort. A curve of the type $y=a*10^{-bx}$ was then adjusted to data points, the production being given by the integral of this function. Total production within a period of 407 days was determined by summing the different cohort production estimates. Production was estimated at 27.219 $g.m^{-2}.yr^{-1}$ or 272.19 $Kg.ha^{-1}\ yr^{-1}$.

Average biomass

Daily total biomass values were estimated from the sum of the products of densities and average weights of recognisable cohorts.

Using appropriate software, a polynomial function was then adjusted to these data:

$$y = 1.899992+3.01*10^{-2}x+2.63*10^{-4}x^{2}+1.90*10^{-6}x^{3}+2.70*10^{-9}x^{4}$$

Where "y" corresponds to the biomass values and "x" corresponds to the time passed from the beginning of sampling. The average biomass was calculated dividing the integral of this function within the interval by 407, and the obtained value was 5.409$g.m^{-2}$ (54 $kg.ha^{-1}$). The $P/\overline{B}$ ratio was then estimated at 5.03.

2nd and 3rd sampling programs

In 1992-93 a total of 337 crayfish were captured, with an average POCL of 3.19 cm, an average weight of 17.18 g and average sex ratio of 0.34. Values of the condition factor for males and females were not separated due to scarcity of data. Minimum average condition values were recorded in September/October 1992 (0.42) and

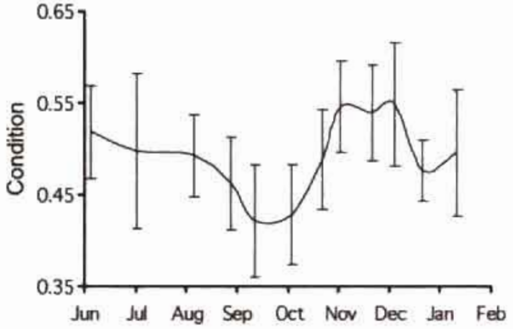

Fig. 3. Fulton's condition factor for crayfish (*Procambarus clarkii*) in the Lower Mondego river valley, Portugal, during the years of 1992/93. Crayfish of both sexes pooled together. Standard deviations are presented. N= 337.

maximum average values were recorded at the end of November/December 1992 (0.55) (Fig. 3). Average condition for all crayfish was 0.49. Size (POCL) was not significantly correlated with condition (r = 0.1, P>0.05).

During 1993-94, 2,149 crayfish were captured, with an average POCL of 2.94 cm an average weight of 15.39 g and average sex ratio of 0.47. Average POCL, sex ratio, and the proportion of Form I males (sexually active) was not constant. For this reasons data were divided into four groups: all males, females, Form I males, and Form II males. There were statistically significant differences in the average condition of these groups (ANOVA, P<0.01). Form I males had the highest average condition factor (0.595) followed by all males (0.550), females (0.529) and Form II males (0.519). Average condition factor for all crayfish captured was 0.537.

Without pooling the data by date, correlations of POCL vs. Condition were low, but statistically significant (P<0.05) for: all crayfish (r = –0.098, N = 2,149), females (r = 0.17, N = 1,450), Form I males (r = –0.18, N = 287), and Form II males (r = –0.29,

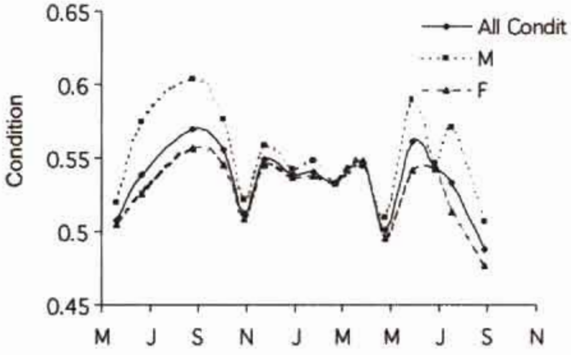

Fig. 4. Fulton's condition factor for crayfish (*Procambarus clarkii*) in the Lower Mondego river valley, Portugal, during the years of 1993/94. Results are presented for all crayfish (All condit, N=2149), females (F, N= 1450), and males (M, N=696).

N = 409); and non significant for all males (r = 0.01, N = 699). Correlations of average POCL vs. average condition values per sampling date were non-significant for any groups (P>0.05). Results for each group were: all crayfish (r = 0.19, N = 15), females (r = 0.02, N = 15), Form I males (r = 0.2, N = 14), Form II males (r = 0.43, N = 15); and all males (r = 0.28, N = 15).

In 1992-93 a decrease in condition was observed in September/October and in December/January. During 1993-94 lower average condition values were found in May 1993 and 1994, in November 1993, and September 1994 (Fig. 4). Higher average values were found in September 1993, December 1993, and June 1994.

Average sex ratio (males/females) was 0.47 and no seasonal trends were observed. Form I males were abundant (over 50% of the males) from May to October. Annual variations in the proportion of Form I males (Fig. 5) were quite similar to those of the condition factor for the overall male population. Nevertheless they were not statistically correlated (Pearson's correlation coefficient, r = 0.415, P > 0.05). There was a tendency for changes in form to start slightly before the changes in overall male condition.

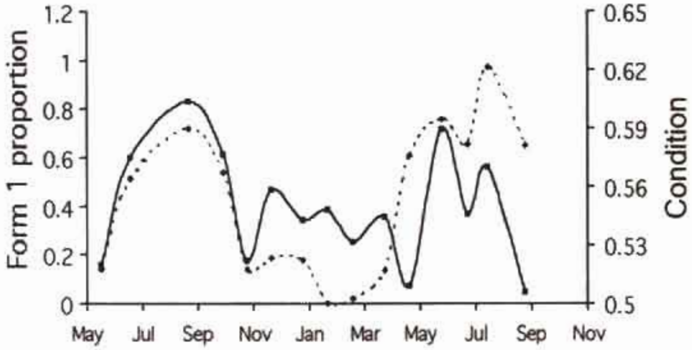

Fig. 5. Fulton's condition factor for all male crayfish (*Procambarus clarkii*) and proportion of Form I males (broken line).

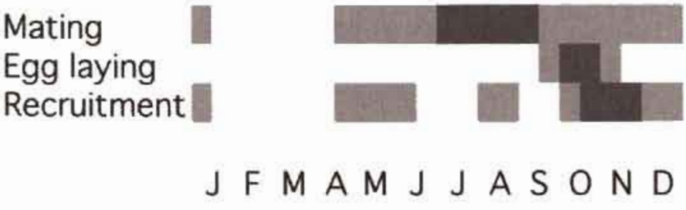

Fig. 6. Diagram representing the biological cycle of the Red swamp crayfish (*Procambarus clarkii*) in the Lower Mondego river valley, Portugal. Darker areas correspond to a more intense process. It is possible that egg laying occurs also during other periods.

Discussion

There are several peculiarities in the crayfish *P. clarkii* populations in the Lower Mondego river valley. As an example, the sex ratio differed from the values provided in the literature. In Louisiana and Kenya the number of females is slightly inferior or equal to the number of males (Table 1). Moreover, although sex ratio favourable to females have been previously reported for this species their values were usually higher than ours. Apparently there is an increase in the female proportion within the populations with increasing latitude and this may be one of the species reproductive strategies.

Table 1. Values of the sex-ratio (males/females) of crayfish *Procambarus clarkii* available in the literature.

Location	Sex ratio	Source
Kenya	2:1 in low waters. The proportion moves progressively to 1:1 as depth increases.	Oluoch, 1990
U.S.A. (Louisiana)	Average value of 1:1. Decrease on female numbers during the hottest months of the year.	Penn, 1943
U.S.A. (Louisiana)	Average value of 1:1.	Huner, 1978
Southern Spain	Approximately 1:2.	Gállego and Ocete, 1985
Central Spain	Variation during lifetime. 0.453 for smaller than 40 mm. 0.558 for individuals between 40 and 50 mm. 0.652 for individuals bigger than 50 mm.	Guerra and Niño, 1990
Portugal (Évora)	Considerable variation along the year. Proportion almost always favorable to the females.	Adão and Marques, 1993
Portugal (Elvas)	Approximately 1:1.	Correia, 1990
Portugal (Lower Mondego river valley)	Average value of 0.218.	Anastácio, 1993
Japan	Lesser than 1. Juveniles born under laboratory conditions present a value of 1:1	Sukô, 1956

It is known that in favourable conditions red swamp crayfish may have three generations per year (Huner 1981). In the lower Mondego region growth rates were relatively low compared to other areas (Table 2), which suggests that the reproductive stage is attained either after a longer period and/or at smaller sizes. According to Wenner et al. (1974) different growth rates influence the size at maturity and faster growing animals should get sexually mature at a larger size. In the present case it is considered that sexual maturity is attained at small sizes.

Thorp and Wineriter (1981) demonstrated a direct relationship between temperature and moult frequency and Sukô (1956) stated that temperatures lower than 10°C would inhibit embryonic development. Nevertheless, in the lower Mondego River region, although temperatures in early winter were lower than this limit, some newly released individuals were still found. This suggests that the population studied may have developed increased resistance to low temperatures, allowing embryonic development to occur at lower temperatures. An alternative explanation could be that a proportion of the youngest animals stopped growing, maintaining a small size for a longer period than normally observed.

P/B ratios ranging from 0.87 to 1.51 were found by other authors for P. clarkii (Momot and Romaire 1981). Nevertheless, most of the literature on the ecology of P. clarkii concerns artificial environments in Louisiana, and under natural conditions $P/\bar{B}$ ratios might differ. Production and average biomass estimated for the Mondego region might have been biased due to insufficient data on adult population density on the 1st sampling program. Assuming that the density of the adult population was underestimated, then the real $P/\bar{B}$ ratio may be lower than 5.032.

Romaire et al. (1977) alerted to the dangers of determining regression coefficients (in size/weight regressions) for *P. clarkii* in a single sampling occasion. Fulton's condition factor is a type of a size-weight relationship and it is also variable throughout the year. Although Huner et al. (1985) obtained a linear relation for the proportion cephalothorax length / abdominal muscle weight in *P. clarkii*, Lathi's (1988) findings for male *A. astacus* were different. Relative muscle weight was length dependent. Moreover, size-weight relationships for crayfish of the genus Cherax (Lake and Sokol 1986) and also for *P. clarkii* are known to be size-dependent. In our study, there was a slight tendency for crayfish condition to depend on POCL, but seasonal variations in average POCL were not significantly correlated with average crayfish condition.

The fluctuations of the condition factor in 1992-93 were not exactly the same as those of 1993-94. Crayfish condition from late June 1992 until the end of January 1993 was not very good. Although no division into groups was performed, the reason for the inclusion of the 1992-93 data in the paper was the parallel found in the seasonal patterns for all the groups in 1993-94. The differences observed between the two sampling programs may be explained by normal environmental variations.

Fernandes et al. (1994), in a biochemical composition study carried out in 1992-93 in the lower Mondego River valley crayfish suggested that accumulated energy was used in preparation for reproduction. Data obtained in the present study during the same years (1992-93), reveals optimal condition at the same periods. It might be interesting to consider that a simple approach such as the use of a condition factor

Table 2. Growth rates of crayfish *Procambarus clarkii* found in the literature.

Author		Growth (mm/week)			Kind of research
		Body measurements			
		TL	CEF	POCL	
Lutz and Wolters. 1989	minimum	2.1	1.1	0.915	Laboratory work on character inheritance.
	maximum	7	3.68	3.05	
Craig, 1985 quoted by Lutz and Wolters, 1989		3.6	1.89	1.57	Experimental tanks with a food supply of rice.
Clark et al, 1974 quoted by Lutz and Wolters, 1989		2.8	1.74	1.21	Experimental tanks.
Huner, 1978	minimum	2.5	1.32	1.09	Monitoring aquaculture ponds (Louisiana)
	maximum	5	2.63	2.17	
Goyert and Avault. 1979	minimum	1.8	0.95	1.610.78	Experimental study to determine effect of recipient size on body growth.
	maximum	3.7	1.94	1.61	
Romaire et al, 1979	minimum	1.18	0.62	0.51	Study made on experimental tanks with crayfish from stunted populations.
	maximum	1.98	1.04	0.86	
Sommer, 1984		1.807	0.952	0.787	Juvenile growth rates on Spring and Winter (California)
Correia, 1990	minimum	0.75	0.395	0.33	Study on wild populations on Elvas region (Portugal).
	maximum	2.75	1.45	1.19	
Anastácio, 1993		1.16	0.61	0.5	Study on wild populations on the lower Mondego river valley region (Portugal).
Day and Avault, 1986	minimum (rice)	1.89	0.99	0.82	
	maximum (rice)	7.84	4.13	3.41	Study made on aquacultural ponds in order to compare the effects of different types of forage upon growth.
	minimum	0	0	0	
	maximum	12.18	6.41	5.3	

• TL – Total Lenght. CEF – Cephalotorax lenght with rostrum; POCL - Post Orbital Carapace Lenght.

may provide reliable information on the internal condition of crayfish. Moreover, our data corroborate the idea that there is a relation between condition and reproduction especially in what regards the male population. In the lower Mondego River region mating takes place from May to August, and egg-laying happens mainly in September-October. Minor recruitment periods are found in May and August. Most eggs reach the final stages of development between the end of July and the beginning of September. It seems natural that stored energy might be used for reproductive activity in males and for the growth of ovaries in females.

The division of the 1993-94 data into four groups revealed significant differences in the average condition factor. Form II and Form I males have, respectively, the lowest and the highest average condition factor. However, there was a similar seasonal pattern of variation for females, all males, reproductive males (Form I) and non-reproductive males (Form II). A decline of condition was observed at the end of the reproductive season, i.e., from September to November, depending on the years. This was followed by a recovery in the average condition factor before the winter, similarly to what was observed for *Astacus astacus* (cf. Lindqvist and Lahti 1983). The general trend from December to May seems to start as a gradual decrease in condition, ending as a steep decrease. A possible explanation is the occurrence of a massive molting of immature to reproductive forms. There is in fact some evidence to support this, since in May the proportion of Form I males suffered a tremendous increase. It seems that molting and condition are related. Nevertheless, from our findings it is not possible to predict mass molting to reproductive forms just by using the condition index.

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ROLE ASSESSMENT OF AN EXOTIC FISH IN RICE FIELDS OF THE LOWER MONDEGO RIVER VALLEY: LIFE HISTORY, POPULATION DYNAMICS, PRODUCTION AND DIET OF EASTERN MOSQUITOFISH, *Gambusia holbrooki* (PISCES, POECILIIDAE)

Abstract

The introduced population of *Gambusia holbrooki* from the rice fields of the Lower Mondego River Valley, Portugal, was studied for 15 months, relating their life cycle and population dynamics with its production and diet, in order to assess the role of the species in the energy flow and secondary production in this type of agro-ecosystem. Two main annual cohorts (1995 and 1996 cohorts) were identified. The females outnumbered males and the average female/male-ratio was 4. The inspection of ovary developmental stages of this viviparous fish, revealed that the most important reproductive period was between April and August. The first recruits were recorded from June and were present thereafter until October. Females reached greater sizes, had a higher growth rate and lived longer than males. Annual production was estimated at 3.101 g m^{-2} year^{-1} (ash-free dry weight, AFDW), the average biomass at 2.896 g m^{-2} (AFDW), and the P/B ratio was 1.071. *G. holbrooki* feeds mainly on copepods, cladocerans and rotifers. Surface insects, such as aphids, collembolans, adult (imago) chironomids and other dipterans, are additional food. Large *G. holbrooki* consumed greater amounts of cladocerans and adult chironomids than other smaller size groups, while small fish prefered rotifers. Gravid females ate copepods, cladocerans, adult chironomids and other dipterans in significantly greater amounts than immatures, males, and non-gravid females. A combination of life history, population dynamics, production and ecological traits (e.g. fast growth, reduced longevity, viviparity, high productivity, an intermediate position in food chain, plasticity and adaptability in its food use, and no special habitat requirements for reproduction) clearly show that the populations of *G. holbrooki*, introduced into rice fields all over the world, may play an

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important role in the structure and functioning of the biological communities of these important agro-ecosystems.

Introduction

Eastern mosquitofish, *Gambusia holbrooki* (Girard) (Cyprinodontiformes: Poeciliidae), is native to the coastal region of the eastern United States, but was been widely introduced into warm temperate and tropical regions all over the world through mosquito control programs (Cech et al. 1992, Haynes and Cashner 1995, Homski et al. 1994, Hoy 1985, Lydeard and Belk 1993, Schaefer et al. 1994, Wurtsbaugh et al. 1980). The species was introduced in the Iberian peninsula in 1921 (Albuquerque 1956) and has invaded the lowest stream sections, wetlands and coastal lagoons (Vargas and Sostoa 1996). This viviparous fish is well known for its consumption of insect larvae, zooplankton and other invertebrates (Blaustein 1992, Cabral et al. 1998, Colwell and Schaefer 1983, Crivelli and Boy 1987, Daniels and Felley 1992, Hubs 1990, Hurlbert and Mulla 1981) but also as a threat to native fishes in habitats where mosquitofish have been introduced. Rupp (1996) reported that the harmful consequences of Gambusia introductions ranged from eating the eggs of economically desirable fishes to endangering rare indigenous species. Viviparity and a high reproductive effort may give such exotics an advantage over native oviparous species, because fry are larger, feed at birth, grow more quickly, and become predators faster (Rupp 1996). In Portugal, the most serious conservation problem for endemic fishes has been introductions of exotic species dating from the time of the Roman occupation of Iberia (Almaça 1995).

In the Lower Mondego River Valley (Western Portugal), the mosquitofish is very abundant, namely in rice fields (Cabral and Marques 1999). Rice fields are complex ecological systems with a variety of plant and animal species (Linden and Cech 1990). In the Lower Mondego River Valley, rice fields are linked by drainage and irrigation channels spread across the whole valley with other ecosystems, such as rivers, streams and wetlands (Anastácio and Marques 1995).

Mosquitofish is presently one of the most widely distributed species of freshwater fish in the world, and is believed to be the most widely disseminated natural predator in the history of biological control (Botsford et al. 1987). In the Iberian peninsula, there have been studies on the reproductive biology and population dynamics of *G. holbrooki* in rice fields (Fernández-Delgado 1989, Franca 1953, Franca and Franca 1954) and lagoons (Fernández-Delgado and Rossomano 1997, Vargas and Sostoa 1996), but its productivity has received little attention. This study addresses this information gap by relating life cycle and population dynamics of the mosquitofish with its production (Cabral and Marques 1999). The integration of this information with the existent knowledge of the relationships between mosquitofish and its main prey in rice fields of the Lower Mondego River Valley (Cabral et al. 1998), is an important requirement to assess the role of the species in the energy flow and secondary production in non-native rice fields.

Material and methods

Study site

The Lower Mondego River Valley (figure 1), in central Portugal (40°10'N, 08°41'W), consists of approximately 15,000 ha. The main agricultural crop is rice, occupying about 60% of the farmable area. Non-cultivated areas, such as swamps, appear in the periphery of the valley, and have characteristic wetland fauna and flora. Drainage channels are spread across the whole valley, constituting biological reservoirs for rice fields (rice paddies and irrigation channels) (Anastácio and Marques 1995).

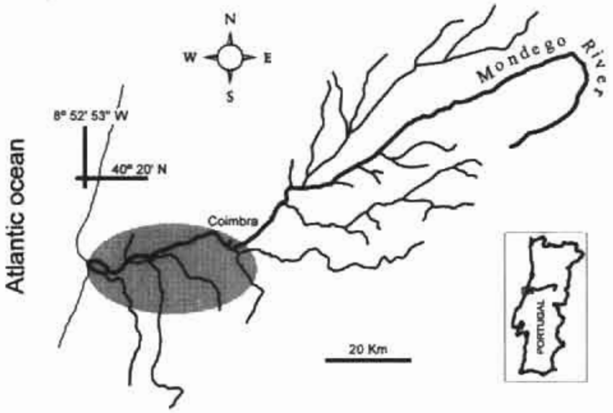

Figure 1. Location of the Lower Mondego River Valley (shaded area).

In the chosen study site, mosquitofish occur in the rice paddies and irrigation channels. However, since rice fields are exposed to dramatic manipulation of the water level, the paddies are dry or have little water during much of the year, whereas the main irrigation channels always have enough water to support a population of mosquitofish. The sampling program was therefore focused in the irrigation channels.

Field program

The sampling program was carried out in a main irrigation channel from April 1996 to June 1997, fortnightly during the most important mosquitofish reproduction period (April - July), and monthly in the remaining period. Samples of mosquitofish, zooplankton, and macroinvertebrates, both benthic and associated with aquatic vegetation, were taken.

During each sampling event, mosquitofish were electrofished in three randomised areas confined by nets laid transverse across the irrigation channel. A semi-portable generator supplied a rectified DC current (350-600V). Sampling always took place between 10 a.m. and 1 p.m., corresponding to the most active mosquitofish feeding period (Crivelli and Boy 1987). Sampled areas ranged from 3 to 16 m^2 and were shocked during a period of 30 to 40 minutes, enough to catch virtually all the fish present in each area. All mosquitofish caught were immediately preserved in 4% neutralised formaldehyde, while other fish were returned to the irrigation channel.

Prey samples were collected from the primary microhabitats in the irrigation channels, including the sediment, water phase, and aquatic vegetation. Three replicates were randomly sampled for each microhabitat as described by Cabral et al. (1998).

Laboratory procedures

In the laboratory fish were washed, counted, and preserved in 70 % ethanol. A total of 5,003 fish were examined. All individuals were measured and sorted by standard length (SL) to the following size groups: 1 (till 10 mm), 2 (11-15 mm), 3 (16-20 mm), 4 (21-25 mm), 5 (26-30 mm), 6 (31-35 mm), 7 (36-40 mm) and 8 (41-45 mm). Moreover, fish were classified as (a) immature (normally with 15 mm or less, if sex could not be determined externally), (b) males (normally no longer than 30 mm and identified by the presence of a gonopodium), (c) non-gravid females and (d) gravid females. All females were dissected. Ova and embryos were removed from the ovaries, measured (maximum diameter) with an ocular calibrated micrometer, counted, and assigned to one of five developmental stages: (1) opaque, white non-fertilised eggs about 100 µm in diameter (Thibault and Schultz 1978); (2) intermediated between immature ova (partially yolked) and mature ova with a clear amber color (complete complement of yolk but no embryonic structures visible) (Meffe 1987, Thibault and Schultz 1978); (3) a stage from the primitive streak to the early embryo (eyes not fully formed; little dorsal pigmentation); (4) middle embryo (eyes fully formed; heavier pigmentation; moderate amount of yolk remaining); and (5) late embryo (little or no yolk remaining; ready for parturition) (Meffe 1987). The females in the developmental stage 2 or in older stages were considered as gravid females. Sex-ratios were calculated as the number of females per male.

Five individuals per size group and from each sampled area were examined for gut contents, except for size groups 3, 4, and 5, where females and males had coincident sizes. In this case, five females and five males were examined per group. For each fish the gut tube was excised from the esophagus up to the point where it bends ventrally and dissected. Prey items were recorded and identified.

Length-weight relationships were determined to estimate production. One hundred fifty-six individuals were collected during the summer, the only period in which all size classes were present, to provide a single regression equation for SL-AFDW (ash-free dry weight in g) conversion. The following equation was obtained: $AFDW = 1.56E^{-6} * SL^{3.24}$ ($r^2 = 0.96$, $p<0.001$). Specimens were dried in an oven for 48 hours at 60 °C and weighted, and ignited in a muffle furnace for 8 hours at 450 °C to obtain AFDW. Weight determinations were carried out with a 10^{-5} g precision.

Data analysis

Growth rates were deduced by tracking recognisable cohorts along size-frequency distributions (1 mm SL classes) from successive sample dates. All fish born during the same reproductive period were assigned to the same cohort (Fernández-Delgado and Rossomano 1997). Mosquitofish length-frequency histograms, complemented with scale readings, allowed a visualisation of the population structure throughout the year. Since there is genetically-based size polymorphism in Gambusia, the growth process is different for each sex. Female mosquitofish have indeterminant growth and may attain a size greater than 50 mm SL, representing therefore a continuous range of growth from the first immature stages, whereas males tended to stop growing upon reaching maturity, approximately with 20 mm SL (Cabral and Marques 1999, Vondracek et al. 1988). Therefore, we considered separately immature and females combined (with continuous growth) and males because population structure analysis becomes difficult if males are considered together with the remainder of the population. The monthly average and standard deviations of SL to cohorts detected were calculated, which established the annual growth pattern for each sex. Since growth rates are normally not constant through the year, seasonal variations were taken into consideration using a model proposed by Gaschütz et al. (1980).

Size-frequency analysis also allowed an estimate of the density of each cohort at each sampling date. Daily mortality rate was estimated by the adjustment of a negative exponential curve to the densities of the cohort detected from the recruitment period, when it reached the maximum density value, to the period where all the individuals belonging to this cohort disappeared (Anastácio and Marques 1995, Cabral and Marques 1999). The instantaneous mortality rate (Z) was also calculated for mean age and mean life span estimations. Z is equal to the natural logarithm (with sign changed) of the complement of the annual mortality rate (Ricker 1975). For exponential mortality at a constant rate, mean age and mean life span are equal and, except when growth is exponential, both are equal to the reciprocal of the instantaneous mortality rate (Allen 1971).

The length of the reproductive season was determined by calculating the time interval between 1 month before the presence of the first immature, since a typical gestation period is about 4 weeks (Meffe 1990), and the presence of the last newborn/small immature.

To identify the preferential prey items caught by mosquitofish we used the Ivlev's electivity index for fishes (Ivlev 1961), defined as $E = (r - p) / (r + p)$, where r = proportion of the number of a given prey in the mosquitofish gut content, and p = proportion of the number of the same organism in field samples. Positive values of E (0 to 1) indicate a preference, negative values (-1 to 0) indicate little or no representation in the gut content. We used the value -0.5 as lower limit to identify "preferential" prey.

The Kruskal-Wallis one-way analysis of variance by ranks followed by a non-parametric multiple comparisons test with unequal sample sizes (Zar 1984) was used to assess the significance of differences in the number of preferential prey items between the gut contents of different mosquitofish size and sex groups.

Production was estimated using Allen curves (Waters 1977). A year-to-year (or cohort-to-cohort) stability was assumed and the method was used for the mixed-age population. In these cases, a single cohort production will be equal to annual production of all population (Waters 1977). The production estimate for the population was achieved by determining the average AFDW and density of mosquitofish, both against time. Then, density was plotted against average AFDW for each sampling date, where production was given by the integral of the curve adjusted to this plot.

The average biomass was determined by the ratio of the biomass integral within a given time interval and the time interval (Allen 1971). Assuming the same cohort-to-cohort stability presupposition used for production estimation, the total biomass in a given time was the product of individual average weight (AFDW) and the density of the mixed-age population.

Results

Population structure and reproduction

It was possible to recognise and track two annual cohorts for both sexes, the 1995 and 1996 cohorts. Recruitment occurred from June through October, at which time the last newborns were collected. Females outnumbered the males during the entire study period, with an average sex-ratio of 4:1 (Cabral and Marques 1999). Inspection of the developmental stage of the female intra-ovarian cycle revealed that the most important period for reproduction falls within the time interval between April and August. The proportion of non-gravid females (stage 1) decreased until the early June, whereas gravid females (specially stages 3, 4 and 5) increased (figure 2). After the beginning of recruitment the inverse trend occurs, and in August only a very small proportion of females were gravid. The average brood size (embryos) reached its maximum in July 1996 (32 embryos per female) for the 1995 cohort, at which time there were almost no unfertilised eggs in the ovaries, and in early June 1997 (34 embryos per female) for 1996 cohort (our last data).

Figure 2. Changes in the percentage of female *Gambusia holbrooki* bearing eggs/embryos in each developmental stage (1, 2, 3, 4 and 5, as defined in Material and Methods) during the most important reproductive period (1402 females were examined). The initials of the months with numbers (1 and 2) correspond to samples made fortnightly.

Growth

The average monthly lengths and their respective standard deviations depict a seasonal variation in growth for immature and females combined and males (figure 3). Therefore, we took seasonal variations into consideration, and growth data were used to calibrate a growth model proposed by Gaschütz et al. (1980). Two main cohorts were tracked for both sexes during a total period of 411 days. Since the study period was not sufficient to contain the entire life cycle of a single cohort, we adjusted a growth curve for each sex to the original data of 1996 cohort merged with the last data on 1995 cohort. The fit was compared based on r^2 values. The growth-data-fitted Gaschütz's model predicted well for immature and female combined ($r^2 = 0.96$ for 17 data points), but not for males ($r^2 = 0.67$ for 13 data points). The growth pattern of males is different since some of them essentially stop growing after maturation (figure 3). Therefore, we used this method only for immature and females (figure 4), the fraction of the population with a continuous growth. The model parameters for this group were estimated as described by Cabral and Marques (1999).

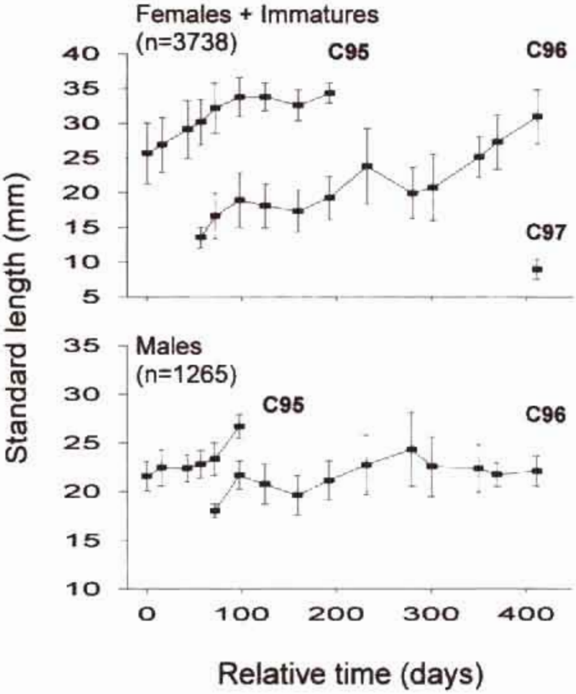

Figure 3. Annual growth pattern in females and immature combined and males from the 1995 (C95), 1996 (C96) and 1997 (C97) cohorts of *Gambusia holbrooki*, based on monthly averages of standard length (mm). The standard deviations from the mean are also indicated.

There was an initial stage with notable growth (0.076 mm day-1), corresponding to the months with mild temperatures (early first summer) and initial recruitment of a new cohort (figure 4). This period was followed by a second stage of extremely slow growth (0.014 mm day^{-1}) between the end of summer and the "cold months" from autumn to mid-winter, and a final phase, with a recovery in growth (0.052 mm day^{-1}) until the end of the second summer (figure 4). For males, we assumed a growth rate similar to the first stage until reaching maturity (approximately with 20 mm SL), and thereafter the second stage growth rate for the remain male life cycle.

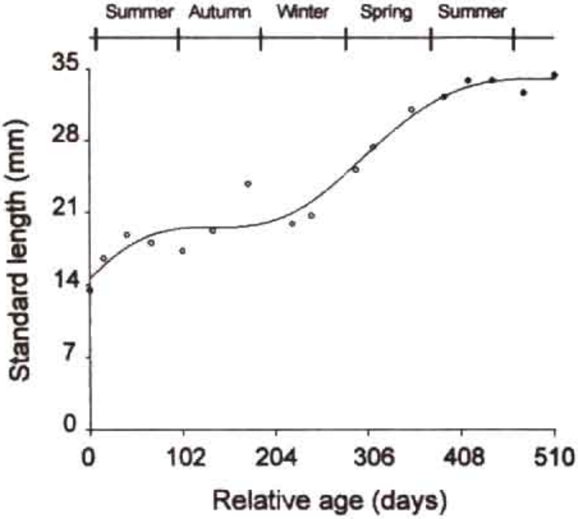

Figure 4. Gaschütz's growth model of standard length (mm) for the 1996 female+immature cohort, merged with the last data on 1995 cohort, of *Gambusia holbrooki*. Data points used to calibrate the model are also plotted (the last five black data points belong to 1995 cohort).

Density, mortality, mean age and mean life span

For all mixed-age population, the mosquitofish showed the same density patterns throughout the study period for the three main "sexual" categories considered: females (non-gravid and gravid), males and immature. The density of the 1996 cohort on each sampling date allowed an estimate of the daily mortality rate for both sexes. This cohort was merged with the last data on 1995 cohort, for the same reason explained for growth analysis. Since the sex-ratio at birth in mosquitofish is 1:1 (Krumholz 1948), we assumed that each sex contributed 50% of the immature density. The daily mortality rate was estimated by the adjustment of a negative exponential curve ($y = a*10^{-bx}$) to the densities of each sex detected from the recruitment period, when they reached its maximum density value, to the period where all the respective individuals disappeared (figure 5). The mortalities, mean age and mean life span parameters estimated for both sexes from the density equations are given in table 1. Females were longer-lived, with smaller mortality rates than males.

Table 1. Estimated parameters for mortalities, mean age and mean life span of both sexes from the functions describing the evolution of a *Gambusia holbrooki* cohort density. 50% of the immature density was considered as belonging to each sex. b is the parameter of the density equations of the type $y = a*10^{-bx}$. D is the number of deaths during the year and N_0 is the number of fish present at the start of a year.

Parameters	Equations	Females + 0.5 (immatures)	Males + 0.5 (immatures)
Daily mortality rate (**m**)	$m = \lvert -b*\ln 10 \rvert$	**0.00746**	**0.00836**
Annual mortality rate (**A**)	$A = D/N_0$	**0.93432**	**0.95268**
Instantaneous mortality rate (**Z**)	$Z = -\ln(1-A)$	**2.72304**	**3.05081**
Mean age and mean life span (**L**)	$L = (1/Z)*365$	**134.04 days**	**119.64 days**

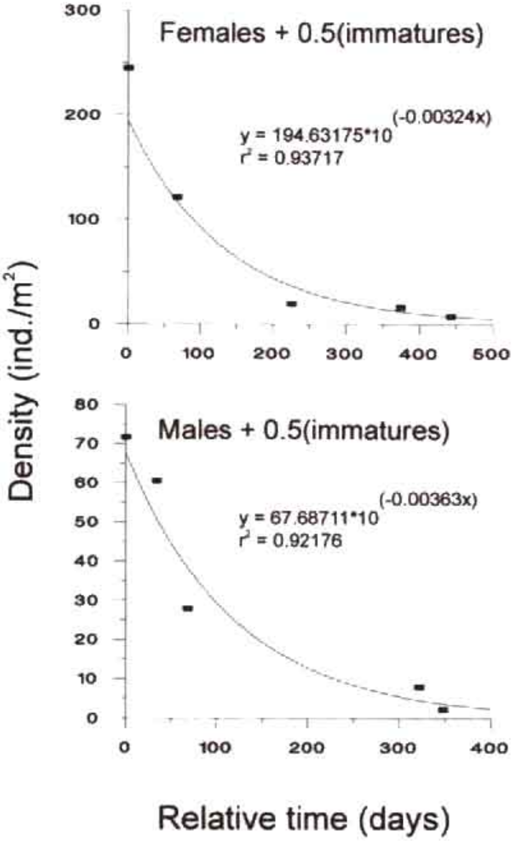

Figure 5. The survivorship curve for both sexes of a *Gambusia holbrooki* cohort (1996 cohort merged with the last data on 1995 cohort). A negative exponential curve ($y = a*10^{-bx}$) was adjusted to the densities throughout time. x and y are time in days and density (individuals m^{-2}), respectively. 50% of the immature density was considered as belonging to each sex. The respective functions and r^2 values are also indicated.

Production estimates, average biomass and P/B ratio

Production was estimated from a plot of the relationship between the density and the average weight of the mixed-age population (Allen curve method). A curve of the type $y = a*10^{-bx}$ was then adjusted to data points. The resulting figure is basically a growth-survivorship curve with the density of survivors plotted against mean individual ash-free dry weight (AFDW) (figure 6a), and the total production within the study period (411 days) being given by the integral of this function. Production (P) was then estimated as 3.10 g m^{-2} $year^{-1}$ (AFDW).

Total biomass values for each sampling date were estimated from the products of densities and average weights (AFDW) of the mixed-age population. A fourth order polynomial function was then adjusted to these data (figure 6b), and the average biomass (B) was calculated by dividing the integral of this function within the study interval by 411. The obtained value was 2.90 g m^{-2} (AFDW). The P/B ratio was then estimated as 1.07.

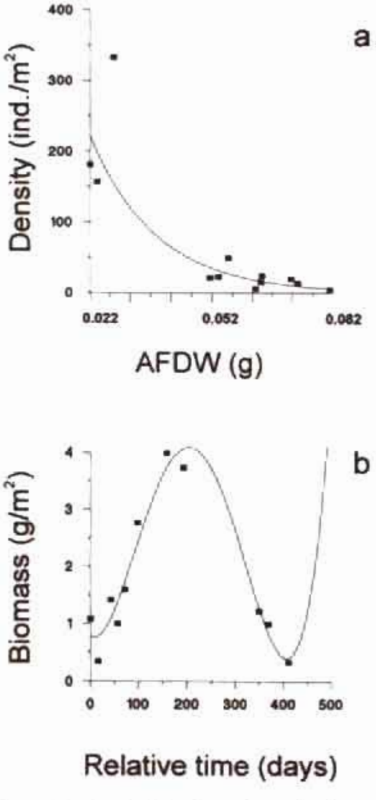

Figure 6. Models used to estimate *Gambusia holbrooki* total production and average biomass, respectively: a) adjustment of the Allen curve to the relationship between the density and the average ash-free dry weight (AFDW) of mixed-age population; and b) adjustment of a polynomial curve to the estimated values for total biomass throughout the study period. Data points used to calibrate the models are also plotted.

Diet

Twenty-three invertebrate large groups were collected in the three microhabitats considered. For the present purposes it was considered enough to take into account high taxonomic levels. The most abundant invertebrates in the water phase were copepods and cladocerans. In the macrobenthos, oligochaets and chironomid larvae were the most abundant invertebrate groups. Adult hydrophilids, aphids, chironomids larvae, and crayfish *(Procambarus clarkii)* were very abundant on the aquatic vegetation, being found during the whole study period.

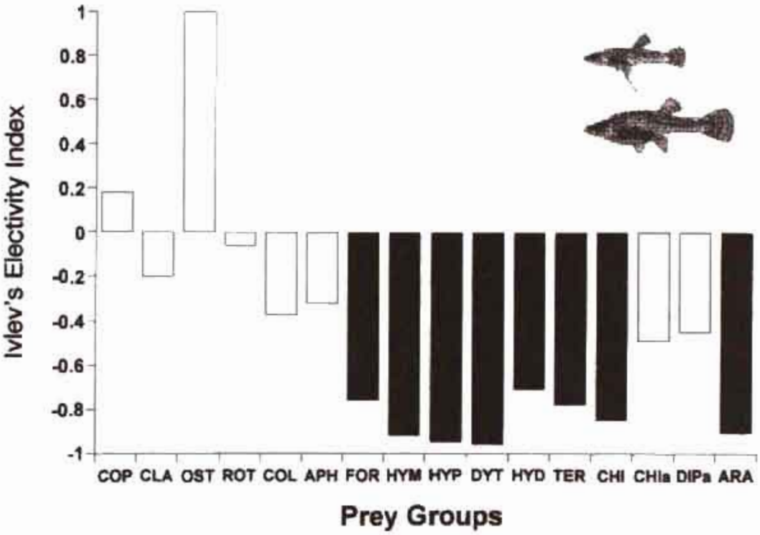

Figure 7. Prey groups Ivlev's electivity index values calculated for the total population sampled of *Gambusia holbrooki*. The white polygons indicate a prey preference, taking into account the lower limit selected to identify "preferential" preys (-0.5). The codes of the sixteen prey groups are respectively: Copepods (COP), Cladocerans (CLA), Ostracods (OST), Rotifers (ROT), Collembolans (COL), Aphids (APH), Ants-Formicidae (FOR), other Hymenopterans (HYM), Hydrophilids (HYP), Dytiscids (DYT), Hydraenids (HYD), Terrestrial-Coleopterans (TER), Chironomids larvae (CHI), adult Chironomids (CHIa), other adult Dipterans (DIPa), and Arachnids (ARA).

The 16 groups found as prey items in the mosquitofish gut contents are given in figure 7. Taking the Ivlev's electivity index values into account, for the population considered as a whole (figure 7), zooplankton (copepods, cladocerans, ostracods and rotifers) constituted the main feeding option for mosquitofish, followed by the insect groups aphids and collembolans, adult chironomids and other dipterans. Ostracods appeared as a preferential prey, although they were found in only 7.8 % of the fish analysed (Cabral et al. 1998). However, ostracods were excluded from the statistical analysis because of the lack of information regarding their occurrence in the environment. The absence of ostracods in the samples can be explained as a function of our sampling methodology for benthic organisms (Cabral et al. 1998). Copepods were the only other group with a positive Ivlev's index (figure 7).

A Kruskal-Wallis one-way analysis of variance by ranks was used to assess the significance of differences in the numbers of preferential prey items between the gut contents of different mosquitofish size and sex groups. Only cladocerans, rotifers and adult chironomids were caught in significantly different quantities by distinct size groups (table 2). Since we analysed only a few fish from the peripheral size groups 1 and 8, they were pooled with groups 2 and 7 respectively. The multiple comparison test showed that large mosquitofish size groups (7+8) clearly consumed greater amounts of cladocerans and adult chironomids than the other size groups, whereas small and medium fish consumed greater amounts of rotifers (table 2). There were also differences between the sex groups regarding the type of prey caught (table 3). Copepods, cladocerans, adult chironomids and other dipterans were caught in significantly greater amounts by gravid females than by immature, males, and non-gravid females. Nevertheless, males and non-gravid females fed more on other adult dipterans than immature, showing also significant differences with respect to their diet (table 3). Non-gravid females ate collembolans in significantly greater quantities than any other sex group.

Discussion

Like other populations of *Gambusia holbrooki* previously studied in the Iberian peninsula (Fernández-Delgado and Rossomano 1997, Vargas and Sostoa 1996), the population in the rice fields of the Lower Mondego River Valley consisted of two main annual cohorts with a cohort substitution during the reproductive season.

In the Lower Mondego River Valley, the reproductive season (April-October) was similar to those of populations in other areas of the Iberian peninsula (Fernández-Delgado and Rossomano 1997, Franca and Franca 1954, Vargas and Sostoa 1996).

In our study, the average sex-ratio of 4:1 (females per male) was similar to the value described for the Canal Vell lagoon (4:1) by Vargas and Sostoa (1996) in NE Spain, and smaller than the value described for Águas de Moura rice fields (5:1) by Franca and Franca (1954) in Portugal. Since equal numbers of male and female mosquitofish occur in the ovary and at birth (Krumholz 1948), the temporal variation in the adult sex-ratio must be attributed to the differential mortality of the sexes.

Maximum growth occurred during the early summer, when the temperatures become mild and the availability of food, specially zooplankton, increases (Cabral et al. 1998). These conditions are favourable to increase the mosquitofish metabolic rate (Cech et al. 1985) and food consumption (Linden and Cech 1990), promoting high growth rates. Females had a pattern of indeterminate growth with seasonal variation (Cabral and Marques 1999): growth slows during the "cold months" until the mid-winter and then recover with relatively high rates until the end of the second summer. The growth of adult males did not followed this pattern, because it is associated with the onset of maturation, growth cessation, or slow growth, after the gonopodium has been completely formed (Krumholz 1948, Vondracek et al. 1988). The estimated growth rates, taking into consideration seasonal variations by using the model

Table 2. Average number of prey items eaten (Mean ± S.E.) by mosquitofish (*Gambusia holbrooki*) taking into account the size groups collected during the study period. The number of solid circles bellow the values indicates significant differences between size groups for the medians of a given prey group (Kruskal-Wallis and nonparametric multiple comparison tests). ** = $p<0.01$; *** = $p<0.001$ (n.s. = no significant). n is the number of individuals.

PREYS	MOSQUITOFISH SIZE GROUPS							
	1 + 2 (n=39)	3 (n=120)	4 (n=137)	5 (n=101)	6 (n=53)	7 + 8 (n=34)	χ^2	p
Copepoda	16.4 ± 4.0	18.0 ± 3.9	31.6 ± 4.7	40.2 ± 8.5	37.0 ± 10.0	51.1 ± 13.1	9.27	n.s.
Cladocera	6.9 ± 1.9 •	7.9 ± 2.9 •	6.4 ± 1.8 •	7.4 ± 2.7 •	19.3 ± 7.6 ••	30.4 ± 10.1 •••	15.76	**
Rotifera	1.5 ± 1.2 •••	7.8 ± 3.4 •••	13.8 ± 4.8 ••••	0.6 ± 0.3 ••	0 •	0 •	15.36	**
Collembola	0.3 ± 0.2	1.1 ± 0.3	1.0 ± 0.3	1.1 ± 0.4	3.1 ± 1.2	0.8 ± 0.7	3.41	n.s
Hemiptera								
Aphididae	1.8 ± 0.4	1.4 ± 0.2	2.6 ± 0.4	3.2 ± 0.6	3.7 ± 0.9	2.1 ± 1.1	4.52	n.s.
Diptera								
Chironomidae (AD)[1]	0.03 ± 0.03 •	0.02 ± 0.01 •	0.2 ± 0.1 •	0.4 ± 0.2 •	0.6 ± 0.3 •	1.7 ± 0.9 ••	22.22	***
Others (AD)	0.03 ± 0.03	0.07 ± 0.02	0.08 ± 0.04	0.3 ± 0.08	0.08 ± 0.05	0.09 ± 0.05	9.48	n.s.

[1] (AD) = Adult

Table 3. Average number of prey items eaten (Mean ± S.E.) by mosquitofish (*Gambusia holbrooki*) sex groups considered during the study period. The number of solid circles bellow the values indicates significant differences between sex groups for the medians of a given prey group (Kruskal-Wallis and nonparametric multiple comparisons tests). *** = $p<0.001$ (n.s. = no significant). n is the number of individuals.

PREYS	MOSQUITOFISH SEX GROUPS					
	Immature (n=37)	Males (n=138)	Non-Gravid Females (n=193)	Gravid Females (n=116)	χ^2	P
Copepoda	14.6 ± 4.0 •	14.2 ± 2.2 •	24.7 ± 3.8 •	65.8 ± 8.8 ••	39.56	***
Cladocera	6.9 ± 2.0 •	5.8 ± 1.8 •	5.2 ± 1.8 •	24.6 ± 5.0 ••	41.10	***
Rotifera	1.6 ± 1.3	7.3 ± 2.9	5.6 ± 2.1	6.9 ± 4.6	6.52	n.s.
Collembola	0.2 ± 0.1 •	0.8 ± 0.2 •	2.3 ± 0.4 ••	0.03 ± 0.02 •	45.28	***
Hemiptera						
Aphididae	2.2 ± 0.5	2.4 ± 0.4	2.6 ± 0.4	2.2 ± 0.5	2.15	n.s.
Diptera						
Chironomidae (AD)[1]	0.03 ± 0.03 •	0.1 ± 0.04 •	0.02 ± 0.01 •	1.2 ± 0.3 ••	38.30	***
Others (AD)[1]	0.03 ± 0.03 •	0.1 ± 0.04 •••	0.06 ± 0.03 ••	0.3 ± 0.06 ••••	16.71	***

[1] (AD) = Adult

proposed by Gaschütz et al. (1980), were about one order of magnitude smaller than the values described for the Canal Vell (Vargas and Sostoa 1996) and Zoñar lagoons (Fernández-Delgado and Rossomano 1997). The possible cause of this may be related with the fact that the adjustment of the seasonal growth curves to the original data for long periods (several months) may skew the growth rates to lower values than when they are directly estimated for short favourable periods (1-2 months), as performed in the studies mentioned above. Moreover, this type of adjustments to the monthly averages of length at successive cohorts allows correction of some of the bias in data (Ricker 1975).

With regard to mosquitofish annual production and P/B ratio, no estimates are available for other mosquitofish populations from the literature, so we have no idea of general levels to be expected. Nevertheless, the estimated mosquitofish annual production of 3.10 g m^{-2} $year^{-1}$ (AFDW), approximately 12.63 g m^{-2} $year^{-1}$ (wet weight) and 126.26 kg ha^{-1} $year^{-1}$ (wet weight), seem to be an extraordinary value for a very small fish species, much higher than, for instance, the values estimated for other planktivore populations studied in lakes from the ex-U.S.S.R. and reviewed by Waters (1977), ranging from 9-24 kg ha^{-1} $year^{-1}$. The P/B ratio of 1.07, is also elevated when compared with values of those planktivore populations, ranging from 0.7-0.8, indicating in this case that mosquitofish have a faster growth and a shorter life span (Waters 1977).

Mosquitofish normally feed primarily near the surface on zooplankton, specially free-living Cyclopoid copepods and cladocerans (Cabral et al. 1998, Colwell and Schaefer 1983, Crivelli and Boy 1987, Daniels and Felley 1992). Hurlbert and Mulla (1981) and Crivelli and Boy (1987) found that copepods were much less affected than cladocerans by mosquitofish predation. However, we observed exactly the contrary, with copepods constituting the most important prey group during the study period. Cladocerans were clearly less important as prey items, and ostracods were found only in a few guts. The difference in these findings may be a function of the different availabilities of the prey groups to mosquitofish. In fact, among the water phase invertebrates, copepods composed 62.8 % of the samples, whereas the cladocerans accounted for only 16.1 % (Cabral et al. 1998). Although mosquitofish prey selectively on larger zooplankters, rotifers seemed to be a relative important prey group for immature fish as well as for young males and females, which constitute the small-medium size classes (table 2).

Throughout the year, surface insects, like aphids, collembolans, adult chironomids, and other dipterans, were an important additional food source. Nevertheless, mosquito larvae (chironomids and other dipterans), which were relatively abundant in the irrigation channel sediments (Cabral et al. 1998), constituted only a small quantitative fraction of the mosquitofish diet. This observation agrees with the disappointing reports from experiments using mosquitofish for mosquito control around the world (Rupp 1996).

During the non-reproductive season, our results showed a similar diet for immature, males and non-gravid females, which ate very close quantities of the preferential prey groups (table 3). During the reproductive season, from April to July, most of the mature females were gravid. These females captured, in general, more prey items than immature, males and non-gravid females. Moreover, during this period of high reproductive investment, gravid females eat more surface insects, especially adult dipterans, than the other population groups (table 3). This may be explained by the larger size of gravid females that enable them to eat bigger prey items and the need for higher caloric intake (Harrington and Harrington 1961).

A combination of life history, population dynamics, production and eco-ethological traits (e.g., fast growth, reduced longevity, viviparity, high productivity, an intermediate position in food chain, plasticity and adaptability in its food use, and no special habitat requirements for reproduction) show that *Gambusia holbrooki*, introduced into rice fields all over the world, certainly induce an important impact in the structure and functioning of the native biological communities of these important agro-ecosystems, such as the Lower Mondego River Valley rice fields. This question is extremely important to reinforce the recommendation that Gambusia, the backbone of biocontrol for one-quarter of a century (Rupp 1996), not be introduced into new areas.

Acknowledgements

This study was supported by E.U. throughout the R & D project AIR 3-CT 94-2432 and by JNICT (Portuguese National Board of Scientific Research). The authors are indebted to Ana Frias, Ruth Carvalho and Sofia Ávila who assisted in the field and laboratory work.

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THE SPAWNING MIGRATION OF THE SEA LAMPREY (*Petromyzon marinus* L.) IN THE RIVER MONDEGO

Abstract

Presently, sea lampreys (*Petromyzon marinus* L.) are confined to the last 35 km of the River Mondego, downstream from the Açude-Ponte dam. Their spawning migration occurs between December and May-June, with a peak in February-March. During that period, this cyclostome presents a nocturnal behaviour, performing their upstream migration during the night, dawn and early morning periods.

Although the physical obstacles present in the freshwater zone of River Mondego retard the upstream movement, the submerged stone weirs create riffles, used by the sea lampreys as resting areas during the day.

The concentration of nests is higher in the first 2 km downstream from the Açude-Ponte dam. After hatching, the ammocoetes spend four years in freshwater habitats, the higher densities being located near the nest area.

Apart from habitat destruction, resulting from dam construction and channelisation, intense fishing and poaching are the major threats to the survival of the sea lampreys in the River Mondego.

Introduction

The River Mondego watershed is one of the most important Portuguese river basins for the diadromous fish. Four species are commercially exploited in this region, the sea lamprey (*Petromyzon marinus* L.), the allis shad (*Alosa alosa* L.), the twaite shad (*A. fallax* Lacépède) and the eel (*Anguilla anguilla* L.).

Assis et al. (1992) point out that the economic value of the diadromous species is directly related with the predictability of the migratory season and movement route, as well as with the occurrence of large concentrations of animals in a short time-space scale, which makes them an easy target for fishermen and natural predators.

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The sea lamprey is a migratory species whose anadromous form is exploited as a commercial fishery in most of the major rivers in Portugal, particularly in the Central and Northern regions of the country. Sea lampreys are mostly captured when entering the estuaries to initiate their upstream reproductive migration, corresponding to the terminal phase of their life cycle (Beamish 1980, Ducasse and Leprince 1980, Guimarães 1987). As the species is considered a delicacy, its commercial value is rather high, attaining market values of 9500 PTE (≅ € 47) per unit.

P. marinus is considered a "Vulnerable" species in the Portuguese Red Book of Endangered Species (Vários 1991), and the literature is unanimous in stating that the sea lamprey's populations are decreasing in all major Portuguese rivers (Guimarães 1987, Alexandrino 1990, Almaça 1990, Assis et al. 1992, Assis 1994, Ferreira and Oliveira 1996).

The human activities that promote significant changes in the physical and chemical characteristics of running water ecosystems can have major impacts in the sea lamprey population dynamics. Several authors have stressed the direct and indirect impacts on the conservation of diadromous species in general, and on the sea-lamprey in particular, resulting from the construction of dams, land recovery (drainage and flood protection), pollution (agricultural, forestry, industrial and urban use), dredging and gravel extraction, overfishing and poaching (Ducasse and Leprince 1980, Assis et al. 1992, Witkowski 1992, Young et al. 1990).

This paper describes the spawning migration of the sea lamprey in River Mondego, based on information gathered from previous works, and from the data obtained by the authors in a recent research project, involving the study of the migratory behaviour observed by ultrasonic tracking in estuarine and freshwater environments. The major threats to the survival of this species in the River Mondego watershed are identified and discussed.

The spawning migration and freshwater phase of the sea lamprey life cycle

Before dam construction, anadromous fish used to migrate into the upper reaches of River Mondego, entering some of the tributaries, namely the rivers Ceira and Alva. Since the construction of the Açude-Ponte dam, at Coimbra, in the early 80's, the migratory species are unable to pass this obstacle, and are forced to complete their life cycle in the last 35 km of the river (Fig. 1). This lower portion of the river runs in open valleys, in a plain area, has two main tributaries, the rivers Arunca and Pranto, and can be divided into two ecologically different stretches: an estuarine, and a freshwater environment. The separation between this two zones results from the presence of a 3 m height blockstone weir, the Formoselha weir, located 11 km downstream from the Açude-Ponte dam. The tidal effect is no longer visible upstream from this weir, and therefore the estuary is limited to the final 24 km of the river. The 11 km freshwater stretch between the Formoselha weir and the Açude-Ponte dam has 11 riffle areas, resulting from partially submerged stone weirs (transversal sediment retention platforms) installed across the river to avoid downstream sediment transportation.

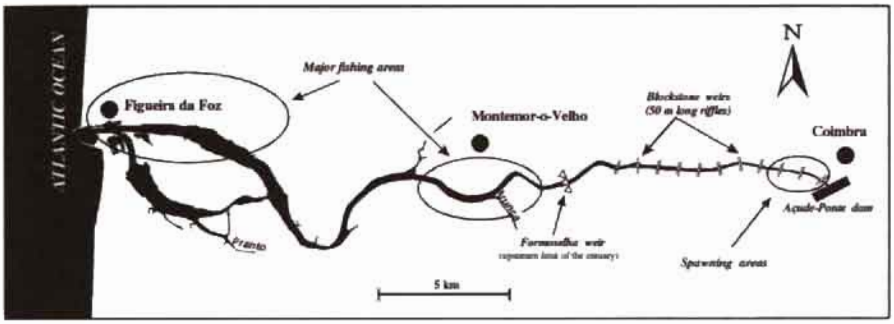

Figure 1. Schematic representation of the River Mondego downstream from the Açude-Ponte dam (adapted from Almeida et al. 2000)

The professional fishermen's activity is restricted to two river stretches (Fig.1) and is only allowed between the 15 January and the 15 April (Anonymous 1972, 1988).

Although the spawning migration of *P. marinus* in the River Mondego starts in late December (Guimarães 1987, Almeida et al. 2000), the period of time spent by these animals in the estuary or in the coastal vicinity, before they engage in their upstream migration, is still unknown. The migration continues until May-June, with a peak in February-March (Guimarães 1987, Alexandrino 1990, Machado-Cruz et al. 1990, Almeida et al. 2000). According to the information obtained from local fishermen, some spawning activity can still be observed in early August.

During their spawning migration, sea lampreys entering River Mondego present a daily migratory behaviour characterised by an alternation between resting periods and continuous upstream movements Almeida et al. (2000). According to these authors, the dusk hours are also associated with an increase in the activity of sea lampreys, but the upstream migration occurs during the night, dawn and early morning periods (*i.e.* between one hour after sunset to 9 a.m.). This nocturnal behaviour has already been observed by Hardisty and Potter (1971), Hardisty (1979, 1986) and Stier and Kynard (1986).

In the studies performed in the River Mondego, Almeida et al. (2000) found that both in the estuarine and in the freshwater environments, the movements of the sea lampreys present identical speeds. The apparently better performances showed by the animals in the estuary probably resulted from the existence of longer river stretches free from physical obstacles, enabling them to present a cruising ground speed of 16.5 body-lengths min^{-1}. This migratory behaviour can be kept for, approximately, nine hours of continuous movement (Almeida et al. 2000).

In freshwater, longer periods of continuous movement resulted in a reduction in the movement speed. The presence of numerous obstacles, namely, sand banks, shallow water sections, riffles and aquatic vegetation, significantly increased the difficulty to reach the upstream stretches of the river (Almeida et al. 2000)

The submerged stone weirs, present in the freshwater section between the Açude-Ponte dam and the Formoselha weir, are responsible for a considerable delay in the upriver progression of the sea lampreys and consequently for an extra energy consumption. On the other hand, these weirs provide the necessary disruption in the habitat continuity, with the formation of riffles, used by these animals as preferential resting areas during the day (Almeida et al. 2000).

The spawning migration ends with the arrival at the spawning site, nest building, and finally, matting and fertilisation. Nest building is mainly performed by males, usually the first to arrive at the spawning sites (Hardisty and Potter 1971, Hardisty 1979). However, Hardisty and Potter (1971) refer that, late in the spawning season, females may assist males in the construction of the nests.

The choice of spawning sites may be influenced by some abiotic factors, specially the existence of an unidirectional water flow, and the presence of a substrate with adequate characteristics to the nest construction (coarse gravel, pebbles and sand) (Ducasse and Leprince 1980, Manion and Hansen 1980, Hardisty 1986). In the River Mondego, besides these factors, the existence of a barrier that cannot be transposed, the Açude-Ponte dam, plays an important role in determining the concentration of nests in the 2 km downstream from the Açude-Ponte dam. These nests are usually located in relatively deep water (50 cm), in the river stretches between the submerged stone weirs.

Males are the first to arrive at the spawning grounds, establishing and protecting a nesting territory (Manion and Hansen 1980). In the River Mondego, poachers take advantage of this behaviour to capture the animals while they are engaged in mating activities (unpublished data).

Like other lampreys, *P. marinus* spends most of its life in freshwater environment (migratory phase and larval stage). Hardisty (1979) believes that the marine phase of the lampreys' life cycle representing only 1/3 to 1/4 of the animal's life, is probably a secondary introduction in the life cycle of a group which primarily lived in freshwater environments.

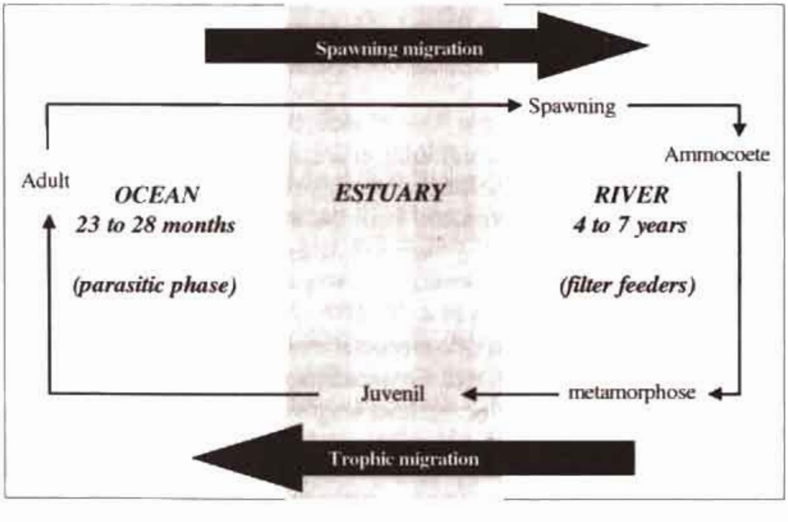

Figure 2. Sea lamprey (*P. marinus* L.) life cycle (adapted from Hardisty and Potter 1971).

The duration of this larval stage depends on the abiotic conditions of the river, and varies between 4 to 7 years (Hardisty and Potter 1971, Sousa 1988, Afonso 1989) (Fig. 2). In the case of the River Mondego, the ammocoetes spend, at least four years in freshwater habitats, before they undergo a process of metamorphosis that will lead them through a macrophtalmic stage to a juvenile form, identical to the adult (Quintella 2000). As a result, the success of a lamprey population is highly dependent on the quality of the freshwater environment, which should provide the appropriate conditions to ensure the spawning success, the development of the eggs and the survival of the ammocoetes.

The authorities responsible for the management of the River Mondego watershed should keep in mind the peculiarities of the sea lampreys' migratory behaviour. From December to June, and at least during the period between dusk and early morning (*i.e.* 1 h before sunset to 3 h after sunrise), the freshwater flow should allow the sea lampreys to reach the upstream spawning grounds with less effort. Furthermore, it is important to maintain the riffle areas. However, some of the physical obstacles (e.g. extensive shallow areas) present in the freshwater zone, upstream from the Formoselha weir, should be eliminated.

Downstream the Açude-Ponte dam the major impacts of man's activities on the aquatic environment result from agriculture-generated wastes, dam construction and channelisation. Among these three factors, dam construction induces the worst consequences to the sea lamprey population. The blocking of longitudinal connectivity drastically reduced the river basin area accessible to the migratory species. Besides, the disturbance of flow regimes downstream from the dams can attenuate the abiotic cues that are usually associated with the entering of the adult sea lampreys in a specific river (Hardisty 1979).

Although eutrophication caused by agriculture-generated wastes and domestic sewage could promote disturbances in the riverine ecosystem, the resulting increase in the phytoplankton production and in the accumulation of particulate organic matter in the sediment, might have been responsible for the good condition factor and fast growth rate of the ammocoete populations (Quintella 2000).

Finally, but by no means less important, the fishing effort from professional fishermen and poachers should be reduced, at least while there is no scientific evidence that the sea lamprey population is recovering.

Acknowledgements

Thanks are due to our colleague Dr. C.A. Assis for the useful comments on early drafts of the typescript.

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CONSERVATION BIOLOGY OF OTTER (*Lutra lutra* L.) IN THE RIVER MONDEGO BASIN

Abstract

The European otter *Lutra lutra* L. populations has a high protection status in Europe. Although little is known on the distribution and densities of this species in Portugal, are considered one of the most stable and widespread of Europe. Nevertheless, the constant loss of habitat and human activities requires an urgent evaluation on its present situation. Here we summarise what is know on the distribution of otters in the Mondego river basin, characterise the actual status populations, identify the main trheats. The otter was generally well distributed in the basin. However, the species has decreased in some areas. We found a strong relation between the presence of otters, and the quality of riparian gallery in Lower Mondego River Valley.

Introduction

In general, otter populations (*Lutra lutra* L.) in Europe have been declining (Marín and Nieves 1999), but some exceptions to this general trend have also bean referred to some zones in France (Bretagne), Spain (Galiza and Asturias) and Portugal (IUCN 1990). This situation has led to the species protection by several International Conventions and, lately, by European Directives (e.g. Habitats Directive - 92/43/CEE). In fact, since 1981 this specie is strictly protected by the Bern Convention being included in its II Annex. The European Union Habitat's Directive too has regarded the species as needing a high protection status and included it in its II Annex. Portugal has signed the Bern Convention and passed it into the National Law, as later did with the Habitat's Directive. Therefore the specie has a high protection status in both Europe and Portugal.

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In spite of its protection status, the otter is under several stresses. Poaching, fishing, water pollution and loss of habitat are the main threats to the species (IUCN 1990) (Mason and MacDonald 1986). Meanwhile, in Portugal the species distribution and abundance is considered as insufficiently known, according to the Portuguese Red Data Book of Vertebrate (SNPRCN 1990). This motivates the need to undertake studies to provide knowledge about the portuguese populations conservation status.

In 1980, S. Macdonald and C. Mason surveyed central Portugal and found a good distribution of the species in the River Mondego Basin (Macdonald and Mason 1982). Other studies provided information to support that conclusion (e.g. Simões Graça and Ferrand de Almeida 1983, Ferrand de Almeida 1987, Silveira and Reis 1991, Trindade et al. 1998). This, as well as the existence of protected areas (Natural Park of Estrela's Mountains, Natural Conservation Site of Carregal do Sal, Natural Reserve of the Marsh of Arzila) and a diversity of habitats capable of being used by the otter, enhances the natural value of the basin.

On the other hand, the basin suffers from major human pressure resulting from the strategic localisation of the basin and the uses of water. Major conflicts with nature conservation in the river and wetland habitats are the consequence of electricity production, industry, agriculture development and urban expansion. Other conflicting activities are the building of small dams, river regularisation, marginal deforestation and inert extraction. These activities are periodically undertaken, with particular expression in the last 10 years. All this generates major stresses to aquatic life, including the otter.

This paper aims to integrate scattered ecological information on the species in the Mondego basin and provide broad guidelines to its conservation.

Materials and methods

Study area

The River Mondego Basin covers an area of 6.644 Km^2 (DGRAH 1981), has a rectangular form with an orientation NE-SW. The Mondego river is the largest totally portuguese river, with 234 Km in length.

Several important natural areas are classified by Ramsar Convention, Habitats Directive (92/43/CEE), Birds Directive (79/409/CEE) and some belong to the National System of Protected Areas.

The Lower Mondego River Valley consists of about 250 Km^2 of lowland alluvial soils. It has an intensive agricultural use, producing mainly corn and rice. In the last 20 years, the valley has been through severe modification processes aiming to the flood prevention and water supply to industry, agriculture and human use. The river course was altered, the drainage system was modified and the field structure was redesigned. Some wetlands were drained and the riparian galleries were reduced in extension.

Distribution of otters in the River Mondego basin

In 1999 a survey was carried out between January and December in order to determine the distribution of otter in the Mondego river basin. The Basin was divided in 10 × 10 Km squares and all major streams in each square were visited in search of otter marks i.e. foot prints and feaces (Martins et al. in press). The site selection was not random as preference was given to easy access places. Visits were made to all major water reservoirs of the basin. The IUCN method was followed (IUCN 1990) for searching otter spraints. The collected data was integrated using Geographic Information System (GIS) software, namely for the spatial distribution graphics.

The quality of the riparian gallery in lower Mondego River section

We assessed the quality of the riparian gallery using data from the Basin Management Plan (MAOT 2000). This classifies stretches of a river according to its bankside coverage, evaluated by aerial photo analysis. The riparian gallery was classified in four categories according with their development (see Table 1). This classification was crossed with the grid used for the distribution survey in Lower River Mondego Valley and corresponding classes were attributed to each square. The entire Lower Mondego Valley was prospected for marks of otter presence (Reis and Tenreiro 1995). The area was divided into 254 squares of 1 × 1 Km. All squares presented potential for otter use including rivers, streams, drainage ditches and different wetlands. Each square was visited at least once, from October 1994 to July 1995. The IUCN method was followed (IUCN 1990). The collected data was integrated using Geographic Information System (GIS) software, namely for the spatial distribution graphics.

Table 1. Classification of the quality of the riparian galleries.

Class	Description
High	Shrubby and arboreal stages well developed on both margins
Median	Shrubby or arboreal stages on both margins
Low	Shrubby and / or arboreal stages well developed on one margin
Incipient	Margins with no shrubby or arboreal stages or shrubby stage in one margin

The diet of otter in the lower Mondego River

The diet of the otter in the Marsh of Arzila was studied by spraint analysis (Silveira and Reis 1991) collected monthly from January to December 1989 following a transept (Silveira and Reis 1991). Simões Graça and Ferrand de Almeida (1983) studied Marsh of Marujal and Casal Novo do Rio (a sector of the old main course of River Mondego). In that study, spraints were collected from November 1980 to June 1981.

The preys identified in spraint analysis were grouped in five categories: fish, amphibia, insects, birds and others. In the latter were included items less preyed such, as reptiles (*Natrix* sp.), crustaceans and mammals. The data was analysed by comparing relative frequencies of occurrence. Seasonal approach was considered, and chi-square method was used to determine significant differences in otter's diet between seasonal prey consumption.

Results

The distribution of the otter in river Mondego basin

In this survey we visited 144 locations, including large and small rivers and reservoirs. At 85 locations (59%) (Fig. 1) we found evidences of the presence of otters. Those locations will be referred as "positive sites". Positive sites covered most of the basin, including artificial hydroelectric power reservoirs, in opposition to Macdonald, S.M. and C.F. Mason (1982). Locations were no evidences for otter were found will be referred as "negative sites". Most of the 59 negative sites (41%) were found in the N-NE region and SW region of the basin (Fig. 2).

In the upper basin sector, Rivers Múceres and Dão had the lowest frequencies (<35%), whereas the highest frequencies were registered in Rivers Pranto and Ceira and in the medium and lower Mondego River, with 100% of positive sites, which is indicative of the importance of the main river for otter population.

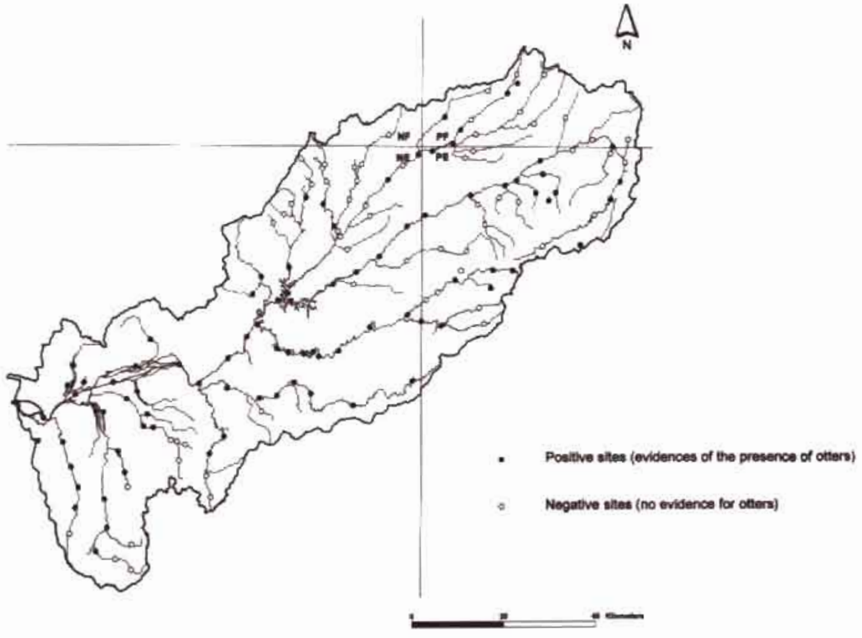

Figure 1. Study area: River Mondego Basin. Distribution of otter in River Mondego Basin based on data from Martins, et. al. (2000).

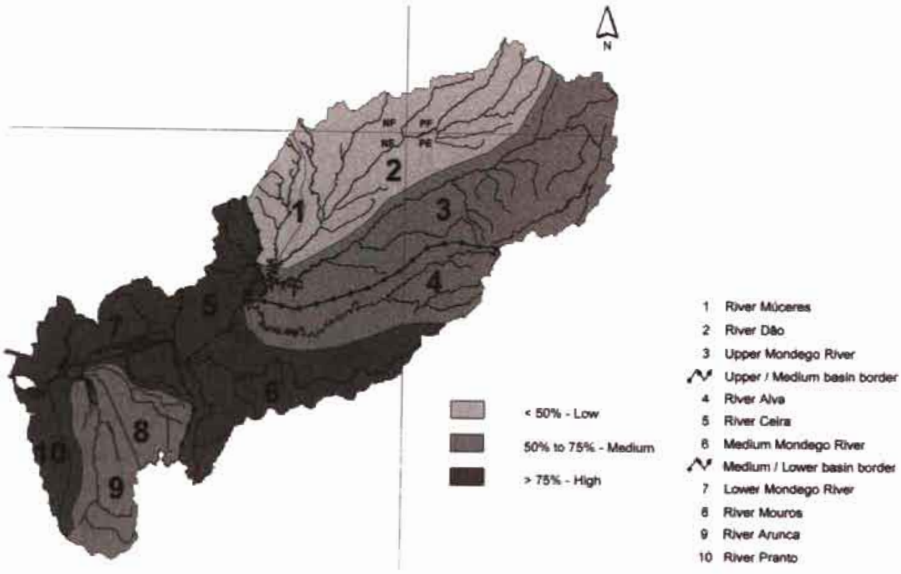

Figure 2. River Mondego Basin: small basins within each Sector. Frequency of positive stations determined for each small basin according to Martins, et. al. (in press).

Otters were not randomly distributed among the Upper, Medium and Low basin Sectors as judged by chi-square analysis ($\chi^2=15.9$; d.f.=2; $p<0.05$), although there was no significant difference between the Medium and Low Stream Sectors ($\chi^2=0.3$; d.f.=1; $p>0.05$). Small basins within each sector were also compared and showed no significant difference for Upper Sector ($\chi^2=4.3$; d.f.=2; $p>0.05$), Medium Sector ($\chi^2=1.9$; d.f.=2; $p>0.05$) and Lower Sector ($\chi^2=1.9$; d.f.=3; $p > 0.05$).

The quality of the riparian gallery in the Lower Mondego Section

We found 103 positive sites in the lower Mondego river section. In this area, the otter uses intensely the secondary basins of the valley (Fig. 3). These basins present 57% of positive sites (in 119) versus 26% of positive sites in the central valley (in 135 sites surveyed). This difference was significant, as judges by the chi-square test ($\chi^2=25.567$; d.f.=1; $p<0.001$).

Crossing positive squares against the quality of riparian galleries suggest a more frequent use of the squares with higher riparian gallery quality (Fig. 3). The otter occurred more frequently when both margins present good coverage (57% for High and 51% for Median) and scarcely when they present incipient coverage (7%) (Fig. 4). These differences were significant ($\chi^2=35.608$; d.f.=3; $p<0.001$).

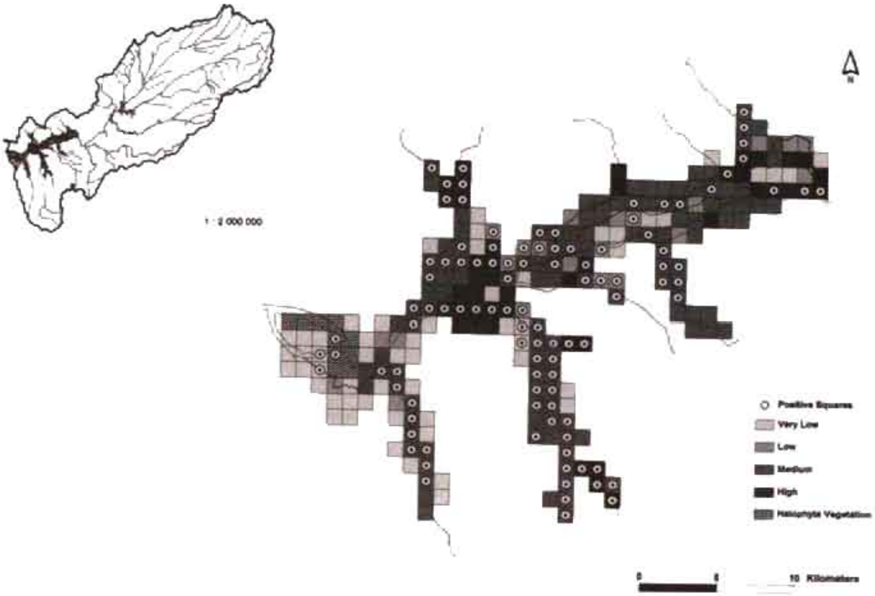

Figure 3. Lower Mondego River sector: otter distribution and quality of riparian gallery.

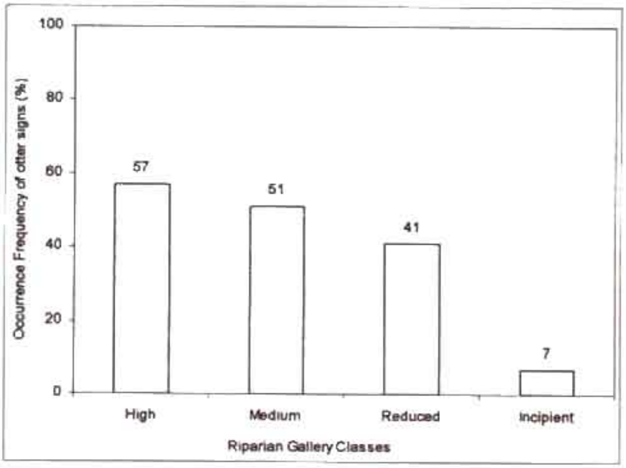

Fig. 4. Occurrence Frequency of otter in four classes of riparian gallery quality (data: MAOT 2000)

The diet of the otter in the lower Mondego River

In all the locations studied the main prey category consumed was fish (Fig. 5). amphibia (mainly *Rana*) and insects (only *Hydrous*) were important prey with similar weight in both marshes. In Casal Novo do Rio amphibia were noticeably more important then insects. Birds (Ralliforms and one Anseriform in Arzila's marsh) were consumed only in the marshes.

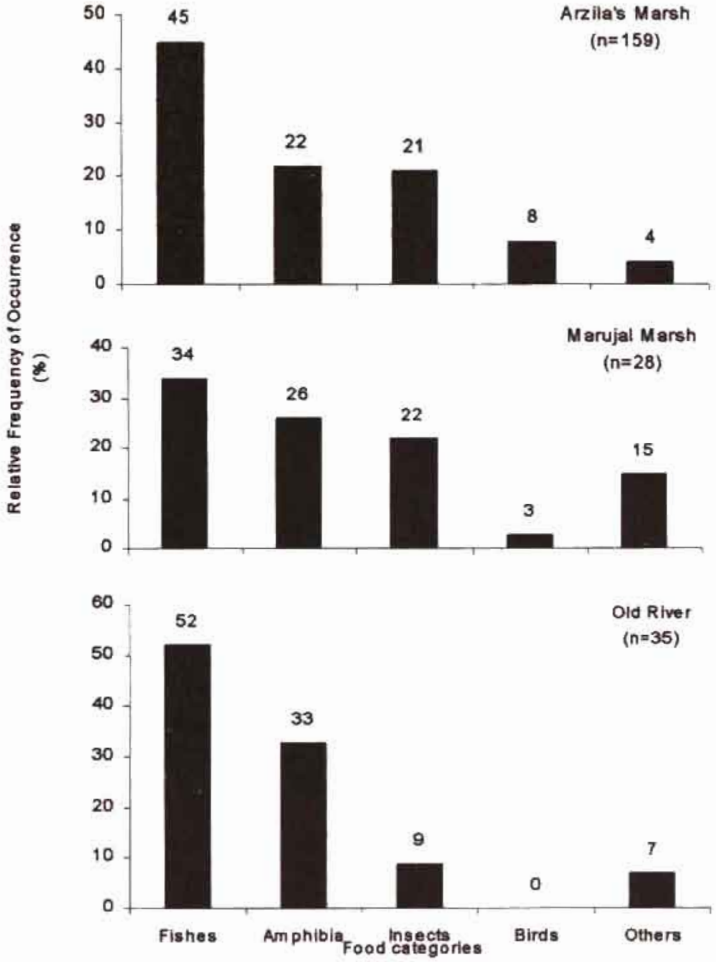

Fig. 5. Diet of otters in Lower Mondego River (Arzila data from, Silveira and Reis 1991, Marujal and Old River from, Simões Graça and Ferrand Almeida 1982)

A seasonal analysis for the Marsh of Arzila (Fig. 6), showed that the frequency of fish in spraints decreased in Autumn. Amphibia and insects do not show significant variation, being consumed throughout the year ($\chi^2=2.261$, d.f.=3, $p>0.05$). Birds were specially consumed in Autumn, being absent from spraints in Spring and Summer.

Ten species of fish were identified in the otter spraints (see Fig. 7). *Gasterosteus aculeatus* and *Anguilla anguilla* were the most frequent taxa (respectively 25% and 23%). Other species had annual relative frequencies <15% each.

Although preyed fish were usually of small size, occasionally remains of large individuals of *Cyprinus carpio* were observed.

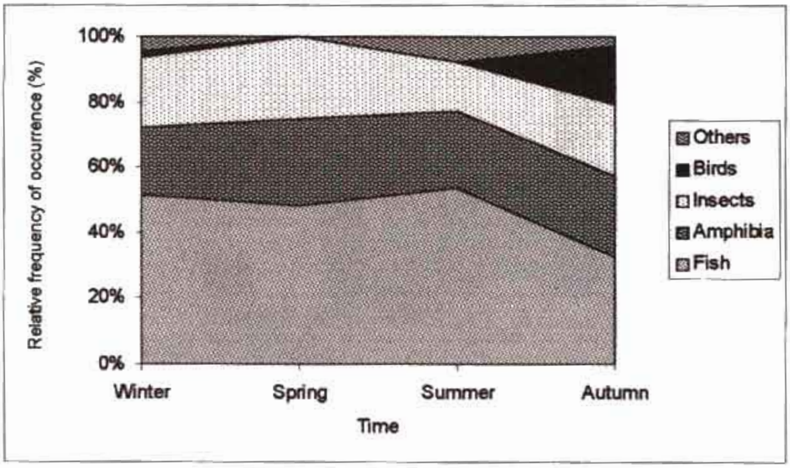

Fig. 6. Seasonality of otter diet in Natural Reserve of Arzila (data: Silveira and Reis 1991)

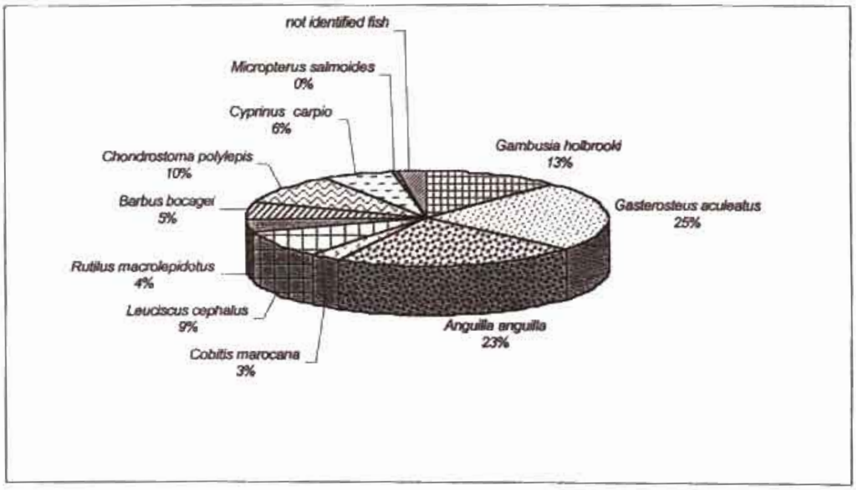

Fig. 7. Relative frequency of fish species in otter diet (data: Silveira and Reis 1991).

Discussion

According with our research, the River Mondego basin supports a widespread population of otters. However, the otter population in this basin is starting to show problems in some of the small basins. There are clear signs of regression especially in the upper parts of the rivers and in River Dão basin where previous positive observations turned negative in the last survey (Fig. 8).

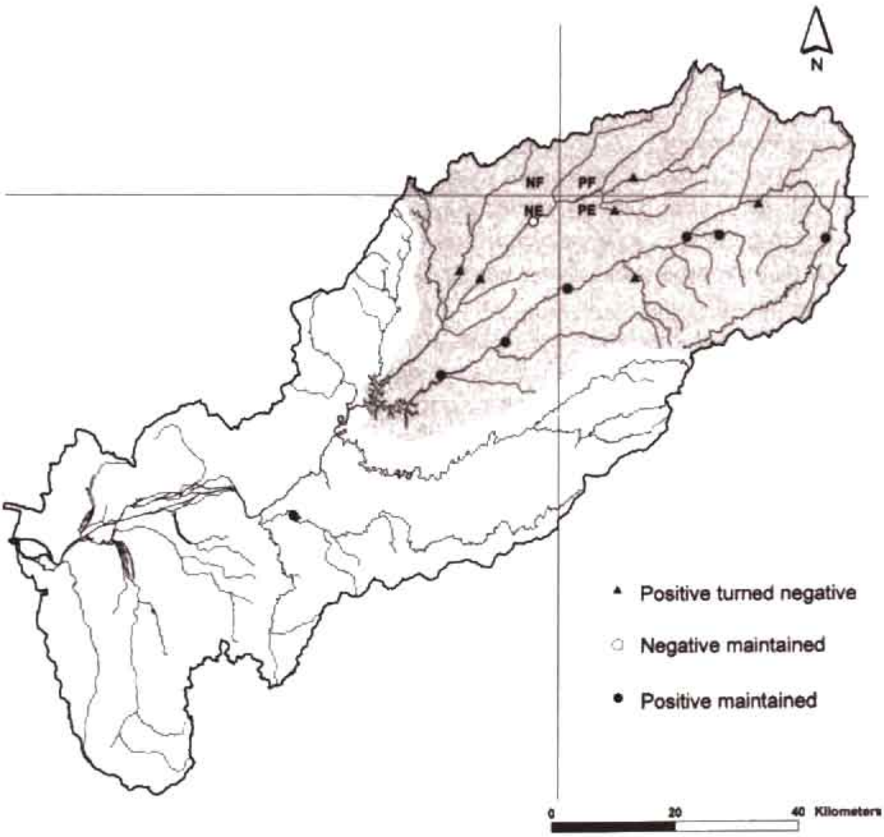

Fig. 8. Comparison and evolution of otter in River Dão and Upper Mondego river based on data from Martins, et al. (in press), Macdonald and Mason (1982).

This regression could be due to several causes, namely the degradation of water quality and destruction of marginal vegetation caused by human activities.

In the upper Mondego river, 2 of the 8 previously positive sites referred in the 1980 survey (Macdonald and Mason 1982) turned out negative in 1999. This could be caused by localised stress factors, nevertheless it shows the need to watch short-term evolution in this particular area.

At the present times, Medium and Lower basin sectors are the most important of the Basin, especially the smaller basins of River Ceira and River Pranto. These areas should have a special protection status to assure the stability of the population and to operate as a birth area from where individuals can recolonise abandoned or regressive areas in the Mondego Basin.

According to Macdonald, S.M. and Mason, C.F. (1982) hydroelectric power reservoirs, were not used by otters. However we did recorded the presence of this species in some reservoirs. It is plausible that the species managed to exploit the artificial lakes, probably due to the high availability of food. Interesting was the existence of latrines in some sites at reservoirs, which is an uncommon behaviour for the species, in this kind of habitat. No latrines were found in other habitats.

The surveys carried out by Trindade, et al. in 1998 and Martins, et al. "In press" analysed, respectively, 12 and 14 sites in the Lower Mondego basin area, both showing 100 % of positive sites. These studies suggest a more extensive use of the valley by the otter population then a survey carried out by Reis and Tenreiro (1995). In fact, in such survey a total of 151 squares prospected (59%) showed no sign of otter. This may suggest that the large-scale surveys tend to show a, not real, more favourable status of the populations.

The different intensity of sprainting activity in the lower Mondego river basin is elucidative of the importance of the secondary basins towards the conservation of otter. It suggests a higher use of the secondary basins, probably a consequence of a more intensive human use of the central valley.

Several studies pointed out a strong influence of the quality of the riparian gallery on the otter distribution and use of habitat (Prenda and Granado-Lorencio 1996). We also found a strong relation between the quality of the riparian gallery and the otter occurrence. In fact, only four squares classified as Incipient riparian gallery were found positive.

As stream and ditch maintenance practices in central Portugal usually involve the clearance of the margins, changes or adjustments to these practices may be regarded as a strong conservation issue. Despite that the more recent maintenance strategies tend to preserve one of the margins, the practices still carry a potential severe impact on the otter conservation. This situation is equivalent to the low class of the riparian gallery quality scale, which presented an occurrence frequency of 41%.

Good ditch maintenance practice should imply the preservation of the riparian galleries and favour its development. When that is not possible, different, less damaging strategies should be developed.

Other factors such as prey availability, water quality and human disturbance, have been less investigated. In the Lower Mondego River, prey availability doesn't seem to be a limiting factor: the Basin Management Plan (MAOT 2000) indicates the existence of an abundant icthyofauna.

In terms of diet, and at the time of the studies, fish were significantly the most important prey category in the three locations. Nevertheless there are differences between the two habitats studied: the river (Casal Novo do Rio as reported by Simões Graça and Ferrand de Almeida 1983) and the marshes (Arzila and Marujal as reported by Silveira and Reis 1991, Graça and Ferrand de Almeida 1983). In both marshes amphibia and insects were consumed similarly (in Arzila's marsh this was verified throughout the year). On the other hand, in Casal Novo do Rio insects were considerable more important a amphibia probably as a result of a difference in prey availability. Birds were only recovered from spraints from the marshes, suggesting a higher availability and easier capture in these areas. On a seasonal approach, in Arzila's marsh, the decrease of fish consume in Autumn may be the result of a higher consumption of water birds, which were not consumed in Spring or Summer. These were invernant species (Anseriforms and Ralliforms) that arrive on the marshes in Autumn, thus suggesting a higher availability on this season. This point out the opportunistic feeding behaviour of otter, and suggests capability to adapt to new prey community structures.

Since these diet studies were made, several modifications occurred on the habitats. Marujal's marsh was drained in the mid eighties. In the early nineties, Louisiana Crayfish *(Procambarus clarkii)* made its appearance in the Valley and on Arzila's marsh. Since then, amphibia populations are considered to be decreasing, by naturalists and the Nature Reserve staff. Another new exotic species, the fish *Lepomis gibbosus*, was also reported in Arzila's marsh (Batista 1997).

These factors must have strong influence on otter population and behaviour. The drainage of Marujal's marsh constitutes a serious loss of important otter habitat. The presence of crayfish may supply an increase of prey to otter, as the species quickly responds to crayfish availability (Delibes and Adrián 1987), spraints being commonly found with its remains.

Nevertheless this may prove negative to otters on the long-term as farmers increased pesticide use in an attempt to control crayfish (Jørgensen et al. 1997, Anastácio et al. 1995). Beside direct negative influences on otter and its prey (namely fish and amphibia), pesticides do accumulate on the surviving crayfish that may be preyed upon by otter.

The kind of diffuse stress factors and threats acting today demand, in short-term, a plan of periodic surveys over the otter population and conservative measures regarding the habitats and its quality. An integrated management policy, which attends the needs of the species as well as farming, industries and water supply, is needed.

Acknowledgements

We are grateful to A. Batista for icthyofauna information, A. J. Veiga Simão, M. Luz and Sónia Talhadas for helping with GIS software. The authors would like to thank the referee for his comments and invaluable suggestions.

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CHARACTERISATION OF THE *Echinogammarus marinus* (LEACH) (AMPHIPODA) POPULATION IN THE MONDEGO ESTUARY

Abstract

A benthic sampling program was carried out in 1985/86, in winter and summer periods, to characterise the intertidal community structure in the Mondego estuary (Portugal). *Echinogammarus marinus* (Amphipoda, Gammaridae) was considered a key species in rocky substrates, particularly in association with *Fucus spp*, and consequently a study on its life cycle and population dynamics was carried out. *E. marinus* showed an aggregated spatial distribution, and the population density changed seasonally, with peaks during spring and summer. No migratory patterns were detected between the estuary and the sea, but migrations inside the estuary might occur. An analysis of the population structure and dynamics was carried out based on the assumption that, although sexual activity was continuous through out the year, it would be possible to recognise and track population groups from their origin up to their total disappearance. Growth was continuous through life, although growth rates were higher during the early phases, and females were morphologically recognisable at smaller sizes than males. In addition, males became larger than females. Females reached sexual maturity before males, producing a succession of broods during the rest of their life. Fecundity increased with the size of females. Life span was estimated to be 10 to 12 months, but females showed higher mortality rates than males. This was probably due to higher costs of reproduction, which might cause a decrease in female survival. Sexual activity showed an increase in spring and summer. In the Mondego estuary, *E. marinus* is a semi-annual species, with three or four generations per year. Reproduction through the year, iteroparous females, high fecundity, and a multivoltine life cycle clearly suggest an r adaptive strategy. Production of *E. marinus* in the algal cover of *Fucus spp.* was estimated. Growth production (P) ranged from 6.36 to 8.81 $g.m^{-2}.y^{-1}$ (AFDW) (76.16 to 105.48 $KJ.m^{-2}.y^{-1}$) and elimination production (E) from 6.33 to 11.44 $g.m^{-2}.y^{-1}$ (AFDW) (75.84 to 137.04 $KJ.m^{-2}.yr^{-1}$). $P/\bar{B}$ ratios ranged from 6,1 to 6,3 and $E/\bar{B}$ ratios from 6,28 to 7,89. The present contribution was mostly based on a previously published paper (Marques and Nogueira 1991).

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Introduction

A benthic sampling program was carried out in 1985/86, to characterise the intertidal community structure in the Mondego estuary (Marques et al. 1993a). Amphipods appeared to have a very important role in the studied communities, both in hard and soft bottoms. Data analysis showed that only 18 of the 105 identified species had a relevant role in the observed structure (Marques 1989, Marques et al. 1993a, 1993b). Of these species, 5 (28%) were Amphipods, (*Echinogammarus marinus* and *Hyale stebbingi* in rocky substrates, *Amphitoe valida, Leptocheirus pilosus,* and *Melita palmata* in soft bottoms), which appeared therefore as a group of primary importance. *Echinogammarus marinus* (Gammaridae), with an extensive distribution and abundance in rocky habitats, was considered a key species in the algal covered habitat of *Fucus spp.*

Actually, *E. marinus* is strongly euryhaline, able to support long emersion periods (Dorgelo 1973, Pinkster and Broodbakker 1980, Marques and Nogueira 1991), and therefore very well adapted to life in estuaries. Its known distribution extends from Norway to the coast of Portugal, and some studies were already carried out on different aspects of its biology and ecology (Maren 1974, 1975 a, 1975 b, Pinkster and Broodbakker 1980, Skadsheim 1982). A study of the life cycle, population dynamics, and production of this species was carried out, from October 1985 to September 1987 (Marques and Nogueira 1991). The present contribution is mostly based on this last work, although several modifications were inserted at the light of new discussions about the same subject that took place since then.

Material and methods

In both arms of the Mondego estuary hard substrates are covered primarily by *Fucus spp.*, which constitutes an eulittoral macroalgae belt. A preliminary survey showed that *E. marinus* abundances were high in this habitat, namely in the south arm (Marques et al. 1993 a) where there is a predominance of fine particles in the sediments. Actually, high densities of this species usually depend on the presence of muddy bottoms. The examination of the population dynamics of *E. marinus* in this estuary, which is the southern limit of its distribution (Marques 1989, Marques and Nogueira 1991), and the comparison with data from other locations, should provide an opportunity to analyse the intra-specific life-history variation of a gammarid along a latitudinal gradient. In addition, it should allow estimate the production of an important warm-temperate estuarine species and to evaluate its importance with regard to energy transfers in the trophic web.

Field program

The population dynamics and field growth rates were examined through a monthly field sampling programme at three stations (figure 1) in the southern arm, for a two year period, from October 1985 to September 1987. It was recognised that such sampling periodicity could be insufficient (Fredette and Diaz 1986 a), but due to logistic constraints it was not possible to sample more frequently. Sampling was conducted during low tide, which facilitated the access to the sampling sites. Nine replicate samples were taken randomly each time. Each sample was obtained by scraping the rocky surface where the algae were attached. In addition, the superficial 1 cm sediment layer around the rocks, under the algae fronds, was also collected to reduce escape of organisms. The sampled surface was often irregular. Thus, the area was estimated by projecting its shape onto a sheet of polyethylene, which was later weighed (the biggest replicate was 0.2334 m^2, and the smallest one was 0.093 m^2). Samples (algae plus sediment) were preserved in 4% buffered formaldehyde, and later sieved into a 0.5 mm mesh bag. Based on the size of newly hatched juveniles this mesh should retain all individuals. The Amphipods were then kept in 70% ethanol. The values of salinity, temperature, oxygen dissolved, pH, nitrites, nitrates, and phosphates of the water were determined on each site. For this purpose, water samples were obtained from puddles near the algae. For the estimation of caloric contents fresh samples were obtained seasonally. In this case the organisms were kept alive up to the laboratory, and subsequently freeze-dried.

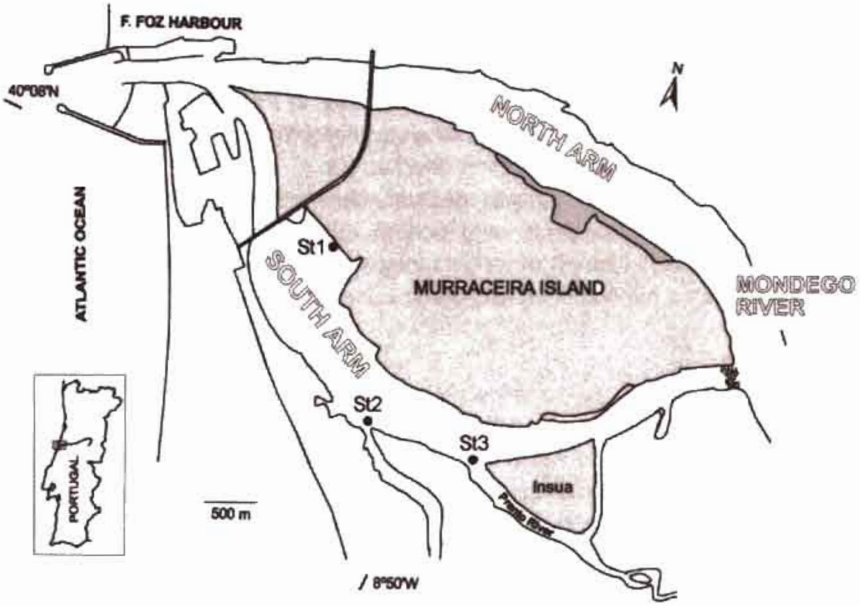

Figure 1. Location of the sampling station in the southern arm of the Mondego estuary.

Laboratory procedures

Amphipods collected were counted, and the cephalic length (L_C) was measured to the nearest 0,02 mm. Since the bodies of *E. marinus*, like most amphipods, are comma-shaped the total length (L_T) is not a very convenient standard to judge age and growth. On the contrary, the cephalic length (L_C) (measured between the extremity of the rostrum and the base of the head) is easier to determine. Thus, a sample of 90 individuals (22 males, 20 females, and 58 juveniles) were measured for both lengths, in order to provide a conversion equation: $L_T = -1.211995 + 10.668509\ L_C$ ($r = 0.986$) (Marques and Nogueira 1991).

The determination of the sex was based on the presence or absence of oostegites and/or broods (females), and of genital papillae (males). Animals without these features were considered to be juveniles. Maturity of non-brooding females was determined by the presence of setae on the oostegites. When broods were present eggs were counted, to estimate the fecundity, measured, and examined to determine the development stage. Taking into consideration several resembling criterions (Goedmakers 1981, Skadsheim 1982, Steele and Steele 1969), five stages were considered: **A** – Newly laid, eggs grouped and looking like a gelatinous mass; **B** – Eggs well separated, internally homogenous; **C** – Embryo comma-shape and initiation of pereopods already visible; **D** – Constriction of the comma clearly visible, appendages segmented and looking slender, eyes visible, cephalotorax orange-red; **E** – Hatched and free juveniles. These may stay for some days in the brood pouch, particularly if conditions are unfavourable. Since eggs became oval in shape during development, an average of their length and width was taken as measurement of their size.

Measured amphipods were then dried at 60o C for 72 h and weighed to the nearest 0.01 mg. Percentage ash was assessed after heating samples for 3 h at 550° C. In both cases small amphipods were pooled to obtain measurable values. Caloric contents (expressed in Joules) of winter, spring, summer, and autumn populations were determined with a NEWHAM micro-bomb calorimeter.

Data analysis

Abundance of amphipods in the algal covers was related both to sampled area and algal density (Fredette and Diaz 1986 a, Marques 1989, Marques and Nogueira 1991). To adjust this potential bias the algae were separated from the samples, dried at 60 °C, and weighed to determine biomass. A moving average (using prior, present, and following dates) was applied to this value. This decreased the variability between intervals and gave better estimates of the algal standing stock. The amphipods abundance was then estimated by multiplying A (number of amphipods per gram of algae in each sample) by B (mean grams of algae per square meter on a sample date).

An index ($\mathbf{I} = \mathbf{S}^2/\overline{X}$; S^2 is the variance of abundances; $\overline{X}$ is the mean abundance) (Elliot 1977) was used to analyse the population spatial dispersion.

Multiple regression models for *E. marinus* were developed: abundance, sex ratio, percentage of ovigerous females, fecundity, and percentage of juveniles in the population being correlated with the environmental parameters. The fitted regression models were expressed as:

$$Y' = a + b_1X_1 + b_2X_2 + ... + b_kX_k$$

Y' – are the values of a given dependent variable (e.g. abundance) predicted by the equation;

$X_1, X_2...X_k$ – are independent variables (e.g. salinity);

The solutions are the estimate of the regression coefficients a, b1, b2...bk. The significance of fitted regressions was tested by using analysis of variance technique (**F**), and the **t** test for the regression coefficients, as described in Edwards (1985). The models were fitted with data by the method of least squares and normal equations were solved by the matrix inversion method.

Although *E. marinus* sexual activity was continuous throughout the year, with just a short pause in late winter, it was assumed (Marques and Nogueira 1991) that it would be possible to recognise and track population groups from their origin up to their total disappearance. Despite the fact that this assumption could be controversial, this approach was considered suitable and growth rates were estimated by tracking recognisable population groups with size-frequency distributions (0.02 mm length classes) from successive sample dates (Marques and Nogueira 1991). The probability paper method (Harding 1949), as performed by Cassie (1954, 1963), was used to carry out size-frequency modal analysis. We used both the χ^2 and the **G** tests ($P \leq 0.05$) (Fisher 1950, Sokal and Rohlf 1981) to test the reliability of the method. Computations were done using the ANAMOD software (Nogueira 1992), that was then under development.

Growth rates are usually not constant along the year. Thus, to express field growth rates we used a model that takes into consideration seasonal changes (Gaschütz et al. 1980 in Marques et al. 1994), expressed as:

$$Lt = L\infty\left[1 - e^{-kD\,(t-t0)\,+\,C\,(KD/2\pi(t-ts)}\right]^{1/D}$$

- **Lt** – length of the organism at a given moment t;
- **L∞** – maximum possible length of the organism;
- **t** – given instant;
- t_0 – instant when the organism would have a length = 0;
- t_s – time interval between growth start (when t=0) and the first growth oscillation; growth is expressed by a sine curve which the period is 1 year;
- **k** – intrinsic growth rate;
- **C** – constant, which the values can change from 0 to 1;
- **D** – Parameter that expresses metabolic deviations from the Von Bertalanffy's 2/3 rule.

Production estimates were based upon population group's recognition. Growth increments or production increments (P) and elimination production (E) were calculated with a method derived from Allen (1971), (Dauvin 1986, Marques et al. 1994). Approximate values of P and E for each population group during a time interval are expressed as:

$$P=\left[(N_t+N_{t+1})/2\right]\left(\overline{W}_{n+1}-\overline{W}_t\right) \quad for \;\; W_{t+1}>W_t$$

$$E=\left[(\overline{W}_t+\overline{W}_{t+1})/2\right]\left(N_1-N_{t+1}\right) \quad for \;\; N_t>N_{t+1}$$

N – density of the population group in each sample date;
$\overline{\mathrm{W}}$ – mean individual biomass in each sample date;
t and **t+1** – consecutive sample dates;

Total values of P and E for each population group are expressed as:

$$P=\sum_{t=0}^{t=n}\left[(N_t+N_{t+1})/2\right]d\overline{W}$$

$$P=\sum_{t=0}^{t=n}\left[(\overline{W}_t+\overline{W}_{t+1})/2\right]dN$$

Total values of P and E for the population are expressed as:

$$P=\sum_{n=1}^{N}P_{cn}$$

$$E=\sum_{n=1}^{N}E_{cn}$$

Pcn and **Ecn** are the growth and elimination production of the population group n. **P/$\overline{\mathrm{B}}$** and **E/$\overline{\mathrm{B}}$** ratios were determined. $\overline{\mathrm{B}}$ (mean population biomass) is expressed as:

$$\overline{B}=\left(1/T\right)\sum_{n=1}^{N}\left(\overline{B}_n t\right)$$

T – period of study;
N – number of successive population groups in the period T;
$\overline{B}_n$ – mean biomass of the population group n;
t – duration of the population group n.

Results

Abundance and spatial dispersion

E. marinus showed an aggregated spatial distribution in the algal covered habitats of *Fucus spp* (the values of I, were always significantly higher than 1). Total abundance was lower from October 1985 to June 1986 than in the following period. In addition, it showed a clear seasonal variation, with peaks during spring and summer (figure 2). Inter-annual differences in abundance were important. In addition, abundance was quite variable between sampling sites, which might be due to the occurrence of migrations inside the estuary, although the available data is not sound enough to answer this.

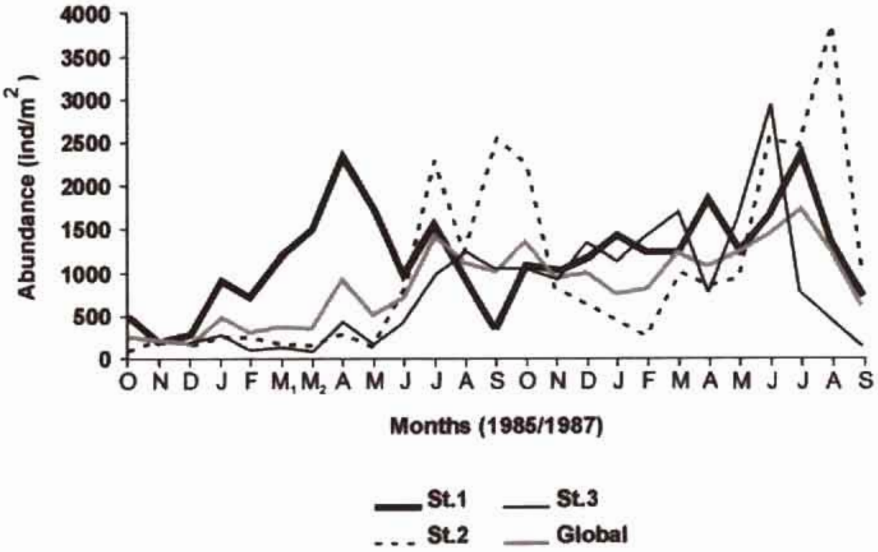

Figure 2. Variation of population abundance from October 1985 to September 1987: global values (average of all samples) (GL) and values for each sampling station (stations 1, 2, and 3) are given.

Period of reproduction

The *E. marinus* population was sexually active all over the year in the Mondego estuary. However, taking into consideration the percentage of ovigerous females over the total female population (figure 3), the sexual activity was higher from early spring (March/April) to the middle of summer (August), and then decreased until early winter (December), exhibiting therefore a clear seasonal pattern of variation. A slight increase was observed in January 1986 and 1987, followed by a new decrease until the spring population bloom. In February/March 1986 sexual activity almost stopped. The variation of the percentage of juveniles in the population (figure 3) was parallel to the ovigerous females variation. Thus, the higher abundances in spring and summer were clearly related to an increase in recruitment. In addition, sexual activity was less intense in the winter 1985/1986 than in 1986/1987, which might explain lower abundances during winter and spring 1985/1986.

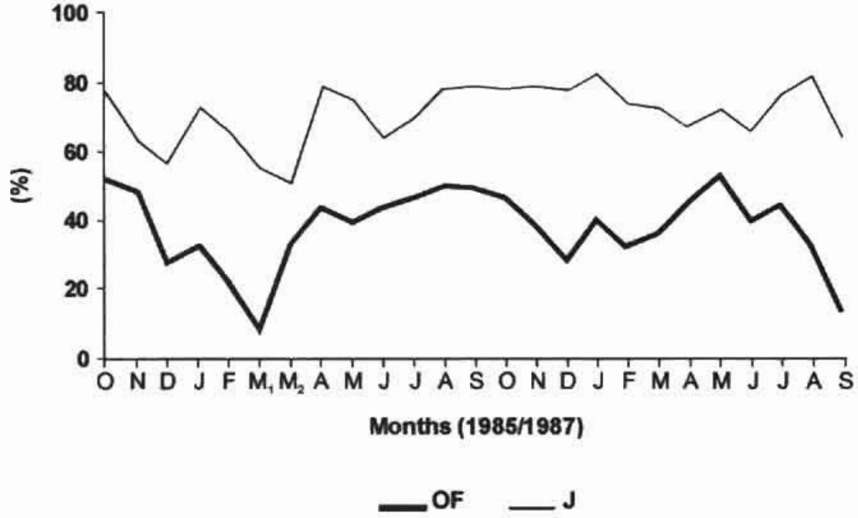

Figure 3. Variation of the percentage of ovigerous females in the total female population (% OF) and the percentage of juveniles in the population (% J), from October 1985 to September 1987.

Sex ratio

The sex ratio (males/females) was almost always favourable to females (figure 4). Still, peaks in the proportion males occurred in January/February and April/May, in 1986 and 1987, and in August 1987. These peaks were normally followed by increases in the percentage of females at sexual rest (oostegites present, with or without setae, but no broods) (figure 4). This was probably due to higher mortality rates of older females after laying eggs in winter, spring, and in summer (at least in 1987). Thus, males appeared to live longer than females.

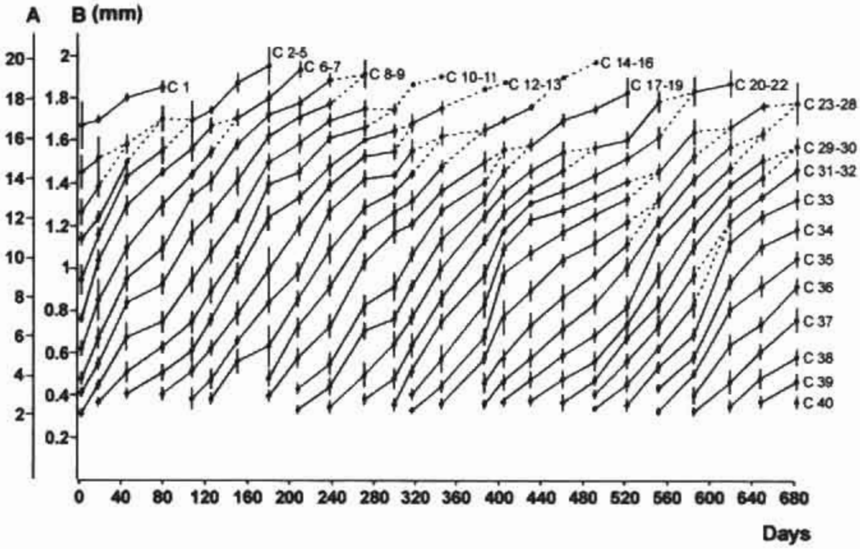

Figure 4 Variation of the sex-ratio and of the percentage of females in a state of sexual rest (oostegites present, with or without setae, but no broods) (% FSR), from October 1985 to September 1987.

Fecundity

The fecundity of females of similar sizes was quite variable. Although a significant correlation was found between number of eggs (NE) within the brood pouches and size of females. The fitted regressions were:

Stage A: – NE = -46.936 + 55.122 L_c (80 females; r = 0.8220)
Stage B: – NE = -33.570 + 44.662 L_c (257 females; r = 0.5684)
Stage C: – NE = -31.421 + 41.222 L_c (82 females; r = 0.6239)
Stage D: – NE = -33.673 + 39.153 L_c (151 females; r = 0.5500)

We did not take into consideration embryos in stage E, which can freely get in and out of brood pouches. Lost of eggs during development were therefore estimated as ≈ 28%, which might be due to incomplete fertilisation, disease, parasitism, or other accidental causes.

Since Amphipods have synchronised embryonic development, all the eggs in a given brood pouch are normally at the same stage. Measurements of eggs (table I) showed that, during development, they increase 36% in mean ovular diameter and 84% in volume. Since losses of eggs were ≈ 28%, an increase of 32% in the brood pouch volume might be enough to allow the observed 84% augmentation in eggs volume.

Table I. Average diameter and standard deviation of eggs at development stages A, B, C, and D, for *Echinogammarus marinus* in the Mondego estuary.

Stage	Diameter (mm) Average ± standard deviation
A	0.512 ± 0.049
B	0.537 ± 0.032
C	0.613 ± 0.025
D	0.695 ± 0.029

Influence of environmental parameters

The influence of salinity, temperature, dissolved oxygen, pH, nitrites, nitrates, and phosphates of water on abundance, sex ratio, percentage of ovigerous females, percentage of juveniles in the population, and fecundity was analysed (table II).

Abundance was positively correlated with temperature and dissolved oxygen (P ≤ 0,05). As organisms were sampled at low tide, higher concentrations of oxygen in the water might have favoured higher abundances of *E. marinus*. During emersion periods, however, organisms depend essentially on water retention. In addition, higher abundances in spring and summer are probably related to higher temperatures, and an increase in recruitment. Higher temperatures, effectively, determine shorter periods of embryonic development (Borowsky 1980).

The sexual activity, measured by the percentage of ovigerous females, which is obviously related with the percentage of juveniles in the population, was positively correlated with salinity and temperature. This explains the increase of abundance in spring and summer.

Table II. Multiple regression models for *Echinogammarus marinus*: abundance (D), sex ratio (males/females), percentage of ovigerous females in the total female population (% OF), percentage of juveniles in the population (%J), and fecundity (NE) are correlated with salinity (SAL), temperature (T), oxygen dissolved (OXYG), pH, nitrites (NTI), nitrates (NTA), and phosphates (PHOS). R – multiple regression coefficients. Values of significant tests (F and t) are given.

Multiple regression models (degrees of freedom: 66 in the numerator; 73 in the denominator)

	SAL	T	OXYG	pH	NTI	NTA	PHOS	Ryy'	F	
D	0.003	0.250	0.365	0.411	-0.212	0.068	-0.092	-0.072	0.5104	4.673
Males/females	0.000	-0.207	0.365	0.307	0.002	-0.319	-0.004	-0.151	0.2953	1.996
% J	0.002	0.180	0.007	0.155	-0.037	0.008	-0.107	0.068	0.2282	1.573
NE	0.028	0.237	0.180	-0.124	0.122	0.145	-0.240	-0.336	0.4812	4.143

Values of t for the regression coefficients (66 degrees of freedom)

	SAL	T	OXYG	pH	NTI	NTA	PHOS
D	1.887	2.996	3.378	1.925	0.573	0.727	0.581
Males/females	1.411	0.050	2.268	0.014	2.407	0.032	1.096
% J	1.203	2.029	1.211	0.296	0.648	0.751	0.493
% OF	2.084	0.203	0.960	0.633	0.848	0.975	0.616
NE	1.758	1.456	0.997	1.085	1.196	1.880	2.665

The sex ratio was positively correlated with the percentage of dissolved oxygen, and negatively with the nitrite concentration. This might indicate that males are less tolerant than females to water oxygen depletion in the water column and to higher concentrations of nitrites.

The number of eggs per female was negatively correlated with the concentration of phosphates in the water. Although concentration values were always low (maximum was 0.086 mg/l), this might indicate a negative influence of this parameter over fecundity.

Growth and life span

Size-frequency distributions were analysed for recognisable population group. In October 1985 we identified 10 population groups, and 30 new ones were recognised during the study period. Minimum average L_c of new population groups ranged from 0.315 to 0.487 mm (2.15 to 3.98 mm L_T). This might have depended on the number of days from hatching to recognition of the new population group, and therefore on sampling periodicity. Maximum mean L_c of population groups ranged from 1.86 to 2 mm (18.62 to 20.13 mm total length). The biggest male had 2 mm L_c (20.16 mm L_T), and the biggest female 1.86 L_c (18.63 mm L_T) (figure 5).

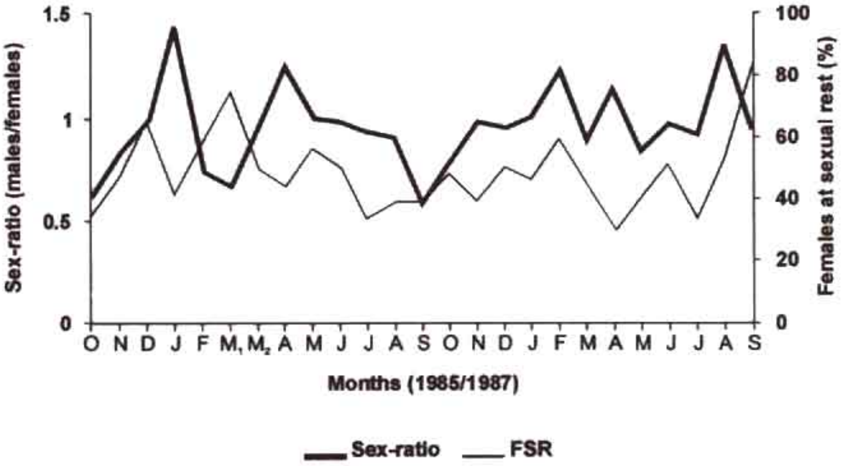

Figure 5. Growth of population groups (average cephalic length ± standard deviation), from October 1985 to September 1987. A: total length; B. cephalic length. Broken lines indicate probable evolutions.

Field growth rates were estimated from the average size of recognisable population groups. Growth was found to be continuous through life, although growth rates were higher in early phases. Mean life span was estimated at 338 ± 29 days (confidence interval; $P \leq 0.001$), that is to say 10 to 12 months. However, as we said before, males seem to live longer than females.

A growth model (Gaschütz et al. 1980) was used on data from population groups 10, 13, 17, and 21, firstly recognised in autumn, winter, spring, and summer sample dates (figure 6). In all cases a decrease in growth rate corresponding to the winter period become evident. This decrease was probably due to lower metabolism caused by lower temperatures, and also less available nutrients.

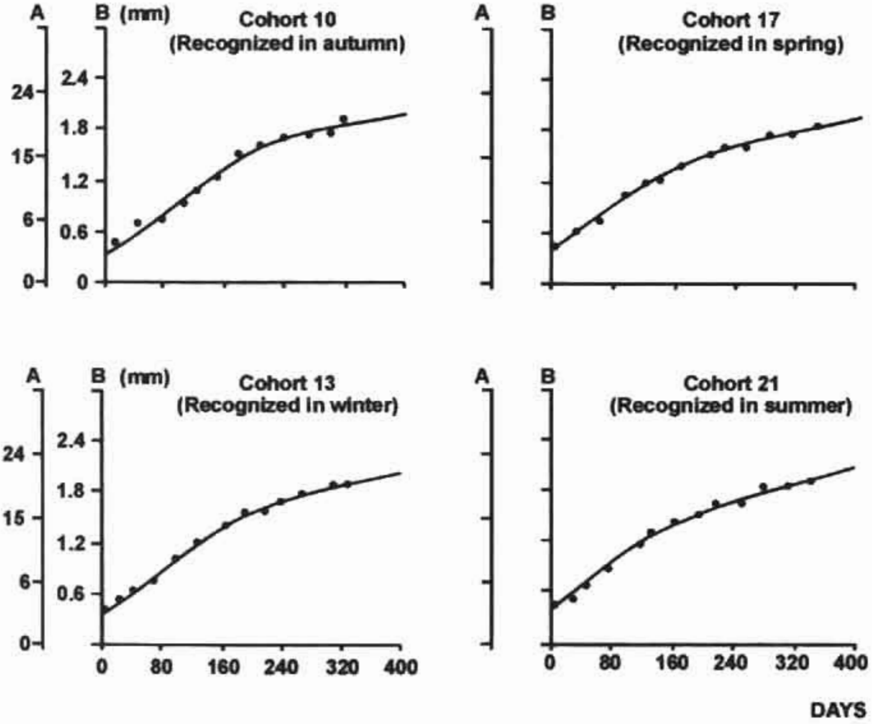

Figure 6. Graphic adjustment of the growth curves of population groups 10, 13, 17, and 21. Values of average cephalic length estimated for each given instant are also plotted. A: total length; B: cephalic length; r: correlation between the adjusted curves and the plotted values.

Males were recognisable with 0.86 ± 0.06 mm L_C (standard deviation) and females with 0.79 ± 0.04 mm L_C (7.95 ± 0.66 and 7.25 ± 0.42 mm L_T), that is to say with a minimum of 9 to 11 and 8 to 10 weeks respectively. Thus, sexual differentiation seems to occur earlier in females than in males, although this could also be due to higher growth rates in males.

Life cycle

Ovigerous females were present in the population throughout the year, with peaks in spring and summer, and therefore juveniles were always an important element of the population. The smallest ovigerous females had 1.12 ± 0.074 mm L_C (10.68 ± 0.8 mm L_T), which corresponded to an age of 14 to 19 weeks.

Percentage of ovigerous females in each population group (figure 7) was determined from size frequency analysis. We estimated that each female may have a minimum of three and a maximum of six or seven broods (iteroparous females; semi-annual species). Therefore, three or four generations per year occurred in the Mondego estuary (multivoltine life cycle).

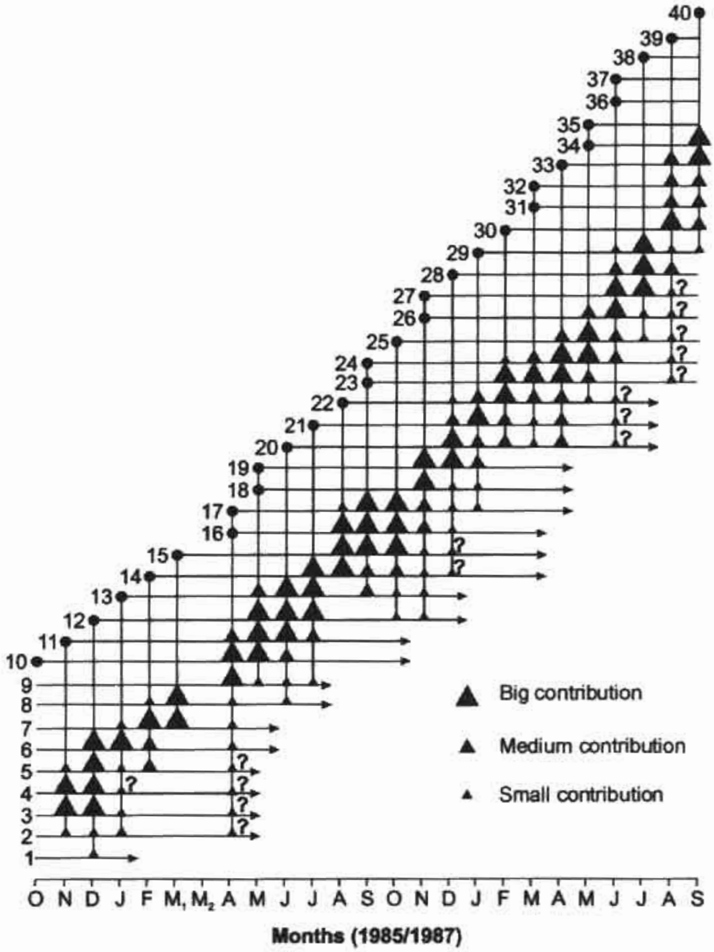

Figure 7. Analysis of sexual activity: The contributions of the females of each population group are indicated. Big contribution: more than 20% of total ovigerous females; medium contribution: from 10 to 20%; small contribution: less than 10%; ? indicates a possible contribution.

Production estimates

Length-weight relations in each season were not different on the basis of an analysis of variance and a t test ($P \leq 0,05$). Thus, the entire data set was combined to provide a single regression expressed as:

$W = 1,5929 * Lc^{2.9344}$ (W – ash free dry weight)

Microbomb combustion of 941 individuals showed that juveniles had caloric contents slightly inferior to adults (11 KJ/g AFDW). In addition, females have caloric contents higher than males (12.3 and 11.5 KJ/g AFDW respectively). Growth production (P) ranged from 6.36 to 8.808 $g.m^{-2}.year^{-1}$ (AFDW) (76.16 to 105.48 $KJ.m^{-2}.year^{-1}$) and elimination production (E) from 6.33 to 11.44 g.m-2.$year^{-1}$ AFDW (75.84 to 137.04 KJ. m^{-2}. $year^{-1}$). $P/\overline{B}$ ratios ranged from 6.1 to 6.3 and $E/\overline{B}$ ratios from 6.28 to 7.89.

Discussion

E. marinus showed a contagious distribution and the population abundance showed clear seasonal variations, with peaks in spring and summer, due to an increase in recruitment. It was not possible to detect any migratory patterns between the Mondego estuary and the sea. This does not agree with the observations of other authors (Pinkster and Broodbakker 1980), and therefore this type of migration might depend on particular environmental conditions of each estuarine system. Nevertheless, migrations inside the estuary may occur, although the available data is insufficient to confirm this. Therefore, despite the significant correlation found between abundance and temperature, which is consistent with the observed seasonal variations, there is a probability that changes in abundance in each site might not depend only on population blooms, but also on any migratory patterns, as observed for *Echinogammarus beriloni* (Goedmakers 1981).

Growth is continuous throughout the life. Females are morphologically recognisable and reach sexual maturity before males, and then produce a succession of broods during the rest of the life. In addition, fecundity increases with the size of females. Although both males and females survived for at most a year, females showed higher mortality rates, and therefore males lived longer, as observed in several *Gammarus* species (Steele and Steele 1969, 1970). This was probably due to the intense sexual activity of females and the consequent phenotypic costs of reproduction. This might have caused a decrease in female parental survival, as observed for example in *Gammarus lawrencianus* (Steele and Steele 1986).

The sexual activity was continuous through the year, with a clear increase in spring and summer, but recruitment was found to be discontinuous. The recognition of new population groups was dependent on sampling periodicity and, probably, on the retention of newly hatched juveniles within brood pouches.

With regard to life cycles it is commonly accepted that the reproductive patterns of Gammarids evolved in relation to environmental constraints (Steele and Steele 1975, Wildish 1982). In the Mondego estuary, which is the southern limit of its known distribution, *E. marinus* is semi-annual, with three or four generations per year (multivoltine life cycle). Other populations of this species show very different life cycles, respectively univoltine in Denmark, in a cold temperate to sub-polar climate (Skadsheim 1982), and bivoltine in Normandy, in a maritime temperate climate (Pinkster and Broodbakker 1980).

Assuming that the observed life cycles are evolutionary stable (Maynard Smith 1974), we must conclude that Gammarids can have large intraspecific variations in reproductive patterns. This feature might have an important role in marine amphipod speciation. In addition to reproduction through out the year, iteroparous females, high individual fecundity, and a multivoltine life cycle revealed an *r* adaptive strategy of *E. marinus* in the Mondego estuary. This is to be expected in a warm-temperate estuary with physically controlled communities, and where opportunistic strategies normally occur.

Both growth production (P) and elimination production (E), when compared with values found in literature (e.g. Birklund 1977, Carrasco and Arcos 1984, Dauvin 1988 a, 1988 b, 1988 c, 1988 d, Fredette and Diaz 1986 b, Hastings 1981, Möller and Rosenberg 1982, Van Senus and McLachlan 1986) can be considered elevated. Data on *E. marinus* production from the Mondego reinforces the generalised notion of estuaries as highly productive systems. It also reflects the important role of amphipods concerning the production of habitats colonised by them.

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LIFE CYCLE, BIOLOGY AND PRODUCTION OF AN ESTUARINE ISOPOD *(Cyathura carinata)*

Abstract

The life cycle, population dynamics and production of *Cyathura carinata* (a keystone species in the Mondego estuary, Portugal) is described from an intertidal mudflat along a spatial gradient of eutrophication. New information is presented concerning the role of macroalgal blooms in the biology, dynamics and production of the estuarine isopod *C. carinata*. A previous hypothesis that large and extensive macroalgal blooms could bring about the disappearance of this species from the intertidal area was not supported. Moreover, it seemed that large biomasses of the macroalgal *Enteromorpha* sp. could favour the population in the Mondego estuary. Just one annual cohort was produced in June, in opposition to previous studies. Our results suggest that *C. carinata* is an annual species in the Mondego estuary, with univoltine life cycle.

Growth production (P) of *C. carinata* during the algal bloom was 10.543 $g.m^{-2}$ 4 $month^{-1}$, and 19.623 $g.m^{-2}$ $year^{-1}$, and without macroalgae 7.718 $g.m^{-2}$ 4 $month^{-1}$, and 11.795 $g.m^{-2}$ year-1. $P/\overline{B}$ and $E/\overline{B}$ ratios were always lower during the macroalgae blooms, which is consistent with the idea that the energy flow is smaller in these periods because the standing stock is much higher. Moreover, this paper reveals that conclusions based on just one annual cycle can be incorrect. Longer sampling programs are needed to have a better understanding of the real dynamics of macrobenthic species in very dynamic systems.

Introduction

Most European estuaries are affected by organic pollution, which often gives rise to eutrophication (Dijk et al. 1994, Flindt et al. 1997, Hickel and Mangelsdorf 1993, Marques et al. 1997, Martins et al. 2001, Pardal 1998, Pardal et al. 2000, Raffaelli et al. 1998, Yeates 1993). Eutrophication, as a response to nutrient enrichment, may commonly cause proliferation of opportunistic green macroalgae, such as

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Chaetomorpha, *Enteromorpha* and *Ulva*, which can cover extensive areas of the estuarine intertidal zones. For example, it has been observed that benthic eutrophication in coastal areas may drive a shift from rooted plants communities, dominated by slow growing species, (e.g. *Zostera*), towards faster growing macroalgae (Hartog 1994, Martins et al. 2001, Pardal 1998, Pardal et al. 2000), as well as changes in the biomass and species composition of macroalgae (Lavery et al. 1991, Pardal 1998). This shift in primary producers may of course determine changes in species composition at other trophic levels (e.g. macrofaunal composition) originating a new trophic structure (Pardal 1998).

Seasonal intertidal macroalgal blooms (mainly of *Enteromorpha* spp.) have been reported in the South Arm of the Mondego estuary for several years (Cabral et al. 1999, Flindt et al. 1997, Lillebø et al. 1999, Marques et al. 1993a, 1993b, 1997, Martins et al. 1997, 2001, Pardal et al. 1993, 2000, Pardal 1998). Nevertheless, such macroalgal blooms may not occur in exceptionally rainy years (e.g. year 1994) due to the occurrence of low salinity values and strong water currents for long periods, as a result of Pranto River discharge (Martins et al. 2001, Pardal 1998, Pardal et al. 2000).

The isopod *Cyathura carinata* is one of the most abundant macrofaunal species in the Mondego estuary and extensively distributed throughout intertidal mudflats especially in the South Arm (Marques and Nogueira 1991, Marques et al. 1993a, 1993b, 1994, Martins et al. 1997, Pardal 1998).

Cyathura carinata is very well adapted to life in estuaries and is widely distributed along the European coast, from the Baltic to the Mediterranean. Several studies on the biology and ecology of *C. carinata* were made (Bamber 1985, Burbanck and Burbanck 1975, Eslava 1994, Jadzewski 1969, Legrand and Juchault 1963, Marques et al. 1994 Martins et al. 1997, Ólafsson and Persson 1986), but the impact of the macroalgal blooms on the life cycle, population dynamics and production have received little attention. (Martins et al. 1997, Pardal 1998).

Materials and methods

Study site and sampling

The Mondego estuary is a warm-temperate coastal system (Fig. 1). It consists of two arms, north and south, with very different hydrologic characteristics. The northern arm is deeper, while the southern arm is largely silted up, especially in upstream areas, which causes most of the fresh-water discharge to flow through the northern arm. Consequently, the water circulation in the southern arm is mainly dependent upon tidal activity and on the, usually small, fresh-water input of the tributary, the Pranto River that is controlled by a sluice.

Although a large part of the intertidal area of the southern arm still remains more or less unchanged, macroalgae blooms of *Enteromorpha* spp. have been regularly observed during the last 15 years. This is probably a result of excessive nutrient release into the estuary, coupled with longer persistence of nutrients (nitrogen and

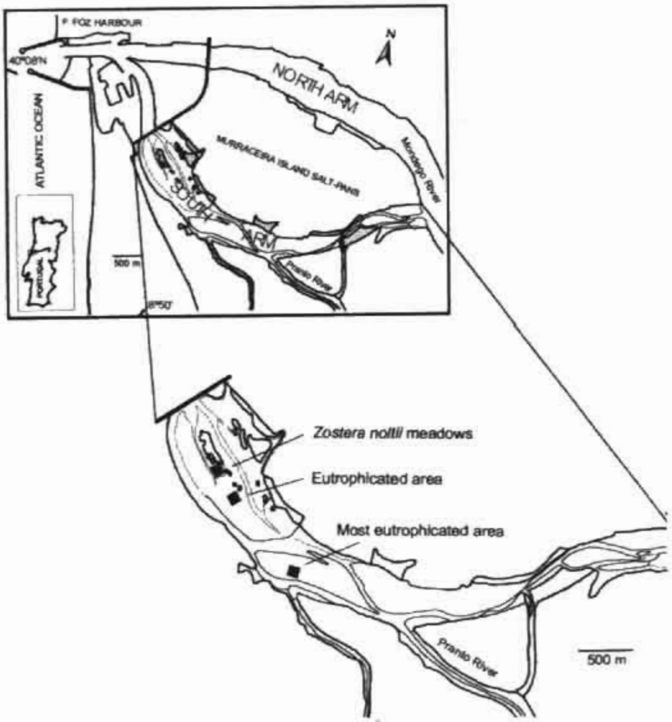


Fig. 1. Location of the sampling stations along a spatial gradient of eutrophication in the Mondego estuary, Portugal.

phosphorous) in the water column (Flindt et al. 1997, Marques et al. 1997, Martins et al. 2001, Pardal 1998, Pardal et al. 2000). Nevertheless, such macroalgae blooms may not occur in exceptionally rainy years (e.g. year 1994) due to the occurrence of low salinity values and strong water currents for long periods, as a result of the Pranto river discharge (Martins et al. 2001, Pardal 1998).

The population of *C. carinata* was monitored for 18 months, from January 1993 to June 1994. Samples of macrophytes, macroalgae, and associated isopods were taken fortnightly at three different sites, during low water, along an estuarine gradient of eutrophication in the south arm of the estuary (Fig. 1). From a non eutrophicated zone where a macrophyte community *(Zostera noltii)* was present, up to a heavily eutrophicated zone, in the inner areas of the estuary, from where the macrophytes have disappeared and *Enteromorpha* spp. blooms have been observed there over the last decade. In this area, as a pattern, *Enteromorpha* spp. biomass normally increases from early winter (January/February) up to July, when an algal crash usually occurs due to anoxia and most of the biomass is washed out into the Atlantic.

Each sampling date at each site, 10 cores (with 141 cm^2) were taken randomly to a depth of 15 cm. Each core was placed in a separate plastic bag and sieved within an hour of sampling by washing it in seawater through a 500 μm mesh sieve.

Laboratory procedures

Isopods were identified, counted, measured and sexed. Due to the difficulties inherent in measuring Total body Length (T_L) accurately in such a small species, an alternative and more accurate length was measured; the Cephalic Length (C_L). In the present paper we used the conversion equation C_L-T_L ($T_L = 0.745 + 9.01C_L$) and length-weight relationships ($W = 0.01906\ C_L^{2.7182}$) for production estimates determined in a previous study also made on the Mondego estuary (Marques et al. 1994). Individuals were dried at 60 °C for 72 h and weighed to the nearest 0.01 mg. AFDW was assessed after combustion of samples for 8 h at 450 °C. The same procedure was used to quantify the macroalgae and *Zostera noltii* biomasses.

The determination of sex was based on the presence or absence of oostegites in pereiopods and/or broods in females, or the presence of an appendix masculina on the second pair of pleopods in males. Animals without these features were considered to be juveniles. When broods were present, eggs were counted to estimate the fecundity, measured and examined to determine the development stage. Taking into consideration several similar criteria (Bamber, 1985, Jadzewski, 1969, Marques et al, 1994) three stages were considered: a) newly laid eggs, which are slightly oblong, internally homogeneous, and have a double membrane; b) embryos which are clearly elongated, possess developing pereiopods and visible antennae; c) hatched juveniles contained in the brood pouch.

Data analysis

An index $I = S^2X / \overline{X}$; was used to analyse the population's spatial dispersion, where S^2 is the variance of abundance and $\overline{X}$ is the mean abundance (Elliot, 1977). Stepwise, least squares multiple regression models for *C. carinata* were developed, to examined the relationship between the density and biomass of *C. carinata* and the biomass of macroalgae, salinity and water temperature. All variables except temperature and salinity were subjected to a $ln\ (x + 1)$ transformation prior to analysis in order to minimise heteroscedasticity. Only variables with partial effects significant at $P < 0.01$ level were retained in the regression model. Regressions were performed using the Stepwise Forward Analysis method performed by the STATGRAPHICS 4.0 statistical package.

Growth rates were estimated as in previous papers (Lillebø et al. 1999, Marques and Nogueira 1991, Marques et al. 1994, Pardal 1998, Pardal et al. 2000) by tracking recognisable cohorts with size frequency distributions from successive sample dates.

Production was estimated as in previous papers (Lillebø et al. 1999, Marques and Nogueira 1991, Marques et al. 1994, Pardal et al. 2000, Pardal 1998) and was based upon cohort recognition. Growth increments or net production (P) and elimination production (E) were estimated with a method derived from Allen (Allen 1971), as described in Dauvin (1986).

Total values of P and E for the population are expressed as:

$$P = \sum_{n=1}^{N} P_{cn}$$

$$P = \sum_{n=1}^{N} E_{cn}$$

P_{cn} and E_{cn} are the growth and elimination of the cohort n.

$P/\overline{B}$ and $E/\overline{B}$ ratios were determined. $\overline{B}$ (mean population biomass) is expressed as:

$$\overline{B} = \left(\tfrac{1}{T}\right)\sum_{n=1}^{N}\left(\overline{B}_{n}t\right)$$

where *T*: period of study; *N*: number of successive cohorts in the period *T*; B_n: mean biomass of the cohort *n*; *t*: duration of the cohort *n*.

Results

Macrophyte and macroalgae biomasses

As expected, *Zostera noltii* biomass exhibited a clear seasonal variation. During autumn and winter total biomass depended essentially on the rhizomes, and during spring and summer total biomass increased due to the growth of leaves (Fig. 2A).

The specific composition of macroalgal biomass changed very much along the eutrophication gradient. Red macroalgae, essentially *Gracilaria* sp., were relatively abundant at the *Zostera noltii* meadows, decreasing along the eutrophication gradient, while green macroalgae (*Enteromorpha* sp.) showed exactly the opposite pattern (Fig. 2).

In the eutrophicated area small amounts of green macroalgae were present in both spring seasons, but the biomasses estimated could not be considered a typical bloom.

In the most eutrophicated area *Enteromorpha* sp. presented a typical spring bloom in 1993, with biomass reaching 413 g.m^{-2} (AFDW) in April. In early summer an algal crash occurred which had a severe impact on the macrofauna in the area attained by the phenomenon (Figs. 2 and 3). In 1994 no macroalgal blooms were observed because it was a very rainy year and the sluice of the Pranto river discharged large amounts of freshwater direct in the estuary causing low salinities and strong water currents. Such conditions inhibited *Enteromorpha* growth, since salinity was below 10‰ for several months.

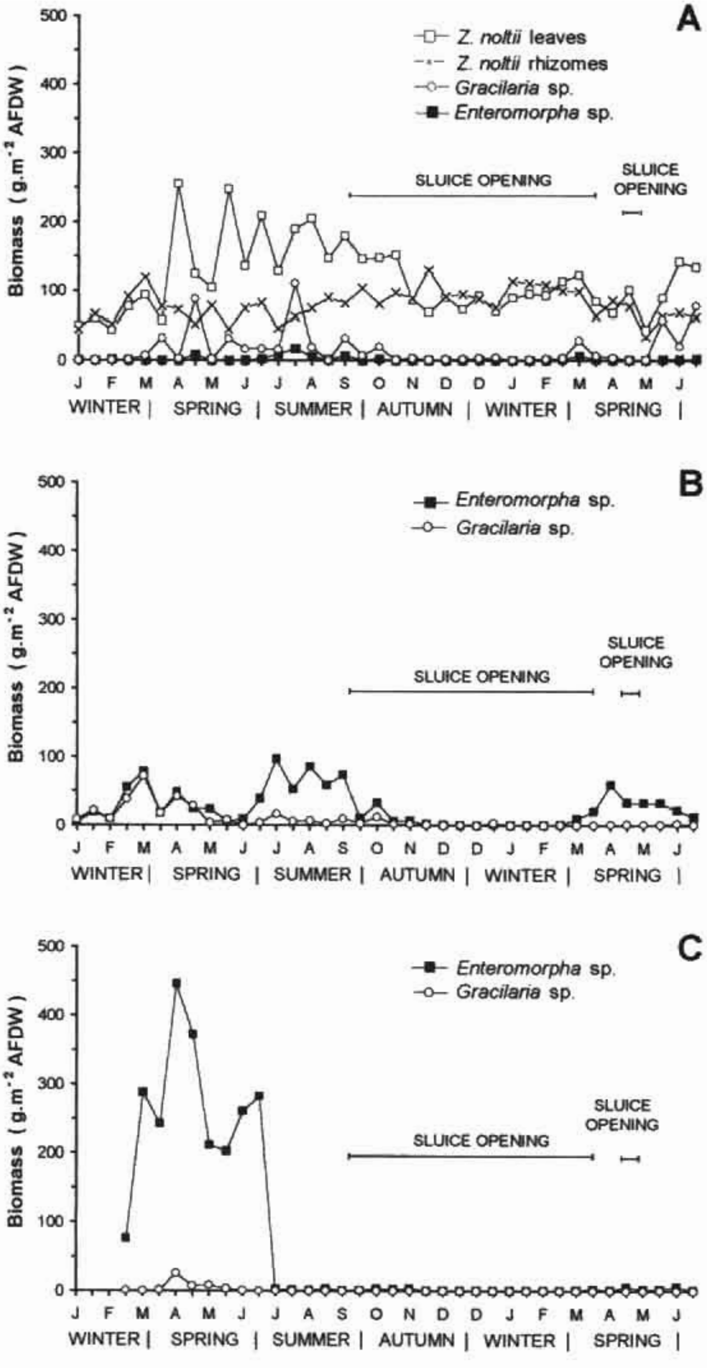


Fig. 2. Seasonal changes in plant biomass along the eutrophication gradient. (A) *Zostera noltii* meadows; (B) Eutrophicated area; (C) Most eutrophicated area.

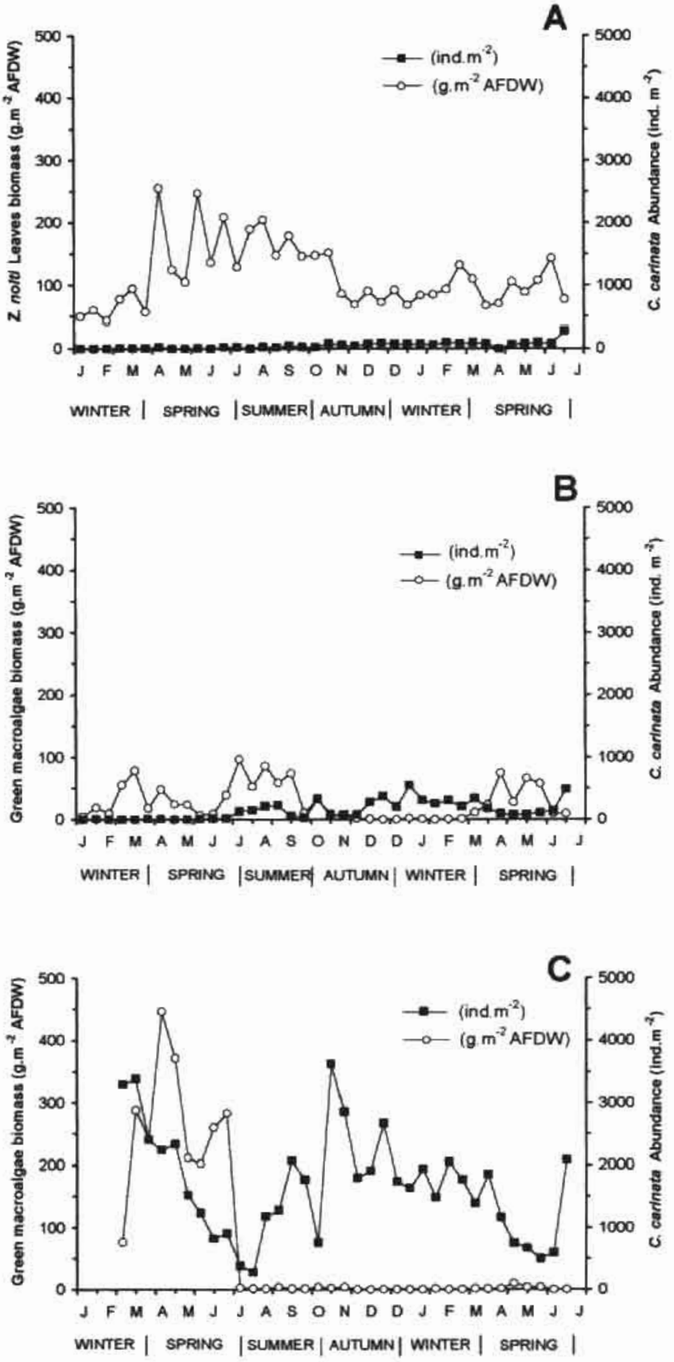


Fig. 3. Abundance of *C. carinata* along the eutrophication gradient. (A) *Zostera noltii* meadows; (B) Eutrophicated area; (C) Most eutrophicated area.

Spatial distribution and abundance

Cyathura carinata showed an aggregated spatial distribution in the three study areas along the eutrophication gradient. Estimated values of I were always higher than 1 (Elliot, 1977). Population density changed throughout the period of study but the pattern of variation was not the same at the three sampling stations (Fig. 3). At the most eutrophicated area densities decreased gradually from winter to late spring, increased from late spring through mid fall, and appeared to decline slightly from the end of fall. This means that during the algae bloom in 1993, isopod density was parallel to the surrounding macroalgal biomass. But the same pattern was observed in the following year where no macroalgae was found (Fig. 3C). At the other two areas the densities of *C. carinata* were always low (but always increasing through time) and did not allow the use of statistical tools.

Stepwise multiple regression analysis indicated that abundance (A) and biomass (B) of *C. carinata* in the most eutrophicated area were negatively correlated with temperature but biomass was also positively correlated with green macroalgae biomass (Ent):

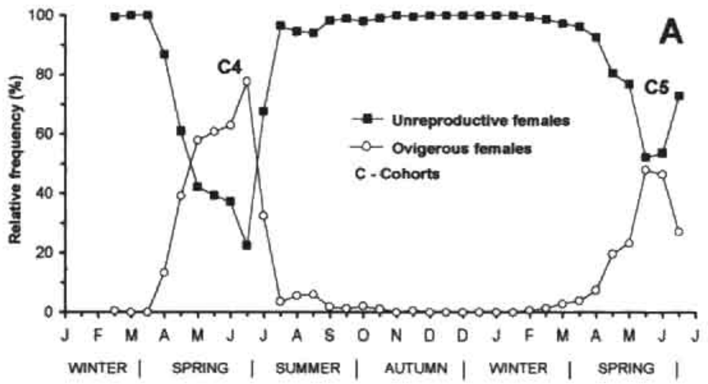
$$ln\,(1 + B) = 2.625 + 0.227\, ln\,(1 + \mathrm{Ent}) - 0.059\,(\mathrm{Temp})$$
$$ln\,(1 + A) = 8.304 - 0.0496\,(\mathrm{Temp})$$

Reproduction, sex-ratio, and fecundity

Females carrying developing embryos in brood pouches were first observed in early spring of both years (Fig. 4A). The percentage of juveniles in the population was clearly related to recruitment. Two peaks of free-living juveniles occurred from June to August (Fig 4B) which corresponded to two new cohorts.

European populations of *C. carinata* exhibited protogynous hermaphroditism, a metamorphosis from female to male form (Burbanck and Burbanck 1974, 1975, Legrand and Juchault 1963, Marques et al. 1994, Martins et al. 1997). In the Mondego estuary this is very clear because males only appear in the population by late winter, approximately 1.5 months before the first observations of females carrying eggs. The analysis of the sex-ratio variation (Fig. 5) indicates that all individuals are initially females, and in the reproduction period (spring season) 20 to 30% of the females will metamorphose to males. Recruitment then occurs continuously from June to August.

At the Mondego estuary the fecundity of females of similar size was quite variable, and no correlation between the number of developing embryos and female size was found. The difference in time between peaks of eggs in the first stage of development (A) and the last one (C) provided us with an estimation of the duration of the embryogenic development (Fig. 6). In both years (with and without macroalgae blooms) the estimation was 45 days.



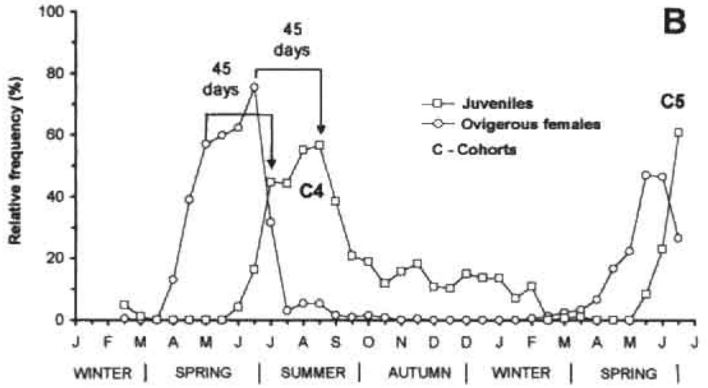


Fig. 4. Biological features of the population. (A) Variation of females in different physiological stages in relation to the female population; (B) Variation of ovigerous females in the total female population and of juveniles in the population.

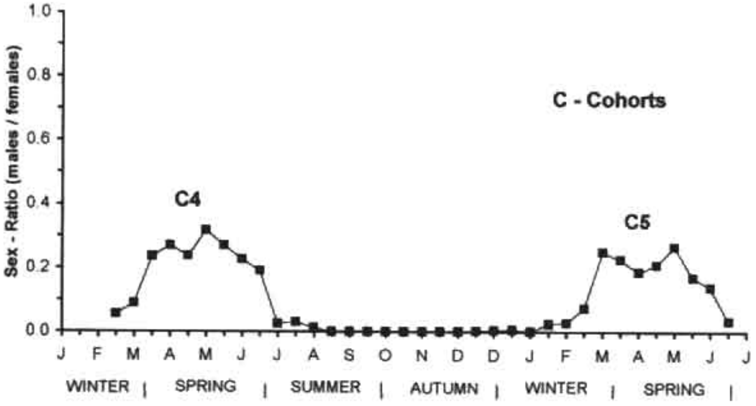


Fig. 5. Sex-ratio variation during the period of study.

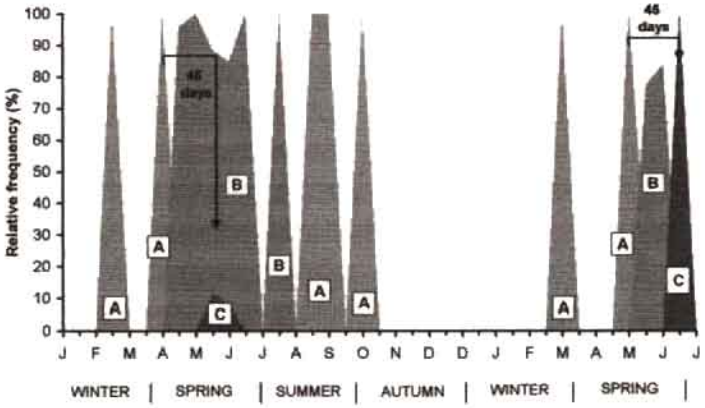


Fig. 6. Proportion of embryos in different developmental stages (definitions given in the text). Estimation of the duration of the embryogenic development in two consecutive years.

Growth and life span

Size frequency polymodal distributions were analysed for recognisable cohorts at the most eutrophicated areas (Fig. 7). Three cohorts (C1, C2 and C3) could be identified from data from the first sampling in February 1993. From modal analysis two new cohorts corresponding to small cohorts (C4 and C5) were clearly identified (χ^2 and G not significant, $P \leq 0{,}05$) on 6 June 1993 and 15 June 1994, respectively. Minimum average cephalic length (CL) of cohorts C4 and C5 was 0,152 mm (2,115 mm TL) and 0,2349 mm (2,862 mm TL) (Fig. 8). These values might have depended upon the number of days from hatching to cohort recognition, and therefore on sampling periodicity. The maximum average CL of cohorts ranged from 0,7714 mm (7,696 mm TL) to 0,976 mm (9,539 mm TL). Males were always bigger than females. The average CL of the 10 biggest males was 1,158 mm (11,179 mm TL) and of the 10 biggest females was 1,067 (10,359 mm TL).

Growth was found to be continuous through the life span of *Cyathura carinata*, although growth rates varied with size and temperature. Actually growth rates were clearly higher in early phases, from early summer to mid fall (see cohort C4) (Fig. 8), but by midfall growth rates decreased considerably, as the average size of individuals decreased, practically ceased in winter (see cohorts C3 and C4), and gradually increased again in spring. Lower growth rates during winter were probably a function of lower temperatures and lower organic matter content of sediments in the estuary. Life span was estimated by taking into consideration the recruitment period, growth rates and the disappearance of cohorts (table 1) and it was possible to see that different cohorts had quite different life spans (23-36 months). Based on present data it seems that when extensive macroalgal blooms occur the cohorts have longer duration (e.g. Cohorts C1 and C2).

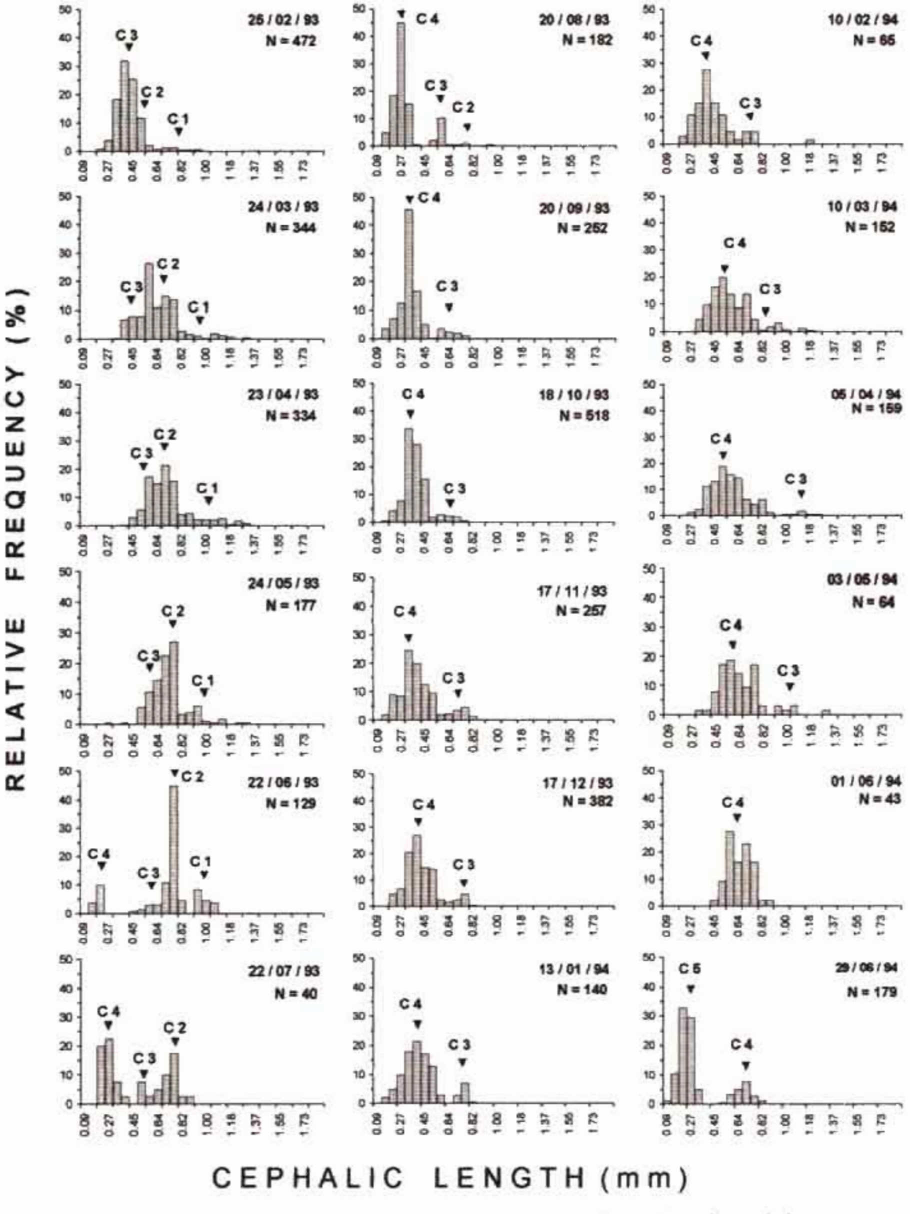


Fig. 7. Size-frequency polymodal distribution at the most eutrophicated area. Arrowheads indicate average cephalic length of numbered cohorts; (N) number of individuals.

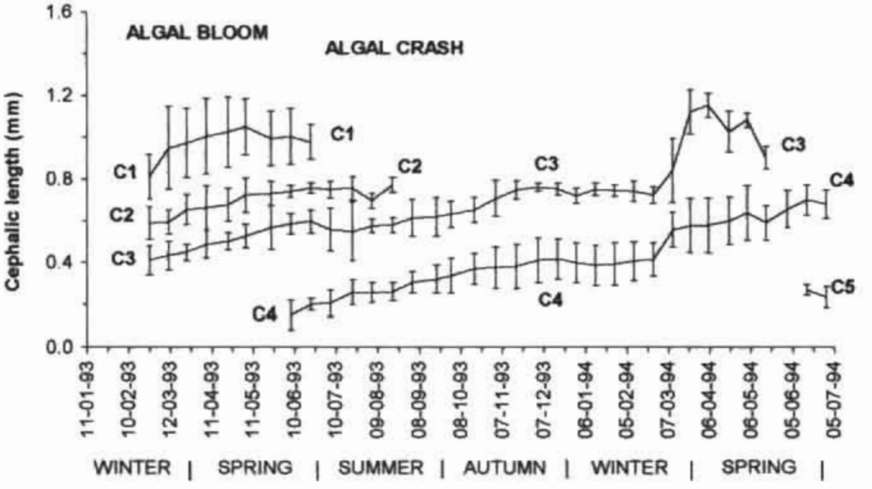


Fig. 8. Estimated growth (field growth data) of cohorts ± standard deviation at the most eutrophicated area.

Table 1. *Cyathura carinata*. Life span of cohorts 1, 2 and 3, in the most eutrophicated area

Cohorts	begining	end	Life span
C1	June 1990	June 1993	36 months
C2	June 1991	August 1993	27 months
C3	June 1992	May 1994	23 months
C4	June 1993	-	-
C5	June 1994	-	-

Production estimates

Previously established length-weight relationships were used in the production estimates on the most eutrophicated area (Table 2). The results clearly showed that growth productivity (P), elimination productivity (E) and the standing stock ($\overline{B}$) were much higher in the presence of extensive macroalgae blooms, but the relations P/$\overline{B}$ and E/$\overline{B}$ were much lower in the same conditions. It is also possible to see that the estimates of growth production and elimination production during the macroalgae bloom (4 months) correspond to nearly 60 % of the total yearly estimate taking the same period into account. Moreover the same values (of only 4 months) are very similar to the yearly estimations when the algae was not present (Table 2).

Based on the present results it is reasonable to assume that the large biomasses of macroalgae that usually grow in the south arm of the estuary are correlated in time with a great increase in the growth and elimination production of *Cyathura carinata* populations.

Table 2. Production estimates of the isopod *Cyathura carinata* at the most eutrophicated area taking into account different periods of time with different ecological conditions.

	P ($g.m^{-2}$ time^{-1})	$\overline{B}$ ($g.m^{-2}$)	P/$\overline{B}$	E ($g.m^{-2}$ time^{-1})	E/$\overline{B}$
18 months (January 1993 to June 1994)	22.078	5,585	3,953	53,718	9,618
12 months **(with a macroalgal bloom)** (February 1993 to February 1994)	19,623	6,192	3,169	47,892	7,734
12 months **(without macroalgae)** (June 1993 to June 1994)	11,795	3,444	3,422	29,253	8,494
4 months **(with a macroalgal bloom)** (February 1993 to June 1993)	10,543	13,001	0,811	25,325	1,948
4 months **(without macroalgal bloom)** (February 1994 to June 1994)	7,718	3,690	2,092	11,649	3.157

Discussion

Abundance

From previous data (Marques et al. 1994, Martins et al. 1997), the abundance of *Cyathura carinata* was expected to be high in inner areas of the south arm of the Mondego, especially in sectors lacking vegetal cover. Examination of the population dynamics of this species in relation to different ecological conditions (with or without macroalgae blooms) provide an opportunity to analyse its intraspecific life history variation. In addition, production estimates will contribute to evaluating the role of this species in the trophic dynamics.

Population density clearly changed along the eutrophication gradient as a result of preferences for bare sediment (without plant coverage) and more sandy sediment. So large and stable populations were found in the inner areas of the estuary. In the eutrophicated area and in the *Zostera noltii* meadows, the densities observed were always very low but with a slight increase throughout the period of study. This was probably due to the increased eutrophication of the estuary that induced a decline in the macrophyte meadows. The areas occupied previously by *Z. noltii* are now starting to become more similar to the most eutrophicated one, in terms of disappearance of plant coverage and increased sand percentage in sediments. This is a natural feature because it is known that the leaves and rhizomes of macrophytes tend to stabilise the sediment and increase the organic matter content. So the general direction of change with increased eutrophication in the estuary would be the disappearance of the *Z. noltii* meadows and an increase in areas occupied by *Cyathura carinata* originated by movement of adult individuals inside the estuary together with new recruitments taking place in the area. It seems therefore that this species has a larger potential for dispersion inside the estuary then it was stated before (Marques et al. 1994, Martins et al. 1997).

In the most eutrophicated area the density of *Cyathura carinata* exhibited a seasonal pattern throughout the year. Density increased during summer as function of recruitment, and the decline from mid fall to late spring corresponded mainly to the death of older animals.

Our results (density variation along the eutrophication gradient and multiple regression analysis), based on 18 months of sampling, suggest that in the Mondego estuary, the abundance and biomass of *C. carinata* are not negatively affected by the large blooms of green macroalgae, as stated before (Martins et al. 1997). On the contrary, it seems reasonable to conclude that to a certain extent the development of macroalgae biomass favours *C. carinata* and that extensive blooms affecting the whole area will increase in time its areas of occupation.

Reproduction, growth and life cycle

Data showed that in spring females always outnumbered males and that all males disappeared by the end of summer, immediately after recruitment. Our results strongly suggest and confirm the occurrence of protogynous hermaphroditism stated by other authors (Cléret 1960, Jadzewski 1969, Legrand and Juchault 1963, Marques et al. 1994, Martins et al. 1997, Ólafsson and Persson 1986), with approximately 20-30% of adults changing from females to males.

The duration of the embryogenic development in peracarids is very long and can reach 6 months in extremely cold climates (Bregazii 1972). In contrast, in temperate coastal waters it is much faster, taking usually only a few weeks (Marques et al. 1994, Moore 1981, Pardal et al. 2000, Powell and Moore 1991, Steele and Steele 1973). In our case embryogenic development was fast, 45 days, in both years. This estimate is quite different from the one made before (32 days) for the same population (Marques et al. 1994). These authors simply counted the number of days between two peaks of embryos in stage C and accepted it as a rough estimation for the duration of the embryogenic development. In the present study we used a more accurate but simple method which consisted of counting the number of days between peaks in embryos from stage A to stage C.

Summer and spring growth rates were much higher than during colder months, which was also observed frequently in other peracarid species (Hastings 1981, Marques et al. 1994, Ólafsson and Persson 1986, Pardal et al. 2000, Wilson and Parker 1996). The previous life span estimate for *C. carinata* in the Mondego estuary was 18-22 months (Marques et al. 1994). Our new estimates are much bigger and range from

23 to 36 months (table1). These new estimates are in agreement with more northern populations (Amanieu 1969, Bamber 1985, Jadzewski 1969, Ólafsson and Persson 1986). Nevertheless, all populations studied (Baltic Sea, English Channel, French west coast and Mondego estuary) present univoltine life cycles (a single generation per year). Therefore, as stated before, (Marques et al. 1994), intraspecific variations of the reproductive patterns as a function of latitude do not seem to be very significant in *C. carinata* in opposition to other peracarid species (eg. Amphipods) (Marques and Nogueira 1991, Pardal et al. 2000, Sudo and Azeta 1996) that tend to increase voltinism in ecosystems with warmer temperature regimes.

According to the stability-time theory (Sanders 1969) in environmentally stressed systems, such as eutrophic estuaries, we should expect the evolution of opportunistic adaptative strategies to take place. An example includes *Ampithoe valida* (Amphipoda) in the same system (Pardal et al. 2000) which appeared as an r strategist, with iteroparous females (except summer generation), a multivoltine cycle, high individual fecundity and recruitment throughout the year. This is the most common pattern in epifaunal species (van Dolah 1980) from physically controlled communities. In contrast, females of *C. carinata* may be able to reproduce two times during life and the population produces a single generation per year, which is not a typical r adaptive strategy. Since populations of *C. carinata* are capable of maintaining the same localities for long periods (Burbanck and Burbanck 1975, Marques et al. 1994) or even disperse to new others (present study), results suggest that for a given estuarine environment large stable populations might be more k-selected.

Production estimates

Taking into account the whole period of study, P (growth productivity) and E (elimination productivity) as well as $\overline{B}$ (standing stock) showed significantly higher values than the ones estimated before for the same species in this estuary (two times more). Moreover, these values are far higher than the ones estimated for other peracarid species in other estuarine systems (Birklund 1977, Fredette and Diaz 1986, Marques and Nogueira 1991, Moller and Rosenberg 1982, Ólafsson and Persson 1986, Pardal et al. 2000). Nevertheless, this difference does not reflect the entire reality. In fact, we must look carefully at the dynamics of the estuarine system in the two different years. For a short period (during the macroalgal bloom) P, E and were much higher than in the same period of the following year, and represent more than 50% of the yearly production estimation in 1993. Moreover they present similar values as for the total yearly estimation without macroalgae (year 1994). This means that during the macroalgae bloom, the population was not stressed as stated before (Martins et al. 1997) but clearly was favoured, probably due to resource availability.

As in other intertidal areas of Europe, *C. carinata* is quite accessible to aquatic predators. High values of P and E found in the most eutrophicated areas during the algal bloom, suggest that this species may play a very important role in the trophic dynamics.

As a whole, the present work reinforces the generalised notion that estuaries are highly productive systems, and reflect the important role of epifaunal macrobenthic species in the productivity of habitats colonised by them. Moreover this paper reveals that the conclusions based on just one annual cycle data can be incorrect. So longer sampling programs are needed to have a better understanding of the real dynamics of macrobenthic species in very dynamic systems.

Acknowledgements

This paper was supported by the programme MAST II (MAS 2 CT 92 0036 and by FCT - Portuguese Foundation for Science and Technology. The authors are indebted to all colleagues from the Coimbra University who assisted in field and laboratory work.

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THE EFFECT OF PRIMARY PRODUCERS DYNAMICS (MACROPHYTES AND MACROALGAE) ON *Hydrobia ulvae* POPULATION DYNAMICS

Abstract

Eutrophication in the Mondego estuary gave rise to qualitative changes in the benthic community, involving the replacement of eelgrass, *Zostera noltii*, by green algae, like *Enteromorpha* sp. and *Ulva* sp.. *Hydrobia ulvae*, is a dominant species concerning abundance and biomass. The objective was to compare to what extent differences in primary producers dynamics might affect *H. ulvae* life cycle, production and population dynamics. During the study period *H. ulvae* population exhibited both temporal and spatial density variations. The settlement was equal in the presence of both primary producers, and took place in March, June, July and September. However, the population was denser in the *Zostera noltii* meadows, when compared to the eutrophied area, were macroalgae where present. Life span was estimated as 21 months ± 3 months. Growth productivity (P) was estimated as 93.7 g. AFDW m^{-2} year^{-1} in the *Z. noltii* meadows, and 30.3 g. AFDW m^{-2} year^{-1} in the eutrophied area. Elimination productivity (E) was estimated as 300 g. AFDW m^{-2} year^{-1} in the *Z. noltii* meadows, and 97.5 g. AFDW m^{-2} year^{-1} in the eutrophied area. The average annual biomass ($\overline{B}$) (standing stock) of the population was estimated as 70.2 g. AFDW m^{-2} in the *Z. noltii* meadows, and 7.4 g. AFDW m^{-2} in the eutrophied area. P/$\overline{B}$ and E/$\overline{B}$ ratios were estimated as 1.3 and 4.8 in the *Z. noltii* meadows, and 4.5 and 13.2 in the eutrophied area, respectively. There is evidence that *H. ulvae* population structure and annual production are affected by primary producers dynamics, particularly by macroalgae blooms dynamics.

Introduction

As in most estuaries all over the world, eutrophication largely increased in the Mondego estuarine system since the eighties, probably as a result of excessive nutrient release into coastal waters (Pardal 1998). These processes are most related to the increase availability of growth limiting nutrients, namely nitrogen and phosphorus (e.g.

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Flindt et al. 1997, Pardal 1998, Martins 2000). The most visible feature of eutrophication in the Mondego estuary is the occurrence of seasonal green macroalgae blooms (*Enteromorpha* sp.) followed by the drastically reduction of *Zostera noltii* (Hornem) community (Marques et al. 1997, Pardal 1998, Martins 2000). This process has been giving rise to qualitative changes in the benthic community, and might be followed by changes in the species composition and trophic structure at other levels (e.g. macrofauna composition), (Marques et al. 1997, Pardal 1998). Through time, such changes may determine a selected new trophic structure (Marques et al. submitted).

The eutrophication process in the south arm of the Mondego estuary has been reported for several years (e.g. Marques et al. 1993a, b, 1997, Múrias et al. 1996, 1997, Flindt et al. 1997, Pardal 1998, Pardal et al. 2000, Martins et al. 1997, 1999, 2001, Martins 2000, Cabral et al. 1999, Lillebø et al. 1999, Lopes et al. 2000). A number of studies were carried out on the benthic communities (e.g. Pardal 1998, Lillebø et al. 1999), in which *Hydrobia ulvae* (Pennant 1777) was identified as a dominant species in the *Zostera noltii* meadows, exhibiting a stable population and significant biomass all over the year. Moreover, this species was found in the inner areas of the estuary, were the green macroalgae blooms usually occur (Pardal 1998, Lillebø 1996, Lillebø et al. 1999, Lopes et al. 2000, Shories et al. 2000), showing a clear dependence on the occurrence of macroalgae (Lillebø 1996, Lillebø et al. 1999). Moreover, a study concerning *H. ulvae* production along an eutrophication gradient in the south arm of the estuary (Lillebø et al. 1999) showed that *H. ulvae* standing stock decreased as a function of increasing eutrophication, while $P/\overline{B}$ and $E/\overline{B}$ ratios increased following the same gradient. Results also suggested that *H. ulvae* might be suffering a change in its adaptive strategy, becoming closer to a typical r strategist in the eutrophied areas (Lillebø et al. 1999).

The purpose of the present work was to evidence the effect of primary producers (macrophytes and macroalgae) dynamics on *H. ulvae* life cycle and production. Taking into account data from Lillebø et al. (1999), we compare *H. ulvae* population dynamics in a *Zostera noltii* meadows and in a eutrophied area, were usually macroalgae blooms of *Enteromorpha* sp. occur, in the south arm of the Mondego estuary.

Material and methods

Study Site

The Mondego estuary is a warm-temperate coastal system on the western coast of Portugal (Fig. 1). It consists of two arms, north and south, with very different hydrologic characteristics. The northern arm is deeper, while the southern arm is largely silted up, especially in upstream areas, which causes most of the fresh-water discharge to flow through the northern arm. Consequently, the water circulation in the southern arm is mainly dependent on tidal activity and on the usually small fresh-water input of a tributary, the Pranto River, which is controlled by a sluice (Pardal 1998).

Although, a large part of the southern arm intertidal area still remains more or less unchanged, exhibiting sand muddy bottoms covered by *Spartina maritima* and

Scirpus maritimus marshes and *Zostera noltii* meadows, macroalgae blooms of *Enteromorpha* sp. have been regularly observed during the last two decades (e.g. Marques et al. 1997, Pardal 1998, Lillebø 2000, Martins 2000). This is probably a result of excessive nutrient release into the estuary, coupled with longer persistence of nutrients (nitrogen and phosphorous) in the water column (Flindt et al. 1997, Marques et al. 1997, Pardal 1998). Such macroalgae blooms may not occur in exceptionally rainy years due to the occurrence of low salinity values for long periods, as a result of the Pranto river discharge (Pardal 1998). As a general trend, *Enteromorpha* biomass increases from early spring (February/March) up to July, when algae crash usually occurs. After summer a second smaller biomass peak may normally be observed in September followed by a decrease in winter (Pardal 1998, Martins et al. 2001).

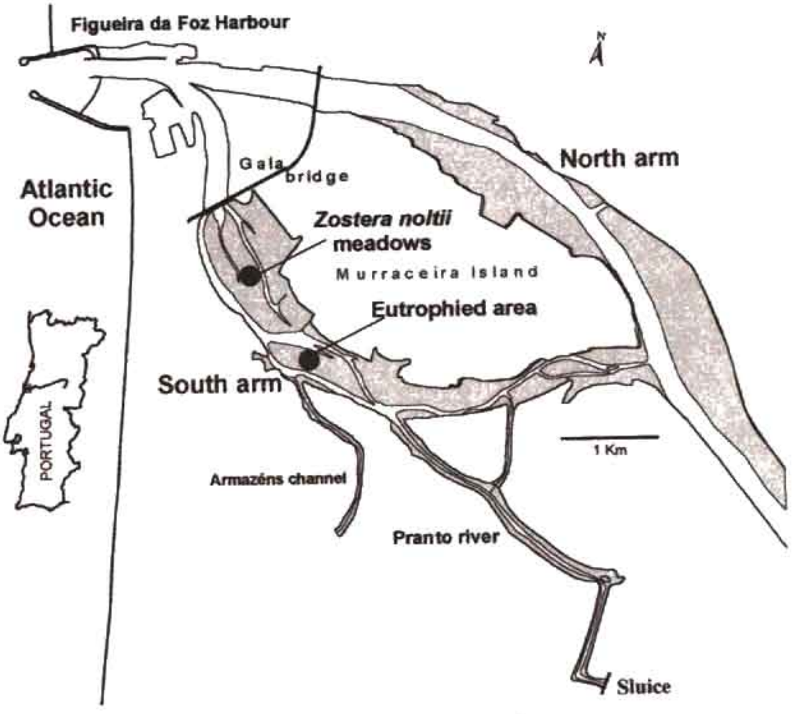

Fig. 1. The Mondego estuary with indication of the two study sites, *Zostera noltii* meadows and eutrophied area, were *Enteromorpha* sp. spring blooms usually occur, in the south arm.

Field Program

The benthic communities were sampled fortnightly from March 1993 to March 1994, at two study sites: *Zostera noltii* community and a eutrophied area, from where macrophytes disappeared while *Enteromorpha* blooms may occur (Lillebø et al. 1999). At each station 10 random samples were taken using a manual core, each replicate corresponding to 141 cm^2 and approximately 3 l of sediment. Samples were preserved in 4% buffered formalin, and later sieved through a 0.5 mm mesh size sieve. Animals were separated and kept in 70% ethanol. At each station and sampling date, salinity, temperature, and dissolved oxygen were measured in situ in low water pools. Sediment samples were also collected and subsequently analysed for organic matter content and granulometry. The organic matter was determined by loss on ignitions (8h at 450 °C), for each sampling date, and the granulometry was analysed seasonally and classified according to the nomenclature proposed by Brown and McLachlan (1990).

Laboratory Procedures

Hydrobia ulvae individuals were counted and their shell height measured to the nearest 0.1 mm. As a number of individuals had the shell damaged we determined other measures, such as the maximum width and maximum length unto the last spire, which were then converted to shell height. These conversions were based on the biometrics relationships of 339 individuals with intact shells (Figs. 2A and 2B), (Lillebø et al. 1999). The relationship between total length and ash free dry weight was also established to be use in production estimates. There were not any significant differences between equations established for each season based on analysis of variance (P = 0.05). Therefore, the data set of 191 individuals measured and weighted throughout the study was used to provide a single regression equation (Fig. 2C), (Lillebø et al. 1999). Individuals were dried at 60 °C for 72 h and weight to the nearest 0.01 mg. Ash-free dry weight was assessed after combustion of the samples for 8 h at 450 °C.

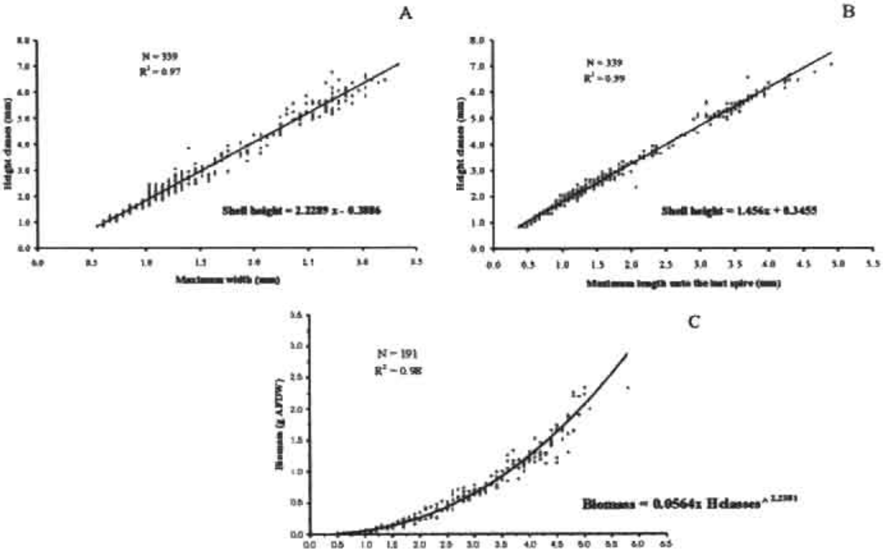

Fig. 2. *Hydrobia ulvae* biometric relationships.

Data analysis

Multiple regression models were developed to relate the variation of *Hydrobia ulvae* biomass with salinity, temperature, dissolved oxygen, organic matter content in sediments, and macroalgae and macrophytes biomass (Lillebø et al. 1999). The fitted regression models were expressed as: $Y' = a + b_1X_1 + b_2X_2 + \ldots + b_kX_k$.

Y' - are the values of a given dependent variable (e.g. biomass) predicted by the equation;

$X_1, X_2 \ldots X_k$ - are independent variables (e.g. salinity).

The solutions are the estimate of the regression coefficients a, b_1, $b_2 \ldots b_k$. The significance of fitted regressions was tested by using analysis of variance (F), and the t test for the regression coefficients, as described in Edwards (1984, 1985). Regressions were estimated using the Stepwise Forward Analysis method performed with the STATGRAPHICS 4.0 statistical package.

Growth rates were estimated by tracking recognisable cohorts along size-frequency distributions (0.02 mm classes) from successive sample dates. Size-frequency analysis was performed using the ANAMOD software package (Nogueira 1992), in which the analysis reliability was tested using the χ^2 and G tests (P= 0.05).

We estimated both growth (P) and elimination (E) production as derived by Allen (1971). Values of P and E for each cohort for a given time interval are expressed as:

$$P = [(N_t + N_{t+1})/2]\ (\overline{W}_{t+1} - \overline{W}_t) \qquad \text{for} \qquad \overline{W}_{t+1} > \overline{W}_t$$

$$E = [(\overline{W}_t + \overline{W}_{t+1})/2]\ (N_t - N_{t+1}) \qquad \text{for} \qquad N_t > N_{t+1}$$

N = number of individuals from cohort at each sample date; = mean individual biomass for each sample date; t and t+1 = consecutive sample dates.

Total values of P and E for each cohort are expressed as:

$$P = \sum_{t=0}^{t=n} [(N_t + N_{t+1})/2]\ \Delta\overline{W}$$

$$E = \sum_{t=0}^{t=n} [(\overline{W}_t + \overline{W}_{t+1})/2]\ \Delta N$$

Total values of P and E, for the population, are expressed as:

$$P = \sum_{n=1}^{N} P_{cn} \qquad \text{and} \qquad E = \sum_{n=1}^{N} E_{cn}$$

P_{cn} and E_{cn} are the growth and elimination production of the cohort n. $P/\overline{B}$ and $E/\overline{B}$ ratios were determined. $\overline{B}$ (mean population biomass) is expressed as:

$$\overline{B} = (1(T) \sum_{n=1}^{N} (\overline{B}_n\, t)$$

T = period of study; N = number of cohorts in the period T; $\bar{B}_n$ = mean biomass of the cohort n; and t = duration of the cohort n.

Results

Macrophytes and macroalge biomass

Z. noltii biomass exhibited a seasonal variation in biomass. During fall and winter total biomass consisted essentially of rhizomes, and during spring and summer total biomass increased due to the growth of shoots (Fig. 3).

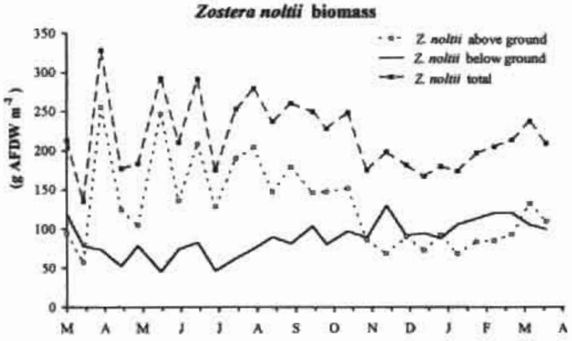

Fig. 3. *Zostera noltii* biomass in the south arm of the Mondego estuary between March 93 and March 94.

The composition of macroalge biomass was distinctive in each study site. Red macroalgae, essentially *Gracilaria* sp., were relatively abundant at the *Z. noltii* meadows (Fig. 4). In the eutrophied area, green macroalgae presented a typical spring bloom, with *Enteromorpha* sp. biomass reaching 413.19 g AFDW m^{-2} (April 7th 1993), followed by an algae crash in early summer (July 6th 1993) (Fig. 4). However, no algae bloom was observed in spring 1994 due to the fact that this was a particularly rainy year.

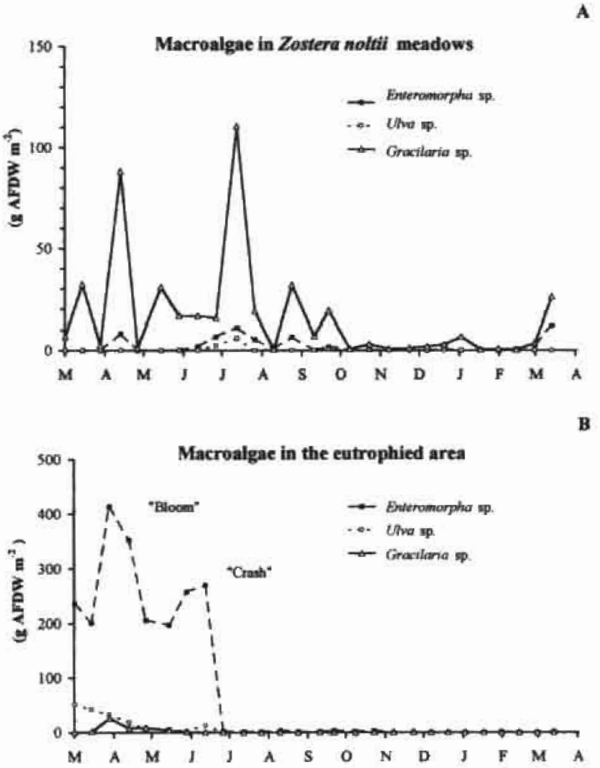

Fig. 4. Macroalgae biomass in the south arm of the Mondego estuary between March 93 and March 94. A – *Zostera noltii* meadows; B – eutrophied area.

Abundance

Population density changed throughout the period of study but the pattern of variation was distinctive for each type of dominant primary producer (macrophytes/ macroalgae), (Fig. 5). Density was consistently higher at the *Zostera noltii* meadows, increasing gradually from November 1993 up to a maximum during spring in 1994 (approximately 288 000 ind. m^{-2}, March 23rd 1994). The population density in the eutrophied area reached a maximum of approximately 167 000 ind. m^{-2} (April 7th 1993), during the algae bloom, and a minimum of 699 ind. m^{-2} (July 6th 1993), after the algae crash (Fig. 5).

Environmental factors

Silt and clay were the main constituents of sediments at the *Z. noltii* meadows, while in area covered with algae, sediments mainly consisted of fine sand (table 1).The multiple regression analysis was performed taken into account *H. ulvae* biomass, primary producers biomasses, salinity, temperature and dissolved oxygen (Lillebø et al. 1999).

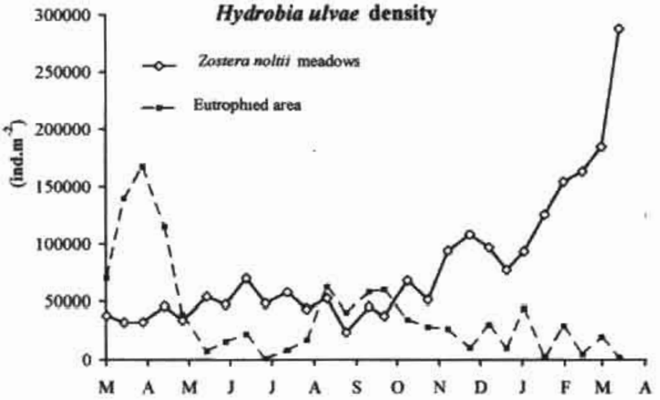

Fig. 5. Abundance of *Hydrobia ulvae* at *Zostera noltii* meadows and eutrophied area, in the south arm of the Mondego estuary between March 93 and March 94.

Table 1. Relative frequency of sediment granulometry classes determined seasonally in *Zostera noltii* meadows and in the eutrophied area, were *Enteromorpha* sp. spring blooms usually occurs, in the south arm of the Mondego estuary.

	Zostera noltii meadows			*Eutrophied* area		
Classes (mm)	June	April	Dec.	April	June	Dec.
> 2	0.29	0.94	3.86	0.42	0.11	0.03
> 1< 2	1.10	1.03	1.44	0.62	0.57	0.33
> 0,500 < 1,0	1.36	4.28	2.27	2.06	1.72	1.34
> 0,250 < 0,500	14.79	1.11	16.23	7.71	10.78	15.08
> 0,125 < 0,250	17.21	22.35	19.99	36.86	33.13	40.02
> 0,063 < 0,125	19.48	63.10	19.30	49.43	28.12	26.19
> 0,038 < 0,063	31.34	4.54	27.85	1.80	20.45	9.87
< 0,038	14.43	2.65	9.06	1.10	5.12	7.14

In the *Zostera noltii* meadows, *H. ulvae* biomass (g AFDW) was positively correlated with green algae (*Enteromorpha* sp.+ *Ulva* sp.), biomass (log+1) and negatively correlated with salinity and *Z. noltii* below ground biomass; (P=0.000; r^2=0.70).

H. ulvae biomass = 105 + 15.0 (ln+1 green algae) – 1.38 (Sal.) – 0.282 (Z. below ground)

Regarding the eutrophied area, we analysed separately the algae bloom and post-algae crash scenarios (Fig. 6). During the algae bloom *H. ulvae* biomass appeared positively correlated with green algae biomass, with organic matter content in sediments, and with dissolved oxygen, ($P=0.007$; $r^2=0.94$):

H. ulvae biomass = -74.2 + 0.0333 (green algae) + 0.150 (o.m.) + 1.89 (oxyg)

After the algae crash *H. ulvae* biomass appeared positively correlated with green algae biomass (*Enteromorpha* sp.+ *Ulva* sp.; ln+1) and dissolved oxygen ($P=0.001$; $r^2=0.63$):

H. ulvae biomass = - 53.6 + 27.0 (ln+1 green algae) + 4.71 8 (oxyg.)

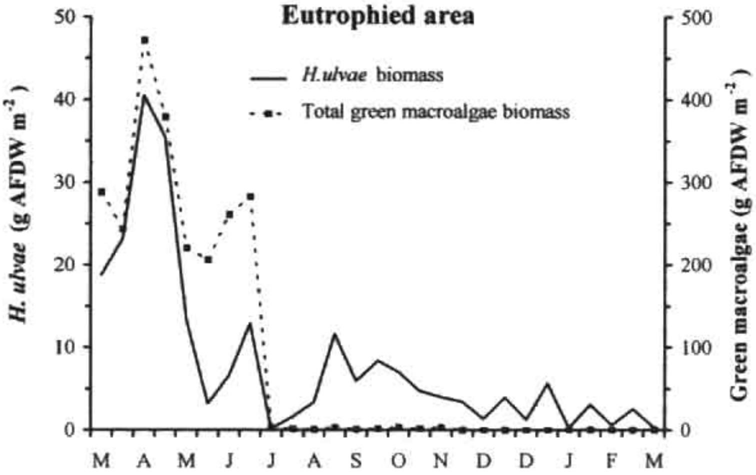

Fig. 6. Green macroalgae biomass and *Hydrobia ulvae* abundance variations in the eutrophied area, in the south arm of the Mondego estuary between March 93 and March 94.

Growth and Life Span

Size-frequency polymodal distributions were analysed for *H. ulvae* recognisable cohorts (Fig. 7). Significant differences in the population structure were observed for each type of dominant primary producer, respectively *Z. noltii* and *Enteromorpha* sp.. Individuals reached larger dimensions at the *Z. noltii* meadows when compared to the eutrophied area. Nevertheless, the same settlement pattern was observed, respectively in March, June, July and September (X^2 and G not significant, $P \leq 0.05$). New cohorts were detected with an average shell height of 1.11 mm at the *Z. noltii* meadows, and 1.10 mm at the eutrophied area.

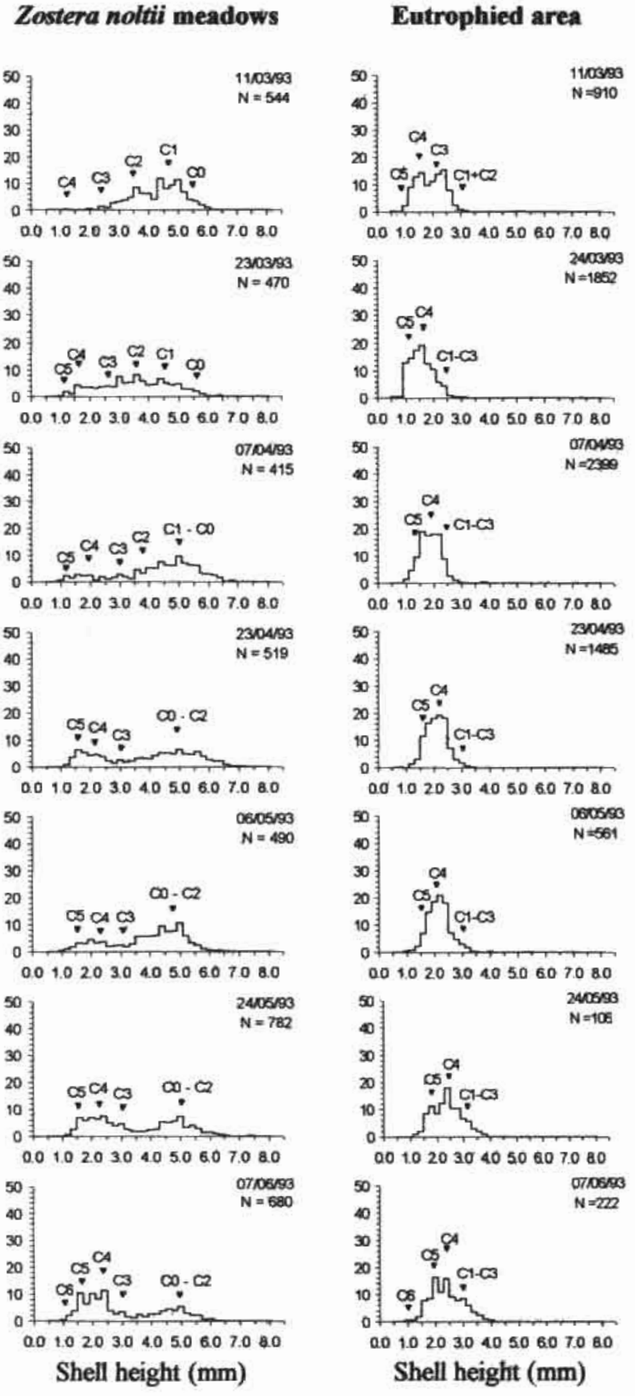
Zostera noltii meadows
Eutrophied area
11/03/93
N = 544
C4
C3
C2
C1
C0
11/03/93
N =910
C5
C4
C3
C1+C2
23/03/93
N = 470
C5
C4
C3
C2
C1
C0
24/03/93
N =1852
C5
C4
C1-C3
07/04/93
N = 415
C5
C4
C3
C2
C1 - C0
07/04/93
N =2399
C5
C4
C1-C3
23/04/93
N = 519
C5
C4
C3
C0 - C2
23/04/93
N =1485
C5
C4
C1-C3
06/05/93
N = 490
C5
C4
C3
C0 - C2
06/05/93
N =561
C5
C4
C1-C3
24/05/93
N = 782
C5
C4
C3
C0 - C2
24/05/93
N =106
C5
C4
C1-C3
07/06/93
N = 680
C6
C5
C4
C3
C0 - C2
07/06/93
N =222
C6
C5
C4
C1-C3
0
10
20
30
40
50
0.0 1.0 2.0 3.0 4.0 5.0 6.0 7.0 8.0
Shell height (mm)
Shell height (mm)

Fig. 7a

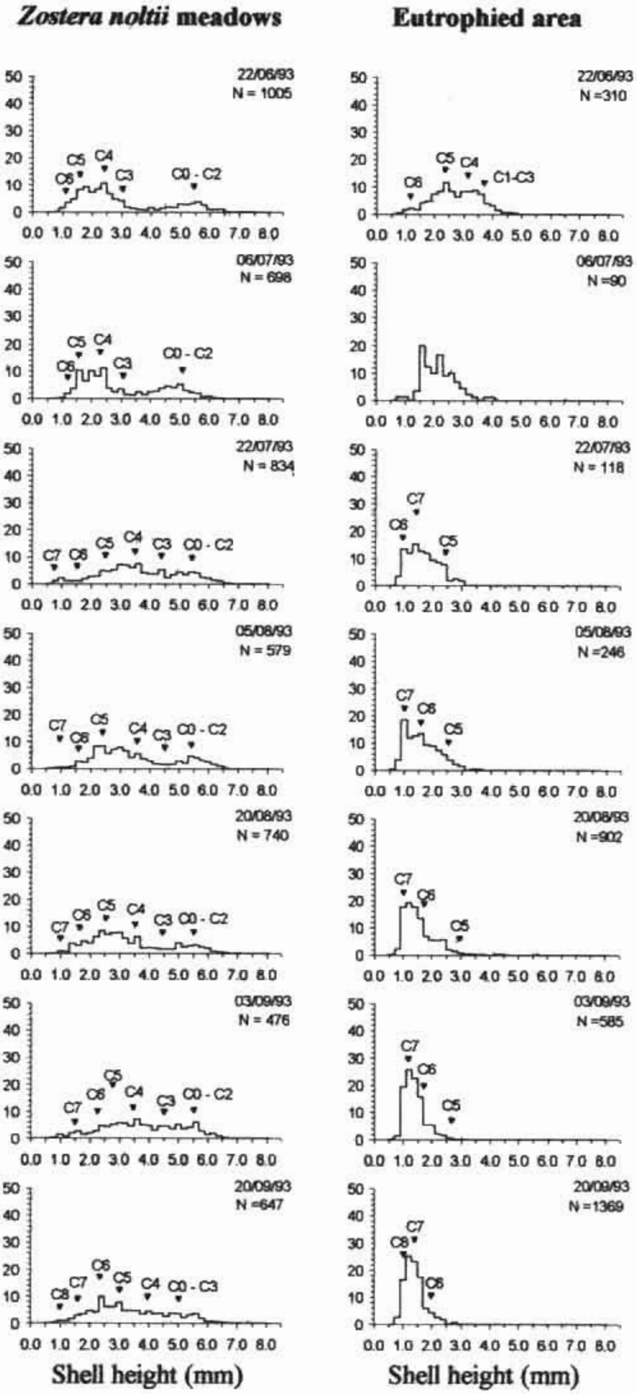
Zostera noltii meadows
Eutrophied area
22/06/93
N = 1005
C6 C5 C4 C3 C0 - C2
22/06/93
N =310
C6 C5 C4 C1-C3
06/07/93
N = 698
C6 C5 C4 C3 C0 - C2
06/07/93
N =90
22/07/93
N = 834
C7 C6 C5 C4 C3 C0 - C2
22/07/93
N = 118
C6 C7 C5
05/08/93
N = 579
C7 C6 C5 C4 C3 C0 - C2
05/08/93
N =246
C7 C6 C5
20/08/93
N = 740
C7 C6 C5 C4 C3 C0 - C2
20/08/93
N =902
C7 C6 C5
03/09/93
N = 476
C7 C6 C5 C4 C3 C0 - C2
03/09/93
N =585
C7 C6 C5
20/09/93
N =647
C8 C7 C6 C5 C4 C0 - C3
20/09/93
N =1369
C8 C7 C6
0 10 20 30 40 50
0.0 1.0 2.0 3.0 4.0 5.0 6.0 7.0 8.0
Shell height (mm)
Shell height (mm)

Fig. 7b

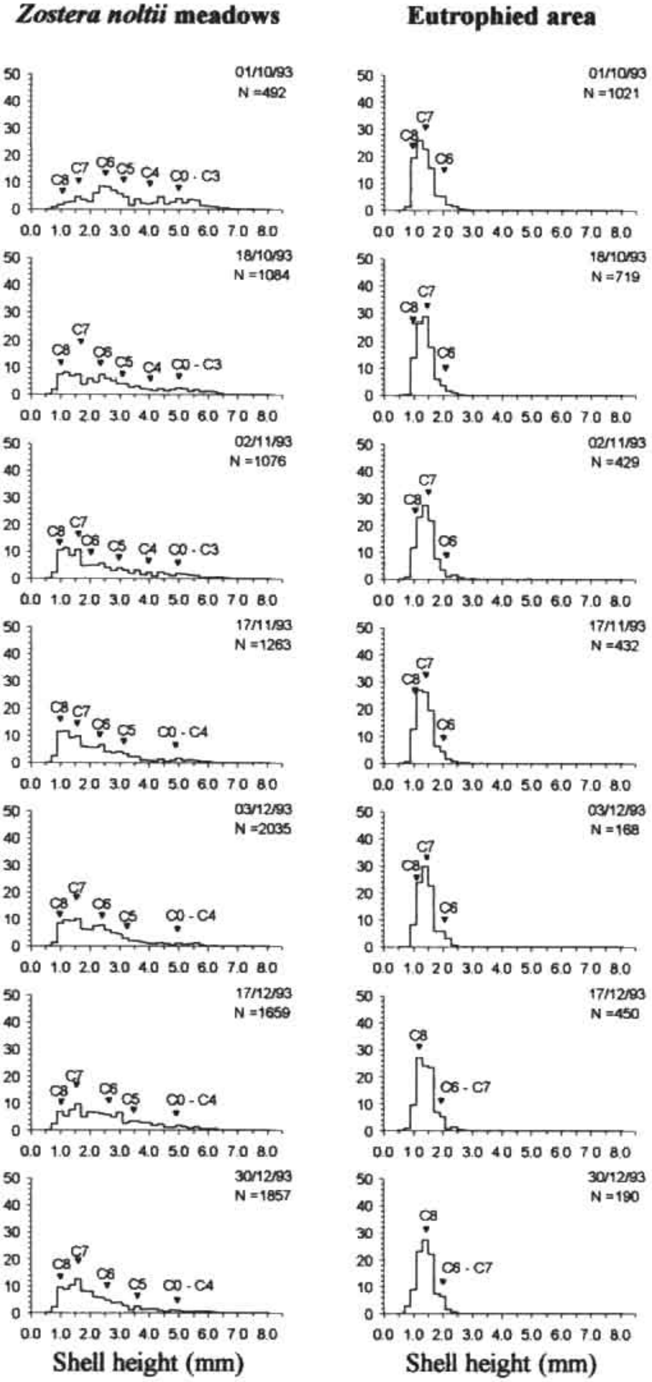
Zostera noltii meadows
Eutrophied area
01/10/93
N =492
C8
C7
C6
C5
C4
C0 - C3
01/10/93
N =1021
C8
C7
C6
18/10/93
N =1084
C8
C7
C6
C5
C4
C0 - C3
18/10/93
N =719
C8
C7
C6
02/11/93
N =1076
C8
C7
C6
C5
C4
C0 - C3
02/11/93
N =429
C8
C7
C6
17/11/93
N =1263
C8
C7
C6
C5
C0 - C4
17/11/93
N =432
C8
C7
C6
03/12/93
N =2035
C8
C7
C6
C5
C0 - C4
03/12/93
N =168
C8
C7
C6
17/12/93
N =1659
C8
C7
C6
C5
C0 - C4
17/12/93
N =450
C8
C6 - C7
30/12/93
N =1857
C8
C7
C6
C5
C0 - C4
30/12/93
N =190
C8
C6 - C7
0
10
20
30
40
50
0.0 1.0 2.0 3.0 4.0 5.0 6.0 7.0 8.0
Shell height (mm)
Shell height (mm)

Fig. 7c

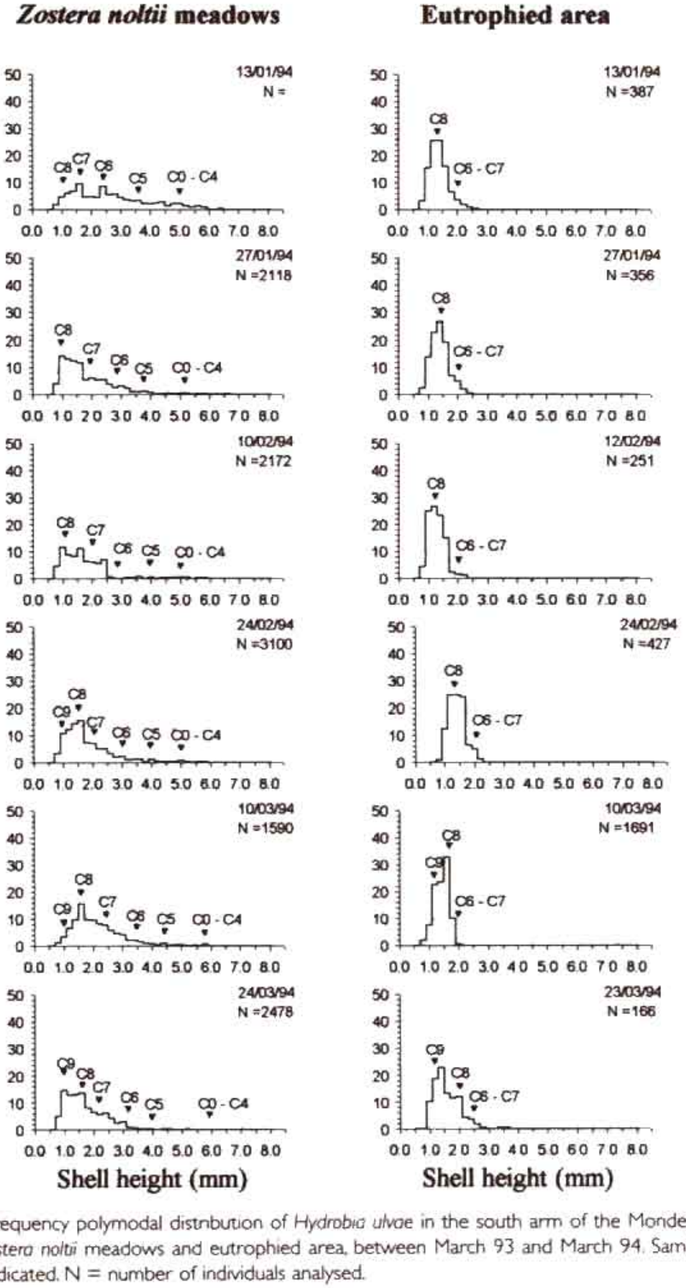

Fig. 7d. Size-frequency polymodal distribution of *Hydrobia ulvae* in the south arm of the Mondego estuary at *Zostera noltii* meadows and eutrophied area, between March 93 and March 94. Sampling dates are indicated. N = number of individuals analysed.

Growth was continuous through life (Fig. 8). Nevertheless, growth rates were highest in spring, decreased from early summer to mid fall, practically ceased in winter (Fig. 8), and gradually increased again until spring. Lower growth rates during winter were probably a result of lower temperatures. During the rest of the year, higher growth rates were probably a function of higher temperatures and also increased food resources.

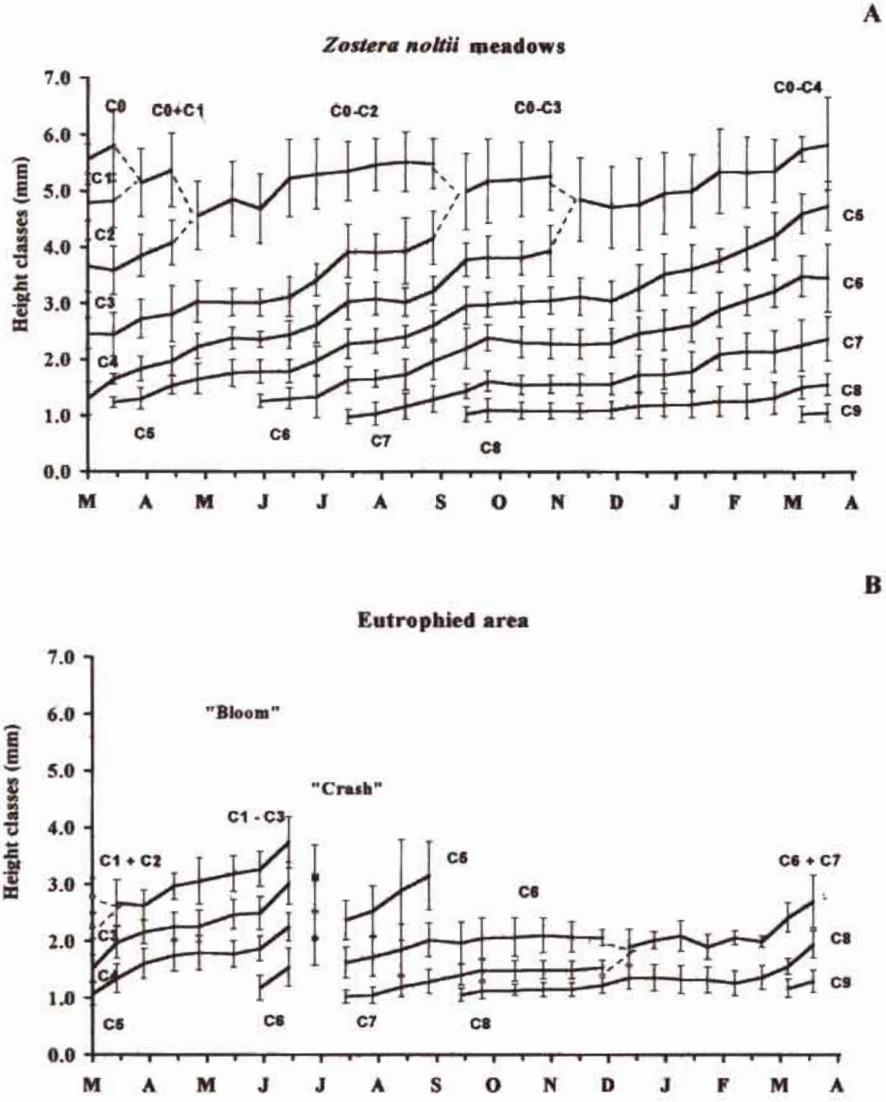

Fig. 8. Estimated growth (field growth data) of *Hydrobia ulvae* cohorts or groups of cohorts, average total length ± standard deviation during the study period. Broken lines indicate probable cohort merging. A - *Zostera noltii* meadows, B – eutrophied area.

Life span was estimated at 21 months ± 3 months. Individuals presented a mean shell height between 3.78 mm (std ± 0.30) and 4.75 mm (std ± 0.43) after the first year, and 5.84 mm (std ± 0.82) after 21 month.

Life Cycle

The reproductive period was studied taking into account the occurrence of egg masses, which are fixed on live shells of it congeners (Fish and Fish 1974). The percentages of the population carrying egg masses in the two study sites were low (Fig. 9). Therefore, data from three study sites, along an eutrophication gradient in the south arm (Lillebø et al. 1999), were also pooled together for the analysis (Fig. 9). Two main reproductive peaks are recognisable in the Mondego estuary population, in spring and in late summer.

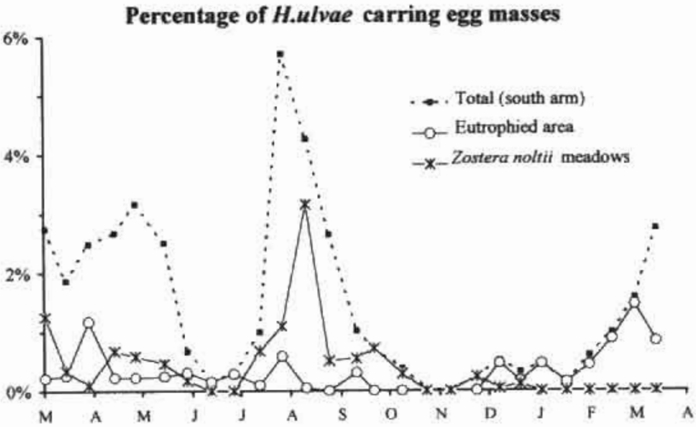

Fig. 9. Percentage of *Hydrobia ulvae* caring egg masses in the south arm of the Mondego estuary between March 93 and March 94.

Production Estimates

Relationships between height and ash free dry weight (g AFDW) (Figure 2C) were used to estimate production taking into account cohorts growth and mortality. Growth productivity (P) was estimated at 93.7 g. AFDW m^{-2} year^{-1} in the *Z. noltii* meadows, and 30.3 g. AFDW m^{-2} year^{-1} in the eutrophied area. Elimination productivity (E) was estimated at 300 g. AFDW m^{-2} year^{-1} in the *Z. noltii* meadows, and 97.5 g. AFDW m^{-2} year^{-1} in the eutrophied area. The average annual biomass ($\overline{B}$) (standing stock) of the population was estimated at 70.2 AFDW g. m^{-2} in the *Z. noltii* meadows, and 7.4 g. AFDW m^{-2} in the eutrophied area. $P/\overline{B}$ and $E/\overline{B}$ ratios were estimated respectively at 1.3 and 4.8 in the *Z. noltii* meadows, and 4.5 and 13.2 in the eutrophied area. Taking into account the two scenarios considered at the eutrophied area, we estimated $P/\overline{B} = 5.3$ and $E/\overline{B} = 14.4$ during the algae bloom, while after the algae crash $P/\overline{B} = 3.3$ and $E/\overline{B} = 11.4$.

Discussion and conclusions

The comparative study of *H. ulvae* population dynamics at *Zostera noltii* meadows, and at a eutrophied area during a typical *Enteromorpha* sp. spring bloom and a post-algae crash situation, provided an opportunity to assess the effect of different primary producers dynamics in the life cycle and production of this species. Moreover, production estimates contributed to evaluate the role of this species with regard to estuarine food web, and the effect of primary producers dynamics to the energy flow in the system.

Spatial distribution and abundance

At the Mondego estuary, densities were clearly higher than, for instance, those observed by Curras and Mora (1990) in "ria del Eo" (Spain), and Sola (1994, 1996) in Bidasoa estuary (Spain), which may be due to the fact that these authors used a 1.0 mm mesh size sieve. In fact, this mesh size is not effective in capturing individuals smaller than 1.5 mm (Bachelet and Yacine-Kassab 1987). Nevertheless, Planas and Mora (1987) observed a maxinum abundance of 100 028 ind. m^{-2} in "ria de Pontevedra", Spain.

Hydrobia ulvae densities were clearly different in the presence of each type of dominant primary producer, probably as a function of *Z. noltii* and *Enteromorpha* sp. dynamics. The *Z. noltii* meadows presented a stable and dense *H. ulvae* population thought the study period, which is consistent with results from other authors (Walters and Wharfe 1980), possibly as a result of the greater stability provided by macrophytes, in comparison to green macroalgae. Moreover, *Z. noltii* meadows are constituted by muddy sediments, with higher organic matter content, which may regulate distribution and abundance of *H. ulvae* (Barnes 1979).

At the *Z. noltii* meadows, *H. ulvae* density increased from early winter 1993 to a maximum in spring 1994. This is not in agreement with observations carried out by other authors, e.g. Curras and Mora (1990) in the "ria del Eo", Spain, where the maximum density occurred in spring and summer and the minimum during winter. Nevertheless, our data may be related to the fact that no algae bloom occurred at the eutrophied area in spring 1994. As a result, the usual alternative macroalgae habitat was not available and juvenile's byssus-drift dispersion (Armonies 1992) probably did not occur. This may as well explain the low population density observed in the eutrophied area in spring 1994. In fact, there were two distinctive scenarios in the strongly eutrophied area. During the algae bloom the population density was much higher (167 510 ind. m^{-2} in April 7th 1993) than in the *Z. noltii* meadows in the same period. This may be related to habitat heterogeneity and food resources (Soulsby et al. 1982, McLusky 1988, Everett 1994). During the algae crash, when anoxic conditions occurred in the sediment surface, the population suffered a sudden and drastic reduction, from 21 818 ind. m^{-2} (June 22nd) to 699 ind. m^{-2} (July 6th). After the algae crash, although the population was able to recover (62 863 ind. m^{-2} in August 20th), it could not come to the initial values again. Such recovery may have resulted from new settlements and/or the byssus-drift dispersion of *H. ulvae* juveniles (Armonies 1992, Armonies and Drake

1995). In the present study, *H. ulvae* showed a remarkable capacity to re-colonise the disturbed area. Furthermore, other authors have reported its dispersion capacity (Sola 1994, 1996) and tolerance to adverse conditions (Planas and Mora 1987, Sola 1994).

Environmental factors

H. ulvae biomass at the *Zostera noltii* meadows was positively correlated with green algae biomass and negatively correlated with *Z. nolti* below ground biomass and salinity. Although, *Enteromorpha* sp. and *Ulva* sp. biomass were very low this positive correlation can be explained by the fact that an organic enrichment may favour algae development (Hull 1987, Lavery et al. 1991, Hardy et al. 1993, Shories and Reise 1993, Everett 1994, Pardal 1998). In addition, grazing activity on periphyton and epiphyte may favour the macrophytes (Howard and Short 1986, Philippart 1995) and therefore food resources were always available for *H. ulvae* (Fenchel et al. 1975, Jensen and Siegismund 1980, Graham 1988, Morrisey 1988). The negative correlation with *Z. noltii* below ground biomass may be explained by the fact that leaves and root-rhizomes showed opposite peaks during the study period. Furthermore, Asmus and Asmus (1985) observations in the Wadden Sea showed that epiphytic diatoms density, which constitute a main food item for *H. ulvae* (Fenchel et al. 1975), always followed *Z. noltii* leaves biomass. The negative correlation with salinity cannot be explained as a cause-effect relationship, since *H. ulvae* tolerates a wide salinity range (10 to 35) (McLusky 1988). In fact, *H. ulvae* abundance and biomass increased from winter 93 to spring 94 and as this was a very rainy period salinity values decreased, being lower in spring 94.

At the eutrophied area and during the algae bloom, *H. ulvae* biomass was positively correlated with the green macroalgae biomass, with organic matter content in sediment and with oxygen concentration. While, after the algae crash it was positively correlated with *Enteromorpha* sp. and *Ulva* sp. biomass and oxygen concentration. A positive correlation with macroalgae biomass makes sense since algae covered habitats can provide food resources and shelter (Hull 1987, Walters and Wharfe 1980, Everett 1994). In fact, Drake and Arias (1995) showed that algae biomass was the most important factor for hydrobiid species temporal and spatial patterns in the Bay of Cadiz, Spain. The positive correlation between the sediment organic matter contents and *H. ulvae* biomass can be related to the fact that in this area organic matter could be a limiting factor (Hull 1987). A possible explanation for the results regarding the oxygen concentration in this area resides in the fact that, during the bloom, due to respiration, a huge green macroalgae biomass may have induced anoxic conditions during nocturnal periods, which strongly affected the *H. ulvae* population, causing its decline. As we always measured oxygen concentrations during the day such conditions could not be detected but it was clear that *H. ulvae* biomass followed the decrease of green macroalgae biomass. In fact, Everett (1994) found dissolved oxygen concentration in the algae-sediment water interface to be significantly lower than in overlying water column, and Hull (1987) observed that sediments under algae matts had significantly lower redox potentials, when compared to bare bottom sediment.

Also, in the Bay of Cadiz, Drake and Arias (1995) found that *H. ulvae* abundance was highest in the sites with the greatest water renewal.

Growth, life span, and productivity

The settlement pattern of *H. ulvae* in the Mondego estuary was alike at the two study sites, with settlements occurring in March, June, July and September. This is not entirely coincident with results obtained by Bachelet and Yacine-Kassab (1987), Curras and Mora (1990) and Sola (1994, 1996), but differences may arise from contrasting geographic environmental conditions. Another possible explanation could be related to sampling periodicity, to the fact that we used 500 mm mesh size sieve, while the other authors used 1000 mm, which may cause a certain bias, or even to the methodology applied in cohorts detection. Nevertheless, it is possible to establish some correspondence between the settlements attending to the mean shell height of the cohorts.

Two main reproductive peaks, but very small when compared two other authors (Fish and Fish 1974, Drake and Arias 1995), were recognisable in the Mondego estuary population. Although, this result may not be consistent with the settlement pattern of *H. ulvae*, only a very small proportion of egg capsules were observed on snail shells. In the Mondego estuary *H. ulvae* egg capsules were mainly observed on macroalgae and *Z. noltii* leaves, but they were not quantified. Nevertheless, contrasting with the scarcity of egg capsules, *H. ulvae* abundance and population structure shows a successful reproduction and recruitment of this species.

Through cohort recognition and tracking it was possible to assess growth and to estimate life span at 21 months ± 3 months. The estimate for the Mondego estuary population was similar to the estimate for the Medway estuary, U. K. (Walters and Wharfe 1980). But slightly higher than for the "ria de Pondevedra", Spain (Planas and Mora 1987) and for the "ria del Eo", Spain (Curas and Mora 1990), and lower than estimates for the Dovey estuary, U. K. (Fish and Fish 1974) and for the Girond estuary, France (Bachelet and Yacine-Kassab 1987).

The *H. ulvae* standing stock was lower at the eutrophied area, while $P/\overline{B}$ and $E/\overline{B}$ ratios were higher at the eutrophied area. This was obviously related with the size of individuals found in each sampling area, in relation with the dynamics of macroalgae mats. Probably as a result from higher exposure to predators (Múrias et al. 1996), *H. ulvae* individuals were considerable smaller than in the *Z. noltii* meadows.

The *H. ulvae* $P/\overline{B}$ estimates in the Mondego estuary were of the same order of magnitude as estimates for the same species in similar habitat, *Z. noltii* meadows (Sprung 1994). Nevertheless, in the eutrophied area these estimates were higher. In this area, and considering the two distinctive situations, $P/\overline{B}$ and $E/\overline{B}$ estimates reflect that macroalgae dynamics induce unstable environmental conditions, which can be expressed as: exposure to anoxic conditions during the algae crash and predators, and consequently, *H. ulvae* population structure is altered probably as reduction of life expectancy.

In the Mondego estuary *Hydrobia ulvae* production was very high when compared to Ria Formosa, Portugal (Sprung 1994) and also when compared with estimates of secondary production of whole intertidal benthic communities (Sprung 1994). Nevertheless, *H. ulvae* $P/\bar{B}$ in the *Z. noltii* meadow can be compared to Sprung (1994) estimate for the whole intertidal benthic communities, attending to the fact that *H. ulvae* represents more than 80% of the total benthic macrofauna biomass in the Mondego estuary (Pardal 1998).

The present results show the generalised notion that macrophytes beds form highly productive systems, which have an important role in estuarine productivity. Moreover, evidence is shown that *H. ulvae* population structure and annual production were affected by the induced instability of macroalgae bloom dynamics.

Acknowledgements

The present work was supported by the Programme MAST II (MAS 2 CT 92 0036) and by JNICT (Portuguese national institution for scientific research) by conceding a MSc grant (BM/ 1760/ 94) on the scope of Praxis XXI program. A very special thanks goes for all the colleagues from IMAR - Coimbra.

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IMPACT OF EUTROPHICATION ON AMPHIPODS *Melita palmata* AND *Ampithoe valida* IN THE MONDEGO ESTUARY

Abstract

The life cycle, population dynamics and production of *Ampithoe valida* and *Melita palmata* were studied in an intertidal mudflat in central Portugal. Sampling was carried out in eutrophicated areas, where macroalgae blooms of *Enteromorpha* spp. usually occur from January to early summer, and also in non-eutrophicated areas characterised as *Zostera noltii* meadows. Both amphipod species showed a contagious distribution, and the populations densities clearly changed during the study period along the eutrophication gradient. No migratory patterns were detected between the estuary and the sea, but dispersion inside the estuary might occur in these amphipods. Both species presented a two-generation life cycle involving a short-lived fast growing summer generation (7 months for *A. valida* and 4-5 months for M. palmate) and a longer-lived, slower growing generation that overwinters (9 months for *A. valida* and 6-7 months for *M. palmata*). Along the eutrophication gradient no differences were found regarding the biology of the species. Besides these features, differences were observed between eutrophicated and non eutrophicated areas in regard to productivity.

In a certain extent, the increase of macroalgae biomass may favour these herbivorous amphipods populations, but extensive blooms affecting the whole area of distribution of these species will determine its disappearance.

This Chapter includes new information and information already published in Marine Ecology Progress Series (2000, 196:207-219).

Introduction

Eutrophication appears to be a common phenomenon in coastal waters around the world. The replacement of rooted macrophytes by opportunistic green macroalgae (*Chaetomorpha*, *Enteromorpha* and *Ulva*) seam to be also a major feature in these ecosystems when they suffer from organic pollution. (Hickel et al. 1993, Yeates 1993, Dijk et al. 1994, Hartog 1994, Flindt et al. 1997, Marques et al. 1997, Rafaelli et al. 1998).

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In the south arm of the Mondego estuary, seasonal intertidal macroalgae blooms (mainly of *Enteromorpha* spp.) have been reported for several years (Marques et al. 1993a, 1993b, 1997, Pardal et al. 1993, Pardal 1998, Flindt et al. 1997, Lillebø et al. 1999, Martins et al. 2001) due to nutrient enrichment of estuarine waters (Marques et al. 1997, Pardal 1998, Martins et al. 2001). As a consequence, *Zostera noltii* beds, which represent the richest habitat with regard to productivity and biodiversity, have been drastically reduced in the south arm of the estuary, presumably out competed by *Enteromorpha* (Rafaelli et al. 1991, Hodgkin and Hamilton 1993, Pardal 1998).

Ampithoe valida and *M. palmata* are grazers that can be found on rocky substrata and on muddy bottoms, often in the presence of green macroalgae or macrophytes (e.g. *Zostera noltii*). Both species present a wide distribution but they received little attention (Borowsky 1983, Pardal et al. 2000). Despite that there are just a few studies on the impact of macroalgae blooms on grazer amphipod species population dynamics (Drake and Arias 1995, Pardal et al. 2000). Such knowledge may definitely contribute for a better understanding of dynamic processes related with shift in primary producers a usual phenomenon in European and north America coastal waters.

Materials and methods

In the south arm macroalgal blooms of *Enteromorpha* spp. have been regularly observed during the last 15 years (Flindt et al. 1997, Marques et al. 1997, Pardal 1998, Lillebø et al. 1999, Pardal et al. 2000, Martins et al. 2001). This is probably a result of excessive nutrient release into the estuary, coupled with longer persistence of nutrients (nitrogen and phosphorous) in the water column. Nevertheless, such macroalgae blooms may not occur in exceptionally rainy years (e.g. year 1994) due to low salinity for long periods, as a result of the Pranto river discharge (Pardal 1998, Martins et al. 2001).

The amphipods were monitored in the Mondego estuary during 18 months, from January 1993 to June 1994. Samples were taken fortnightly at three different sites along an estuarine gradient of eutrophication in the south arm of the estuary (Fig. 1). From a *Zostera noltii* meadow, up to a heavily eutrophicated zone, in the inner areas of the estuary, where *Enteromorpha* spp. blooms have been observed during the last two decades.

Each time at each site ten cores (with a 141 cm^2) were taken to a depth of 15 cm and sieved through a 500 μm mesh sieve.

Amphipods were identified, counted, measured and sexed. Due to the difficulties in measuring Total body Length (T_L) accurately in such a small organisms that are typically comma-shaped, an alternative and more accurate length was measured: The Cephalic Length (C_L) (measured between the extremity of the rostrum and the base of the head). So for both species an equation for C_L-T_L conversion was determined ($T_L = -0.1355 + 9.4233*C_L$, $n = 262$, $r = 0.965$ for *A. valida* and $T_L = -0.62503 + 9.8771*C_L$, $n = 323$, $r = 0.955$ for *M. palmata*). Length-weight relationships were determined for production estimates. A single regression equation was determined for each species ($W = 0.00085*C_L^{3.631}$, $n = 95$, $r = 0.983$ for *A. valida* and $W = 0.00196*C_L^{3.6535}$, $n = 72$, $r = 0.966$ for *M. palmata*).

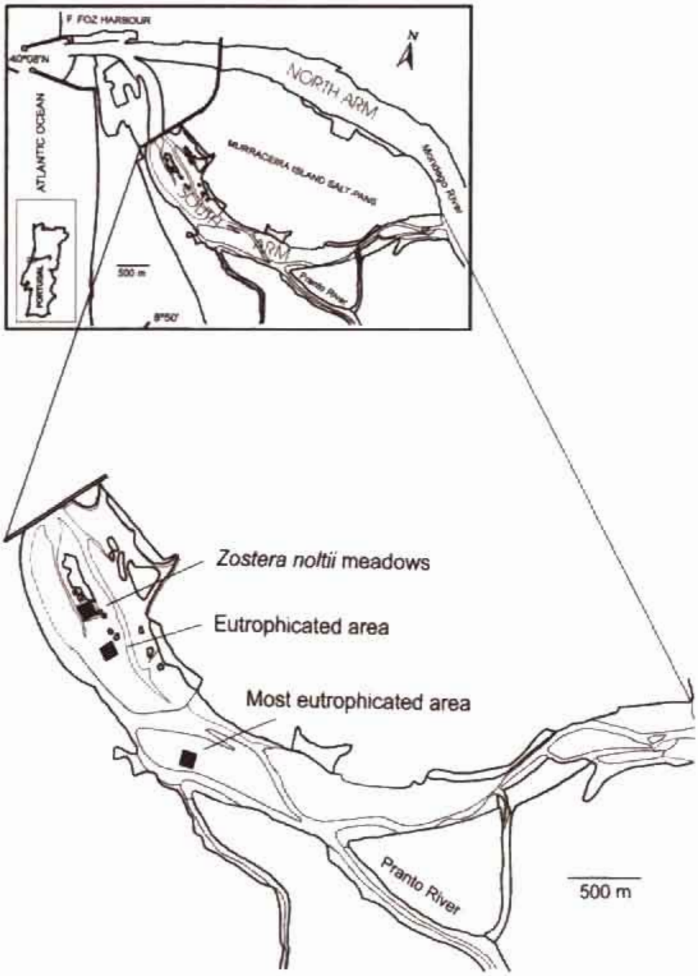

Fig. 1. Location of the sampling stations along a spatial gradient of eutrophication in the Mondego estuary.

For the determination of sex we used the same methodology described in Pardal et al. (2000, 2001). When broods were present, eggs were counted (to estimate the fecundity), measured and examined to determined the development stage. Taking into consideration several similar criteria (Goedmakers 1981, Skadsheim 1982, Steele and Steele 1969, Marques and Nogueira 1991, Marques et al. 1994, Pardal et al. 2000) five stages were considered: a) newly laid, eggs grouped and resembling a gelatinous mass; b) eggs well separated, internally homogeneous; c) embryo comma-shape, pereopods starting to be visible; d) constriction of the comma clearly visible, appendages segmented and looking slender, eyes visible, cephalotorax orange-red; e) hatched and free juveniles.

All the statistical analysis, concerning: a) population spatial dispersion; b) multiple regression models between the density and biomass of amphipods and biomass of green macroalgae, salinity and water temperature; c) estimation of field growth rates; d) estimation of production (net production (P) and elimination production (E), were developed following the procedures and techniques explained in Pardal et al. (2000; 2001).

Results

Macrophytes and macroalgae biomass

The *Zostera noltii* biomass exhibited a clear seasonal variation. During autumn and winter total biomass depended essentially on the rhizomes, while during spring and summer total biomass increased due to the growth of leaves (Fig. 2A).

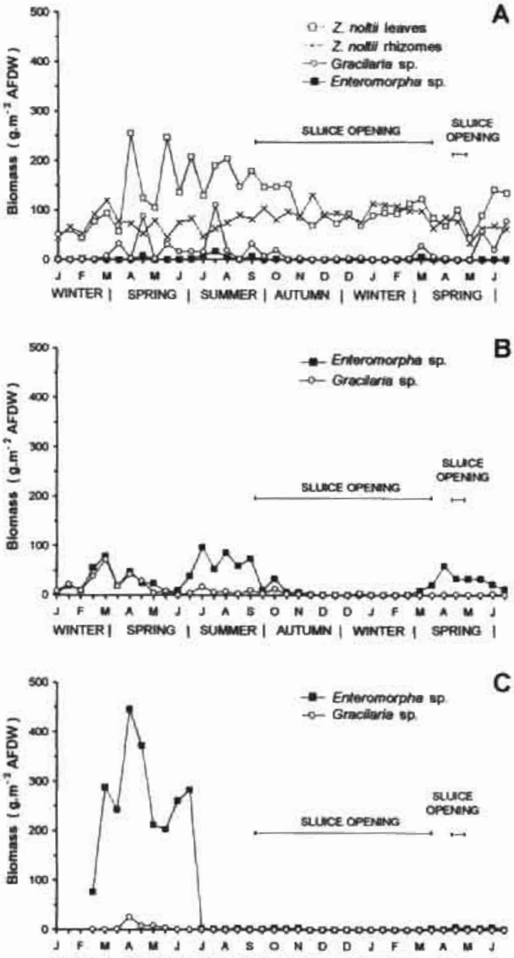

Fig. 2. Seasonal changes in plant biomass along the eutrophication gradient. (A) *Zostera noltii* meadows; (B) Intermediate eutrophicated area; (C) Most eutrophicated area.

In the inner areas of the estuary that corresponds to the most eutrophicated area *Enteromorpha* sp. exhibited a typical spring bloom in 1993. In the end of June an algal crash occurred causing a severe impact on the macrofauna (Figs. 2, 3). In 1994 no macroalgal bloom occurred because it was a very rainy year in central Portugal which increased freshwater discharges of the Pranto river that kept salinity low. Such conditions inhibit *Enteromorpha* growth, since salinity was below 10 ‰ for several months.

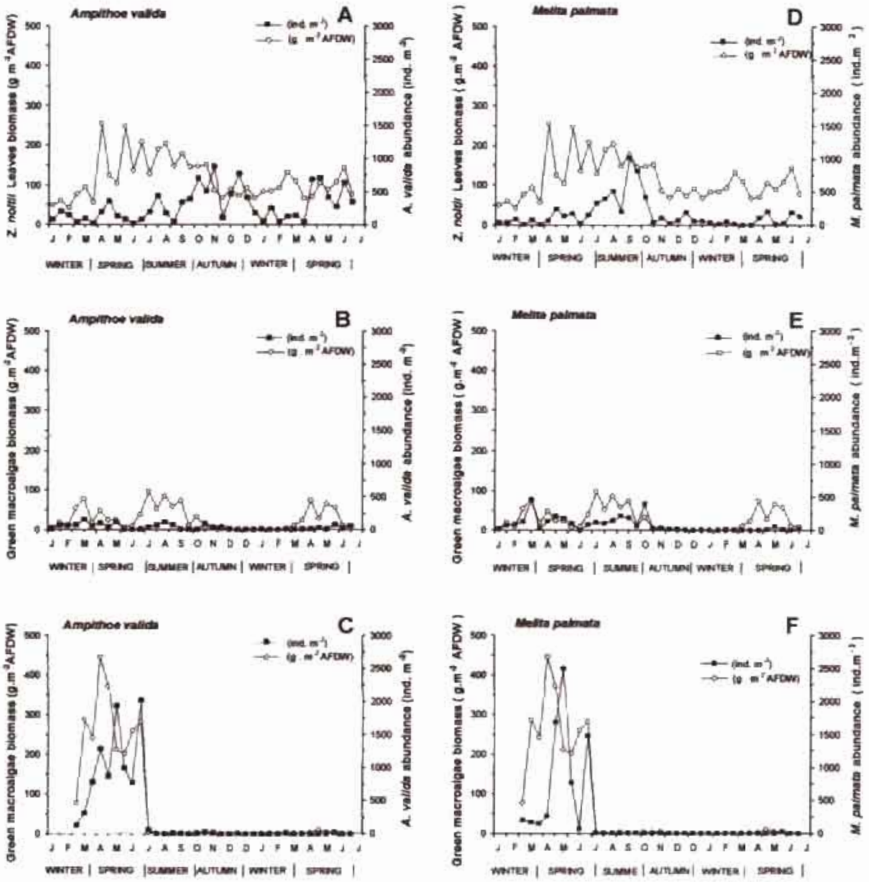

Fig. 3 Abundance of *A. valida* and *M. palmata* along the eutrophication gradient. *Zostera noltii* meadows (A and D); Eutrophicated area (B and E); Most eutrophicated area (C and F)

Spatial distribution and abundance

Both amphipod species showed an aggregated spatial distribution in the three study areas along the eutrophication gradient (Elliot 1977). Population density of *A. valida* and *M. palmata* changed throughout the period of study but the variation pattern was not the same along the eutrophication gradient (Fig. 3). In the most eutrophicated area densities were consistently higher during the algal bloom. After the algae crash density of both species declined sharply and remained low, or organisms even disappear throughout the study (Fig. 3C and 3F). In the intermediate eutrophicated area the variation in density was correlate with the variation in macroalgal biomass ($r = 0.87$, $N = 26$ for *A. valida*), but densities never reached so high abundances as in the previous case (Fig. 3B). With such low abundances most of the statistical analysis that we performed in the present paper were not possible in this area. At the *Zostera noltii* meadows, as a pattern for both species, the population density increased during summer and fall (1993) and also during spring (1994) (Fig. 3A and 3D).

Stepwise multiple regression analysis indicated that abundance (A) and biomass (B) of both amphipods in the most eutrophicated area was positively correlated with *Enteromorpha* sp. biomass (ENT), *Gracilaria* sp. biomass (Grace) and temperature of the water (TEMP):

A. valida

$\ln(1 + A) = 0.350 + 1.142 \ln(1 + ENT)$

$\ln(1 + B) = -0.030 + 0.030 \ln(1 + ENT) + 0.096 \ln(1 + Grace)$

M. palmata

$\ln(1 + A) = -4{,}633 + 0{,}660 \ln(1 + ENT) + 0{,}248\ (TEMP)$

$\ln(1 + B.) = -0{,}238 + 0{,}017 \ln(1 + ENT) + 0{,}012\ (TEMP)$

In the *Z. noltii* meadows only for *M. palmata* were found significant correlations between these population parameters and leaves biomass (LEA) and temperature of the water.

M. palmata

$\ln(1 + A) = -3{,}784 + 0{,}660 \ln(1 + LEA)$

$\ln(1 + B) = -0{,}88 + 0{,}007\ (TEMP)$

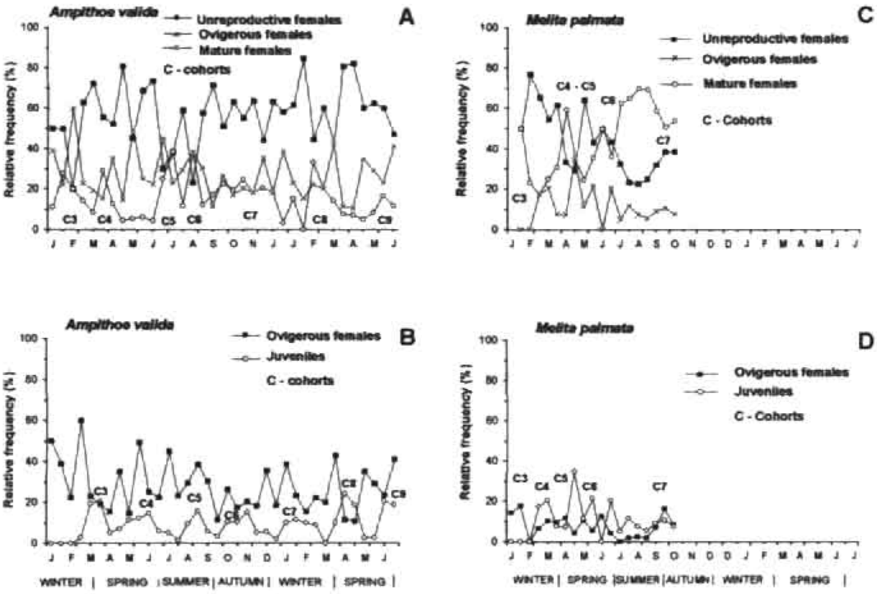

Fig. 4. Biological features of *A. valida* and *M. palmata* population. (A and C) Variation of females in different physiological stages in relation to the female population; (B and D) Variation of ovigerous females in the total female population and of juveniles in the population.

Reproduction, sex-ratio, and fecundity

The *A. valida* and *M. palmata* populations were sexually active throughout the year in the south arm of the Mondego estuary. However, taking into consideration the percentage of ovigerous females over the total female population (Fig. 4), sexual activity was higher from late spring to late summer. The variation of the percentage of juveniles in the population was clearly related to increases in recruitment of both species.

In *A. valida* and *M. palmata* females were usually more abundant than males overall, and no significant seasonal changes of this pattern were observed (Fig. 5). The fecundity of females of similar size was quite variable, and, no correlation between the number of developing embryos and female size was found in any of the studied species. Measurements of eggs in different developmental stages (Table 1) showed that, egg volume increase changes seasonally. For instance on *A. valida* an increase of 361% was estimated in summer, 452% in spring and 492% in autumn. It was also possible to recognise that for the same developing stage eggs' volume was greater in colder periods, while the average number of eggs per female was smaller (table 1 and 2).

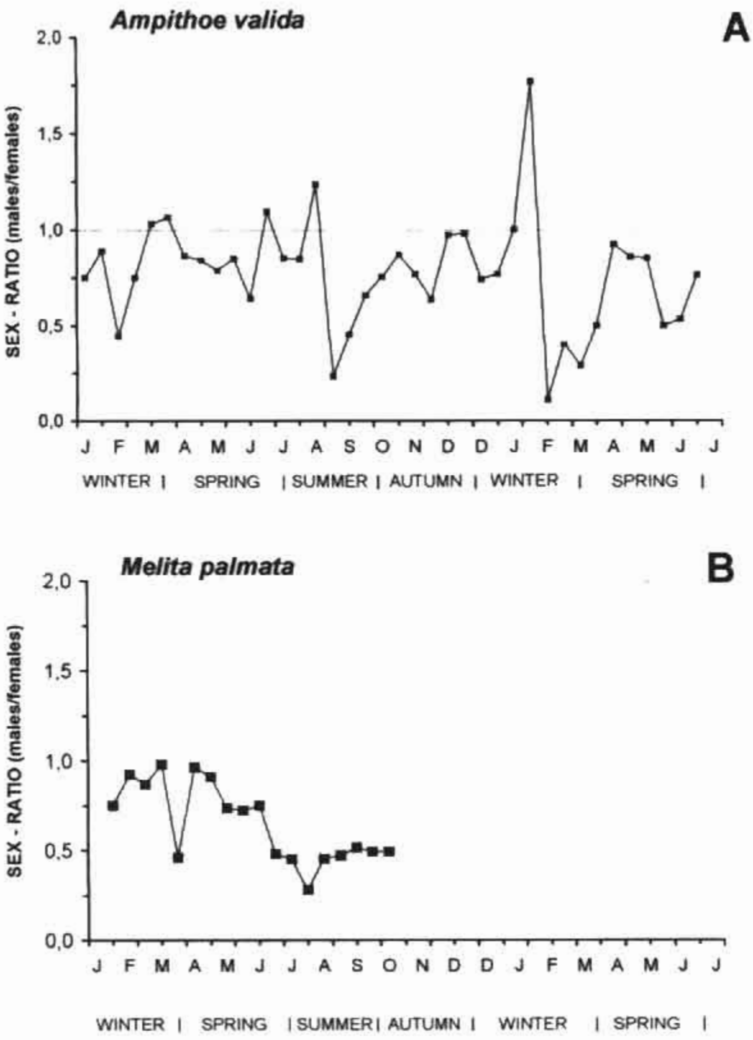

Fig. 5. Sex-ratio variation during the period of study. (A) *Ampithoe valida*; (B) *Melita palmata*.

Table 1. Mean number (N) and volume (Vol) of eggs per female of *Ampithoe valida*, at development stages AB, C, D and E, in the Mondego estuary.

	AB		C		D		E	
	N	Vol (mm^3)	N	Vol (mm^3)	N	Vol (mm^3)	N	Vol (mm^3)
Winter	18	0,205	12	0,218	16	0,384	7	-
Spring	16	0,151	24	0,186	18	0,214	9	0,682
Summer	11	0,155	-	0,170	-	0,167	-	0,56
Autumn	6	0,218	11	0,232	-	0,233	-	1,073

Table 2. Mean number (N) and volume (Vol) of eggs per female of *Melita palmata*, at development stages A, B, C, D and E, in the Mondego estuary.

	A		B		C		D		E	
	N	V (mm³)	N	Vol (mm³)	N	Vol (mm³)	N	Vol (mm³)	N	Vol (mm³)
Winter	1,5	0,71	2,8	1,34	-	-	8	1,57	2,5	3,46
Spring	1	0,60	4,7	0,97	3,5	1,35	3,3	1,99	4,25	2,24
Summer	3	0,98	3,8	0,96	2	1,35	2,5	1,58	1,7	2,82
Autumn	-	-	-	-	2,5	1,26	4,5	1,89	1	3,63

The difference in time between peaks of eggs in the first stage of development (A) and the last one (E), provided us an estimation of the duration of the embryogenic development quite similar for both species (Fig.6). During spring and summer the embryogenic development was faster (30-45 days) than in autumn or winter (60 days).

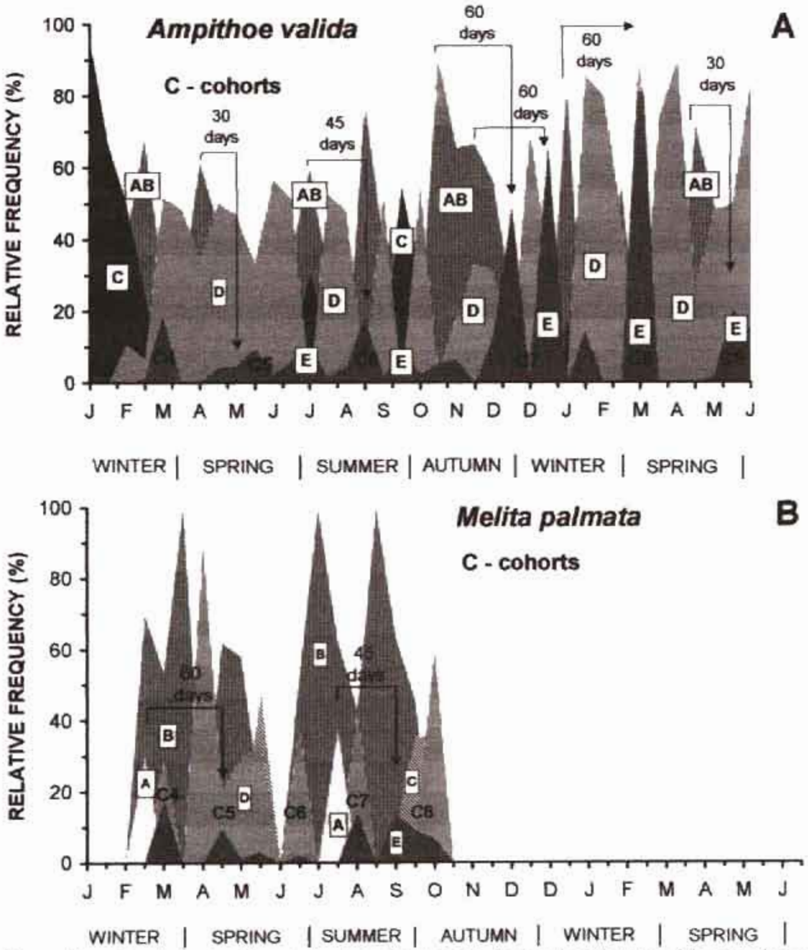

Fig. 6. Proportion of embryos in different developmental stages. Seasonal estimation of the duration of the embryogenic development. (A) *Ampithoe valida*; (B) *Melita palmata*.

Growth and life span

Size frequency polymodal distributions were analysed for recognisable cohorts, allowing in first place to identify significant differences in the population structure on the *Zostera* beds and in the most eutrophicated area. As a pattern for these two species, Individuals reached larger dimensions at the most eutrophicated area in comparison with the other two areas (*Z. noltii* meadows and intermediate eutrophicated areas). Nevertheless, in the first months the recruitment pattern was the same all along the eutrophication gradient. Following the algae crash both populations disappeared from the most eutrophicated area and cohorts tracking became only possible at the *Z. noltii* meadows. For instance, for *A. valida* in January of 1993 three cohorts were identified, and six new ones were recognised during the study period (χ^2 and G not significant, $P \leq 0.05$). Minimum average C_L of cohorts ranged from 0.327 (spring) to 0.397 mm (winter) corresponding to 2.946 and 3.606 mm of total length.

For both species growth was continuous through life (Fig. 7). Nevertheless, growth rates were higher during spring and summer and decreased during winter. Lower growth rates during winter were probably a function of lower temperatures and lower biomasses of macroalgae (food resources).

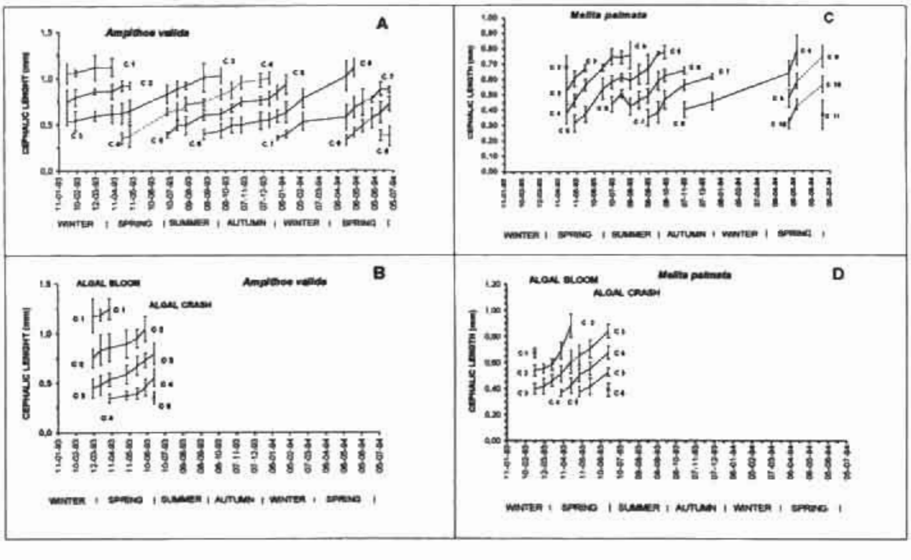

Fig. 7. Estimated growth (field growth data) of cohorts ± standard deviation of *Ampithoe valida* and *Melita palmata*. (A and C) *Z. noltii* meadows; (B and D) Most eutrophicated area.

Life spans were estimated for *A. valida* at 191 ± 30 to 238 ± 15 days for summer and spring cohorts and at 242 ± 30 days for the autumn cohort and for

M. palmata at 120 ± 30 to 148 ± 15 days for summer and spring cohorts and at 182 ±. 30 for the autumn cohort. Similarly, for both species the age at which females and males matured were lower in spring and summer cohorts than in the autumn-winter cohorts. Nevertheless, during the yearly cycle, males always reached maturity before females.

Production estimates

Length-weight relationships previously established (Pardal 1998, Pardal et al. 2000) were used in production estimates in the most eutrophicated area and in the *Z. noltii* meadows (Tables 3 and 4). Taking into account the whole period of study, results clearly showed that growth productivity (P), elimination productivity (E) and the relations P/$\overline{B}$ and E/$\overline{B}$ were much higher in the *Z. noltii* meadows. But in the most eutrophicated area during the algal bloom P, E as well as population density, were clearly higher than in *Z. noltii* meadows (Tables 3 and 4).

Table 3. Production estimates for *Ampithoe valida* at *Z. noltii* meadows and at the most eutrophicated area taking into account different periods of time with different ecological conditions.

	P (g.m^{-2} time^{-1})	$\overline{B}$ (g.m^{-2})	P/$\overline{B}$	E (g.m^{-2} time^{-1})	E/$\overline{B}$
Z. noltii (18 months) (January 1993 to June 1994)	0.635	0.106	5.988	1.316	12.41
Z. noltii (13 months) (January 1993 to February 1994)	0.409	0.112	3.646	0.853	7.603
Z. noltii (13 months) (May 1993 to June 1994)	0.531	0.092	5.756	0.921	9.972
Z. noltii (3 months) (April 1993 to June 1993)	0.036	0.486	0.074	0.082	1.685
Eutrophicated area (3 months) (with a macroalgal bloom) (April 1993 to June 1993)	0.411	0.343	1.20	0.945	2.758
Eutrophicated area (4 months) (with a macroalgal bloom) (March 1993 to June 1993)	0.444	0.312	1.424	0.945	3.033

Table 4. Production estimates for *Melita palmata* at *Z. noltii* meadows and at the most eutrophicated area taking into account different periods of time with different ecological conditions.

	P ($g.m^{-2}$ $time^{-1}$)	$\overline{B}$ ($g.m^{-2}$)	P/$\overline{B}$	E ($g.m^{-2}$ $time^{-1}$)	E/$\overline{B}$
Z. noltii (14,5 months) (April 1993 to June 1994)	0.473	0.063	7.561	0.767	12.26
Z. noltii (13 months) (June 1993 to June 1994)	0.456	0.064	7.144	0.734	11.49
Z. noltii (2 months) (April 1993 to June 1993)	0.059	0.029	2.030	0.062	2.131
Eutrophicated area (2 months) (with a macroalgal bloom) (April 1993 to June 1993)	0.496	0.397	1.248	0.683	1.720
Eutrophicated area (4 months) (with a macroalgal bloom) (March 1993 to June 1993)	0.728	0.236	3.088	0.702	2.977

Discussion

Abundance

For both species population density clearly changed along the eutrophication gradient as a result of the macroalgal annual dynamics, like observed before for *Microdeutopus gryllotalpa* (Drake and Arias 1995). In fact, in the most eutrophicated area, the occurrence of a macroalgal bloom in the first year prepared the conditions for the presence of large densities of *A. valida* and *M. palmata*. The algal crash, which acted as a catastrophic event (Marques et al. 1997) and the non occurrence of an algal bloom in the second year had a strong effect on the population's density in the inner parts of the estuary. Consequently, since in the second spring (1994) there were no macroalgae habitats available, *A. valida* and *M. palmata* individuals seemed to occur only in the *Z. noltii* meadows.

During the macroalgae bloom the population densities of both species were much higher than in the *Z. noltii* meadows in the same period. This may be related to habitat protection against potential predators (crabs, fishes and birds) and food resources (Greeze 1968, Duffy and Hay 1991), since they can feed directly on the algae. Next, during the algae crash, when anoxic conditions occurred, the amphipods population suffered a sudden and drastic reduction. After the algae crash the amphipods population was never able to recover since no food resources were available. On the contrary, in the *Z. noltii* meadows both species increased in number after the algae crash. A possible movement of adult individuals inside the estuary moving to avoid the extreme conditions of anoxia, is the most probable explanation, together with new recruitments taking place in this area. It seems therefore that both species have a large potential for dispersion inside the estuary, avoiding as much as possible stressing situations caused by macroalgae blooms in early summer. It seems reasonable to conclude that in a certain extent the development of macroalgae biomass favours *A. valida* and *M. palmata* populations, but extensive blooms affecting the whole area of distribution of this species will determine its disappearance.

Reproduction, growth and life cycle

Data showed that females of both species almost always outnumbered males, which is a common feature in amphipod populations (Hastings 1981, Dauvin 1988a, 1988b, Marques and Nogueira 1991, Morrit and Stevenson 1993, Covi and Kneib 1995, Sudo and Azeta 1996).

In our case embryonic development was fast but showed seasonal variations, from 30 days in spring up to 60 days in autumn and winter. In colder months the developing embryos (in fewer number) present larger volumes, which allow to optimise chances of survivorship since they contain higher nutritive reserves. Such volume increase was higher than the one observed by Moore and Wong (1996), although they were similar to the ones calculated by Marques and Nogueira (1991) for *Echinogammarus marinus*. Moreover, in spring and summer sexual maturity was reached at smaller sizes in comparison with autumn cohorts, which agrees with previous observations on other amphipods (Moore 1991, Powell and Moore 1991, Sudo and Azeta 1996).

Summer and spring growth rates were much higher than during colder months. This was also observed for other amphipod species in temperate areas (Hasting 1981, Dauvin 1988a, 1988b, Franz 1989, Marques and Nogueira 1991, Uitto and Sarvala 1991, Drake and Arias 1995, Wilson Jr. and Parker 1996), and growth rates estimated by us were similar to other species at the same latitude (Ali and Salman 1987, Marques and Nogueira, 1991, Drake and Arias, 1995).

Like other amphipod species, *A. valida* and *M. palmata* are semi-annual with short-lived spring and summer generations and long-lived autumn and winter generations (Moore 1981, Powell and Moore 1991, Beare and Moore 1994, Sudo and Azeta 1996).

Both species appeared as *r* strategists, with iteroparous females, multivoltine cycles, high individual fecundity and recruitment all over the year. This is the most common pattern in epifaunal species (van Dolah 1980) from physically controlled communities according to the stability-time theory (Sanders 1969). In environmental stressed systems like estuaries we should expect the evolution of opportunistic adaptive strategies to take place.

Production estimates

Taking into account the whole period of study P (growth productivity) and E (elimination productivity) as well as $\overline{B}$ (standing stock) showed significantly higher values in the *Z. noltii* beds than in the most eutrophicated area. Nevertheless, this difference does not reflect the entire reality. In fact we must look carefully to the dynamics of the two areas. In the most eutrophicated area for a short period (during the macroalgae bloom) P, E as well as $\overline{B}$ were higher than in the *Z. noltii* meadows. This means that during the macroalgae bloom the most eutrophicated area was clearly the preferential habitat for the Amphipods probably due to resources availability.

The results clearly suggest that *A. valida* and *M. palmata* population dynamics and spatial distribution may change as a function of environmental changes through adaptative behavioural mechanisms. Nevertheless, it appears that macroalgae may represent mainly a good additional resource for population with a stable habitat like *Z. noltii* beds. But the macroalgae mats by it self can not sustain stable populations of amphipods due to its own dynamics. A conclusion would be that the total replacement of *Z. noltii* by macroalgae mats would negatively affect this kind of grazers.

The present results suggest that these species may play a more important role in the trophic dynamics than we might expected from their standing stock biomass. As pointed out by Fredette and Dias (1986), our data show that in warm shallow marine habitats relatively low biomass of benthic invertebrates can result in large production estimates (Kalejta and Hockey 1991).

As a whole, in the Mondego estuary, although macroalgae seem to be favourable to amphipods populations, if we look to the following effects of the algae crash it is reasonable to conclude that more extensive blooms, affecting the whole area of distribution of the species will on the contrary have a strong negative impact and could imply its disappearance.

Acknowledgements

This paper was carried out in the scope of the WET-project (Wetland, Ecology and Technology), financed by the European Scientific TMR Program (ERB 4061 PL 95-0832). The authors are indebted to all colleagues who assisted in field and laboratory work.

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THE SUPRABENTHIC MYSID *Mesopodopsis slabberi* (VAN BENEDEN) IN THE MONDEGO ESTUARY

Abstract

The biology, production, biochemical composition and energy content of the mysid *Mesopodopsis slabberi* (van Beneden) are described. Significant positive correlations between total length (TL) and cephalic length (CL) ($TL = 2.5\ CL + 0.012$) and between dry weight (DW) and total length ($LnDW = 3.0298\ LnTL - 6.0229$) were found. The annual production was 13.17 $mg.m^{-2}year^{-1}$, and the P/B ratio was 9.32. This turnover rate strength the hypothesis that *M. slabberi* plays an important role in the Mondego estuary food web. Protein, carbohydrate, chitin, lipid, phospholipid, and cholesterol contents were determined from freshly caught juveniles, males, and females throughout the year. Energy equivalents were calculated using conversion factors. Statistical analysis revealed significant seasonal differences in biochemical composition, and also between juveniles, females, and males. The nutritional cycle (environmental conditions: trophic conditions) and reproductive cycles appeared both as the main processes influencing the biology of *M. slabberi*.

Key words: Mysids, secondary production, biochemical composition, energy content.

Introduction

Mysids play an important role in the functioning of soft-bottom benthic ecosystems (San Vicente and Sorbe 1995). The population of *M. slabberi* is an important component within the suprabenthic communities (Azeiteiro and Marques 1999) and nectobenthic communities or benthopelagic zooplankton. Due to the abundance of *M. slabberi* in the Mondego estuary we studied several aspects of the species biology. The knowledge of the species population dynamics and secondary

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production (Azeiteiro et al. 1999), biochemical composition (Azeiteiro et al. 2000, in press a) and energetics (Azeiteiro et al. in press b) allows the analysis of an important fraction of the suprabenthic and nectobenthic trophic stock and the comparison among various mysid populations in their respective distribution areas.

Material and methods

Five sampling stations were located along the south arm of the estuary in order to represent the whole subsystem (Fig. 1). Although we were aware from former studies (Gonçalves 1991, Azeiteiro 1999) that the *M. slabberi* population was particularly abundant in the mid-areas of the south arm, it was decided to survey the entire south arm. Suprabenthic and crepuscular-time plankton samples were collected monthly at the five stations, during spring tides, following always the same sequence, from June 96 and July 97. Mysids samples were taken from sub-surface waters using a WP2 modified net (60 cm diameter and 335 µm mesh) (sub-superficial tows) and a suprabenthic net (50 cm diameter and 500 µm mesh) (suprabenthic tows).

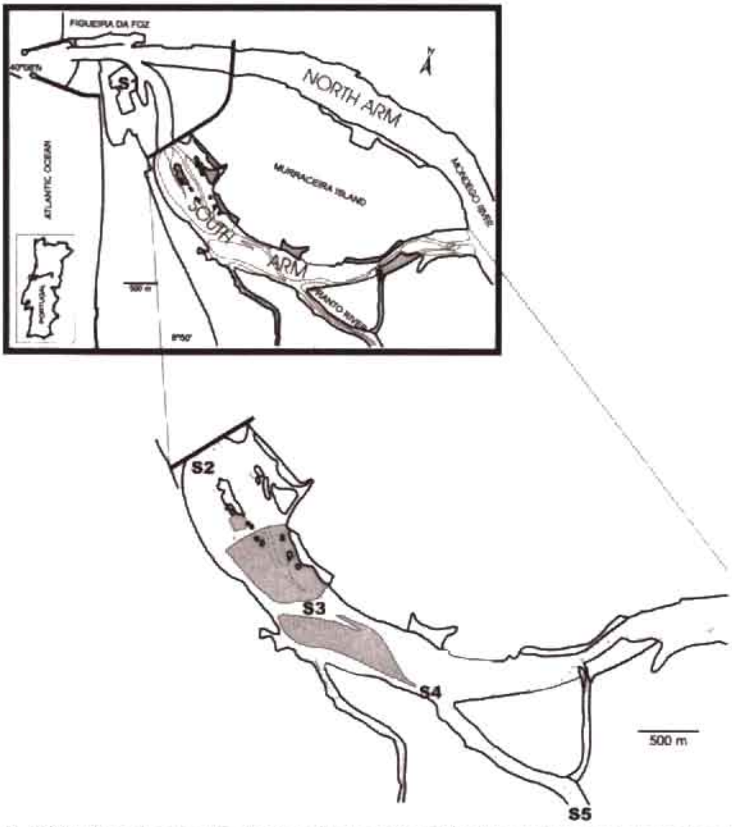

Fig. 1. Map of Mondego river estuary showing the locations of the five sampling stations in the southern arm.

Laboratory procedures

All samples were transported back to laboratory in good condition within 2h of collection. Those freshly caught animals were kept in ice at ±4 °C before they were classified and separated in juveniles, females, and males. Samples from each group were lyophilised, weighted, and kept at −30 °C; smaller portions of this material were later weighed and utilised for each analysis.

Production procedures

Specimens were measured (to the nearest 0.02 mm) from the anterior tip of carapace to the posterior tip of telson, as well as between the anterior and posterior tips of the carapace (Wooldridge 1986, Azeiteiro et al. 1999).

Individuals were classified into the following categories (Mauchline 1980, Sorbe 1984, Azeiteiro et al. 1999):

1. Juveniles: Secondary sexual characteristics absent;
2. Males;
3. Females (Immature females: secondary sexual characteristics in process of development; mature incubant females: marsupium fully developed and carrying eggs or embryos; and mature empty females: with empty expanded marsupium from where young have recently emerged).

To estimate mysid standing stock (mg of dry mass.m^{-3}) it was necessary to determine the relationships between cephalic length and dry weight and total length and dry weight. For this purpose, a set of specimens were measured and then dried for 24 h at 60 °C (Wooldridge 1986, Azeiteiro et al. 1999), after what they were individually weighted (to the nearest 0.01 mg) with a Mettler microbalance. According to Matthews (1973) and Jørgensen and Matthews (1975) we pooled the entire set of specimens, belonging to the different categories, in order to estimate morphometric relationships considered valid for the whole population (Azeiteiro et al. 1999).

Production was estimated using the Hynes (1961 *in* Sorbe 1984) average cohort method, modified by Benke (1979) with particular significance for multivoltine populations and called the size-frequency method by Waters and Hokenstrom (1980). The Hynes method for estimating aquatic invertebrates production involves first an estimation of the total number of individuals that developed into each size class and then the calculation of losses in numbers between size classes. Production is then estimated as the sum of biomass losses between successive size classes. The Hynes method does not require the recognition and tracking of individual cohorts. It is therefore suitable for populations with continuous reproduction and no synchronisation of larval release and egg extrusion (Sorbe 1984, Wooldridge 1986, Azeiteiro et al. 1999).

Biochemical analyses

Protein - Lyophilised material was homogenised in the proportion of 0,5 mg to 3 ml of pure water into different 10 ml test tubes. The water soluble protein content was analysed by the Lowry et al. (1951) method, modified by Fernandes et al. (1994) (Azeiteiro et al. 2000, in press a, b).

Carbohydrate - we prepared the samples for analysis following basically the same procedure as for proteins, but higher test tubes were used. The homogenates were analysed by the Raymont et al. method, described in Bamstedt (1976) and Omori and Ikeda (1984), using 1ml of 5 % phenol solution and 5 ml of concentrated sulphur acid (Azeiteiro et al. 2000, in press a, b).

Chitin – the analysis was performed using the Bamstedt (1976) method for dried homogenised material. However, instead of being incinerated, the chitin final product was analysed for its content in carbohydrate, using the method described above (Azeiteiro et al. 2000, in press a, b).

Total lipid – the analysis was performed following the method described by Lehtonen (1996), but we have done two washes instead of one. Approximately 15 mg of lyophilised material were weighed and homogenised in 0.5 ml of a chloroform/methanol (2:1) solution, and then centrifuged during 30 seconds. The precipitate was washed a second time with 0,5 ml chloroform:methanol (2:1) and centrifuged again for 30 seconds. We added 20 % volume of 0.9 % NaCl solution to the chloroform/methanol (2:1) solution from both washes and centrifuged again. The chloroform phase containing the lipids in solvent solution were placed into tared cups and the solvents were evaporated. Following the evaporation of the solvent solution, the cups were weighed and the weight of the lipids was calculated (Azeiteiro et al. 2000, in press a, b).

Lipid extracts were analysed for their phospholipid content through the quantification of phosphate. For this purpose we used the Bartlett (1958) phosphate determination with Fiske and Subbarow reducing agent, according to the method described in Sidney and Nathan (1969). All volumes were nevertheless reduced to one half of the indicated in the original description and spectrophotometer readings were done at 830 nm (Azeiteiro et al. 2000, in press a, b).

Lipid extracts were also analysed for their cholesterol content using the method described by Fernandes et al. (1994). The lipid chloroform extraction was evaporated to completely dryness. Lipids were dissolved with 20 ml of acetic acid and allowed to react with 1 ml of Liebermann-Burcherd reagent described by Huang et al. (1961), adapted to tissue by (Fernandes pers. comm.). We didn't find necessary to separate cholesterol from other lipids (Fernandes et al. 1994) because there wasn't any pigment interfering with spectrophotometer readings (Azeiteiro et al. 2000, in press a, b).

Energy equivalents calculation

From the biochemical analysis performed, energy equivalents were calculated using the conversion factors given by White et al. (1973): 4.1 cal/mg for protein, 4.3 cal/mg for carbohydrate, and 9.5 cal/mg for lipid (Azeiteiro et al. in press b).

Data analysis

The estimation of variation coefficients (CV) ($CV = SD \times 100 / X$) (Bamstedt 1978) was use to provide an index of the relative variability for each biochemical component.

The first step in data analysis consisted of rejecting observations using a Q Test, defined as the ratio of the divergence of the discordant value from its nearest neighbour to the range of the values (Skoog and West 1972).

Secondly we performed an angular (arcsine) transformation of the data before analysing it. The arcsine transformation was chosen since it is recommended that data transformation is not warranted for analysis of variance with binomial data unless the largest sample size is more than five times greater than the smallest, and the smaller variances are associated with the smaller samples (Zar 1996). Now, it is known from statistical theory that percentages from 0 to 100 % or proportions from 0 to 1 form a binomial, rather than a normal, distribution, the deviation from normality being great for small or large percentages (0 to 30 % and 70 to 100 %). So, if the square root of each proportion, p, in a binomial distribution is transformed into its arcsine (i.e., the angle whose sine is the square root of p), the resultant data will have an underlying distribution that is nearly normal (Zar 1996).

Finally, we performed an Analysis of Variance (ANOVA) to test differences between sexes and between months for all the components considered. An ANOVA Two Factor or factorial analysis of variance was carried out in first place to test for interaction among factors (Zar 1996). Since interaction was found the means of levels shouldn't be compared (Zar 1996). It was therefore necessary to perform a One-Way ANOVA for each factor, in order to reveal significant differences among the levels of a factor. Then we performed multiple comparison procedures (Zar 1996) following two methods: (1) the Tukey test (Tukey 1953 *in* Zar 1996), and (2), the Newman-Keuls test (Newman 1939 and Keuls 1952 *in* Zar 1996).

Results

The following body size/weight relationships were estimated for freshly caught specimens of *M. slabberi* (Figure 2): $TL = 2{,}5 \times CL + 0.012$, $LnDW = 3.0298 \times LnTL - 6.0229$.

Production estimates are summarised in Table 1. The annual net production was calculated at 13.17 $mg.m^{-3}.year^{-1}$, and the P/B ratio was estimated at 9.32.

Protein – It was the primary body component all over the year (Figure 3), constituting in average more then half of the dry weight. Protein contents varied between 58.2 and 74.8 %, for juveniles, between 61.7 and 83.8 %, for females, and between 58.1 and 78.7 % for males (Table 2). In general we could observe that, except for the winter season, the protein contents in juveniles was lower than in females and males. Juveniles showed a small decrease in protein contents in November, followed by a slow increase until the beginning of May. By the end of May a new and more

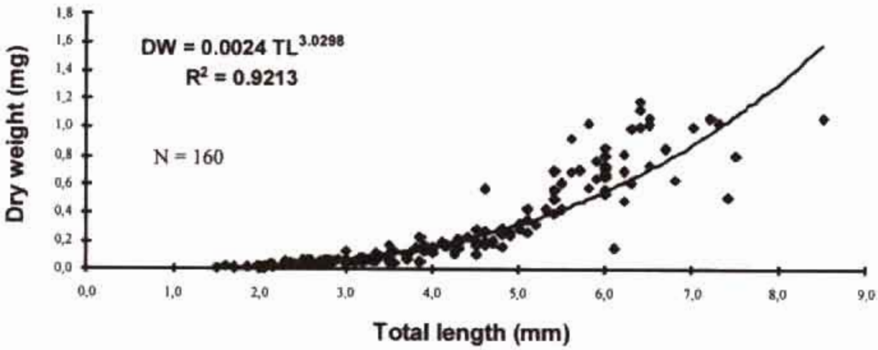

Fig. 2. Regression model for weight-length relationships. Correlation coefficients (R) and sample size (N) are indicated.

Table 1. Production estimates for *M. slabben* in the Mondego estuary.

Total Length							Biomass	
(mm)	Year Density	dm	dm_i-dm_{i-}	W (mg)	(mg/m^2)	$(W_i W_{i-})^{1/2}$	Lost Biomass	P (*18)
1,450	0,67	5,5E-02		7,390E-03	4,098E-04			
1,825	4,41	3,7E-01	-3,1E-01	1,484E-02	5,458E-03	1,047E-02	-0,0033	-5,888E-02
2,200	12,30	1,0E+00	-6,6E-01	2,614E-02	2,680E-02	1,970E-02	-0,0129	-2,330E-01
2,575	19,41	1,6E+00	-5,9E-01	4,212E-02	6,815E-02	3,319E-02	-0,0197	-3,541E-01
2,950	20,15	1,7E+00	-6,2E-02	6,360E-02	1,068E-01	5,176E-02	-0,0032	-5,750E-02
3,325	14,47	1,2E+00	4,7E-01	9,140E-02	1,102E-01	7,624E-02	0,0361	6,504E-01
3,700	13,13	1,1E+00	1,1E-01	1,263E-01	1,383E-01	1,075E-01	0,0119	2,150E-01
4,075	10,04	8,4E-01	2,6E-01	1,693E-01	1,416E-01	1,462E-01	0,0377	6,788E-01
4,450	7,54	6,3E-01	2,1E-01	2,210E-01	1,388E-01	1,934E-01	0,0404	7,266E-01
4,825	3,62	3,0E-01	3,3E-01	2,824E-01	8,513E-02	2,499E-01	0,0816	1,469E+00
5,200	4,27	3,6E-01	-5,5E-02	3,543E-01	1,261E-01	3,164E-01	-0,0173	-3,106E-01
5,575	4,00	3,3E-01	2,3E-02	4,376E-01	1,458E-01	3,938E-01	0,0090	1,615E-01
5,950	2,17	1,8E-01	1,5E-01	5,330E-01	9,621E-02	4,829E-01	0,0737	1,327E+00
6,325	1,30	1,1E-01	7,2E-02	6,414E-01	6,937E-02	5,847E-01	0,0423	7,615E-01
6,700	1,19	9,9E-02	9,2E-03	7,638E-01	7,555E-02	6,999E-01	0,0065	1,162E-01
7,075	0,49	4,0E-02	5,8E-02	9,008E-01	3,648E-02	8,294E-01	0,0485	8,723E-01
7,450	0,35	2,9E-02	1,1E-02	1,053E+00	3,073E-02	9,741E-01	0,0110	1,986E-01
7,825	0,10	8,6E-03	2,1E-02	1,222E+00	1,047E-02	1,135E+00	0,0234	4,209E-01
			8,6E-03			5,882E-06	0,0000	9,068E-07
				Sum(biom.) =	1,412E+00		Sum(p)=	6,583E+00
			P =	13,167	mg m^{-2} year^{-1}	P/B = 9,32		

accentuated decrease was observed, followed again by a slow increase. Female protein contents exhibited a permanent variation throughout the year, with the lowest and highest values being observed in May, respectively in the beginning and in the end of the month. A small decrease was also observed in November. Males never showed a great variation in protein contents, except for a clear decrease observed in December and a smaller one observed in June.

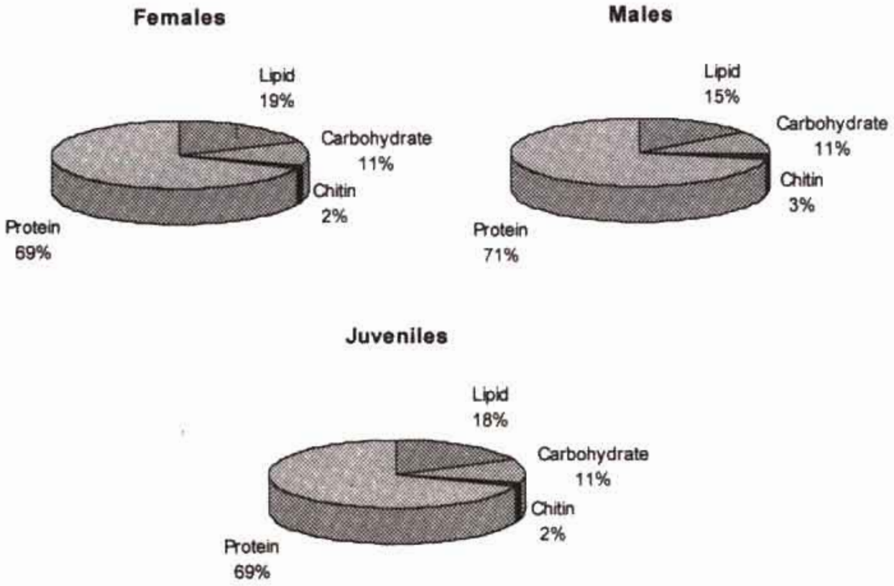

Fig. 3. Average annual composition of biochemical components (expressed as a % of tissue dry weight) in *Mesopodopsis slabberi* from the Mondego estuary.

Table 2. Seasonal variation in protein contents in *Mesopodopsis slabberi* from the Mondego estuary.

Protein (% Dry Weight)

	Juveniles		Males		Females	
	Average	Standard Deviation	Average	Standard Deviation	Average	Standard Deviation
October	74,8	7,9	70,0	5,2	74,6	7,6
November	64,3	2,4	73,8	6,6	61,7	3,0
December	67,7	4,9	58,1	1,9	70,3	5,1
March	69,7	2,9	74,0	2,5	67,6	2,9
May (9)	72,0	3,1	74,4	4,5	83,8	3,9
May (24)	58,2	4,7	75,2	3,7	61,8	2,7
June	61,0	3,3	70,8	3,1	69,4	6,5
July	70,3	1,7	78,7	1,5	72,0	2,1

Carbohydrate – Contrary to what we observed for proteins, juveniles consistently presented higher carbohydrate contents than females or males, which showed a similar variation (Table 3). Actually, carbohydrate contents varied between 6.24 and 16.12 %, for juveniles, between 4.86 and 28.99 % for females, and between 5.2 and 30.89 % for males. Juveniles showed the lowest value in November and the maximum by the end of May. With regard to males, we also observed the lowest values during winter, with a minimum in November, and the highest ones during spring, with a maximum in October and the beginning of May.

Chitin - The variation of chitin contents was basically similar in juveniles, females, and males, the average varying between 0.48 and 7 % (Table 4). The highest values were observed in December and the lowest ones during spring.

Table 3. Seasonal variation in carbohydrate contents in *Mesopodopsis slabberi* from the Mondego estuary.

Carbohydrate (% Dry Weight)

	Juveniles		**Males**		**Females**	
	Average	Standard Deviation	Average	Standard Deviation	Average	Standard Deviation
October	10,32	1,11	30,89	39,18	28,99	31,47
November	6,24	2,16	5,20	0,66	12,35	4,14
December	11,94	1,64	8,39	1,24	6,02	0,55
March	11,81	2,80	6,79	1,75	7,24	2,69
May (9)	11,04	4,17	9,54	2,42	11,06	2,21
May (24)	16,12	3,40	8,73	1,06	9,40	1,60
June	10,93	1,76	8,57	2,56	4,86	1,53
July	8,64	2,35	8,64	1,42	9,27	1,78

Table 4. Seasonal variation in chitin contents in *Mesopodopsis slabberi* from the Mondego estuary.

Chitin (% Dry Weight)

	Juveniles		**Males**		**Females**	
	Average	Standard Deviation	Average	Standard Deviation	Average	Standard Deviation
November			2,25	0,52	3,66	1,04
December	4,06	0,96	7,00	2,58	5,44	2,72
March	3,14	1,67	3,64	1,83	2,11	0,55
May	0,98	0,34	0,91	0,23	0,81	0,21
June	0,75	0,15	0,48	0,16	0,66	0,2
July	0,61	0,19	0,71	0,23	0,66	0,24

Lipids – The variation in lipid contents was significantly different between juveniles and females, on one side, and males on the other side. In fact, lipid contents varied from 7 to 42 % in juveniles, between 10 and 43 % in females, and from 8 to 15 % in males (Table 5). Juveniles showed the highest values in November, then a clear decrease, with low values being kept during the winter and early spring, followed by a new increase in June. Female lipid contents exhibited a peak in March. As for males, there was almost no variation through the year, although slightly higher values could be found during the winter.

Table 5. Seasonal variation in total lipid contents in *Mesopodopsis slabberi* from the Mondego estuary.

Lipid (% Dry Weight)

	Juveniles	**Males**	**Females**
November	42	15	25
December	10	11	19
March	12	10	43
May (9)	11	8	14
May (24)	7	9	10
June	22	8	11
July	15	10	10

Phospholipids – There was little variation in phospholipids contents through the year in all population groups. Phospholipids contents ranged between 1.1 and 2.12 % in juveniles, from 1.12 to 2.17 % in females, and between 1,1 and 1,63 % in males (Table 6). Juveniles exhibited higher values in November and in June, while the lowest ones were recorded in May. Female phospholipids contents showed slightly higher values in November and again in March and July. Finally, in males, there is no conspicuous variation through the year.

Table 6. Seasonal variation in phopholipids contents in *Mesopodopsis slabberi* from the Mondego estuary.

Phopholipids (% Dry Weight)

	Juveniles		**Males**		**Females**	
	Average	Standard Deviation	Average	Standard Deviation	Average	Standard Deviation
November	2,12	0,55	1,63	0,32	2,17	0,29
December	1,75	0,08	1,10	0,15	1,30	0,08
March	1,54	0,42	1,19	0,14	1,59	0,11
May (9)	1,52	0,05	1,18	0,09	1,18	0,04
May (24)	1,10	0,35	1,24	0,08	1,24	0,05
June	1,82	0,08	1,51	0,12	1,12	0,12
July	1,43	0,05	1,57	0,13	1,48	0,25

Cholesterol – Yearly variation was clearly more evident in juveniles than in the adults. In fact, cholesterol contents ranged between 0.39 and 1.68 % in juveniles, while in females and males it ranged respectively between 0.41 and 0.61 % and from 0.57 to 0.73 % (Table 7). Therefore, while adults presented basically always the same cholesterol contents, juveniles exhibited a strong variation through the year, with the highest values in December, early May, and June, and the lowest ones in March, end of May, and July.

Table 7. Seasonal variation in cholesterol contents in *Mesopodopsis slabberi* from the Mondego estuary.

	Cholesterol (% Dry Weight)					
	Juveniles		**Males**		**Females**	
	Average	Standard Deviation	Average	Standard Deviation	Average	Standard Deviation
November	0,75	0,03	0,65	0,18	0,56	0,04
December	1,03	0,05	0,59	0,08	0,55	0,06
March	0,73	0,03	0,57	0,06	0,61	0,13
May (9)	1.68	0,15	0,59	0,02	0,58	0,07
May (24)	0,39	0,03	0,62	0,06	0,41	0,06
June	1,09	0,28	0,69	0,10	0,55	0,07
July	0,73	0,04	0,73	0,11	0,60	0,05

The largest variations occurred for carbohydrate and lipid contents, while protein contents were the most unchanging ones. The estimated CV values were as follows: in juveniles, protein 5.7 %; carbohydrates 23.4 %; phospholipids 16 % and cholesterol 20.8 %; in females, protein 6 %; carbohydrates 23.7 %; phospholipids 11.3 % and cholesterol 18.1 %, and, in males, protein 5 %; carbohydrates 19.6 %; phospholipids 8.2 % and cholesterol 18.7 %;

The protein caloric contents make the largest contribution to the total energy, remaining fairly uniform throughout the year (Table 8). The caloric content of the lipid fraction exceeded the values calculated for carbohydrates, suggesting that lipids are the major energy storage reserve (Table 8). The carbohydrate caloric energy makes a modest contribution to the total available caloric energy, remaining low throughout the year (Table 8).

Table 8. Seasonal changes in energy content in *Mesopodopsis slabberi* from the Mondego estuary.

	Energy content (cal/mg Dry Weight)								
	Proteins			**Carbo hydrates**			**Lipids**		
	Females	Males	Juveniles	Females	Males	Juveniles	Females	Males	Juveniles
October	3.133	2.940	3.142	1.218	1.298	0.433			
November	2.591	3.100	2.701	0.517	0.218	0.260	2.375	1.406	4.000
December	2.953	2.440	2.843	0.252	0.353	0500	1.843	1.026	0.912
March	2.839	3.108	2.927	0.302	0.286	0.496	4.123	0.969	1.102
May (9)	3.520	3.125	3.024	0.466	0.399	0.462	1.292	0.779	1.064
May (24)	2.596	3.158	2.444	0.395	0.305	0.676	0.950	0.865	0.637
June	2.915	2.974	2.562	0.193	0.445	0.601	1.017	0.741	2.109
July	3.024	3.305	2.953	0.361	0.479	0.424	0.931	0.960	1.397

The total caloric content (Table 9) was higher in females as compared with males in November, December, March and May (9). The total caloric content was higher in females as compared with juveniles in December, March, May (9), and May(24). The total caloric content was higher in males as compared with juveniles in May (24). In females the total caloric contents increased during June to July (summer), decreasing after March until May (24) (spring). In males the total caloric contents remain fairly uniform throughout the year except for a decrease in December (winter). In juveniles the total caloric contents after a decrease in May (24) increased notoriously in June (summer).

Table 9. Seasonal changes in total energy content in *Mesopodopsis slabberi* from the Mondego estuary.

Energy content (cal/mg Dry Weight)

	Females	Males	Juveniles
October			
November	5.483	4.724	6.961
December	5.048	3.819	4.255
March	7.265	4.363	4.525
May (9)	5.278	4.303	4.550
May (24)	3.940	4.388	3.757
June	4.125	4.160	5.272
July	4.316	4.744	4.773

The most pronounced differences in the total caloric contents between females and males were found in March, between females and juveniles were found also in March, and between males and juveniles were found in June (Table 9). The most pronounced differences in the total caloric contents between months were found in females between March and May (9) (Table 9).

ANOVA results are summarised in Tables 10 and 11. The factors tested were sex (considering juveniles, females, and males) and months (with 8 levels coincident with sampling dates: October, November, December, March, May - 09, May - 24, June and July). We performed an Analysis of Variance (ANOVA) to test differences between sexes and between months for all the components considered. An ANOVA Two Factor or factorial analysis of variance was carried out in first place to test for interaction among factors. Since interaction was found the means of levels shouldn't be compared. It was therefore necessary to perform a One-Way ANOVA for each factor, in order to reveal significant differences among the levels of a factor. Then we performed multiple comparison procedures following two methods: (1) the Tukey test and (2), the Newman-Keuls test.

Table 10. Analysis of variance: the variables under consideration are proteins, carbohydrates, chitin, phospholipids and cholesterol proportions (expressed as of total dry weight), in juveniles, females, and males of *Mesopodopsis slabberi* over a thirteen months period. The factors tested were sex (considering juveniles, females, and males) and month (with 8 levels coincident with sampling dates*: October, November, December, March, May - 09, May - 24, June and July).

ANOVA: Two-Factor with replication for testing interaction between factors: Sex groups X Months groups					ANOVA: Single Factor for testing interaction between all groups for each biochemical component					
Biochemical Component	*df*	*F calc*	*P-value*	*F crit*	Source of Variation	*df*	*MS*	*F calc*	*P-Value*	*F crity*
Proteins	14	5.5085	4.4655E-08	1.7750	Between Groups	23	0.0340	7.6636	1.391E-14	1.6197
					Within Groups	120	4.439E-3			
Carbohydrates	14	4.3935	2.7809E-06	1.7750	Between Groups	23	0.0235	4.1492	1.411E-07	1.6197
					Within Groups	120	5.675E-3			
Chitin	10	8.0821	3.5119E-09	1.9376	Between Groups	16	0.0242	7.8510	4.974E-11	1.7639
					Within Groups	85	3.088E-3			
Phospholipids	12	2.9989	1.2150E-03	1.8455	Between Groups	20	0.0019	4.0721	1.115E-06	1.6714
					Within Groups	105	4.712E-4			
Cholesterol	12	21.3605	1.1673E-20	1.8693	Between Groups	20	0.0010	34.2887	4.318E-32	1.6968
					Within Groups	84	3.001E-3			

* Specimens were collected only in these sampling dates. F calc = calculated F-value; F crit = critical F-value ($p \leq 0.05$).

Table 11. Multiple comparison of biochemical components average values: proteins, carbohydrates, chitin, phospholids, and cholesterol proportions (expressed as % of total dry weight) in juveniles, females, and males of *Mesopodopsis slabberi*, over a thirteen months period.

		Proteins		Carbohydrates		Chitin		Phospholipids		Cholesterol	
		P1	P2	P1	P2	P1	P2	P1	P2	P1	P2
October	Females	*	*	*	*						
	Males	*	*	**	**						
	Juveniles	*	*	**	**						
November	Females	**	**	*	**	*	*	*	*	***	***
	Males	*	*	**	***	*	**	*	*	***	***
	Juveniles	**	**	**	***			*	*	***	***
December	Females	*	*	**	***	*	*	*	*	****	****
	Males	**	***	**	**	*	*	*	*	***	***
	Juveniles	*	*	**	**	*	*	*	*	**	**
March	Females	**	**	**	**	*	*	*	*	***	***
	Males	*	*	**	**	*	*	*	*	****	****
	Juveniles	**	**	**	**	*	*	*	*	**	**
May (09)	Females	*	*	**	**	**	**	*	*	***	***
	Males	*	*	**	**	**	**	**	**	***	***
	Juveniles	*	*	**	**	**	**	*	*	*	*
May (24)	Females	**	***	**	**	**	**	*	*	*****	*****
	Males	*	*	**	**	**	**	*	*	***	***
	Juveniles	**	***	*	**	**	**	**	**	****	*****
June	Females	**	**	**	***	**	**	**	**	***	***
	Males	*	**	**	**	**	**	*	*	***	***
	Juveniles	**	**	*	**	**	**	*	*	**	**
July	Females	*	*	**	**	**	**	*	*	***	***
	Males	*	*	**	**	**	**	*	*	***	***
	Juveniles	*	**	**	**	**	**	*	*	***	***

Tests employed were: P - Tukey test and P' - Newman-Keuls test

Discussion

Body size/weight relationships were consistent with values previously reported for other mysid species (Ladurantaye and Lacroix 1980, Allen 1984, Sorbe 1984, San Vicente and Sorbe 1993, 1995, Chigbu and Sibley 1996).

The *Mesopodopsis slabberi* population has continuous reproduction in the Mondego estuary (Azeiteiro et al. 1999) like other mysids in other systems, namely, *Siriella armata* Milne Edwards, 1837, *Anchialina agilis* (G.O. Sars 1877), *Leptomysis gracilis* (G.O Sars 1864), *L. lingvura*, *Paramysis arenosa* (G. O. Sars 1877), *P. bacescoi* Labat. 1953, *P. nouveli* Labat, 1953, *Schistomysis kervillei*, *S. ornata*, *S. spiritus*, *Praunus flexuosus* (Muller 1776), *P. inermis* (Rathke 1843), *Neomysis integer* (Leach 1814) (Nouvel and Nouvel 1939, Labat 1957, Mauchline 1965, 1969, 1971 a, b, c, Parker and West 1979, Sorbe

1980) The *Mesopodopsis slabberi* population exhibited clear spatial and temporal (tidal and seasonal) migration patterns (Azeiteiro et al. 1999). This type of migration has been described in other estuaries (Greenwood et al. 1989, Webb and Wooldridge 1990) for *M. slabberi* and other mysid species (Ladurantaye and Lacroix 1980, Allen 1984). Marine populations of *M. slabberi* may undergo onshore/offshore migration (Mauchline 1980, Webb and Wooldridge 1990, Azeiteiro et al. 1999). Moreover, Collins and Williams (1982) considered *M. slabberi* to belong to a more estuarine-marine community in summer (April-August) and to a euryhaline-marine community in winter (January). Such seasonal onshore/offshore migrations may have underlying salinity-related reproductive significance (Greenwood et al. 1989). Although reproduction and recruitment were continuous throughout the year, main peaks were observed in late summer/autumn and late spring/early summer (Azeiteiro et al. 1999). A smaller peak was also recorded in early winter (Azeiteiro et al. 1999). Such a recruitment pattern suggests the occurrence of two (bivoltinism) or three (trivoltinism) generations per year (Azeiteiro et al. 1999). Spring females and males die after the late spring/early summer recruitment period (Azeiteiro et al. 1999). In fact, large mysids disappeared from the population in June as their progeny, the first summer generation, matured (Azeiteiro et al. 1999). A similar pattern was reported for *Mysidopsis bigelowi* (W.M. Tattersall) in a temperate estuary (Allen 1984), and for *Anchialina agilis* (G.O. Sars) in temperate neritic waters (Sorbe 1984). This common biological model among littoral and neritic mysids between 25° and 50° of latitude is also refered to *Schistomysis kervillei*, *S. spiritus* and *Neomysis integer* in the temperate West European Atlantic coasts (Mauchline 1967, 1971 a, Bremer and Vijverg 1982, San Vicente and Sorbe 1990). Other observations (Sorbe 1984, 1991) confirm that the mysid voltinism is directly influenced by temperature (Pezzack and Corey 1979). The yearly cycle, similarly to other estuarine/neritic temperate species, appears to cope with seasonal changes in each particular environment (Mauchline 1980, Sorbe 1984, Johnston and Northcote 1989). Temperature, salinity, oxygen and chlorophyll a biomass are determining factors affecting *M. slabberi* population dynamics and production in the Mondego estuary (Azeiteiro et al. 1999). Marques et al. (1994) claimed that the prevailing conditions in the Mondego estuary, namely eutrophication, should result in the development of opportunistic adaptive strategies among invertebrate species. This might be related to the fact that the production of *M. slabberi* was found to be relatively low as compared to other species in other systems but the P/B ratio is higher.

The P/B of the available measurements for zooplankton present a modal turnover rate about 10-20 times a year (Valiela 1995). The few available P to B rates of meiofauna indicates a turn over about 10 times per year, considerably larger than those for the larger macrofauna (modal macrobenthic turnover rate is one to two times a year) (Valiela 1995) (Table 12 provides a comparison between our results and data on the annual P/B ratios of different mysids). The high specific production –what we referred as the P/B- of *M. slabberi* population makes them important secondary producers. The faster turnover of smaller organisms means that although the biomass of small-sized species may be smaller than that of larger species, the P/B of smaller species makes them proportionately more important producers than larger species

Table 12. Annual P/B ratios for other mysid species

	Reference	annual P/B ratios
Neomysis americana	Richards and Riley 1963	3.66
Neomysis americana	Richards et al. 1967	3.66
Gastrosaccus spinifer	Arntz 1971	2.00
Mysis relicta	Hakala 1978	3.0-3.8
Mysis relicta	Sell 1982	2.2-3.3
Neomysis integer	Bremer and Vijverberg 1982	4.00
Rhopalophthalmus terranatalis	Wooldridge 1983	8.66
Mesopodopsis wooldridgei	Wooldridge 1983	8.00
Rhopalophthalmus terranatalis	Wooldridge 1986	7.85
Anchialina agilis	Sorbe 1984	4.29
Schistomysis ornata	Sorbe 1984, 1991	6.09
Schistomysis kervillei	San Vicente et al. 1990	9.38
Schistomysis parkeri	San Vicente and Sorbe 1993	9.73
Schistomysis spiritus	San Vicente and Sorbe 1995	6.77

(Vernberg and Cull 1974, Valiela 1995). *M. slabberi* production and turnover rate justify the hypothesis that this species plays a relevant role in the energy flow in the food web (Azeiteiro et al. 1999).

The marine invertebrates show a seasonal variation on biochemical composition. The protein, lipid and carbohydrate accumulation cycles show their maximums in spring (Ansell 1974, 1980, Newell and Bayne 1980, Lethonen 1996) appearing to be related with the nutritive disponibility, breeding cycle and temperature variations (Clarke 1977). Carbohydrates, lipids and proteins exhibited maximums in late autumn (November) and spring. The species appeared to accumulate lipids in the beginning of the phytoplankton maximum (February) (Azeiteiro 1999), females showed the lipid accumulation peak in March, and the juveniles in November, after a massive recruitment period occurring in late summer/autumn and spring/early summer (Azeiteiro et al. 1999), and the third phytoplankton minimum (September and October) (Azeiteiro 1999). Females appear to restore their body mass after the main recruitment periods when phospholipids peaks. Sex influence can be realised because a certain disinchrony in reaching spring maximums becomes evident (Azeiteiro et al. 1999).

Our results were basically consistent with information provided by literature on the biochemical composition of Crustaceans (Raymond et al. 1964, 1968, Bamstedt 1975, 1976, 1978, Omori and Ikeda 1984, Lethonen 1996), namely regarding other mysid species like *Boreomysis arctica* (Kroyer) and *Neomysis integer* (Leach). Although there are not many studies on mysids to compare our results, these were nevertheless consistent with data obtained for a number of benthic and suprabenthic peracarid species (Raymond et al. 1964, Bamstedt 1975, 1976, 1978, Johnson and Hopkins 1978, Omori and Ikeda 1984, Lethonen 1996) and for the Louisiana red swamp crayfish (Fernandes et al. 1994, Mendonça 1995).

Proteins were the main body component all over the year, and also the least changeable one. In other groups (Moss and Lawrence 1972, Ortega et al. 1984) the slightly fluctuations in the protein content were related to the hydric condition, what may suggest that protein variation reflects seasonal fluctuations in the hydration level of the tissues (Ortega et al. 1984). The minimum values obtained in November, for juveniles and females, and in December, for males, months of adverse environmental conditions, may suggest that lost in weight during winter months (Azeiteiro et al. 1999) may be sustained also by proteins. On the contrary, carbohydrates and lipids, namely the lipids, suffered apparent seasonal variations. The carbohydrates showed a great temporal variability which indicates their rapid accumulation and also depletion (easy accessible reserve) (Stryer 1988). Although a great accumulation occurred in autumn (October), it decreases towards the winter (starvation period), namely the females. Juveniles showed the lowest value in November and the maximum by the end of May. With regard to adults, we also observed the lowest values during winter, with a minimum in November in the males and December in females, and the highest ones during spring, with a maximum in May which follows the higher chlorophyll a concentration months (Azeiteiro 1999). The variation of chitin contents were basically similar in juveniles, females, and males. The highest values were observed in December and the lowest ones during spring, which might not be an absolute variation in chitin but a changing relation between the area and volume of the individuals as they pass from a winter starvation period to a spring one well nutrished (Azeiteiro et al. 1999). Although we haven't those results we believe they would confirm the statement above because of protein variation results we achieved. Protein as the main body component is the most important component for weight variations, and has its lowest values in winter months. Lipid variation is a function of metabolism and reproductive strategy, depending therefore on the species yearly cycle (Ruizverdugo et al. 1997). In fact, many life history traits of aquatic invertebrates depend on investments in depot lipids (Lehtonen 1996, Ohman 1997). We could observe seasonal variations in cholesterol and phospholipids. Phospholipids showed a continuous raise from December to July, in males, June to July, in females, and May to June, in juveniles, which may indicate their structural role. Yearly variation in cholesterol was clearly more evident in juveniles than in the adults. Therefore, while adults presented basically always the same cholesterol contents, except for a small decrease in June in females, juveniles exhibited a strong variation through the year, with the highest values in November, December, March, and June, that are also peak recruitment months (Azeiteiro et al. 1999) which may indicate their role as a precursor of growth hormones. Seasonal changes in lipids, namely triglycerides, and carbohydrates, i.e., the major cellular components under which energy is stored (Stryer 1988), appear to be mainly a function of the nutritional cycle (since they peak after favourable environmental trophic conditions) (Azeiteiro 1999).

Changes in phospholipids and cholesterol appear to be directly related to the reproductive cycle. Actually, as structural components of cell membranes, phospholipids forcibly change depending on cells proliferation and degeneration (Cullis and Hope 1991). On the other hand, cholesterol plays a double role as a structural component of cell membranes (Bloch 1991) and as a precursor of sexual hormones involved in the reproductive control of crustaceans (Sastry 1983, Goddard 1988).

During early spring, *M. slabberi* females in the Mondego estuary stores energy. This accumulated energy is utilised in preparation for reproduction peaks, after that they recover. The results achieved indicate also that *M. slabberi* does not accumulate energetic reserves for use during winter. The storage of energy in *M. slabberi* females during March (early spring) may be primarily a function the synthesis of reproductive products. Actually, adult females requiring large amounts of energy for egg production store considerably during March (late winter to early spring) as compared with relatively low storage in adult males with no break in spring months.

In *M. slabberi*, like in other crustaceans, biochemical changes apparently resulted from metabolic purposes in relation with the nutritional cycle and/or synthesis of reproductive products (Chaisemartin 1979, Sastry 1983, Kabre 1983, Kabre and Chaisemartin 1987, Zekhnini et al. 1991). Therefore, both environmental and trophic conditions appear to play an important role in determining seasonal changes in biochemical composition and then the biology of the mysid species in the estuary.

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Isabel Domingos [1]

GLASS EEL MIGRATION AND FISHERIES IN THE MONDEGO ESTUARY – FUTURE PERSPECTIVES

Abstract

The fishery for glass eels (*Anguilla anguilla* L.) in the Mondego estuary started in the 1950's and its importance has increased since then, mainly due to a constant demand and the attractive price it has reached in the market. In 1990 the price was around 60 € per kilo and in 1999, during Christmas, it reached 300 €. The migration occurs all year round, the most intense period is from October to March - April, depending on the weather conditions, and the official fishing season, although variable, usually extends from November to February. This paper summarises the results of a study on glass eel migration, along the Mondego estuary, between 1988 and 1990, presents official data from captures (DGPA) and discusses the future of this fishery in Mondego, Portugal and in Europe. The probable causes for the decline in recruitment are analysed, and possible solutions are presented. The implications of the results with respect to the commercial fishery, population abundance in the watershed, and the contribution of Mondego eels to the European stock and recruitment are also discussed.

Introduction

Eel recruitment, eel stocks and eel fisheries have all declined since the 1970's and the future of the eel fisheries of Europe is an important matter that has seriously concerned fishery managers and scientists (Moriarty 1997). In fact, this concern expressed by so many people, led to the establishment of the working group EC Concerted Action AIR A94-1939, to pursue the project "*Enhancement of the European eel fishery and conservation of the species*" funded by the EU (Moriarty and Dekker 1997). The species Anguilla anguilla is classified as commercially threatened (CT) in Portugal (SNPRCN 1991).

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In Figueira da Foz the glass eel fishery started around 1950, when some representatives of a Spanish company visited the place with the intention of developing a system to capture glass eels and sell them to Spain. In the 1960's the glass eels were still very abundant and their fishery involved hundreds of fishermen, but in the 1970's, there was a decline in recruitment. It was then that the hand net was replaced by a type of fyke net, the portuguese botirão (Jorge and Sobral 1989).

The botirão (total length varying from 45 to 80 m) is a wing net consisting of two wings with a variable length (15 - 22.5 m) and height (2 - 4 m), attached to a conical net (length from 15 to 20 m) with a cod end in which the glass eels are trapped. The mouth of the net can measure 8 m. Although this net provides higher catches for fishermen, the considerable bycatch of postlarvae and juvenile of crustacean and other fish (unpublished data) does inevitably have an impact on the other fisheries both in the estuary and in the adjacent coastal area. The bycatch of this net can reach extremely high biomasses as happened in December 1987 (7.5 kg) and March 1988 (12.5 kg) in the Mondego estuary (Jorge and Sobral 1989).

Nowadays, the fishery for glass eels in the Mondego estuary is an activity that includes professional fishermen as well as many poachers using those wing nets which are illegal. Apart from the use of illegal nets, they also fish out of the official fishing season which, despite being variable, usually extends from November to February. All their catches are sold to brokers who afterwards transport them to Spain, the only European country where there is substantial human consumption of glass eels, considered a delicacy which can reach extremely high prices at the restaurants. Nevertheless, the business does not end in the restaurants. Most of the glass eels are exported to third parties, in other countries, for aquaculture purposes.

Glass eel migration

A study on the fluctuation of glass eel migration and its relation with some environmental parameters (lunar phase, salinity, temperature, rainfall and river flow) was done in the lower part of the Mondego estuary between 1988 and 1990 (Domingos 1991, 1992). Although glass eels are present in the Mondego estuary all year round (Fig. 1), according to these studies, there is a decrease in the amount captured out of the official fishing season, especially during summer months.

During winter, normally the most intense period of migration, temperature ranged from 9.7°C to 17.7°C at the surface and from 10.1°C to 18.3°C near the bottom. However, in July 1988, when there was an unexpected important yield, temperature ranged from 13.8°C to 20.2°C (Domingos 1991). Thus, the migratory activity does not seem to be related with water temperature. This conclusion is supported by Naismith and Knights (1986), and Tongiorgi et al. (1986), who state that the thermal preference of A. *anguilla* glass eel changes in relation to different environmental conditions.

The use of the tide to progress upwards is confirmed by the amount of glass eels captured according to the phase of the moon. The greatest catches were registered during new moon and full moon when the tides have wider ranges (unpublished data).

A research on the relation between glass eels' abundance and tidal evolution was performed, in the Mondego estuary, during a spring tide (March 1989), and it was noted that, after the turning point of the tide, the abundance started to increase and was highest when the water column was vertically homogeneous (Domingos 1991). These results are supported by McCleave and Kleckner (1982) who believe that, in order to ensure rapid landward transport, glass eels adjust their behaviour according to hydrographic conditions, selecting the flood tide to leave their shelter at the bottom.

As Gascuel (1987) and Domingos (1991, 1992) concluded, it seems that the river flow is the most important factor ruling the glass eel migration. The more intense period of migration occurs during winter months which is coincident with the rainy season. However, it was also noticed that heavy rain during the summer months, which is not very common, can also promote a more intense migration, as happened on the 13th July 1988 (Fig. 1), supporting the idea of the importance of river flow in this process.

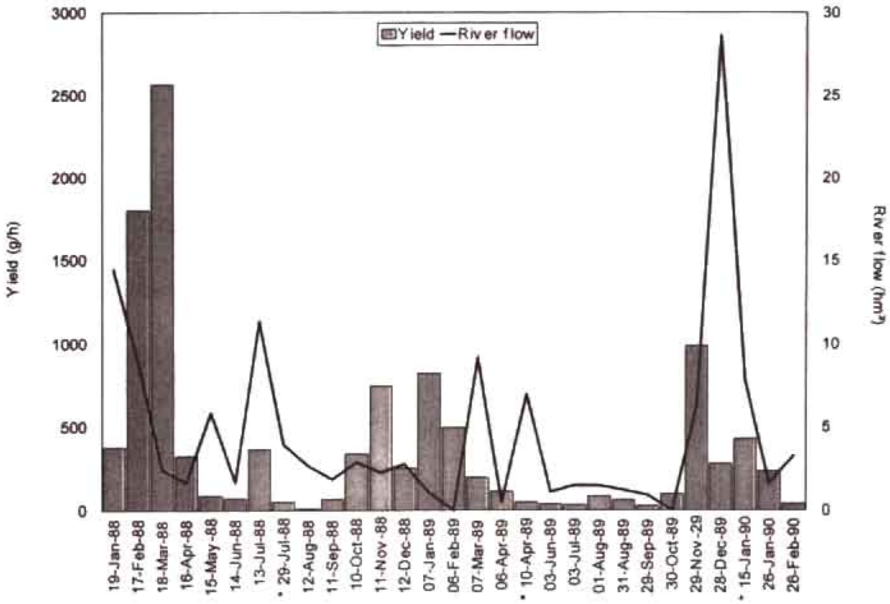

Figure 1. Relation between yield (g/h) resulting from passive fishing at new moon, and freshwater flow (hm3) from Raiva. (*) Fishing not performed at new moon.

The relation between river flow and glass eel migration has been indirectly confirmed by many authors (Deelder 1958, Creutzberg 1961, Crnjar et al. 1992, Tosi and Sola 1993, Sola 1995) who stated that glass eels and elver stages are, in general, attracted towards the freshwater odours.

Heavy rainfall and river flow can, however, have a negative effect on the success of migration, as happened in December 1989 and January 1990, suggesting that the river flow was too strong to enable glass eels to proceed with their migration using the flood tide (Domingos 1992).

Commercial fishery

The first document regulating the glass eel fishery, in national rivers, started to be applied since the 1st December 1985, until a law was published on the 17th of July (Decreto Regulamentar n° 43/87, 17th July). This legislation establishes the rules for this activity restricting it exclusively to inland waters within the maritime jurisdiction area, by means of a hand net either along the bankside or by boat, and obliges fishermen to report their catches. Another regulation (Portaria n° 564/90, 19th July) adds that the permission to fish for glass eels will only be granted to professional fishermen.

The official fishing period, as well as the maximum number of licences to be issued, are established each year by specific regulation, and the fishermen are obliged to return monthly reports on catches.

Official data on catches before 1985 are unknown because the previous legislation did not oblige fishermen to apply for fishing licences or report their catches. Table 1 contains data from those reports between 85/86 - 90/91 and 96/97 - 99/00 periods. It must be noted that until 1990 the number of established licences was rather large. More recently, an effort has been made to restrict the fishery by reducing the number of licences and, despite some extension of the fishing period in 97/98 and 98/99 (Tab. 1), in the last fishing season more strict measures were undertaken resulting in a reduction to only three months of fishing and 34 issued licences.

Table 1. Catch statistics for glass eels, licences and fishing period for each fishing season in Figueira da Foz.

Fishing season	Fishing period	Established licences	Issued licences	Total catches (kg)
	1 Dec - 28 Feb	(?)	94	383.7
1986/1987	1 Nov - 28 Feb	350	179	225.1
1987/1988	1 Nov - 28 Feb	350	29	345.5
1988/1989	1 Nov - 28 Feb	350	151	337.7
1989/1990	1 Nov - 28 Feb	350	260	761.8
1990/1991	1 Nov - 28 Feb	350	67	128.0
1991-1996	(?)	(?)	(?)	(?)
1996/1997	1 Nov - 28 Feb	150	(?)	(?)
1997/1998	1 Nov - 31 Mar	150	72	262.8
1998/1999	15 Nov - 15 Mar	80	52	264.0
1999/2000	15 Dec - 15 Mar	35	35	385.5

Source (DGPA)
(?) Data not available

Figure 2 illustrates the official catches. Despite being clear that total catch has sharply decreased in 1990/1991, it is obvious that there is a fluctuation which does not show any clear trend in glass eel abundance. The variation in the number of fishermen as well as differences in fishing effort, which are unknown, make the analysis more difficult. Nevertheless the catch per fisherman has been higher during the last two seasons than during the period from 88/89 to 90/91, but it looks as if it is almost independent from the total catch.

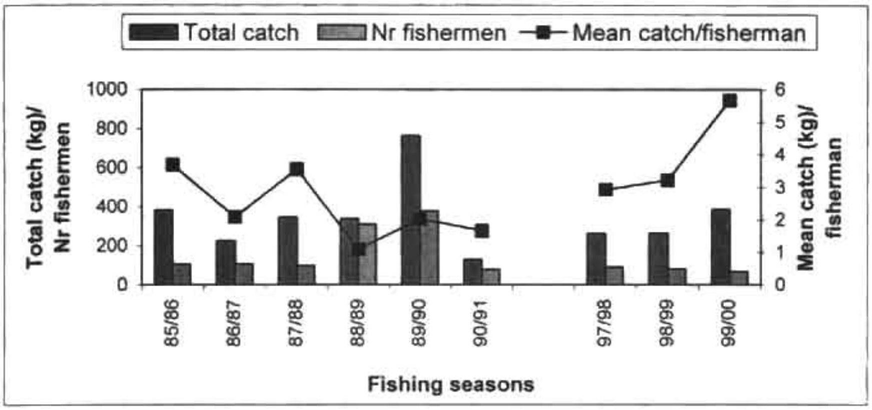

Figure 2. Catch statistics for glass eels, number of fishermen and mean catch per fisherman for the official fishing seasons from 85/86 to 90/91 and 97//98 to 99/00 periods in Figueira da Foz.

Large discrepancies occur between real catches and the amounts reported by the fishermen. As a matter of fact, I had the opportunity to be a witness of that situation, since I was informed by the buyers that, in February 1989, between the 19th and the 24th, 418 kg of glass eels were caught, while the official data, for the whole month, was 171 kg. This way it is clear that the statistics based on official data lead to false conclusions as catches are obviously underestimated.

Although neither table 1 nor fig. 2 suggest a decrease in catch per fisherman, maybe due to a bigger effort in fishing activity, the fishermen state that it is indeed decreasing which is supported by Moriarty (1992) and Castonguay et al. (1994) who state that American eel as well as European eel recruitment have declined dramatically since the 1980's.

Factors affecting the fishery

The fisheries have suffered severe losses from overfishing, mostly with illegal nets, physical modifications of the river, pollution, and parasites.

Overfishing

An effort to control the fishery has been done not only by introducing changes in the legislation, but also through the confiscation of illegal nets. This task has been executed, either by the maritime police, who confiscated 281 "botirões" between 1998 and 9th March 2000 (Harbour Master Office from Figueira da Foz), or by the forest authorities (DGF), who confiscated 83 "botirões" between 1998 and 1999 (Eng. António Grácio, pers. comm.).

According to the same source (DGF), the confiscation of nets has mostly occurred between Ponte de Lares and Quinta do Canal, but some have also been confiscated between Ereira bridge and Quinta do Canal (Fig. 3). The location of the nets in these areas indicate that the tidal influence is noticed far beyond the limit of the maritime jurisdiction area since these nets are set across the river facing the incoming tide. According to the information given by the maritime police from Figueira da Foz, the nets are mostly found near the Cinco Irmãos, very close to the freshwater jurisdiction area.

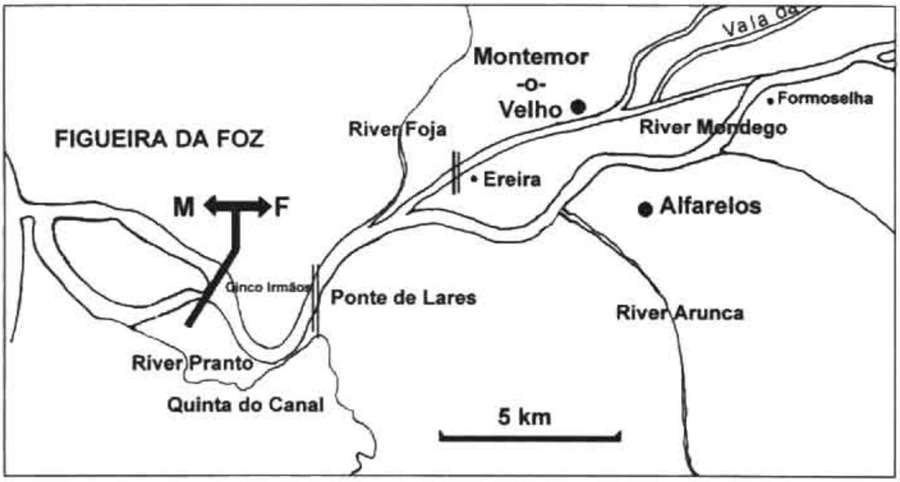

Figure 3. Limits of the maritime and freshwater jurisdiction areas and location of places where the wing nets (botirões) were apprehended. The line close to Cinco Irmãos is the limit of those areas. The arrows indicate the freshwater jurisdiction area (F) and the maritime jurisdiction area (M). The lines in the middle of the river are the bridges of Lares and Ereira.

Overfishing glass eels does most probably contribute to a low recruitment in the watershed, with inevitable implications in the subadult population (yellow eels and silver eels). The capture of yellow and silver eels is regulated in both freshwater and brackish water with definition of minimum size of capture (22 cm), and regulated fishing gear. Longline, fishing rod, "sertela" and "enguieira" are allowed in the Mondego freshwater area (Portarias n° 643/96, November 8th and n° 164/99, March 10th), and longline and fishing rod are the permitted gear for fishing eels in the maritime jurisdiction area (Portaria n° 564/90, July 19th). Apart from this, there is no closed season, and data from those captures are underestimated, or mostly unknown. An evaluation of the stock based on catch statistics is therefore almost impossible.

Water demands and dams

During the last 20 years several human actions have severely changed the River Mondego watershed. The river bed regulation and embanking to prevent the floods, the channelisation of the river and finally hydroelectric schemes have contributed to completely change the river morphology and hydrology. The natural flow regime was also affected by the regulation of the water flow.

The population of *A. anguilla* has suffered severe losses due to anthropogenic factors. Water demands for agriculture, industry, human consumption and hydroelectric power are detrimental for fisheries if not properly managed. Studies to define ecological flows are extremely important in this watershed.

Dams, such as Aguieira and Raiva, have become major obstacles to upstream and downstream migration, and severe regulation of water levels, mainly by these power stations, has almost dried certain parts of the river and decreased the flow needed to attract the glass eels to the estuary. There are no fish passages in these dams and although eels are capable of finding their way up through humid soil during rainy periods, obstacles with a height of 80 metres, as in Aguieira dam, are inevitably discouraging, even for animals with a strong rheotropic behaviour. Upstream these areas fishermen claim that eels have become rare shortly after the dams were built.

Apart from hydroelectric power plants there is another relatively high obstacle downstream from that area, the Açude-Ponte dam, at Coimbra, which has a fish passage that is ineffective. Although some eels manage to pass this obstacle with a height of 4 m, the free colonising area is restricted to the last 35 km of the river.

Size is an important factor in the eels' ability to climb weirs since the climbing of smooth vertical surfaces is restricted to elvers and very small juveniles (Naismith and Knights 1993). As the Açude-Ponte dam is still relatively close to the river mouth, it is possible to find small eels which weight enables them to climb and overcome the obstacle. The probabilities of this happening in dams such as Raiva and Aguieira, further upriver, is much lower.

Another impact of dams on the species is related to the downstream migration of silver eels. Not only they constitute important obstacles, but the passage through turbines of hydropower stations usually provokes a considerable mortality in this migrant population, a problem well known in European rivers (Berg 1986, Hadderingh et al. 1992). Furthermore, loss of available habitat for growth, has reduced the number of females since the distribution of males and females differs from estuary to freshwater with more females in the upper reaches, an observation that is supported by Costa et al. (1993) and Naismith and Knights (1993). The number of females reaching the Sargasso sea is vital for the success of reproduction.

Pollution

The plan for developing agriculture in the Lower Mondego has contributed to poor water quality with eutrophication problems in certain areas. Modern agriculture with its emphasis on the use of chemical fertilisers, herbicides and pesticides which, in the case of rice production are distributed from the air, has lead to an increase in water pollution. This is considered one of the most polluted areas in Portugal (INAG 1995). Industry and domestic sewage can also be a problem in certain points. Textile industry, located in some areas upstream, is another problematic source of pollution (INAG 1995).

Although eels are one of the species least affected by eutrophication and pollution (Dill 1990), human consumption of these animals may become dangerous in terms of public health. In the Thames River commercial fishing effort in the estuary was extremely reduced following the discovery of high levels of pesticide residues in the eels (Naismith and Knights 1993).

Parasites

The nematode *Anguillicola crassus*, a swimbladder parasite originally hosted by *Anguilla japonica*, was brought into Europe in the early 1980's. Although apparently harmless to the native host it appears to be pathogenic in the European eel only (Székely 1996). It causes an impairment of the swimbladder function, which may hamper the success of the spawning migration, as it increases the energy expenditure necessary for the eel to remain at a certain water depth (Würtz et al. 1996). Eels highly infected with this parasite loose their appetite and vitality and become emaciated (Egusa 1979). Although this study was done on cultured eels, it would be important to verify whether the same happens in natural conditions. If that is the case, a new mortality factor may have to be taken into consideration.

This parasite has been recorded in the eels from Mondego basin captured from 1988 to 1990 mostly inside the swimbladder, but they were also found in the mesenteric fat inside the abdominal cavity (unpublished data).

Management proposals

Moriarty and Dekker (1997), in a Concerted Action funded by The EU, have considered three management options for fishery managers: i) Control of fisheries including restrictions on fishing areas, periods or methods, to prevent or reduce exploitation; ii) Stocking strategies, which are vital in catchments with obstacles and in isolated waters suitable for eel, and increase in escapement of silver eels, especially large females in order to enhance the reproductive stock; iii) Use of passes to enhance recruitment into freshwater catchments.

In the River Mondego several threats to the stock have been previously identified. A solution to enhance the stock should include: i) the construction of efficient eel passage devices, which are not necessarily expensive, in all dams up to Aguieira (including this one), allowing the habitat available for the species to increase and return to its original size before the construction of dams; ii) the construction of parallel channels running for some kilometres upstream the dams to prevent migrant silver eels from being sliced in turbines; iii) to increase the river flow, at least during peak migration season, so that glass eels can continue to be attracted to the Mondego estuary; iv) to Improve water quality, especially in the lower Mondego, which hopefully will be achieved with the implementation of the Council Directive for Water Quality and the Waterbasin Management Plan; v) to control and extinguish the black market for glass eels, which promotes illegal fisheries and overfishing contributing to the decline of recruitment; vi) to promote monitoring programmes for recruitment and downstream migration of silver eels, aiming at the evaluation of the stock; vii) to determine the carrying capacity of the watershed in order to develop a model to predict yield, and be able to advise fishery managers on the most efficient management of the eel fishery aiming at a sustainable exploitation of this resource; viii) to promote toxicity and bioaccumulation studies to check whether there is no problem for public health as happened in the Thames, and finally ix) to analyse the level of contamination by *A. crassus*.

All these proposals can be applied, with the necessary adjustments, to most of our watersheds. However, management measures for each watershed or country, despite being a valuable management action in helping to support the European stock, will not solve the problem if there is no international involvement and cooperation.

Eel fisheries have, for many decades, been managed on a local or national basis as if they were represented by independent local stocks. Recently, the question on whether management at a national level can sustain the stock throughout the distribution area has arised because the European eel is a shared marine resource since it reproduces in a common breeding area in the open ocean, and should be treated as such (Moriarty and Dekker 1997).

The EU Concerted Action is of vital importance since the European stock is under severe stress especially with the increasing demands of glass eels for aquaculture from East Asian countries, mainly China, which is competing with Japan in the production of eels. Either severe restrictions in European policy towards glass eels exports are established and exclude countries outside Europe, or the European eel will follow the same steps as *A. japonica* glass eels which recruitment has definitely declined since the 1980's (Gousset 1992), maybe because of overfishing for aquaculture purposes. Another way to solve the question would be to include the species in the CITES Convention. This way, exportation of glass eels to Asia would have to end, and the management of the stock would become less difficult.

In a near future, the European Concerted Action should be enlarged to include countries outside Europe, namely the United States of America, as there is also a decline in recruitment of the American eel *(A. rostrata)*, a species which has not been overexploited. This fact points out that climatic changes may be responsible for some of the decline in recruitment due to their implications on oceanic currents. According to Moriarty and Dekker (1997), further knowledge on the silver eel migration, reproduction, and leptocephalus biology could clarify the oceanic causes for the decline in recruitment.

Finally, a strong financial support to the development of studies on artificial reproduction, larval development and growth could be, in the long run, the solution for both aquaculture demands and conservation of the species in nature. Maybe this way there is hope for the leptocephali to continue travelling along with the oceanic currents.

"The eel as a species may not be threatened, but many of the fisheries have an uncertain future until a European management plan is put into operation. At this stage we leave the contribution that may be done by the scientists and hand the matter over to the economists and politicians." (Moriarty 1997).

Acknowledgements

Thanks are due to my colleagues Dr. C. Assis, Dr. J.L. Costa and Doutor P.R. Almeida who gave valuable comments and criticism to the draft of the manuscript.

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EFFECTS OF *Eucalyptus globulus* PLANTATIONS IN LOW ORDER STREAMS OF CENTRAL PORTUGAL

Abstract

Low order streams derive most of their energy from the riparian vegetation. Therefore, changes in forest cover have the potential to affect stream biota. Herein we assess the effects of *Eucalyptus globulus* afforestation to low order streams by comparing (a) the structure of macroinvertebrate communities between impacted (eucalyptus plantations) and unimpacted (deciduous forest) streams; (b) decomposition rates of eucalyptus leaves and some native species; (c) fungal colonisation and (d) sporulation of decomposing leaves. During autumn/winter, streams flowing through eucalyptus forests contained a lower number of invertebrates and taxa than streams running through deciduous forests. The low number of invertebrate taxa and individuals might be a consequence of hydrology and/or low quality food resource of leaves. Decomposition of leaves and fungal sporulation followed a same pattern: leaves of alder decomposed at a fast rate, followed by chestnut, eucalyptus and oak. The colonisation of eucalyptus leaves by aquatic hyphomycetes was highly limited by the cuticle (and facilitated by stomata). It progressed predominantly in and from the eucalyptus leaf mesophyll to the outside. Eucalyptus oils and, to a lesser extend, polyphenols, were strong deterrents to fungal growth. A possible incorporation of eucalyptus litter into secondary production in a reasonable time span is suggested. We hypothesise that aquatic hyphomycetes play a dominant role in the eucalyptus breakdown process overlapping shredder low consumption or low density.

Introduction

The exotic species *Eucalyptus globulus* (Labill.) was introduced in 1829 in the Iberian Peninsula. Nowadays, this species is common and widespread in Portugal where monocultures occupy over 21% of the forested area. The evergreen eucalyptus was mainly planted for the paper mill industry replacing the original mixed deciduous forest

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and pine plantations. Given the large evidence that low order streams derive most of their energetic requirements from senescent leaves originated in the riparian zone and that leaves of trees differ in their quality, changes in the riparian forest have the potential to affect the streams. Alterations of forest cover or litter input were already documented to affect the aquatic biota (Molles 1982, Stout et al. 1993, Wallace et al. 1999).

Attempts to document the effects of eucalyptus afforestation on the energetics of Portuguese low order streams are few and recent (e.g., Abelho and Graça 1996, Canhoto and Graça 1999). However, changes in the structure and functioning of these ecosystems were, nevertheless, expected.

In order to determine the ecological effects of eucalyptus plantations on Central Portuguese streams we compared the macroinvertebrate community structure of low order streams flowing through three forest types – deciduous, eucalyptus and mixed (eucalyptus with a deciduous riparian vegetation). Moreover, because the physico-chemical characteristics of leaves affect decomposition through their influence on fungal colonisation and invertebrate consumption, we also analysed fungal richness and sporulation rates of fungi colonising decomposing leaves. Finally, we examine mechanisms of fungal invasion of leaves and tested the potential effect of polyphenolics and eucalyptus oils to inhibit fungal growth.

This Chapter includes information already published in Hydrobiology (1996. 324: 195-204, 1996. 333: 79-85) and Microbial Ecology (1999. 37: 163-172).

Materials and methods

Macroinvertebrate community structure

The first question to address was if the invertebrate community differed between perturbed and unperturbed streams. We selected streams running through the forest types classified according to the surrounding vegetation into "deciduous" (dominated by *Castanea sativa* and to a lower extent *Quercus* spp.), "eucalyptus" (in *E. globulus* forests) and "mixed" (flowing through eucalyptus forest but bordered by indigenous deciduous trees: *Salix* spp., *Quercus* spp., and others). We collected samples of macroinvertebrates in three deciduous (D1,D2,D3), three eucalyptus (E1, E2, E3) and four mixed forest (M1, M2, M3, M4) streams. The deciduous forest streams were located at Mata da Margaraça and Fraga da Pena. They were compared with streams at Serra do Caramulo. Some of which run to the Mondego basin whereas other run to the Vouga basin. Six sample replicates were collected from each site with a hand net (0.3 × 0.3m; 0.5mm mesh size, covering an area of 0.3 × 1m). The samples were transported to laboratory to be sorted. Animals were kept in 70% alcohol and identified to the lowest possible taxonomic level. For each sample, the number of individuals and taxa were calculated. Comparisons among three stream types in terms of number of taxa and number of individuals were made by ANOVA.

Decomposition rates

Leaves of alder *(Alnus glutinosa)*, chestnut *(Castanea sativa)*, oak *(Quercus faginea)* and eucalyptus *(E. globulus)* were collected during senescence from individual trees, and stored dry until needed. They were dried (50°C; 48h), weighed and assembled in groups of 3 g in nylon bags (0.5 mm mesh size). A total of 160 packs tied to 10 ropes (4 replicates of each species per rope) were placed in Ribeira do Sobral Cid, Coimbra. At days 0, 3, 7, 14, 21, 28, 42, 56, 70 and 84, one rope was randomly retrieved from the stream. The leaves from the bags were then washed, incubated for sporulation (see below), and dried at 50 °C for 48h. Weight loss was expressed as a percentage of initial weight (n = 3). Decomposition rates were estimated using the exponential decay model $W_t = W_0 e^{-kt}$, where W_t is the remaining weight at time t, W_0 is the initial weight and k is the decay coefficient (Petersen and Cummins 1974). Regression lines (ln transformed data) were compared by Covariance Analysis followed by a Tukey test (Zar 1984).

Before drying the leaves, leaf fragments were individually incubated in petri dishes flooded with sterile water for 2 days (15°C). This time was enough to get abundant detached conidia. Identification of fungal conidia was made under an inverted microscope.

Fungal colonisation: invasion processes and growth limitations

In a second decomposition experiment, changes in leaves nitrogen content (Kjeldahl method; Wilde et al. 1972) and total phenolics (Folin Denis assay; Martin and Martin 1982) were analysed. Eucalyptus leaf fragments were also prepared for observations at light and electron microscopy. To observe cuticle changes and fungal invasive processes small eucalyptus leaf sections were fixed in 2.5% glutaraldehyde, post-fixed in 1% buffered osmium tetroxide and dehydrated in graded ethanol series (20-100%). SEM observations were made on small leaf sections critical-point dried with carbon dioxide as transition fluid, coated with gold and mounted on aluminium stubs. For optical microscopy, the samples were dehydrated and embedded in Spurr's resin (Spurr 1969). Semithin sections were than stained with 0.2% toluidine blue before observation.

To test the effects of eucalyptus secondary compounds on fungal growth, increasing concentrations (0-7.5%) of tannic acid or eucalyptus essential oils were added to a malt extract agar medium. Laboratory cultures of aquatic hyphomycetes involved in leaf decomposition were used to inoculate the agar plates (at least 3 replicates per treatment). We used the following species: *Articulospora tetracladia*, *Heliscus lugdunensis*, *Lemonniera aquatica*, *Lunulospora curvula*, *Tricladium angulatum* and *Tricladium gracile*. Fungal growth was allowed for 15 days and expressed as the increase in the colony size (squared mean diameter – cm^2). Results were treated using one-way ANOVA followed by a Student-Newman-Keuls test. The EC50 values were obtained using a Probit analysis (Finney 1971).

Results

In terms of quantity of invertebrates, the number of individuals was significantly higher in the deciduous forest streams than in the eucalyptus or mixed forest streams ($H = 5.94$, $P < 0.05$). In terms of taxa, higher richness was observed in the deciduous forest streams (Fig. 1) but the difference was just above the significative limit ($H = 5.66$, $P > 0.05$). The proportion of individuals per functional group was similar in all streams with predominance of collectors and shredders.

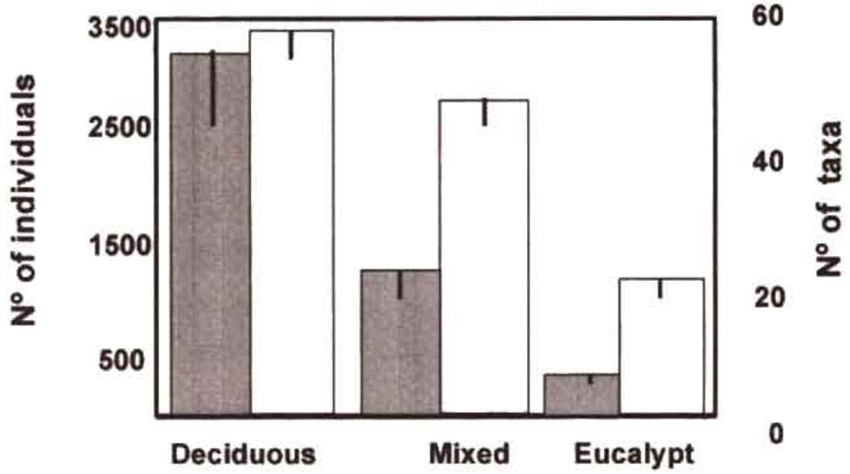

Fig. 1. Number of individuals (dark bars) and number of taxa (light bars) of invertebrates (mean ± SE) collected in the streams flowing through deciduous, mixed and eucalyptus forests (after Abelho and Graça 1996)

Leaves of the four tested species differed in their breakdown rates (ANCOVA; $P < 0.05$). Processing was faster on *A. glutinosa* ($K - 0.016$ day^{-1}) followed by *C. sativa* ($K - 0.008$ day^{-1}), *E. globulus* ($K - 0.007$ day^{-1}) and, finally, *Q. faginea* ($K - 0.004$ day^{-1}) (Fig. 2a).

Initial nitrogen (N), expressed as a % of leaf dry weight, ranged from 0.78% (chestnut) to 2.8% (alder). Oak and eucalyptus presented intermediate values (1.3% and 1.5%, respectively). Following immersion of leaves in the streams, N contents increased with conditioning time in all leaf species to a maximum at 21 days in alder and over 60 days for the other species. Higher initial polyphenolic percentages were observed in leaves of oak (14.1%) followed by chestnut (9.2%), eucalyptus (6.1%) and alder (5%) (Fig. 2b). Soon after immersion, the leaves polyphenolic contents decreased rapidly and, by day 28, it stabilised in about 2%.

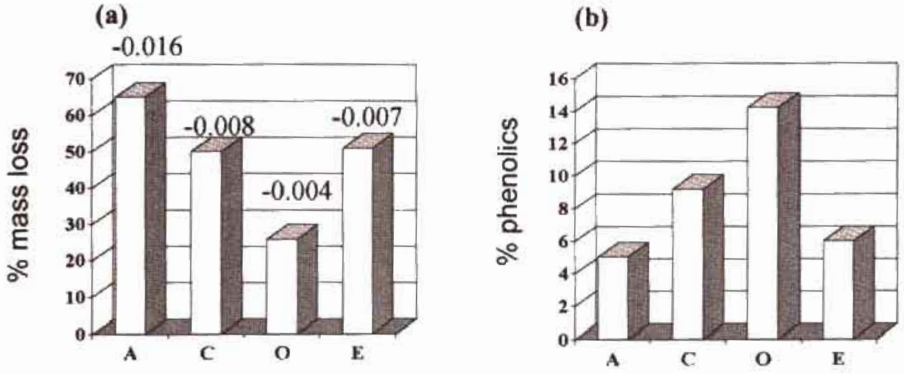

Fig. 2. (a) Percentage of mass loss and k values of leaves of alder (A), chestnut (C), oak (O) and eucalyptus (E) decomposing, over 84 days, in a stream; (b) Initial phenolic content each leaf type (after Canhoto and Graça 1996).

Conidial production of fungi colonising decomposing leaves followed the same pattern as breakdown rates being quicker on the faster processed leaves of *A. glutinosa* and *C. sativa* than in the *E. globulus* and *Q. faginea*. However, a clear increase in the number of fungal species occurred with time for all leaf species (Fig. 3).

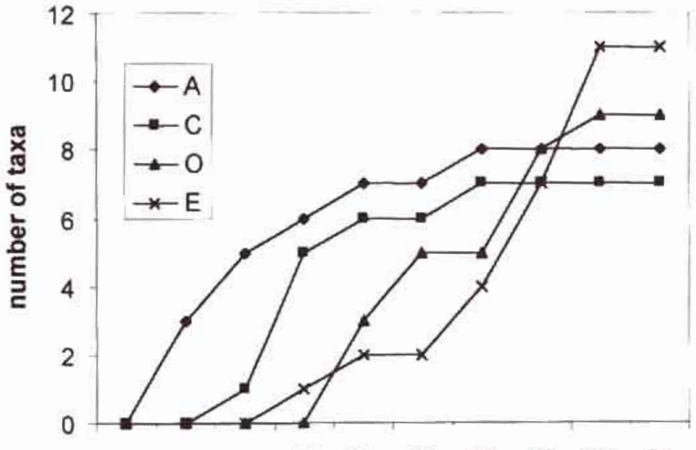

Fig. 3. Cumulative number of taxa of aquatic hyphomycetes in decomposing alder, chestnut, oak and eucalyptus leaves over 84 days. A = alder; C = chestnut; O = oak; E = eucalyptus.

Transverse sections of eucalyptus leaves showed a coherent and resistant waxy cuticle over the epidermis with thinner extensions lining the substomatal cavities. This barrier was practically unaltered through 5 weeks of immersion isolating the leaf parenchyma from the exterior. Mycelial penetration, apparent soon after immersion,

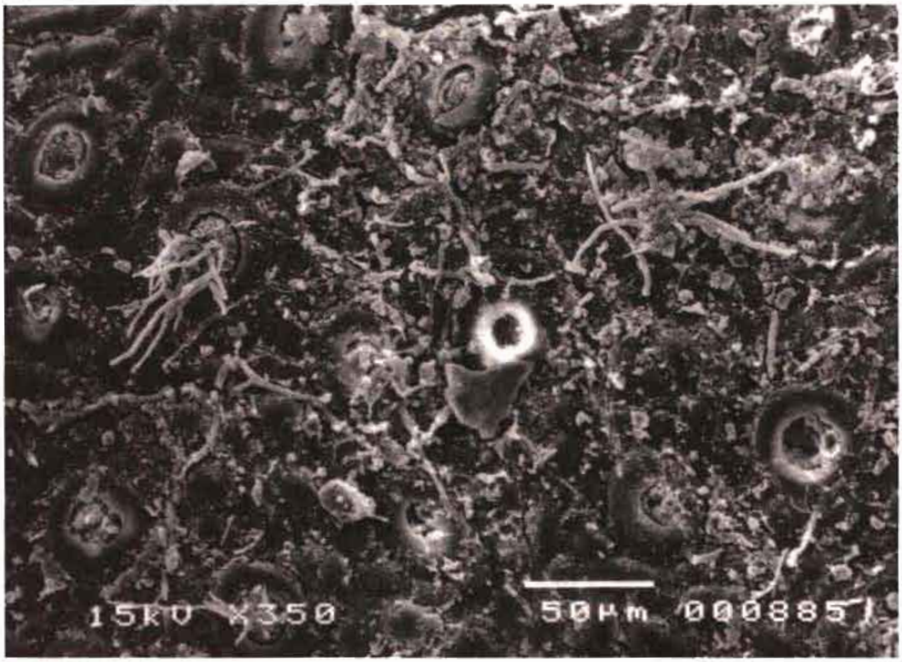

Fig. 4. Scanning electron micrograph of hyphae crossing stomata (×525) of an eucalyptus leaf after 2 weeks immersion in a stream.

occurred mainly through stomata (Fig. 4). The oil vesicles of the eucalyptus leaves largely remain intact over time.

The addition of eucalyptus oils or tannic acid to the solid media completely inhibited or depressed fungal growth (Fig. 5). In fact, *H. lugdunensis*, *L. curvula* and *T. angulatum* growth was significantly decreased by the addition of either oils ($P < 0.001$; $35.96 \leq F \leq 63.14$) or tannic acid ($P < 0.001$; $33.77 \leq F \leq 243.49$) while the other fungal species (*A. tetracladia*, *L. aquatica* and *T. gracile*) did not grow at all.

Higher percentages of tannic acid were always needed to get a significative inhibition of fungal growth (Student-Newman-Keuls test; $P < 0.05$) of fungal growth – 0.75% for *L. curvula*, 0.25% for *T. angulatum* and *H. lugdunensis* (vs. 0.1% oils in all cases). For the same concentration of tannic acid or oils, these species consistently exhibited smaller colonies in the presence of oils. Consistently, smaller amounts of oils were also needed to completely suppress growth of *H. lugdunensis* and *L. curvula*. In fact, low and similar values for EC50 were obtained when oils were present: 0.248 (0.086 – 0.717 95% CL) for *H. lugdunensis*, 0.290 (0.186 – 0.45 95% CL) for *L. curvula* and 0.358 (0.234 – 0.55 95% CL) for *T. angulatum*. *Lunulospora curvula* was the most tolerant species to tannic acid (EC50 = 2.202; 1.56 – 3.104 95% CL) followed by *H. lugdunensis* (EC50 = 1.1914; 0.83 – 4.43 95% CL) and *T. angulatum* (EC50 = 0.528; 0.39 – 0.715 95% CL).

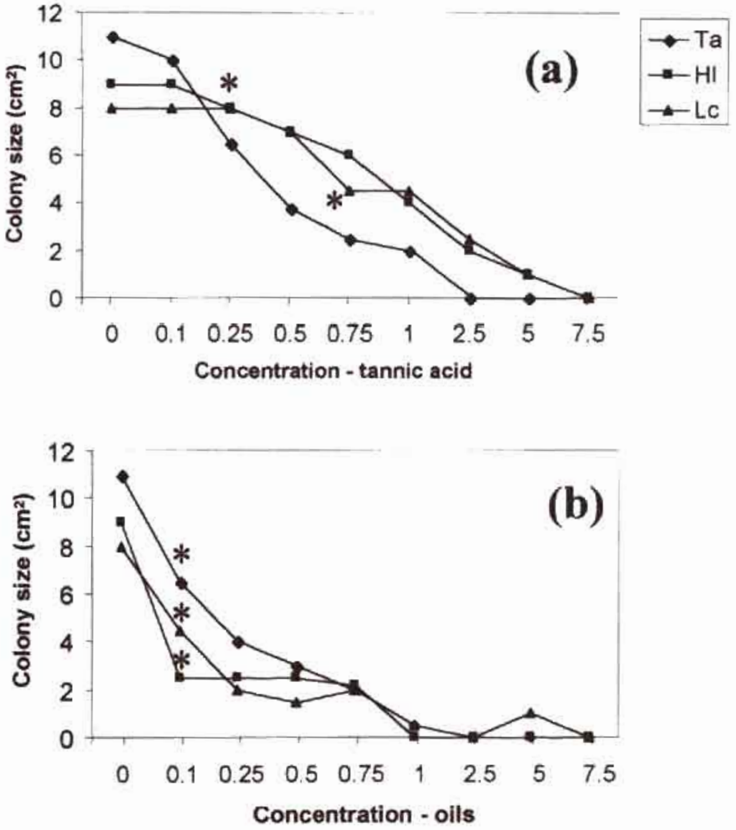

Fig. 5. Growth of 3 species of aquatic hyphomycetes in malt extract agar medium supplied with increasing concentrations of (a) tannic acid and (b) eucalyptus essential oils. Growth was allowed for 15 days. Hl = *Heliscus lugdunensis*; Lc = *Lunulosora curvula*; Ta = *Tricladium angulatum*. The symbol * indicates the lowest concentration of tannic acid or oils that induced a significant ($P < 0.05$) decrease in fungal growth (after Canhoto and Graça 1999).

Discussion

Allochthonous organic matter plays a major role in energy and matter flow in low order streams. Abelho and Graça (1998) showed that perturbations of riparian areas as eucalyptus afforestations induced great changes in the patterns and quality of litter inputs to the streams. Originally dependent on autumn-shed leaf litter as the main energy source, many low order streams of Central Portugal rely now on a similar but continuous supply (with a peak in summer when discharge is minimal) of organic matter of low quality.

Streams running through eucalyptus and deciduous forests differed in macroinvertebrate assemblages. These differences may be related with two main factors: first, a direct result of changes in the seasonality of litter inputs (Abelho and Graça 1996). Life-cycles with larval stages in autumn (when food was abundant) may be at higher risk of being eliminated by the eucalyptus new timing of food supply. Secondly, the leaves themselves may be a low quality resource. Previous papers (Canhoto and Graça 1992, 1995) demonstrated that *Sericostoma vittatum* (Rambur) and *Tipula lateralis* (Meig.), two common shredders in Portuguese streams, were unable to grow and survive in a diet of conditioned eucalyptus leaves. Leaf toughness, high phenolic and oil contents (up to 5% of leaf weight; Costa 1964) have been considered responsible for the low food quality of these leaves (Mellilo et al. 1982, Campbell and Fuchshuber 1995, Canhoto and Graça 1995). A third factor may explain the differences in macroinvertebrate assemblages: changes in the streams hydrological regime. Abelho and Graça (1996) reported higher hydrophobic soils in eucalyptus forests. If this characteristic is a consequence of eucalyptus plantations, than rapid run-off in the rainy season prevents infiltration and therefore less water available in summer with a consequent drying of some small streams.

Eucalyptus leaves have some physico-chemical similarities with the deciduous oak and chestnut. Processed at a medium rate, as chestnut, the colonisation of the exotic leaves by aquatic hyphomycetes seems to be ruled by the particular presence of a resistant waxy cuticle that hardens the leaves and reduces hyphal penetrating capacity. A constrained (stomatal) area of fungal access to the leaf mesophyll is, most probably, the primary cause of the delayed, not decreased, conidia production observed in these leaves. Bärlocher et al. (1995) showed that, given enough time, maximal conidia production in fully conditioned eucalyptus leaves was higher than in alder; furthermore, the cumulative number of species is similar between both leaf species. Nevertheless, distinct patterns of fungal colonisation of eucalyptus from the other leaf species were detected (see Canhoto and Graça 1996) suggesting the possibility of a resource specificity of some fungi (Chamier and Dixon 1982).

The role of phenols and terpenes as defensive compounds against fungi is generally accepted (Bunn 1988, Bennett and Wallsgrove 1994, Bärlocher et al. 1995) and was corroborated by our study. As in marine and terrestrial environments (Steinberg 1988) oils showed a stronger depressing effect on fungal growth than phenols. Protected by the cuticle, the deterrent effects of both leaf defences in eucalyptus leaves seem to last for a long time: a slow initial decrease in polyphenolic leaf contents and an elongated integrity of the oil vesicles in the leaf matrix may inhibit fungal enzymatic activity and limit microbial colonisation (Canhoto and Graça 1999).

In summary, the microbiological breakdown of eucalyptus leaves, highly dominated by aquatic hyphomycetes, seem to proceed predominantly in and from the leaf mesophyll to the outside, primarily because of a resistant cuticle. The predominance of apparently intact but "hollow" leaves, common in eucalyptus streams, suggests that such degradation process may overlap shredders (low) consumption or density (see Canhoto and Graça 1999).

In our experiments, mixed streams had an intermediate number of invertebrates. This is relevant for forest management since it seems to indicate that the presence of an unperturbed riparian corridor may attenuate the effects of eucalyptus plantations. A possible incorporation of eucalyptus litter into secondary production in a reasonable time span was also suggested by our work. However, differences in the timing of supply of eucalyptus detritus, distinct stream hydrology, potential changes in the stream fungal microflora and eucalyptus low quality to some shredders are crucial factors that must be carefully considered.

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EFFECTS OF HABITAT LOSS ON WADERS (AVES, CHARADRII) IN THE MONDEGO ESTUARY: A SYNTHESIS AND FUTURE PERSPECTIVES

Abstract

The destruction of the salines represents one of the major threats to the wader populations in the Mondego estuary (Portugal). Although the actual levels of competition in the intertidal habitats are low, thus allowing for many displaced birds from the salines to settle there, these birds will still lose part of their previous feeding space and time. The ongoing eutrophication process in the estuary will further aggravate this. The conservation of this area, in what the wader populations are concerned, demands an integrated policy of all authorities and individuals involved.

Introduction

Habitat loss is probably the most intensively examined of the factors that are known to potentially affect the survival of estuarine wader populations (Goss-Custard et al. 1996a, b). Most studied cases have focused on the consequences for waders of the direct loss of intertidal low-water feeding areas through land reclamation for industrial, agricultural or water storage purposes (Davidson et al. 1991), and of indirect losses due to the submersion of previously accessible feeding areas following the construction of tidal power and storm-surge barriers or sea-walls, as a consequence of a sea-level raise (Meire et al. 1994, Lambeck et al. 1996). In contrast, there have been few studies dealing with the consequences of the loss of supratidal feeding areas, probably because in north European estuaries these are not heavily used, or critically endangered, although their importance for waders is recognised (Davidson and Evans 1986, Hötker 1994)

The situation may be different in the south European Atlantic estuaries. Supratidal habitats, such as the salines, usually occupy large areas within the estuaries and "rias",

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and seem to be intensively used throughout the tidal cycle, by a lot of species (Rufino et al. 1984, Perez-Hurtado et al. 1991, 1993b). These man-made wetlands are currently more threatened in the southern European estuaries than are the natural intertidal areas, mainly due to their abandonment and/or transformation into fish-farms or rice fields (Rufino and Neves 1992, Neves and Rufino 1995, Perez-Hurtado and Hortas 1993a).

The loss of salines would affect the two broad groups of birds that use them in slightly different ways. One group of waders feed over the low-water period on the intertidal mudflats. If these birds are, however, unable to obtain all they require, they then feed in the salines over high tide when their preferred intertidal flats are no longer available. These birds were called the "intertidal birds". The other group of birds is those which feed in the salines throughout the tidal cycle, both at low and high water, and rarely if ever utilise the mudflats of the intertidal area. These were called the "salines birds" (Múrias 1997).

The removal of the salines would remove all the feeding space presently used by the "salines birds", which would thus lose all the current feeding time, as well. Those birds would probably try to settle in the mudflats (Meire et al. 1994, Lambeck et al. 1996). Their survival in the estuary would then depend on their actual feeding requirements in the salines (i.e. if they are able to get all the food they need within the 8.5 hour limit for feeding at low-water in the mudflats), and of the present level of competition in the intertidal areas (Goss-Custard and West 1997).

The "intertidal birds", on the other hand, would lose the extra feeding time they presently use at high-water, when their main feeding areas in the intertidal mudflats are unavailable. On many occasions, particularly in the more energetically-demanding periods of the year, these birds will not be able to recover this feeding time by extending their foraging at low-water through an increase in the foraging time and/or in the intake rate (Davidson and Evans 1986, Goss-Custard et al. 1996c). Their only chance, if they were to remain on the area would be, therefore, to look for alternative supratidal sites.

The present paper summarises the results of a research study conducted on the problem and potential consequences for waders of habitat loss in the estuary of Mondego, in 1993-95 (Múrias 1997). Three main issues were examined in this study of the potential effects of habitat loss on waders in the Mondego estuary: a) the importance of the salines as feeding habitats; b) the ability of the mudflats to support the displaced birds in case the salines were destroyed, and c) the effects on all this, of the increasing eutrophication process which is actually taking place in the Mondego estuary.

Study Area and Methods

The Mondego is a warm-temperate estuary in a region with a basic Mediterranean climate. The terminal part of the estuary consists of two arms, north and south, that surround an alluvion-formed island, the Morraceira (Figure 1). Due to distinct hydrodynamics in the two arms, the south arm is heavily silted-up, thus providing the most important intertidal areas (134 ha) which, along with the salines of the Morraceira Island, are the most suitable feeding areas for waders in the estuary. There is a north-south gradient of increasingly finer sediments in this arm (Marques et al. 1993). In late spring and summer of some years, the finer sediments are covered with extensive weed mats, which remain in place until the beginning of the winter (Múrias 1997). For the purpose of this study, the intertidal area was subdivided in three naturally delimited sub-areas, of 66.8 ha (upstream section), 30.9 ha (middle section) and 36.1 ha (downstream section).

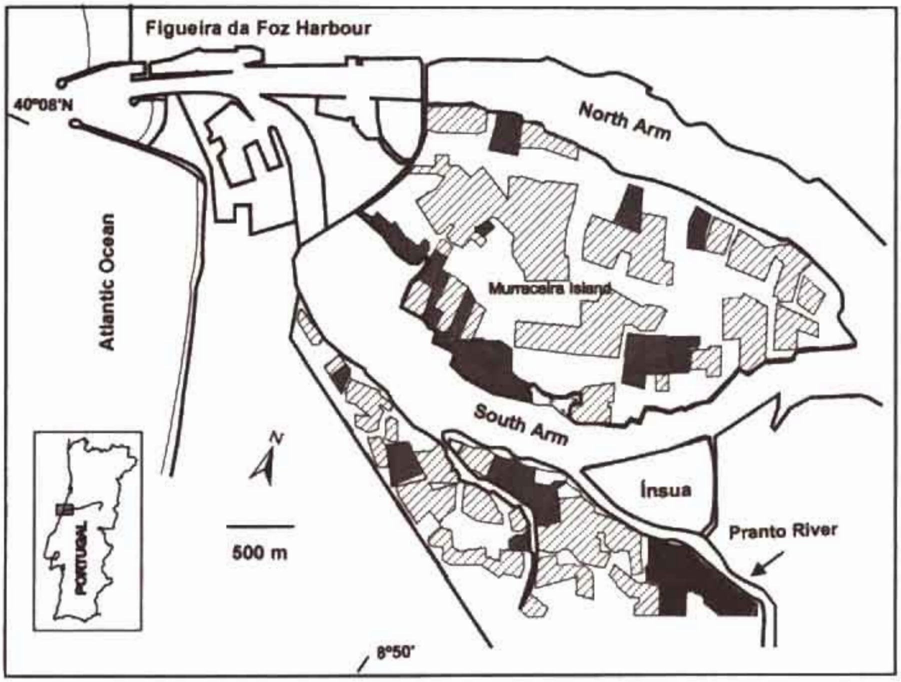

Fig. 1. The estuary of Mondego showing the location of the main area of artisanal salines (stippled), fish-farms (dark-grey) and industrial salines (black). The abanndoned salines of the north arm and the Ínsua are not represented. The main intertidal areas are located along the south arm

The basic method used to estimate bird numbers were high-water and low-water censuses, carried out with 10 × 50 binoculars and a 20-60 × 50 telescope, in the Morraceira's salines and in three fixed plots along the south arm of the estuary. The counts were performed each month, from October 1993 to January 1994 and fortnightly onwards, to May 1995.

Invertebrate samples were taken along fixed transects in the south arm with a corer, 95 cm^2, 5 cm deep, taken to the laboratory and sorted, identified, counted and measured according to the usual procedures (Marques et al. 1993a, Múrias 1997, Múrias et al. 2002).

A more detailed account of the methods used can be found in Múrias et al. (1996) and Múrias (1997).

Results and Discussion

Loss of salines

Overall, the results of this study suggest that, due to time loss, many birds in this estuary could be in trouble were all the salines to be destroyed.

The salines were shown to be intensively used by most wader species at high-water, both as complementary feeding areas to the main feeding places in the intertidal mudflats, but also at low-water, as alternative feeding areas, with a high proportion feeding there at all times (Table 1). However, the number of salines available to the waders in the estuary is rapidly decreasing, due to abandonment and transformation of the ponds into fish farms (Table 2).

Table 1. Percentage of the total number of birds of each species counted in the whole study area at low-water (N) that used the Morraceira's salinas at low and high-water (in parenthesis, percentage of birds that were feeding). Values represent the average number of birds present per count in the estuary over the whole study period (October 1994 to May 1995, maximum n=38 counts).

	N ± SE	(n)	Low-water %		High-water %	
Black-winged Stilt *Himantopus himantopus*	62.4 ± 9.9	(22)	79.7	(68.9)	87.4	(70.0)
Redshank *Tringa totanus*	12.4 ± 3.7	(26)	53.3	(65.0)	69.0	(67.4)
Little Stint *Calidris minuta*	25.3 ± 11.1	(21)	52.9	(59.3)	77.4	(59.3)
Dunlin *Calidris alpina*	451.6 ± 74.4	(37)	20.0	(70.1)	60.0	(70.2)
Kentish Plover *Charadrius alexandrinus*	91.5 ± 10.6	(38)	18.8	(48.3)	59.4	(42.4)
Ringed Plover *Charadrius hiaticula*	66.7 ± 11.3	(34)	12.1	(61.5)	57.7	(50.7)
Whimbrel *Numenius phaeopus*	7.9 ± 3.4	(7)	6.9	(5.0)	25.5	(3.6)
Grey Plover *Pluvialis squatarola*	86.6 ± 10.8	(28)	1.3	(3.8)	14.3	(8.4)
Avocet *Recurvirostra avosetta*	388.6 ± 53.4	(18)	< 0.1	(0.5)	0.1	(3.7)

Note: Only the species with 5 birds per season and per year, or more, were considered

Table 2. The loss of salines by abandonment and transformation into fish-farms in the Morraceira Island up until 1994. Total number of salines in the Morraceira before 1984 was 229, with an area of 305.1 ha. These figures do not include some salines levelled prior to 1984.

	1955 - 1984	1984 - 1994	Total
Number of salines abandoned	26	30	56
Number of salinas transformed	-	22	22
Total number lost	**26**	**52**	**78**
% of total number lost	11.4[1]	25.6[1]	34.0
Rate of loss (salines.year^{-1})[3]	0.9	5.2	1.9
Area abandoned (ha)	35.3	51.0	86.3
Area transformed (ha)	-	19.7	19.7
Total area lost (ha)	**35.3**	**70.7**	**106.0**
% of total area lost	11.6[2]	26.2[2]	34.7
Rate of loss (area.year^{-1})[3]	1.2	7.1	2.5

[1] Calculated in each period by excluding the total number already lost in the preceding period(s).

[2] Calculated in each period by excluding the total area already lost in the preceding period(s).

[3] The number of years is: pre-1984 - 30 years; 1984-94 - 10 years.

Ultimately, this process may force these birds to leave the salines and try to re-establish themselves on the mudflats, if they are still able to accommodate them.

All depends on the present-day level of competition in the mudflats and how close the densities of the birds are, in these areas, to reach the point where their mortality rates, or body condition, become density-dependent. As stressed by Goss-Custard and West (1997), the moment were this point is reached is what really matters to this issue, as it ultimately leads to a reduction in local bird numbers, through intensified competition, well before the carrying capacity is reached, if ever.

At the moment, all data seems to suggest that the effects of competition on the mudflats, either through interference or through resource depletion, are weak (Figure 2). Therefore, a number of the birds that use the salines as alternative feeding areas to the mudflats could eventually re-establish themselves in the intertidal flats at low-water, buffering the loss of low-tide feeding space in the salines. However, even these birds seemed to intensively use the salines for feeding at high-water (Table 1). So, the need for finding supplementary supratidal feeding areas could involve both groups of birds, the mudflat-feeders and the salines-feeders.

The problem arises because no other supratidal habitat in the estuary is likely to offer the good feeding conditions that birds can presently find in the salines. The rice fields are only usable at certain times of the year, at other times being too flooded (in winter) or too dry (in summer). Furthermore, it is unlikely that birds that do not feed on mudflats covered by algae the "bare-sediment" specialists, such as the small plovers (Kentish and Ringed Plovers) would use this habitat at all. Elsewhere, saltmarshes are also commonly used supplementary feeding sites for mudflat feeders, both in Europe

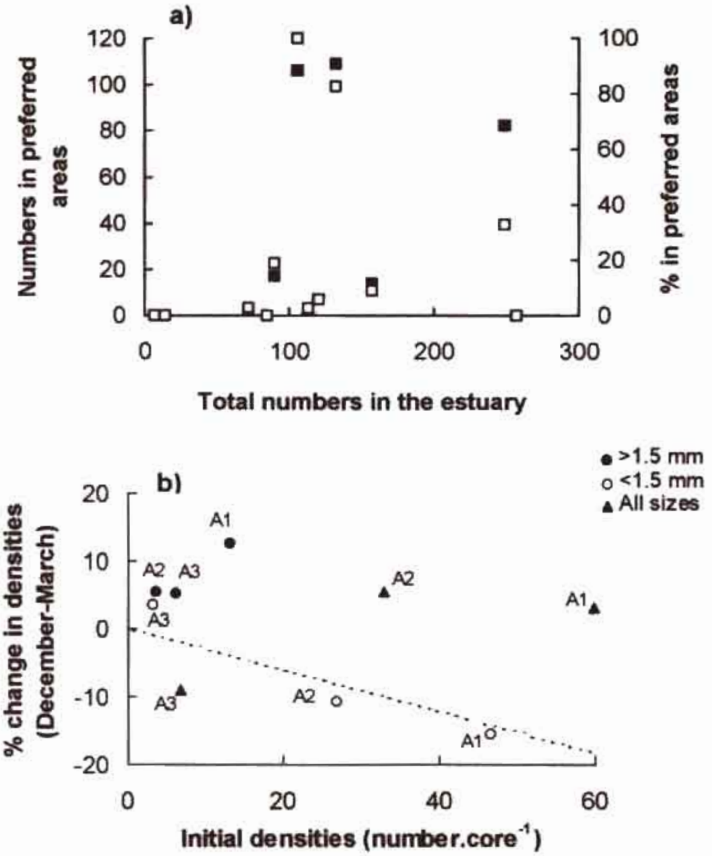

Fig. 2. Graphics showing the absence of competition by (a) interference of feeding birds and (b) resource depletion. In (a) it is illustrated the evolution in bird numbers (■) and in the percentage of these birds in preferred areas (□) for Ringed Plover *Charadrius hiaticula*. The preferred areas are defined as being those areas firstly occupied by birds (Goss-Custard, 1980). In case interference competition was operating, the number of birds in preferred areas should initially increase and then, with the increase in total numbers in the estuary, reach a plateau, indicating that these areas were already full. The percentage of birds in preferred areas, on the contrary, should follow the opposite trends, as more and more birds were driven out from the preferred areas by competition and forced to establish in poorer areas. In (b) is investigated whether the depletion of *Hydrobia ulvae* populations by birds in the estuary during the winter (November-March) was density-dependent (indicated by the dotted line), thus suggesting that the competition for food was high (birds tend to concentrate where the densities of their prey are higher). No such trend was found for the class of prey consumed by the birds (> 1.5 mm), therefore suggesting that no significant depletion occurred during the study period.

and in South Africa (Davidson and Evans 1986, Velasquez et al. 1990, Velasquez and Hockey 1991). However, in the Mondego, they are mainly formed by *Spartina* sp., whose dense stands are known to deter many small species, such as Dunlin (Goss-Custard and Moser 1988). Only the larger species (Grey Plover, Whimbrel, godwits) were occasionally seen using this habitat in the Mondego, but these would probably be the least affected species by the loss of the salines (see Tables 1 and 3).

Table 3. The calculated amount of feeding space (expressed as the percentage of "bird feeding-hours", or BFH - number of feeding birds x number of hours spent feeding) and feeding time lost by several wader species in the estuary of Mondego in case the salines are destroyed, and the predicted increase in the feeding pressure on the mudflats if all birds displaced from the salines try to settle there. Two groups of birds with slightly different strategies were considered: those that used the salines as alternative feeding to the mudflats, throughout the tidal cycle (the "salines birds"), and those that used the salines only at high-water, as supplementary feeding areas to the intertidal mudflats (the "mudflat birds"). Values are means for 1993-94 and 1994-95, averaged for autumn, winter and spring; therefore, N=6 for all species, except for Whimbrel, for which N=2, and Avocet, for which N=5.

	Total feeding (BFH ± 1SE)	"Mudflat"	birds	"Salinas" birds	Predicted increase in BFH in the mudflats (in %)
		Space loss (%)	Space loss[2] (%)	Space loss[3] (%)	
Redshank	199.9 ± 77.2	70.0	25.0	36.9	66.0
Little Stint	334.0 ± 154.3	68.0	-	18.5	90.0
Dunlin	2318.1 ± 1780.7	23.7	37.5	16.7	42.6
Kentish Plover	668.6 ± 71.4	22.1	9.4	16.5	34.8
Ringed Plover	610.6 ± 21.1	9.8	16.3	20.7	26.9
Grey Plover	449.4 ± 62.2	3.5	13.2	-[4]	4.5
Avocet	1256.0 ± 392.2	0.5	-	0.1	1.1
Whimbrel	56.9 ± 6.7	0.0	-	-[4]	0.5
All species	**736.9 ± 272.3**	**24.7**	**20.3**	**15.5**	**33.3**

[1] BFH in the mudflats at low-water + salines at both high and low-water

[2] Calculated as the percentage of feeding time above the 8 hours available for feeding in the mudflats that is currently used by the mudflat birds in the salines at high-water. Maximum feeding time was assumed to be 12.5 hours each tidal cycle, and total feeding time per species were: Redshank - 10 hours; Little Stint - 4.4 hours ; Dunlin - 11.0 hours ; K. Plover - 8.8 hours ; R. Plover - 9.3 hours; G. Plover - 9.1 hours ; Avocet - 5.5 hours; Whimbrel - 7.8 hours

[3] Equivalent to the same amount of time loss, as these birds are assumed to feed in the salinas throughout the tidal cycle

[4] Less than 0.1%

A less suitable but still usable alternative supratidal site to the salines could be provided by the fish-farms. They have the advantage of being encircled by wire fences, thus providing quiet and relatively safe places from attack by raptors. In fact, waders use them now as roosting sites. However, the ponds are usually too deep for waders to feed there even for the long-legged species, and in practice they are used only when they are periodically emptied for cleaning (Perez-Hurtado and Hortas, 1993a, b). In the Mondego, even this periodic usage seems to be infrequent (at least it was never observed during the present study), probably due to the depth and to the narrow dimensions of the ponds.

Blooms of algae

Loss of intertidal habitat could also occur in the estuary, through the increasingly extensive "blooms" of green macroalgae that occur seasonally due to eutrophication (Marques et al. 1993a, b). The presence of contiguous and extensive algae mats would be expected to decrease the abundance of many prey species (see, e.g. Everett 1994), and the subsequent recolonisation after the algal crash, from the small islets of unweeded areas that would remain, would be insufficient to replace the losses (Raffaelli et al. 1989, 1991). Waders could then be unable to find enough food to meet their daily requirements in the previously weeded areas.

In fact, although the effects of the eutrophication at the low levels of the trophic chain can be assessed in a matter of 2-3 years (e.g. Soulsby et al. 1982, Desprez et al. 1992, Everett 1994), its consequences for waders may take more time to be established. Subtle changes in the diet of some waders, when the populations of their main prey are affected, usually appear even before any change in numbers begin to be noted (Desprez et al. 1992). Only in a later stage of the process do waders respond by changing their feeding areas. In the Mondego, no indication was obtained that the waders changed feeding areas due to the presence of algae (see Múrias et al. *Chapter 5.2 of this book*). On the contrary, although circumstantial, there was evidence that some species (e.g Dunlin) could even be attracted to the mats during, or soon after, the algal "bloom", had taken place in early spring.

Apart for some methodological reasons and the time-scale used (Múrias et al. *Chapter 5.2 of this book*), another factor may account for the lack of a relationship between bird numbers and algae in the Mondego. The prey most consumed by waders in this estuary seem to be mobile sediment-water interface feeders, such as some errant polychaetes and *Hydrobia ulvae* (Lopes et al. 1998), which may be favoured, at least in the earlier stages of the season, by the growth of algae (Soulsby et al. 1982, Everett 1994), thus providing enhanced, even though seasonally-limited, food resources for the waders.

Implications for the management of the system

In summary, this study suggested that the main threat to estuarine waders in the Mondego at present is the continuing destruction of the supratidal habitats. It seems that there is some buffering capacity on the mudflats to receive a number of displaced birds from the salinas. However, the lack of sufficient area in the supratidal habitats themselves that would enable birds to recover the feeding time that would be lost with the loss of the salines, could be detrimental to the populations of many species. In the long-term, the effects of the loss of supratidal habitat could be further aggravated if the eutrophication continues to increase at the present rate, thereby perhaps also reducing the available feeding space for waders at low-water, if the growth was severe enough.

From a conservation point of view, there is always the possibility of creating artificial supratidal habitats to replace those that are lost (Davidson and Evans 1986, 1987, Hötker 1994). There are, however, some limitations on the creation of adequate artificial supratidal wetlands. These are (i) the large areas that are required to allow for the settlement of all the displaced birds; (ii) the need to provide similar habitats to those destroyed, particularly in terms of their sediment types and invertebrate faunas, in order to attract the same species that were displaced; and (iii) the need to begin the work some years (2-3) in advance of the destruction of the primary habitat, due to the time required to find an appropriate place, prepare the area and allow the settlement and growth of the invertebrate prey (Davidson and Evans 1987). Even so, there is no absolute assurance that waders will accept the new sites. Hötker (1994) showed that at least two of three artificially-created supratidal habitats in the German and Danish part of the Wadden Sea, that were constructed to compensate for the loss of reclaimed intertidal habitats, did contribute to the increase the number of bird species and densities in the area, but they did not fully compensate the losses due to land claims.

It seems that prevention is still the best way to avoid the more deleterious effects of habitat loss. In the Mondego, there may still be time to reverse the present trend. Many salines have been abandoned, but not yet transformed. Deserted salines are not completely unattractive to waders (Múrias et al. submitted), although they are far less used than the active ones. An effective and relatively inexpensive way of recovering these salines for waders would be to pay their owners to keep them clean and to maintain a permanently controlled water level. This would avoid the salines conversion or drainage. An alternative or, even better, complementary solution would be to impose strict rules on the construction of new fish farms in the estuary, by improving the design of the pond walls, in order to create areas of shallow water, as it was suggested by Rehfisch (1994) for man-made brackish lagoons in England. This could allow even the smaller waders to use the ponds, although some care should be taken to prevent the access of piscivourous birds (e.g. herons).

Regarding eutrophication, any local intervention (e.g. by imposing some form of treatment of the urban, agriculture and fish-farm discharges to the estuary) would not be enough. It would be also necessary to control the urban and agricultural discharges along the whole lower river valley, in which the majority of rice fields and other extensively irrigated lands are located. This is a very difficult task, however, as it requires the involvement of many different official and private organisations.

Besides the obvious need to preserve the estuarine biodiversity and the health of the whole ecosystem, of which waders are an important component, there is another important reason why the quality of the habitat for waders should be maintained or even enhanced in this estuary. Small estuaries like the Mondego, with relatively low number of waders, as compared to the major estuaries of the East Atlantic Flyway (Smit and Piersma 1989), may act as "emergency" sites for some migrating or wintering birds. Emergency sites are areas where, in normal conditions, few birds land, but where, under adverse weather, they may stage in great numbers (Piersma, 1987 *in* Smit and Piersma 1989). This may prevent many birds from starving, avoiding the high mortality rates which otherwise would probably occur. Moreover, the real number of birds of all species that use the estuary of Mondego may have been underestimated, particularly during the migratory periods. As Smit and Piersma (1989) showed for a small Moroccan estuary (Sidi Moussa), the spring migration peak of 7000 birds underestimated by 3 times the real number of individual birds that crossed the area during a two-month period, as investigated by an intensive counting (3-5 days counts) and colour-marking program. If this is also the case in the Mondego, its perceived importance for waders would naturally increase still further.

Acnowledgements

This work was supported by the Portuguese Research Board (JNICT), through grants FMRH/BD/331/93 and PRAXIS XXI/BD/5570/95 (TM). The authors are also indebted to all the colleagues that helped in the fieldwork, and to an anonymous referee for the constructive comments.

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EFFECTS OF EUTROPHICATION ON WADERS (AVES: CHARADRII) IN THE MONDEGO ESTUARY: A MULTI-LEVEL APPROACH

Abstract

The effects of eutrophication on birds are difficult to assess, as the predicted decline in numbers with the spread of algae mats is a long-term process. However, indirect evidences of this phenomenon can be obtained through the study of the predator-prey relationships. In this paper, a multi-scale approach to this problem in the Mondego estuary was adopted, integrating observational and experimental analysis of the relationships between the birds and their invertebrate prey populations in relation to the spreading and abundance of the algae mats. The results suggest that the presence of algae does not influence the distribution of wader species, particularly when algae are abundant, nor their feeding behaviour. This is confirmed by the results of the exclosure experiments, which show that predation by waders on the densities of most prey species is independent of the amount of algae present. The advantages and problems of using the multi-scale approach are discussed.

Introduction

Eutrophication is a widespreading phenomenon in many european estuaries which arises from crescent organic pollution in the estuarine environments (Hickel et al. 1993, Yeates 1993, Dijk et al. 1994). As a response to the nutrient increase, blooms of opportunistic green macroalgae, such as *Enteromorpha* spp. and *Ulva* spp., usually occur, covering large extensions of the intertidal areas in the estuaries (Lillebø et al. 1999a, b). Ultimately, the increase in macroalgae biomass may have profound effects on the underlying invertebrate assemblages through deoxigenation of the sediments, causing the complete substitution of these assemblages and giving origin to a new trophic structure (Azeiteiro and Marques 1999, Desprez et al. 1992, Everett 1994, Hardy et al. 1993, Hodgekin and Hamilton 1993, McComb and Davis 1993, Metzmacher and Reise 1994, Raffaelli 1992, Schories and Reise 1993, Tapp et al. 1993). The energy flow at the secondary and tertiary levels will be affected, which will affect

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the populations from the uppermost trophic level organisms, mostly dominated by wading birds (Grant 1981, Masero et al. 1999, Raffaelli 1992).

The most straigthforward method of measuring the effects of the eutrophication in birds is to document any decline (or increase) in bird numbers over time, as the process goes on (Raffaelli et al. 1989). However, in contrast to the immediate response of the macrobenthic fauna to changes in their environment due to the eutrophication, which can be assessed in the short-term (several months to 2-3 years), the response of the birds is typically seen in the medium or long-term (Raffaelli et al. 1989, Desprez et al. 1992). A long time-series would be needed before any tendencies could be devised.

On the other hand, most species can adapt themselves to feed in the new conditions by slowly changing their feeding habits (e.g., by shifts in the prey type/size classes consumed, or by increasing the exploitation of a preferred prey until its complete depletion), before they are forced to leave the area. The use of the counts alone would thus prevent the detection of any eutrophication effects until they were irreversible, as the process could be occurring long before the numbers made it clear. This is also a major handicap from the estuarine conservation perspective (Raffaelli 1992).

An alternative way of tackling the problem, would be to consider data at different levels (e.g. from global counts to microhabitat use), using both observational and experimental (e.g. Metzmacher and Reise 1994) methods. This paper attempts to explore the use of such approach, by integrating different levels of information, based on studies already published or in course of publication, that took place in the 1993-97 period, within the framework of ongoing research in the estuary of Mondego (west Portugal) ecosystem. Not all the mentioned papers refer to the same period of the year which can prevent a straightforward comparison between them. The present review should thus be seen as a first attempt to explore the viability of the multi-level approach using the data available.

The levels of analysis covered were (1) a whole-estuary study, based on general bird numbers and algae biomass in winter and spring (Múrias et al. 1996), (2) a detailed study of the relationship between bird densities and some behavioural parameters with prey densities and algae densitiy/biomass in marked plots in winter (Cabral et al. 1999), and (3) an experimental analysis of the changes in invertebrate densities according to the presence of algae and predation pressure, using exclosure cages in spring (Lopes et al. 2000).

Study Area and Methods

The study area

The Mondego estuary is warm-temperate in a region with a basic Mediterranean climate. The terminal part of the estuary consists of two arms, north and south, that surround an alluvion-formed island, the Morraceira (Figure 1). Due to distinct hydrodynamics in the two arms, the south arm is heavily silted-up, thus presenting the most important intertidal areas (134 ha) which, along with the salt-pans or salines of the Murraceira Island, are the most suitable feeding areas for waders in the estuary. There is a north-south gradient of increasingly finer sediments in this arm (Marques et al. 1993). In late spring and summer of some years, the finer sediments are covered with extensive weed mats, which remain in place until the beginning of the winter (Múrias et al. 1996). For the purpose of this study, the intertidal area was subdivided in three naturally delimited sub-areas, with 66.8 ha (upstream section), 30.9 ha (middle section) and 36.1 ha (downstream section).

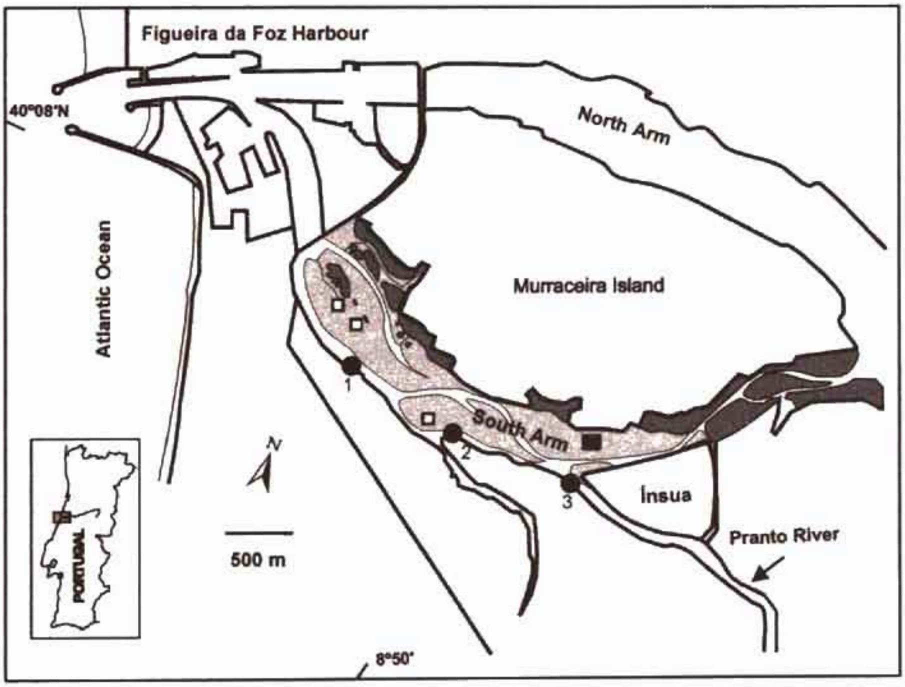

Fig. 1. Map of the study area, showing the intertidal mudflats (light-grey) and the saltmarsh (dark grey) areas of the south arm. Also shown are the positions of the three fixed stations used to census the waders in the south arm. Areas A1, A2 and A3 corresponds to the three main areas of mudflats, from upstream to downstream. The white squares show the study plots used for the behavioural studies, while the black rectangle locates the experimental area in the south arm (see text).

Bird numbers and algae biomass

Census of feeding birds were carried out fortnightly from October 1993 to May 1994, with 10x50 binoculars and a 30-90x60 telescope in three fixed stations, located along the south arm, at both counts and both peak tides (± 2 hours) of spring-water.

The identification and number of all species present, as well as the percentage of birds feeding were noted and plotted in a map, along with the area covered by algae. Similarly simultaneous counts were performed in twenty-one salines of Morraceira island, which represent 57% of the total number of pans in the island.

Algae biomass was directly obtained for the upstream and middle sections of the estuary from a sample of 10-20 weekly cores collected for a simultaneous study since January 1993 (Pardal, pers. comm.). An equation relating the proportion of area covered by algae to its biomass was established from those data ($y=-0.972+58.76x$, $r^2=0.83$, $p<0.01$, $n=11$) and used to predict the algae biomass in the downstream section Biomass, instead of percentage cover was used to estimate the amount of algae present, because the algae mats are not homogeneous (Pardal, pers. comm.)

Macrohabitat use

Census of feeding birds were carried out fortnightly from October 1993 to May 1994, with 10x50 binoculars and a 30-90x60 telescope in three plots marked with wooden stakes, five on spring tides and five on neap tides. Each sequence of five counts was made within 2 hours around dead low-water. These plots represented areas of dense (plot 1), sparse (plot 2) and inexistent (plot 3) eelgrass *Zostera noltii*, periodically covered by green macroalgae (Cabral et al. 1999).

In each count, all birds present were identified and counted. The percentage of birds feeding was estimated and the air temperature was recorded. Between each count, focal observations (30 seconds to 1 minute) of selected birds of the species Grey Plover *Pluvialis squatarola* and Kentish Plover *Charadrius alexandrinus* were made. Plovers feed in a stereotyped "stop-run-peck" manner, which allows a reliable record of feeding behaviour (Pienkowski 1982). Also, these species have a high feeding success, with peck rates close to the real food-intake rates (Baker 1973, Pienkowski 1982). Finally, plovers forage entirely by sight, detecting and catching their prey by exploiting their periods of surface activity, reflecting surface food detectability (Pienkowski 1982, 1983).

A continuous description of the feeding behaviour was recorded on tape for each observed bird and kept for posterior analysis. Data were extracted from the tapes using a multi-event recorder computer program, and pecking rate (number of pecks per unit of time) and movement periods (number of seconds spent walking) determined. It was assumed that the pecking rate is roughly equivalent to the intake rate (Pienkowski 1982). The movement periods are assigned to the detectability of a prey, preceding a successful peck (Pienkowski 1982). The effect of the presence of the algae in both aspects of the feeding behaviour of these species could then be estimated.

Wader faeces were collected in the study area in winter and summer of 1995, stored in alcohol and brought to the laboratory, were they were analysed under a binocular microscope, looking for identifiable fragments (Cabral et al. 1999). The wader species concerned were Ringed Plover *Charadrius hiaticula*, Kentish Plover, Grey Plover and Dunlin *Calidris alpina*. The most consumed items were the polichaete *Nereis*

(Hediste) diversicolor (which occurred in 35.2% to 66.7% of the droppings examined (all wader species concerned), other small polychaetes (11.1% to 33.3%), the gastropod *Hydrobia ulvae* (33.3% to 74.1%), the bivalve *Scrobicularia plana* (2.9% to 30.0%) and the amphipods (11.1% to 44.1%). Less important prey included *Carcinus maenas*, *Cerastoderma edule* and Diptera larvae (see Table I, *in* Cabral et al. 1999).

Data on algae and associated macroinvertebrate densities and biomass were obtained for the same periods, from M. Pardal and co-workers (Pardal 1999).

Field experiments

In spring 1997, an exclusion experiment was carried out in the downstream section of the estuary, aiming to test for the effects of algae and predation on macroinvertebrate densities/biomass (Lopes et al. 2000). The experiment was performed in spring 1997 in a 27 ha mudflat, free from algae cover, in the inner part of the south arm. At this time of the year macroalgae biomass is highest and most waders are using the estuary as a stopover to their northern breeding grounds (Múrias et al. 1996).

To separate the effects of macroalgae cover and wader predation, four treatments were used: NA - open areas with no algae cover; A - open areas with algae cover added artificially; NAE - areas with no algae cover, plus wader exclosures; AE - areas with added macroalgae cover, plus wader exclosures. Each treatment was replicated five times, and the plots consisted of 2 m side squares. Each plot NA and A was simply delimited with four bamboo stakes. All plots were located at the same tidal height in a silted area, with no algae cover and an average emersion period of 8 hours per tidal cycle. Birds were excluded from plots NAE and AE using three plastic strings running horizontally round four wooden corner posts. A nylon mesh (1 cm mesh size) was used as a roof at 0.5 m above the structure (Lopes et al. 2000). This structure was designed both to effectively exclude the birds and to minimise the effects of the structure itself in the experiment (e.g. Raffaelli and Milne 1987, Trush et al. 1994, Sewell 1996). A total of 20 quadrats, disposed in an area of 50 m × 38 m was used. Treatments were interspersed in a rectangular 4 × 5 matrix, with the plots 10 m apart from each other (Lopes et al. 2000). See Lopes et al. (2000) for a detailed discussion on the advantages and drawbacks of the methodology used.

The experiment was initiated in 24 March 1997 and lasted for two months. Invertebrate sampling took place in March, after the algae transplantation, to record the initial conditions, and again in 28 April and 26 May. The quadrats were examined every 5-7 days to look for possible damage of the exclusion structures, evidence of wader use and to clean those quadrats of non-algae treatments from any attached algae or other artifacts (Raffaelli and Milne 1987, Everett 1994, Trush et al. 1994).

Five cores of 24.63 cm^2 were taken in each quadrat. Each core was subdivided into two fractions of up to 5 cm, the probing depth of most wader species (Cramp and Simmons 1983, Kalejta 1993) and 5 cm - 20 cm. In order to compare the initial conditions inside and outside the experimental areas, ten cores (13 cm diameter and 20 cm deep) were randomly taken from the area around the quadrats. Samples were rinsed through a 500 µm sieve and preserved in 4% buffered formalin. Invertebrates were later manually sorted and identified through a binocular microscope.

Statistical analysis

Statistical tests used included parametric multiple regressions (both normal and stepwise), non-parametric correlations (Spearman's r) and ANOVA's, preceded by the usual data transformations, when required (see text). Data were grouped or split up, depending on the particular analyses to be made. For some of the whole-estuary analysis, the data were standardised to a common scale (see, e.g. Goss-Custard 1977), which consisted in expressing each individual value for a given month as the proportion of the total for the three intertidal subareas combined. This method allowed to take into account the concomitant monthly variations in both birds and algae, but was not, unfortunately amenable to formal statistical testing. To assess the probable importance of the correlations, the empirical classification of Guilford (*in* Martin and Bateson 1993) was followed, and all cases where $r>0.4$ were considered a "strong" association. More detailed information describing the statistical methods can be found in Múrias et al. (1996), Cabral et al. (1999) and Lopes et al. (2000).

Results

Effects of algae on the numbers of birds (level 1 analysis)

The quantity of algae present in the three areas varied during the study period from barely none to about 70 g DW.m^{-2}, in 1993-94 and 50 g DW.m^{-2} in 1994-95 reaching peak values from April to October (Figure 2). However, average monthly biomass (24.3±22.4 (s.d.) g DW.m^{-2} and 23.1±14.0 g DW.m^{-2}, for 1993-94 and 1994-95, respectively) did not differ significantly between years (Mann-Whitney U-test, U=74.0, n.s., n_1=11; n_2=16). The maximum percentage of the area covered reached 82.1% in late April of 1994-95, whereas, in 1993-94, coverage did not exceed 36.7% (late May). Average monthly values did not differ significantly between the two years (16.0%±12.4% of total area in 1993-94, and 35.5%±27.4% in 1994-95; Mann-Whitney U-test, U=91.0, n.s., n_1=11; n_2=16). Spatial variations also occurred, more marked upstream area, than downstream, particularly in the first year (Múrias, unpublished results).

The hypothesis that algae deterred the birds was tested for two extreme situations: (1) when the algae was scarce (<5 g DW.m$_2$ per area, in December/January and February/early March in both years) and (2) when it was abundant (>10 g DW.m^{-2} per area, in late March to May). More birds fed on unweeded areas when algae was scarce (in winter) than when it was abundant (in spring) (Table 1). So, the presence of algae, by itself, seemed not to deter the birds. However, all significant results occurred in winter, when bird numbers were higher and the algae abundance was scarce.

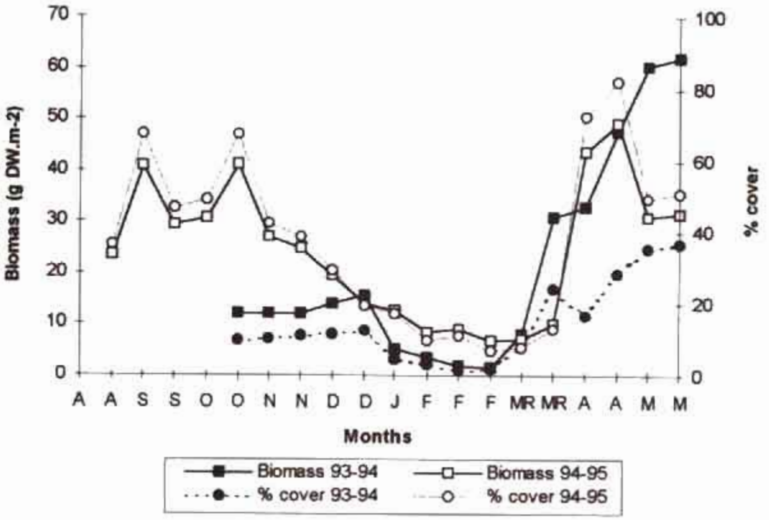

Fig. 2. Seasonal variation in the biomass and the percentage cover of algae on the sediments of the Mondego estuary, in 1993-94 and 1994-95.

Table 1. Number of birds feeding (mean ± 1 SE) in weeded and unweeded areas when algae was scarce (December/January to early March) and when it was abundant (late March to May) in 1993-94 and in 1994-95. n, number of counts, U, Mann-Whitney U-test,: * p < 0.05; ** p < 0.01; *** p < 0.005 (Adapted from Múrias et al. 1996).

	Scarce (< 5 g DW.m^{-2})				Abundant (> 10 g DW.m^{-2})			
	n	No algae	Algae	U	n	No algae	Algae	U
1993-94								
Kentish Plover	7	15.3 ± 4.9	0.3 ± 0.3	49.0*	10	4.5 ± 1.1	4.0 ± 2.5	69.5
Ringed Plover	5	37.4 ± 17.4	5.8 ± 5.8	22.0*	10	8.7 ± 3.9	1.9 ± 0.9	75.0
Grey Plover	10	42.1 ± 9.0	13.8 ± 5.6	71.5	10	8.1 ± 4.8	12.1 ± 5.7	67.5
Dunlin	8	207.1 ± 75.4	14.1 ± 5.9	54.0*	11	223.1 ± 162.1	14.0 ± 7.3	70.0
1994-95								
Kentish Plover	15	35.3 ± 6.3	3.3 ± 2.0	12.0***	21	21.3 ± 6.4	12.4 ± 4.3	167.0
Ringed Plover	13	35.9 ± 6.0	1.8 ± 0.9	9.0***	21	2.5 ± 1.2	9.0 ± 1.8	82.5**
Grey Plover	19	122.7 ± 20.5	9.3 ± 3.2	68.0**	12	9.6 ± 3.4	12.7 ± 2.4	46.0
Dunlin	17	188.6 ± 31.4	55.1 ± 25.2	53.0**	23	28.8 ± 24.8	77.5 ± 24.8	180.0

Even though the algae did not deter the birds, completely, they may have reduced their densities. This possibility was tested both in space and in time by plotting relativised bird densities against relativised algae biomass when this was over 5 g DW.m^{-2} (thus excluding counts from January to early March). Overall, there was no indication of a consistent association between relativised algae biomass and relativised bird densities, when both variables were correlated (Table 2), but most strong associations (r>0.4) were obtained again in winter, particularly in 1993-94.

Table 2. Values of the Spearman correlation coefficient of algae biomass against the total biomass densities of all the species combined and the numerical densities of individual species during the study period of 1993 to 1995, over: (1) the whole period (excluding January and February counts in 1993-94, and late January, February and early March counts in 1994-95), (2) the autumn months (August, September and October only in 1994-95,), (3) the winter months (November and December and, in 1994-95, early January) and (4) the spring months (March -late March, in 1994-95 -, April and May). The number of counts for each period is given in bracketts. Correlation values indicating strong associations for the individual areas (r>0.4, see text for explanation) are shown in bold type. (Adapted from Múrias et al. 1996).

	1993-94				1994-95			
	All year (n =27)	Autumn n[a]	Winter (n =6)[b]	Spring (n =18)	All year (n =46)	Autumn (n =15)	Winter (n =16)	Spring (n =15)
All species	-0.24	-	**-0.43**	-0.07	-0.10	-0.07	-0.03	-0.004
Kentish Plover	+0.18	-	**-0.46**	**+0.55**	-0.10	-0.11	**+0.53**	-0.11
Ringed Plover	+0.05	-	**-0.41**	+0.31	+0.12	+0.06	**+0.40**	-0.06
Grey Plover	-0.24	-	**-0.43**	-0.30	-0.14	-0.32	-0.26	**-0.42**
Dunlin	-0.11	-	-0.09	-0.03	+0.11	+0.13	+0.07	**+0.43**

[a] Only two observations for October of 1993 were available, therefore this periods was not analysed

[b] The Area A2 was not considered in the counts, because no algae were present in the months sampled

The possibility still existed that these results could be due to the influence of other factors that independently affected both the algae and the birds, particularly in winter, the sediment type being a strong candidate. To control for the effect of this, a temporal analysis was performed in an area-by-area basis, using the months when bird densities were stable (winter and early spring). If birds were repelled by algae, the densities in one area should have decreased over time as the abundance of algae in that area increased, taking changes in the algae cover in the other two areas into account. In fact, no clear trend was found for any species or for all species combined, both for the whole estuary and for each area (Table 3). All things considered, there is little to suggest that the birds changed their feeding areas in response to temporal variations in the abundance of algae.

An alternative way of testing whether algae deterred waders was to see if birds moved to the available, always algae-free, supratidal habitats (the salines) when the amount of algae in the estuary was highest. Because the use of these habitats may be linked to the competitive pressure associated with changes in bird numbers (e.g. Goss-Custard 1977), both this factor and the algae were used in a multiple regression analysis, controlling for the year of the study and the season. This allowed to cover both the period (winter) when bird numbers were stable but the algae biomass was less abundant, and the migratory periods, when high variation in bird numbers took place, but algae biomass reached the highest values. Only those species that used both the salinas and the mudflats at low-water were considered.

Again, there was little to suggest that waders as a whole, or individual species, used the salines more when algae were abundant in the mudflats (all $p>0.05$) (Table 4).

Table 3. Values of the Spearman correlation coefficient with algae biomass against: (1) total bird biomass densities for the whole estuary, (2) each of the three intertidal areas (see text) and (3) the relative densities of individual species in each area during the winter (November to February) and early spring (early March) months of the 1993-94 and 1994-95 years of study. n = 7 in 1993-94 and n = 10 in 1994-95. Formal statistical tests were performed for the whole estuary situation (* $p < 0.05$). Otherwise, 'significant' values for the individual areas ($r>0.4$, see text for explanation) are shown in bold type. (Adapted from Múrias et al. 1996).

	1993-94				1994-95			
	All estuary	Area A1	Area A2	Area A3	All estuary	Area A1	Area A2	Area A3
All species	-0.53	+0.29	**-0.89**	**+0.46**	-0.65*	+0.05	+0.38	+0.00
Kentish Plover	-	**+0.74**	-0.29	+0.07	-	-0.06	**-0.57**	-0.39
Ringed Plover	-	+0.14	-0.14	**-0.92**	-	**+0.40**	-0.07	-0.23
Grey Plover	-	-0.26	-0.37	-0.25	-	**-0.49**	+0.03	+0.06
Dunlin	-	**+0.67**	**-0.84**	+0.32	-	**+0.46**	**+0.47**	+0.06

Table 4. Multiple regression analysis of the effect of algal biomass and total number of birds of each species in the estuary (controlling for the year of study and the season), on the proportion of birds feeding in the salinas. Values of the coefficients for each variable are shown, as well as their sign, the r and F values. n = 31, ** $p < 0.01$, * $p < 0.05$

	Algae biomass	Total numbers	Year	Season[1]	Constant	r	$F_{(4,26)}$
All species	+0.07	-0.29	-0.15	-0.05	+35.96	0.31	0.70
Kentish Plover	+0.06	-0.05	-0.14	0.70**	-11.26	0.72	6.03**
Ringed Plover	-0.33	-0.35	-0.16	+0.45*	+25.56	0.51	2.31
Dunlin	-0.33	-0.30	-0.13	-0.01	+60.10	0.30	0.61

[1] Measured as the number of days since the beginning of the autumn (October, in 1993-94, and August, in 1994-95)

The effect of algae on macrohabitat use by waders (level 2 analysis)

Algae biomass was among the main factors that affected the proportion of birds of some species present in selected plots in the south arm (Table 5). In all cases it was a negative effect, suggesting that these species actively avoid feeding in weeded areas. However, the feeding behaviour (pecking and movement rates) of the visual-hunting species was, in most cases, not affected by the algae. In fact, the pecking rate of Kentish Plover was actually enhanced by the presence of algae. Overall, the occurrence of birds in the tidal flats of the south arm seemed to be more related with the other abiotic variables (algae, total density of birds, density of gulls) than with the prey variables.

In order to test the hypothesis that foraging birds were not indifferent to tidal flats distinguished by different sediment characteristics or by the presence of epistructures such as green macroalgae and seagrass meadows, a one-way ANOVA analysis was

Table 5. The regression equations, the coefficient of determination (R2), and F-values and their significance level (**P<0.01, ***P<0.001) for all combinations reported, as selected by stepwise multiple regression. D = density, PEK = pecking rate; MOV = movement periods. Variable codes as: AIR = air temperature; GUL = density of gulls; TOT = total bird densities; ALG = macroalgae biomass; NER = Nereis (Hediste) diversicolor; HUL = Hydrobia ulvae; SPLA = Scrobicularia plana; DIP = Diptera larvae; EDU = Cerastoderma edule; POL = small polychaetes; AMP = amphipods. The effects of algae in each case are depicted in italic. (Extracted from Cabral et al. 1993).

Equations	R^2	F	d.f.
Calidris alpina	0.64	19.51***	47
D = 0.192 - 0.006 (GUL) + 0.004 (TOT) - 0.010 (ALG) + 0.102 (NER)			
Charadrius alexandrinus			
D = 0.373 - 0.003 (GUL) + 0.002 (TOT) - 0.006 (HULV)	0.40	9.88**	47
PEK = 12.232 + 0.089 (ALG) - 3.002 (NER) + 0.521 (SPLA) + 171.655 (DIP)	0.11	4.47**	143
MOV = 32.257 - 0.98 (AIR) - 0.117 (HULV)	0.16	13.56***	143
Charadrius hiaticula			
D = 0.3 - 0.005 (GUL) + 0.003 (TOT) - 0.005 (ALG)	0.40	9.90***	47
PEK = 5.015 + 0.28 (AIR) + 0.342 (SPLA)	0.11	8.62***	148
MOV = 37.635 - 0.022 (TOT) + 0.186 (GUL) - 0.743 (AIR) - 1.688 (EDU)	0.15	6.56***	148
Pluvialis squatarola			
D = 0.339 + 0.001 (TOT) - 0.008 (ALG) - 0.305 (POL) - 0.49 (CAR) + 6.669 (DIP)	0.37	4.89**	47
PEK = 9.973 - 0.343 (AIR) - 12.62 (POL) + 0.574 (SPLA) + 95.372 (DIP)	0.40	21.07***	132
MOV = 42.261 - 1.084 (AIR) - 4.51 (NER) - 23.213 (AMP)	0.12	6.02***	132

conducted to test for bird preferences according to specific sediment characteristics and seagrass presence in each of the three plots.

Only Kentish Plover clearly preferred plot 3 (muddy-sand substrates without *Zostera noltii* meadows, $F_{2,45}=8.17$, $p<0.001$). Considering the muddy substrates covered by vegetation, Kentish Plover preferred plot 2 (sparse *Z. noltii* meadows). Dunlin and Ringed Plover also used preferentially some plots, but it was not possible to assess which ones ($F_{2,45}=3.96$, $p<0.05$ and $F_{2,45}=3.66$, $p<0.05$, respectively). Grey Plover, on the other hand, did not show any preference at all ($F_{2,45}=0.08$, n.s.).

Field experiments: the response of prey species to predation and macroalgae growth (level 3 analysis)

The experimental setup proved to be very efficient in excluding the birds and preventing the artificially implanted algae to be washed off (Lopes et al. 2000).

The most abundant species present in the area at the beginning of the experiment, was the gastropod *Hydrobia ulvae*, by far outnumbering the next six more abundant species (the polychaetes *Amage adspersa, Streblospio shrubsolii, Capitella capitata* and *Hediste diversicolor*, the bivalve *Scrobicularia plana* and the Oligochaetes) (Lopes et al. 2000). Overall, there was no evidence that the total densities of invertebrates were affected by the presence of the predators, either at the beginning, or later in the season. On the contrary, the presence of the algae affected the densities of some polychaete species, either positively (*C. capitata* and *H. diversicolor*) or negatively (*S. shrubsolii*) (Table 6). Some of these results may have been masked by internal variations between the quadrats. A significant interaction between algae and predation, occurred only once in late May, with *H. ulvae*. The densities of this species were higher in areas covered by algae but accessible to the predators (i.e. in the control areas), than in the other areas.

Both the algae and predation did not seem to affect the densities of the macroinvertebrate species in the 0-5 cm depth strata, while only those individuals of H. diversicolor buried deeper in the sediment (5-20 cm), experienced a significant reduction of densities outside the exclosure cages in areas free of algae, although such reduction did not occur in algae-covered areas. (Lopes et al. 2000).

Table 6. Effects of algae and predation on the densities of selected macroinvertebrate species (nested ANOVA, with three factors on log-transformed (log10 (n+1) densities). Degrees of freedom for each factor are: algae, predation and algae x predation = 1,16; quadrat (algae x predation) = 1, 80. Significant p-values (p<0.05) are shown in bold. (Extracted from Lopes et al. 2000).

		Algae		Predation		Algae x predation		Quadrat (A x P)	
Date	Species	F	p	F	p	F	p	F	p
28 April	*Hydrobia ulvae*	0.10	0.758	0.84	0.374	2.40	0.141	0.86	0.620
	Scrobicularia plana	0.12	0.739	0.01	0.925	1.17	0.296	0.83	0.648
	Hediste diversicolor	7.61	**0.014**	0.03	0.866	0.74	0.402	0.69	0.795
	Amage adspersa	16.45	**0.0009**	3.56	0.078	0.25	0.621	1.05	0.420
	Streblospio schrubsolii	12.24	**0.003**	1.45	0.246	0.30	0.589	1.59	0.092
	Capitella capitata	28.63	**0.0001**	0.32	0.577	4.69	0.046	1.84	**0.04**
	Oligochaeta spp.	0.65	0.431	0.17	0.686	0.17	0.685	1.59	0.091
26 May	*Hydrobia ulvae*	0.25	0.624	0.07	0.793	8.71	**0.009**	0.82	0.662
	Scrobicularia plana	3.24	0.091	1.92	0.185	0.75	0.400	0.79	0.691
	Hediste diversicolor	6.27	**0.024**	0.14	0.717	1.43	0.250	1.25	0.249
	Amage adspersa	6.03	**0.026**	0.78	0.389	0.17	0.682	4.50	**0.000**
	Streblospio schrubsolii	6.95	**0.018**	0.37	0.552	0.00	0.971	3.62	**0.0001**
	Capitella capitata	18.15	**0.006**	0.01	0.910	3.24	0.091	1.72	0.059
	Oligochaeta spp.	2.96	0.104	0.98	0.338	0.16	0.693	1.52	0.113

Discussion

It is usually considered that the ultimate effect of the increase of algae on birds in an estuary is to depress their numbers due to a decrease in the quality of their feeding areas (e.g. Raffaelli 1992). However, this is usually the final stage of the process, when the alteration in the feeding conditions for waders are such that they are not able to match them for longer. In fact, waders usually react to changes in the numbers or availability of their prey by developing a set of behavioural adaptations that allow them to buffer the effect of such changes (Desprez et al. 1992, Metzmacher and Reise 1994). The results of the whole-estuary analysis (first level) suggest that the majority of the negative associations between bird densities and algae biomass took place in winter, when algae is generally scarce. This could be explained by a combination of factors (like an high selectivity of the birds and lower food availability) not directed linked to the effect of the algae. In the spring period, on the other hand, the lower density of birds could mask any potential effects arising from the presence of dense algal mats in the flats.

Thus, at present, the results of the whole-estuary analysis (first level) suggest that the eutrophication process in the Mondego, as far as the birds are concerned, is still in its initial phase. This is confirmed by the second-level analysis focusing on the possible effects of the algae in shaping the relationship between the bird densities and feeding behaviour and the densities of their prey, performed at a local scale. This analysis showed that the densities of some species, but not their feeding behaviour, were negatively influenced by the amount of algae present. This suggests that the birds were probably feeding in zones with sparse vegetation, selecting the bare sediments, rather than the weeded ones, as confirmed by the data on habitat selection (Cabral et al. 1999).

The results of the first-level analysis can be partially compared with a recent experimental work performed by Lopes et al. (2000) in the spring of 1997. As is stressed by Lopes et al. (2000), these kind of field experiments may themselves be biased by several factors, and should be treated with caution. However, the experiment partly confirmed the previous analysis, especially the fact that, at least in the spring, the predation pressure is generally not influenced by the amount of algae present, although each factor, by itself, may have an effect in the densities of some infaunal groups. Overall, present data suggests that, the ongoing eutrophication process in the Mondego estuary has not yet affected the wader communities.

The presence of the algae may even be advantageous to the birds, at least in the short-term and to a certain extent, as shown by other studies (Metzmacher and Reise 1994). Certain epibenthic organisms (polychaetes, crustaceans) benefit from the presence of the mats (Soulsby et al. 1982, Raffaelli and Milne 1989, Raffaelli et al. 1991, Desprez et al. 1992, Everett, 1994), a fact which was confirmed in the Mondego by both Cabral et al. (1999) and Lopes et al. (2000) in independent analysis. The problem arises when the mats cover the whole extension of the intertidal areas, thereby preventing the recolonisation of these areas from the vicinity, after the algae crash (Soulsby et al. 1982, Everett 1994). The situation in the Mondego is still far away from that: in the two years monitored, the average amount of intertidal area covered did not exceed 36% of the area (see above), and the tendency in the recent years has been not to increase (Pardal, pers. comm.). Assuming, as Souslby et al. (1982) did, that the changes in infauna related to the presence of the mats occur when 50% of the area is covered, it is evident that a considerable area of the mudflats may still be occupied by algae in the Mondego before the numbers of avian predators exploiting the infaunal species seriously decline. However, the first signs of the process can be noted long before that through subtle alterations in the foraging behaviour (Desprez et al. 1992, Metzmacher and Reise 1994).

The present review was based on different studies performed over different spatial and temporal scales. From this point of view, caution should be taken when comparing them directly. However the potential benefits of an integrated multi-level analysis were made clear. We strongly advise that an approach extending over different temporal and spatial scales, as the one attempted in this review, is likely to be a powerful tool to detect any possible changes that may be occurring. Moreover, the combination of methods presents the advantage of reducing the potential flaws associated with each one.

Acnowledgements

This work was supported by the Portuguese Research Board (JNICT), through grants FMRH/BD/331/93 and PRAXIS XXI/BD/5570/95 (TM), PRAXIS XXI BM/3261/92-IG (JAC) and PRAXIS XXI BM/7232/95 (RL). The authors are also indebted to all the colleagues who helped in the fieldwork, particularly to M. Pardal who also supplied some of his unpublished data, and to an anonymous referee for the helpful comments, which helped to improve the manuscript.

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ELEMENTAL COMPOSITION AND CONTAMINANTS IN SURFACE SEDIMENTS OF THE MONDEGO RIVER ESTUARY

Abstract

Major (Al, Si, Ca, Mg, Fe), minor (Mn) and trace elements (Zn, Pb, Cr, Cu, Ag, Cd), organochlorine (PCB congeners, pp'DDT and metabolites) and tri- and dibutyltin levels were determined in 47 surface sediments from the Mondego River estuary. Concentrations varied within different ranges in river, sand and sandy mud samples. Higher values of Si and Ca were found in sands from the lower estuary while levels of Fe, Mn, Zn, Cu, Cd, Cr, tPCB and tDDT were higher in sandy muds and fluvial sediments. Linear correlations between Fe, Mg, Mn, Zn, Cu, Cr, Cd and Al indicate that some variability in their concentrations may be explained by the Al fraction of the sediments. Poor correlations to Al were found for organic contaminants (tPCB, tDDT, TBT+DBT). The contrasting longitudinal distributions of tDDT Ag and Ca concentrations indicate the marine and river contribution, respectively. In spite of low contamination of sediments, levels of other contaminants seem to reflect the nature of the particles and the local anthropogenic sources.

Introduction

Chemical composition of bottom sediments has been determined in many estuaries and coastal lagoon in Europe and North America (Alderton 1985) and compared to the composition of the crust (Martin and Meybeck 1979). Geographical differences in elemental composition of sediments may be attributed to mixing of river and marine sediments (Muller and Forstner 1975) formation of new particles (Yeats et al. 1979) and post-depositional changes in the upper sediment layers (Sundby and Silverberg 1985, Gobeil et al. 1987). In estuaries surrounded by urban areas and industrial parks, enrichment of trace elements and synthetic organic pollutants in sediments are usually attributed to environmental contamination (Libes 1992). The deposition of contaminated particles are influenced by the sediment dynamics, which may be periodically ruled by the tides (Allen et al. 1980, Gelfenbaum 1983) and the

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episodic high river discharges (Castaing and Allen 1981, Vale et al. 1993). When contaminants are weakly mobilised in the sediments, depth variation of their concentrations elucidates the historical evolution of the contamination in the area (Bruland et al. 1974, Gobeil and Cossa 1993).

The Mondego River estuary consists of two channels separated by an island at about 7 km from the estuarine mouth. The north and south channels have different hydrographic characteristics (Marques et al. 1993). The south channel is almost silted up in the upstream areas and water circulation is mainly driven by the tide. The North Channel is deeper and ended in the Mondego River, which is the main freshwater input. The combination of the tidal rhythm and the regime of river water discharges determine how freshwater mixes with seawater, the salinity structure being profoundly modified during runoff periods in Winter. The Mondego River drains a hydrological basin with intensive agriculture and crosses the urbanised area of Coimbra. The North Channel has been dredged periodically in order to allow the navigation to the harbour facilities in Figueira da Foz. A large volume of sediments has been dredged in the early 90's followed by hydraulic engineering operations in the shorelines of the lower estuary. This paper reports the major, minor and trace element composition of surface sediments along the North Channel of the Mondego estuary and the concentrations of polychlorinated biphenyls (PCBs), DDTs and butyltin compounds after those modifications.

Material and Methods

Surface sediments (0-2 cm) have been collected along the North Channel of the Mondego River estuary in 1994, after a large dredging operation upstream the commercial harbour of Figueira da Foz, and near the mouth of estuary in 1998. Sediments were collected in the middle of the channel and near the shoreline where fine material was deposited. The number of sampling sites was: 2 in the river, 27 in upper and middle estuary, and 18 in the lower estuary (fig 1). Metal concentrations (Al, Si, Ca, Mg, Fe, Mn, Zn, Pb, Cr, Cu, Ag and Cd) in sediments were determined after a total acid digestion of the samples following the procedure of Rantala and Loring (1977) and analysed by flame and furnace atomic absorption spectrophotometry. For tri- and dibutyltin compounds (TBT and DBT) the sediments were acidified and extracted with hexane following procedures originally described by M&T Chemicals, and modified by Bryan et al. (1986) and Langston et al. (1987). Tri- and dibutyltin, were measured, as tin, by atomic absorption in a Perkin-Elmer atomic absorption spectrophotometer with an electrodeless discharge tin lamp at a wavelength of 286.3 nm. For the determination of organochlorine compounds (PCB congeners and pp'DDT and its metabolites) the dry sediments were soxhlet extracted in n-hexane for 16 hours and cleaned up with a Florisil column and sulfuric acid. They were analysed in a Hewlett Packard gas chromatograph with an electron capture detector and capillary column (DB5, J&W, 60m). In this study tDDT means the sum of pp'DDE, pp'DDD and pp'DDT and tPCB the sum of the congeners CB18, CB26, CB31, CB44,

CB49, CB52, CB101, CB105, CB118, CB128, CB138, CB149, CB151, CB153, CB170, CB180, CB183, CB187 and CB194 (IUPAC n°s). International certified standards (AGV-1, GSP-1, G-2, MESS-2 and BCSS-2) and standard solutions were used to control the accuracy of our procedures. For all metals and compounds investigated, obtained and certified values were not statistically different ($p<0.01$).

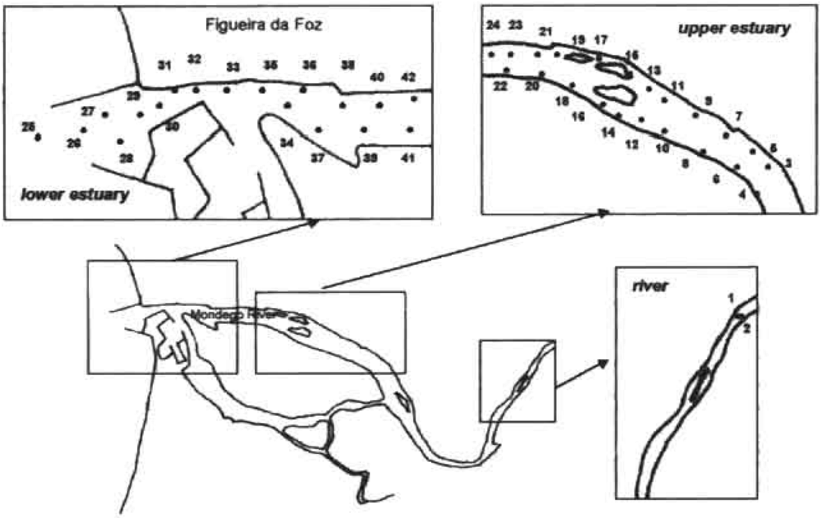

Figure 1. Map of Mondego river estuary with sampling sites.

Results and Discussion

Concentrations of metals, organochlorines and butyltin in surface sediments

Elemental composition and concentrations of organochlorines (pp'DDT and its metabolites and PCB congeners) and TBT+DBT in surface sediments of the Mondego River estuary ranged within broad intervals of values (Table 1). The ranges in fluvial sediments differed from those found in sands and sandy muds. The sands are naturally enriched in Si and presented higher concentrations of Ca and lower levels of Fe, Mn, Zn, Cu, Cd, Cr, tPCB and tDDT than sandy muds and fluvial sediments. The pp'DDT and its most stable metabolite (pp'DDE) are the major contributors to the values of tDDT. Among the analysed PCB congeners the tri- and tetrachlorinated compounds accounted to more than 65% of tPCB concentrations. The more toxic congeners with six to eight atoms of chlorine had a smaller contribution. The concentrations of TBT in lower estuary sediments were relatively uniform, and DBT were presented only in four samples nearby the shipyard factory, showing concentrations comparable to TBT. Levels of trace elements, organochlorines and butyltin compounds were low when compared to values registered in sediments from other estuarine areas of Portugal (Caetano 1998, Castro and Vale 1995, Cortesão and Vale 1995, Cortez et al. 1993, Vale 1990).

Table 1. Concentrations of major, minor and trace elements (% and $\mu g.g^{-1}$) and of tDDT, tPCB and TBT+DBT ($ng.g^{-1}$) in fluvial sediments, coarse sands and sandy mud deposited in the estuarine zone.

Chemical parameters	River sediments (n=2)	Estuarine sands (n= 33)	Estuarine sandy muds (n=13)
Al (%)	6.5 - 9.5	2.8 - 4.6	4.9 - 7.5
Si (%)	19 - 20	12 - 44	14 - 33
Ca (%)	0.60 - 1.1	0.010 - 1.8	0.060 - 2.3
Mg (%)	1.0 - 1.9	0.18 - 0.49	0.26 - 3.7
Fe (%)	2.3 - 3.5	0.30 - 1.1	0.94 - 3.2
Mn ($\mu g\ g^{-1}$)	499 - 893	26 - 433	98 - 1093
Zn ($\mu g\ g^{-1}$)	73 - 105	7 - 74	33 - 159
Pb ($\mu g\ g^{-1}$)	41 - 55	14 - 35	16 - 52
Cr ($\mu g\ g^{-1}$)	46 - 65	2.8 - 28	19 - 62
Cu ($\mu g\ g^{-1}$)	23 - 36	1.0 - 9.5	3.0 - 43
Ag ($\mu g\ g^{-1}$)	1.3 - 1.7	0.010 - 0.060	0.050 - 1.4
Cd ($\mu g\ g^{-1}$)	0.23 - 0.27	<0.010 - 0.070	0.040 - 0.32
tPCB ($ng\ g^{-1}$)	1.1 - 1.9	0.19 - 3.6	0.32 - 5.2
tDDT ($ng\ g^{-1}$)	0.40 - 1.2	0.010 - 0.12	0.030 - 1.7
TBT+ DBT ($ng\ g^{-1}$)	-	8.0 - 11*	8.0 - 21

* n = 5

Relationships to Al

Some samples were mainly constituted by sand and others had a larger fraction of fine particles. In order to assess weather the nature of the particle influences the metal distribution, relationships between element concentrations and Al content were examined. Concentrations of most metals were linearly correlated to Al content (Table 2). The higher correlation was obtained for Fe-Al ($r^2 = 0.93$), suggesting that iron in surface sediments is closely associated with aluminosilicates which are mainly present in fine sediments. Iron oxides that usually precipitated near the sediment surface (Sundby and Silvergerg 1985) appear to be a minor contributor to the total iron concentrations in Mondego sediments. High correlation coefficients were also found for Cr, Cu, Cd, Zn, Mn and Mg, indicating that some variability in their concentrations may be explained by the Al fraction in the sediments. These elements are either incorporated in the aluminosilicates or associated with the fine fraction of the sediments that is expressed by the Al content. The sand and calcium carbonates can thus be considered dilutors of iron and trace metals. The Pb-Al and Ag-Al relationships showed poorer correlations, meaning broader distributions of these elements among the particles. The levels of Ca, tDDT, tPCB and TBT+DBT were not related to Al suggesting that association of these substances to sediment particles is not related with grain size distribution. An illustration of the different situations is depicted in figure 2.

Table 2. Correlation coefficients and levels of significance between Al and major-, minor- and trace-elements, tDDT, tPCB (n= 46) and TBT+DBT (n= 18) in sediments of Mondego river estuary.

	Fe	Ca	Mg	Mn	Zn	Pb	Cr	Cu	Ag	Cd	tPCB	tDDT	TBT+ DBT
r (p)	0.93 (p<0.001)	<0.10 (p>0.1)	0.75 (p<0.001)	0.79 (p<0.001)	0.82 (p<0.001)	0.41 (p<0.005)	0.83 (p<0.001)	0.83 (p<0.001)	0.22 (p>0.1)	0.81 (p<0.001)	<0.10 (p>0.1)	<0.10 (p>0.1)	<0.10 (p>0.1)

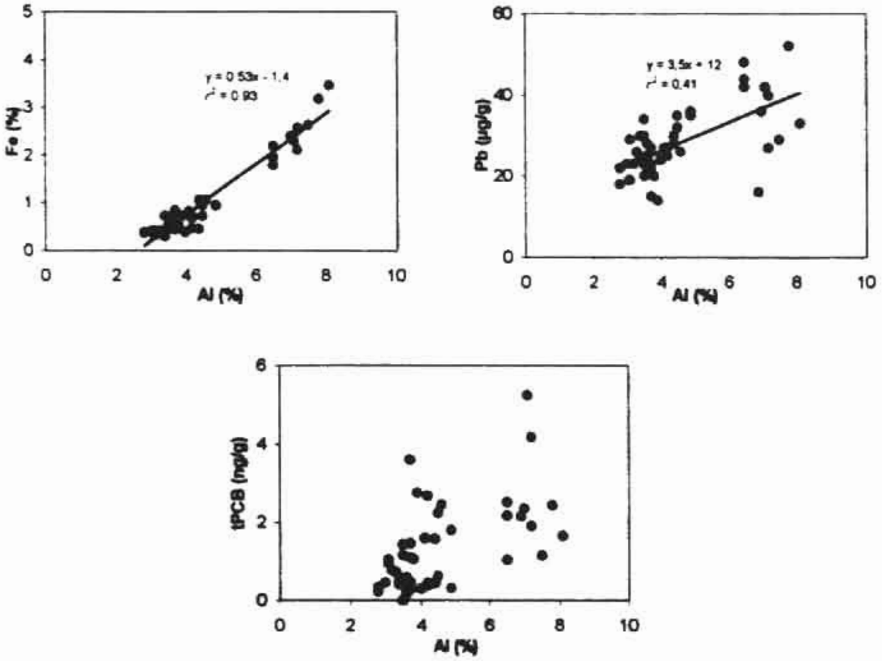

Figure 2. Relationships between Fe, Pb and tPCB with Al in sediments of Mondego River estuary

Longitudinal distribution pattern of marine and fluvial tracers

The longitudinal distribution of Ca exhibits a considerable concentration increase in sands and fine particles of the lower estuary (fig. 3). A similar distribution pattern of Ca was registered in the Tagus and Sado estuaries (Vale 1986). The increase of Ca concentrations in sediments and suspended particulate matter of the lower part of estuaries has been interpreted as the presence of material from marine origin enriched in biogenic calcium carbonates. The drastic differences on Ca content may be useful to distinguish and delimit the marine influence on the sediment sources. It appears that marine-derived sediments are mainly deposited in the lower Mondego estuary and upstream the commercial harbour the sediments are transported by the river, presumably during the periods of runoff. An opposite distribution was found for tDDT and Ag (fig. 3). Levels in the river sediments and in fine material from the upper estuary were ten times the concentrations found in the material deposited in the estuary. High levels of Ag reflect probably the urban source

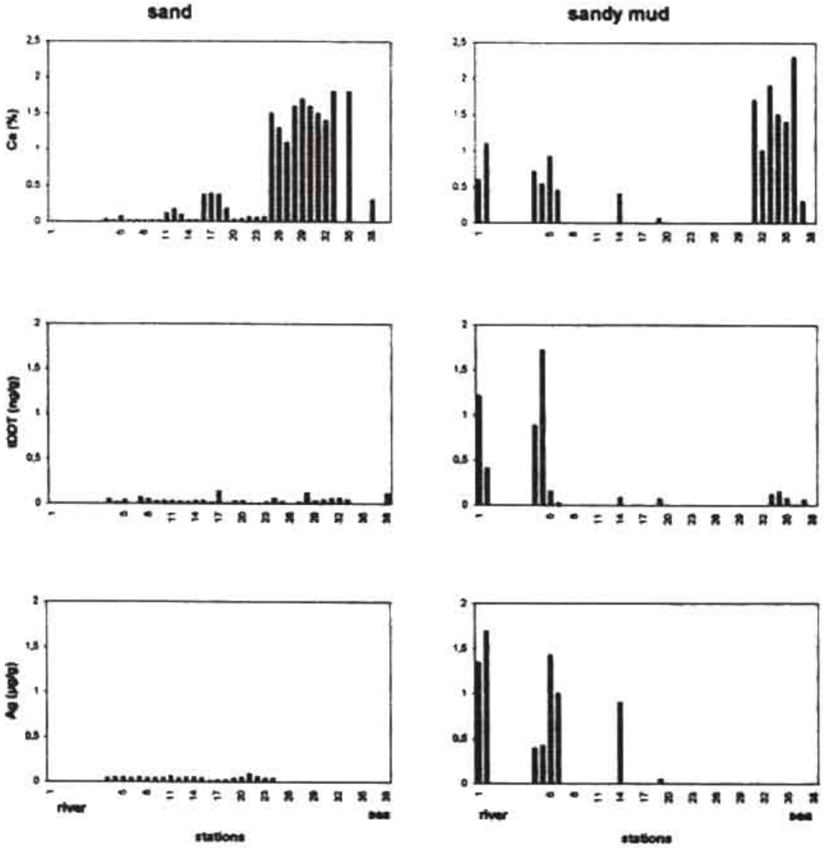

Figure 3. Longitudinal distribution of Ca (%), tDDT (ng.g⁻¹) and Ag (µg.g⁻¹) in sands and fine particles of Mondego River estuary.

from the city of Coimbra. Longitudinal distribution of tDDT, which is a typical contaminant from agriculture areas (Vale et al. 1993), point to the presence of eroded soil particles in river and upper estuarine sediments. Although these values were low in comparison to other estuarine areas (Ferreira and Vale 1995) the longitudinal distributions of their concentrations are indicative of the fluvial influence on the sediments of the Mondego River estuary.

Distribution pattern of contaminants

When element concentration is correlated to Al content its geographic distribution in coastal sediments and suspended particles matter is frequently expressed as element/Al ratio in order to minimise differences related to the nature and grain size of the particles (Windom et al. 1989). The element/Al ratios in sands and sandy muds of Mondego River estuary are shown separately in figure 4. Clearly, Fe, Mn,

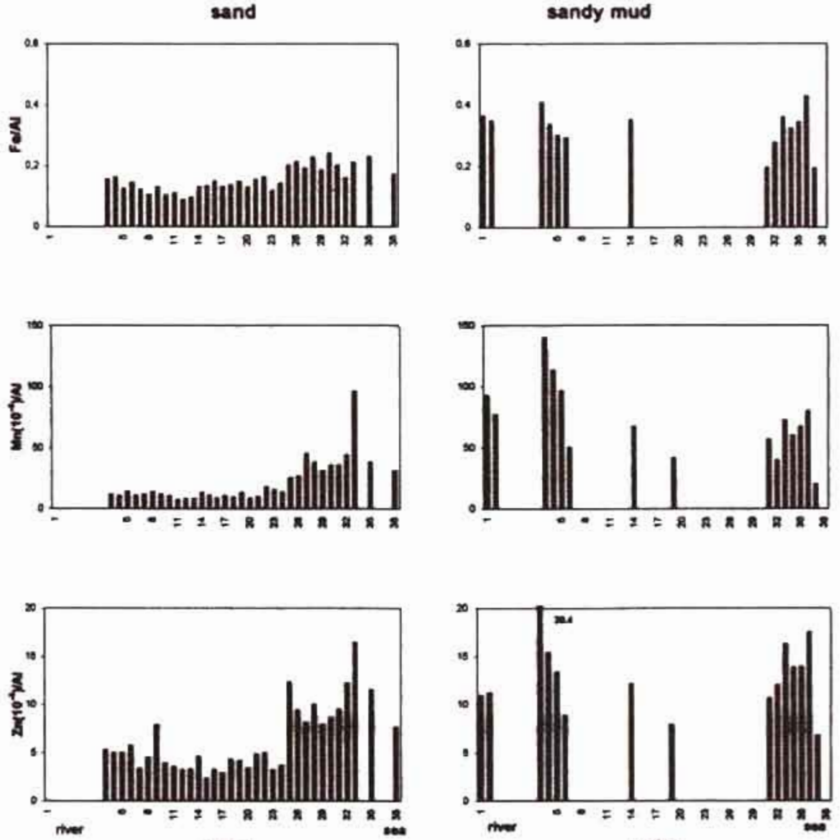

Figure 4. Metal/Al ratios in sands and sandy muds of Mondego river estuary.

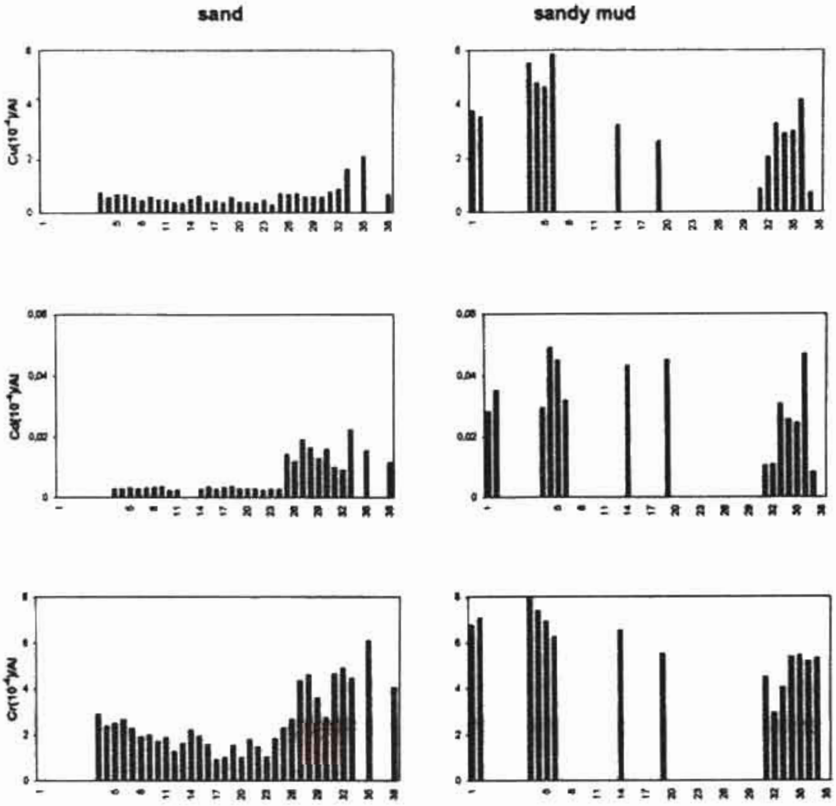

Figure 4. (cont.)

Zn, Cu and Cd ratios to Al were higher in fine particles than in sand. The ratios recorded in river material are comparable to those found in fine particles trapped in North Channel of the Mondego estuary. The Zn/Al, Cd/Al, Cr/Al and Cu/Al ratios were higher in sand from the lower estuary than in coarse material collected upstream. A similar distribution was found for tPCB concentrations (fig. 5). The increments of these metal/Al ratios and tPCB may reflect the incorporation from local anthropogenic sources. The levels of Pb were relatively uniform along the estuary and lower in sands than in fine particles (fig. 5).

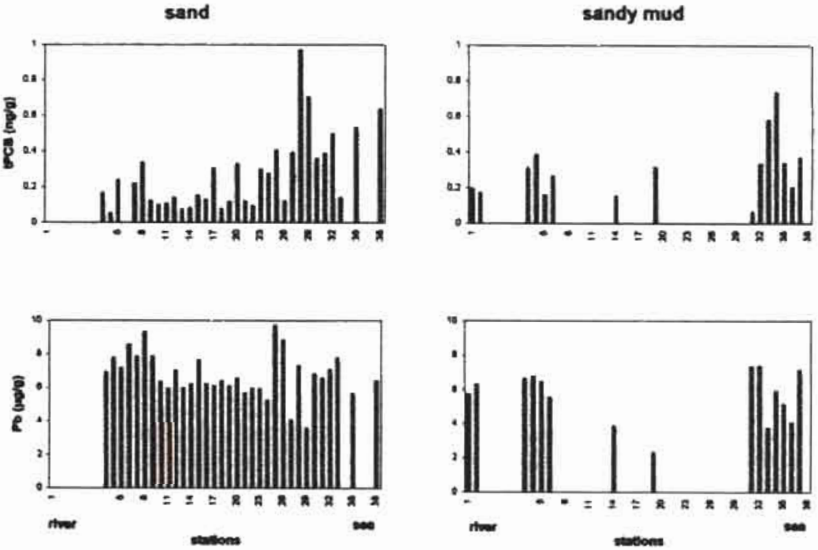

Figure 5. Concentrations of tPCB (ng.g⁻¹) and Pb (µg.g⁻¹) in sands and sandy muds of Mondego river estuary.

Conclusions

In spite of the low contamination of sediments along the Mondego River estuary, its chemical composition indicates that lower estuary sediments are mainly derived from the sea (Ca content) and slightly contaminated by metals, tPCB and butyl tin compounds from anthropogenic sources located around Figueira da Foz. River-derived material exhibited higher concentrations of tDDT and Ag. Geographic distribution of these chemicals and the periodical record will contribute to a better understanding of the sediment dynamics in the Mondego estuary.

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APPLICATION OF HOLISTIC ECOLOGICAL INDICATORS OF ECOSYSTEM'S INTEGRITY: A CASE STUDY IN THE MONDEGO ESTUARY

Abstract

Benthic eutrophication may cause qualitative changes in marine and estuarine ecosystems, for example the shift in primary producers. Subsequently, changes in species composition and trophic structure at other levels may often occur, and through time a new trophic structure might be selected. In structurally dynamic models such changes may be simulated using goal functions to guide ecosystem behaviour and development. The selection of other species and other food web may then be accounted by a continuous stepwise optimisation of model parameters according to an ecological goal function.

Exergy has been applied as goal function in structurally dynamic models of shallow lakes. Hypothetically, exergy is assumed to become optimised during ecosystems development. Therefore, ecosystems are supposed to self organise towards a state of an optimal exergy configuration. Exergy may then constitute a suitable system-oriented characteristic to express natural tendencies of ecosystems development, and simultaneously a good ecological indicator of ecosystems health.

Biodiversity, a powerful and traditional concept, is also an important characteristic of ecosystems structure. We found it suitable to test the intrinsic ecological significance of exergy. Therefore, we examined the properties of exergy (exergy and specific exergy) and biodiversity (species richness and heterogeneity) along an estuarine gradient of eutrophication, testing the hypothesis that they would follow the same trends in space and time. This hypothesis was only partially validated, since exergy, specific exergy and species richness decreased as a function of increasing eutrophication, but heterogeneity behaved mostly in the opposite way. Nevertheless, exergy and specific exergy behaved as hypothesised, providing useful information regarding the studied communities. They appeared therefore suitable to be used as goal functions in ecological models and as holistic ecological indicators of ecosystem

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integrity. Moreover, since exergy and specific exergy showed to respond differently to ecosystem seasonal dynamics, we recommend using both as complementary parameters.

The method proposed by Jørgensen et al. (1995) to estimate exergy, which takes into account the biomass of organisms and the thermodynamic information due to genes, appeared to be operational, but more accurate (discrete) weighting factors to estimate exergy from organisms biomass need to be estimated. We propose to explore the assumption that the dimensions of active genomes, which are primarily a function of the required genetic information to build up an organism, are proportional to the relative contents of DNA in different organisms.

The present contribution was mostly based on two previously published papers (Marques et al. 1997, 1998).

Introduction

Changes in environmental factors often give origin to qualitative modifications in the ecosystems, such as species composition and biodiversity. For example, eutrophication processes may cause a shift in primary producers, which may also determine changes in species composition at other trophic levels, like the faunal composition. Through time, a sequence of modifications may give rise to the selection of a new structure of the trophic network.

To model these types of qualitative changes, which describe the development of ecosystems as a response to changes in external factors, the qualitative trophic alterations through time must be included in the models. The more conventional deterministic models of aquatic ecosystems, although efficient, due to lack of generality are difficult to apply from one system to another. Nevertheless, it seems possible to incorporate the type of change described above through the development of structurally dynamic models (Jørgensen 1993, Nielsen 1992, 1994, 1995), which has recently been started. The dynamic element of the model is needed to account for the adaptational and selective processes. This new generation of models may improve the existing ones, not only in the sense of increasing their predictive capability, but also by gaining a better understanding of ecosystems behaviour, and consequently providing a better tool for environmental management. In this sense, besides the development of structurally dynamic models, it might be necessary to use soft parameter sets.

By applying the principles of optimisation theory in ecology, structurally dynamic models can use goal functions to guide the model simulation of ecosystem behaviour and development (Nielsen 1995). In such a case, in the simulation, the selection of other species and the selection of another food web is accounted for by a change of model parameters according to an ecological goal function. In structurally dynamic models, parameters may be introduced which change as functions of changing forcing functions and conditions of state variables. Therefore, the ability of the ecosystem for instance to move away from thermodynamic equilibrium may be optimised by a stepwise approach.

In ecological models goal functions are assumed to measure given properties or tendencies of ecosystems, emerging as a result of self-organisation processes in their development. But contrary to what happens in the run of structurally dynamic models, natural ecosystem development and adaptation does not pursue a goal, in the teleological sense. Nevertheless, from an environmentally static assessment point of view, the same mathematical expressions may constitute suitable measures of system-oriented characteristics for natural tendencies of ecosystem development, and good ecological quality indicators. Such measures may then act as an adequate interface between modelling, where they are used in the scope of optimisation theory, and empirical ecology, where they are utilised as environmental indicators.

In the last fifteen years several algorithms have been proposed as possible goal functions. Exergy, a holistic concept derived from thermodynamics (Jørgensen and Mejer 1979, 1981), which can be seen as energy with a built in measure of quality, appears to be a promising approach. Actually, in shallow lake models, exergy exhibited a possible role in expressing shifts in species composition and trophic structure (Jørgensen 1988, Nielsen 1990, 1995).

Hypothetically, exergy is assumed to become optimised during ecosystem development. In other words, ecosystems are supposed to self-organise towards a state where this property is optimised (Jørgensen 1992). There are nevertheless theoretical and practical problems to be solved before this concept can be entirely accepted and used in models for the management of nature and as a holistic ecological quality indicator. Three major questions are empirically approached in this paper:

- What are the relationships between exergy and a more conventional ecological indicator like biodiversity?
- In practice, how can estimations of exergy values be made operational?
- To what extent are estimated exergy values abble to add useful information regarding the state of an ecosystem?

The point in analysing the relationships between exergy and biodiversity was that this latter concept, although somehow illusory, is powerful and intuitive, constituting a good available tool to test the ecological significance of goal functions when describing the ecosystem state. For instance, although there might be other factors involved, it is commonly accepted that, within a given ecosystem, polluted areas (e. g. eutrophied zones), will exhibit less complex communities, with a less complex trophic structure, and lower biodiversity than non polluted areas. Biodiversity may then be considered as an indicator of the quality state of the ecosystem. On the other hand, in what way could exergy, as a holistic ecological quality indicator, be effective in discerning between distinct states of the ecosystem when differences are relatively subtle? We empirically tested the following hypothesis: exergy and biodiversity will follow the same trends in space and time along an estuarine gradient of eutrophication. The chosen study site was the Mondego estuary, in the western Atlantic coast of Portugal (Fig. 1).

The present contribution was mostly based on two papers previously published (Marques et al. 1997, 1998).

Materials and Methods

The benthic communities in the Mondego estuary were monitored fortnightly, from February 1993 to February 1994. Samples of macrophytes, macroalgae, and associated macrofauna were collected fortnightly at different sites, during low tide. The organisms were almost always identified to the species level, and their biomass was determined ($g.m^{-2}$ afdw). Corresponding to each biological sample, the following environmental factors were determined: salinity ($g.l^{-1}$), temperature (°C), pH, dissolved oxygen, ammonia, silica, chlorophyll a, nitrites, nitrates, and phosphates ($mg.l^{-1}$), for the water, and organic matter contents ($g.m^{-2}$ afdw), for the sediments.

Sampling was performed along a gradient of eutrophication in the south arm of the estuary (Fig. 1), from a non-eutrophied zone up to a heavily eutrophied zone, in the inner areas of the estuary. A *Zostera noltii* community is present in the non-eutrophied zone and blooms of the green macroalgae *Enteromorpha* spp. have been observed during the last decade in the heavily eutrophied zone. The pattern in the most eutrophied zone is that, *Enteromorpha* spp. biomass normally increases from mid winter to early summer, when an algae crash usually occurs. A second but much less important algae biomass peak may be observed in late summer followed by a decrease in biomass through the fall season.

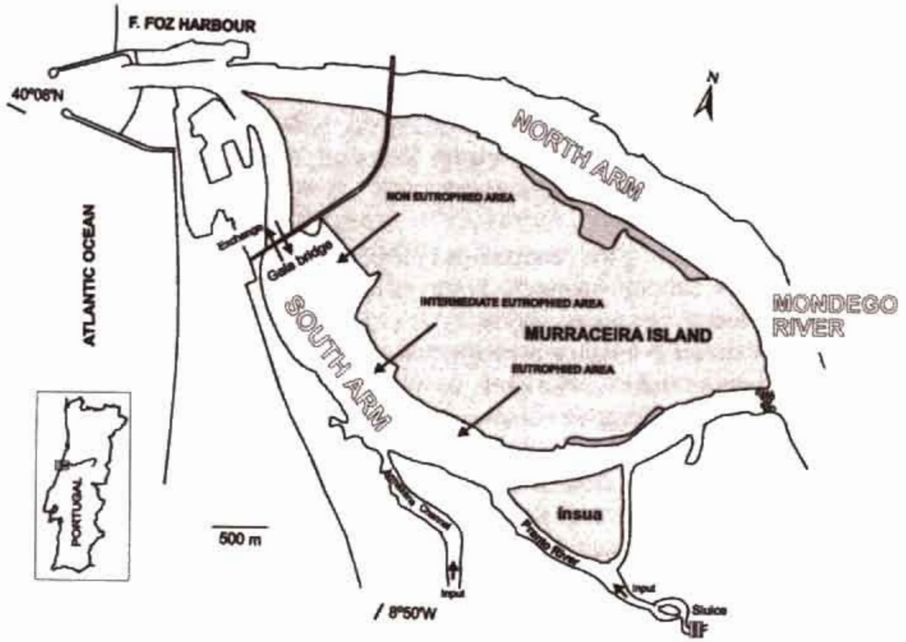

Figure 1. The Mondego estuary: It consists of two arms, north and south, with very different hydrographic characteristics. The southern arm of the estuary is eutrophied, and the gradient of eutrophication is indicated.

These measurements provided a comprehensive field data set on the spatial and temporal variation of benthic communities along the gradient of eutrophication, and was used to make exergy and biodiversity calculations.

To estimate exergy we used the method based upon the thermodynamic information due to genes proposed by Jørgensen et al. (1995). This method is considered the best candidate for exergy calculations of ecosystems, because it takes into account the organisational level of the organisms. Following this approach, an estimation of exergy may be given by

$$Ex = T\sum_{i=1}^{i=n} \beta i \cdot Ci \tag{1}$$

where T is the absolute temperature in Kelvin, Ci is the concentration of component i in the ecosystem (e. g. biomass of a given taxonomic or functional group), βi is a factor which roughly express the quantity of information embedded in the biomass. Detritus is chosen as the reference level, i.e. $\beta i = 1$, and exergy in the biomass of different types of organisms is expressed in detritus energy equivalents.

Consequently, the variation of exergy through time in an ecosystem may be caused from the variation of the biomass and information built in one unit of biomass (expressing the quality of the biomass)

$$\Delta Ex_{tot} = \Delta B_{iom}\, \beta i + \Delta \beta i\, \beta_{iom} \tag{2}$$

If the total biomass (Biomtot) in the system remains constant through time, then the variation of exergy (Ex_{tot}) will be a function of only the structural complexity of the biomass or, in other words, of the information embedded in the biomass. It may then be called specific exergy (SpEx), expressed as exergy per unit of biomass. For each instant, specific exergy is given by

$$SpEx = Ex_{tot} / Biom_{tot} \tag{3}$$

Values of exergy and specific exergy were calculated from the biomass of the different organisms ($g.m^{-2}$ afdw) through the use of weighting factors that are able to discriminate different "qualities" of biomass (Table 1). For this purpose, taking into account the available set of weighting factors, data on organisms biomass was pooled as a function of higher taxonomic levels (e. g. Phylum or Class).

There is nevertheless an obviously lack of discriminating power in the weighting factors given in table 1. The need for more discriminating weighting factors have in fact been discussed (Marques et al. 1997) and new methodologies have been proposed to allow an easier estimation of weighting factors for different types of organisms (Marques et al. 1997, Fonseca et al. 2000). This involves nevertheless difficulties that are not fully understood and overstepped (Fonseca et al. 2000).

Table 1. The evolution of g DNA/cell, number of genes, and number of cell types (approximate figures are given) for different organisms. The concentration of each organism was multiplied by the proposed weighting factor to estimate exergy. The weighting factor accounts for the information embodied in the organism in addition to the simple biomass (g m^{-2} afdw). For this purpose, it is assumed that detritus (organic matter contents in sediments) does not contain relevant structural information. Sources: Li and Grauer (1991) *in* Jorgensen et al. (1995). All the values marked with * were not provided by any source but assumed as reasonable at the present state of knowledge, taking into account the evolutionary level of the groups concerned.

Organisms	10-12 g DNA/cell	Number of genes	Number of cell types	Weighing factor
Detritus	0	0	0	1
Bacteria	0.005	600	1 - 2	2.7
Algae	0.009	850	6 - 8	3.4
Yeast	0.02	2000	5 - 7	5.8
Fungus	0.03	3000	6 - 7	9.5
Sponges	0.1	9000	12 - 15	26.7
Plants, trees	-	10000 - 30000	-	30 to 90 *
Jellyfish	0.9	50000	23	144
Nemertineans	-	-	-	144*
Insects	-	-	-	144*
Crustaceans	-	-	-	144*
Annelid worms	20	100000	60	287
Molluscs	-	-	-	287*
Echinoderms	-	-	-	144*
Fish	20	100000 - 120000	70	287 - 344
Birds	-	120000	-	344
Amphibians	-	120000	-	344
Reptiles	-	120000	-	344
Mammals	50	140000	100	402
Human	90	250000	254	716

Regarding biodiversity, we took into consideration the species richness and the heterogeneity (species richness + eveness). For each date measurements were calculated using data on the species biomass ($g.m^{-2}$ afdw). For this purpose only macrofauna was taken into account. From the considerable assortment of indices designed by ecologists, we considered suitable the use of the Margalef index (I), to compute species richness, and of Shannon-Wiener's index (H') based on the information theory, to compute heterogeneity (Legendre and Legendre 1984, Magurran 1988). The Margalef index is:

$$I = (n-1) / \log_e N \qquad (4)$$

where n is the number of species found and N is the total number of individuals, and the Shannon-Wiener index is given by:

$$H' = \sum_{i=1}^{n} p_i \log p_i \qquad (5)$$

where n is the number of species, and pi is the proportion of the biomass of species i in a community were the species proportions are $p_1, p_2, p_3, ...p_i, ...p_n$.

Exergy and biodiversity values constitute static estimations of dynamic qualities of ecosystems. Therefore, to examine their properties as ecological indicators, a moving average (using the contiguously prior, present, and following dates) was applied to each value, in order to adjust potential bias due to sampling.

Results - Testing the Working Hypothesis

The spatial and temporal variation of exergy, specific exergy, species richness, and heterogeneity were analysed, to test the hypothesis that these ecological indicators would capture changes in benthic communities in such a way that they would provide equivalent information about the ecosystem.

Exergy (Fig. 2 A), as hypothesised, was found to be consistently higher in the *Zostera noltii* community than in the eutrophied areas. Additionally, during the spring and early summer of 1993, exergy values were higher in the most heavily eutrophied area when compared with the intermediate eutrophied area. This was obviously related with the intensity of the *Enteromorpha* bloom, determining much higher values for total biomass in the most eutrophied area (Fig. 2 C).

Specific exergy was also consistently higher in the *Zostera noltii* community than in the eutrophied areas until late spring (Fig. 2 B), in accordance with the working hypothesis. However, the picture changed completely from early summer, when values became higher in the eutrophied areas. This was obviously a function of the macroalgae crash in the eutrophied areas (between the 22 of June and the 6 of July 1993), which resulted not only on a drastic reduction of the total biomass (Fig. 2 C), but also on a change from a primary production based system toward a detritus based food web. Since total biomass after the 6 of July consisted essentially of animals (consumers), primarily deposit feeders and detritic feeders (e. g. annelid worms and crustaceans), it is clear that the abrupt increase of specific exergy in the eutrophied areas after the algae crash does not reflect an augmentation of the structural complexity of the community, but simply the different quality of the biomass involved in the calculations. This becomes evident if we compare the specific exergy estimated for the non eutrophied area (*Zostera* community), taking only the macrofauna into account, with the values found for eutrophied areas (Fig. 2 B). In fact, after the 6 of July, if we account for both primary producers and consumers, specific exergy is lower in the *Zostera* community than in the eutrophied areas. But if we account only for the consumers the specific exergy is clearly higher in the *Zostera* community, following the same pattern from before the algae crash, in agreement with the working hypothesis. We must conclude that the specific exergy may shift very drastically as a function of annual dynamics, like in communities dominated by r strategists, providing spatial and temporal information that may not be related with the long-term evolution and integrity of the system.

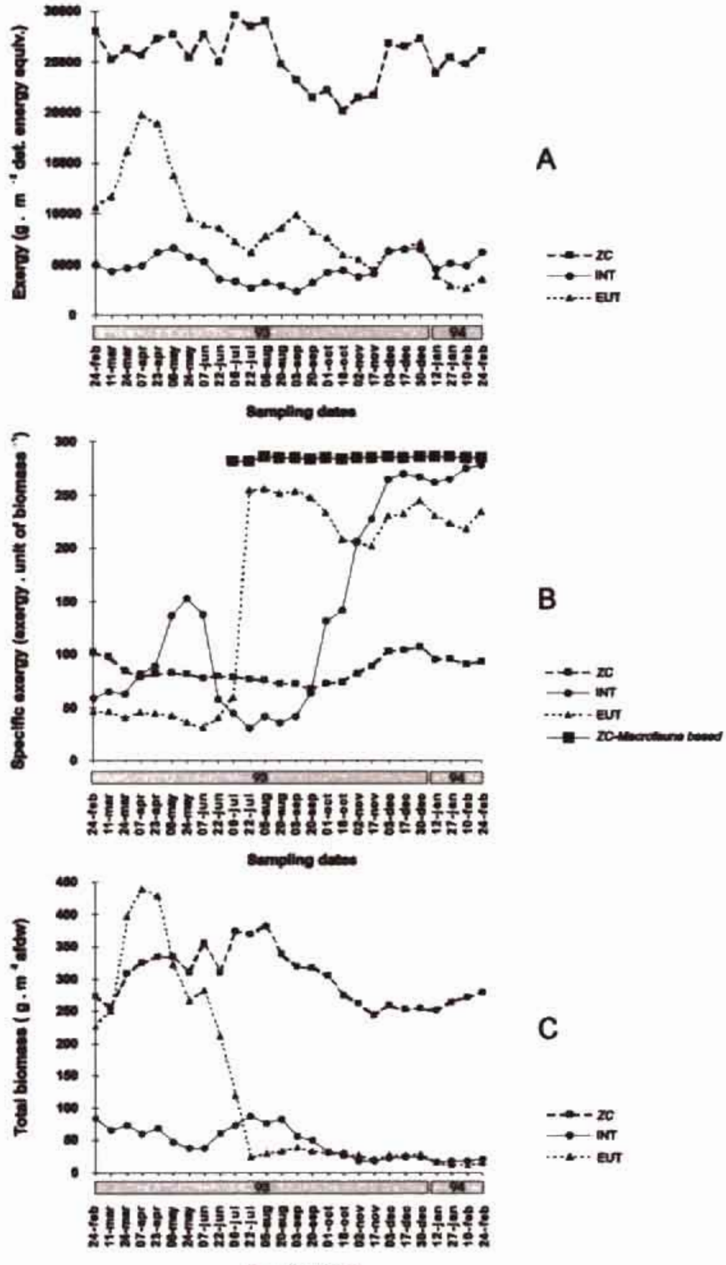

Figure 2. Spatial and temporal variation of exergy (A) specific exergy (B), and total biomass (C) in non eutrophied (*Zostera noltii* community) (ZC), intermediate eutrophied (INT) and eutrophied (EUT) areas. For specific exergy (B), we indicate the values estimated from the total biomass, taking into account the whole period, and the values estimated only from the macrofaunal biomass, for the period from 6 of July 1993 to 24 February 1994.

The variation of species richness and heterogeneity (species richness + eveness) along the gradient of eutrophication provided quite different information (Fig. 3). Through time, as hypothesised, species richness was consistently higher in the non-eutrophied area, corresponding to the *Zostera* community, decreasing along the gradient of eutrophication (Fig. 3 A). On the contrary, heterogeneity was always higher in the eutrophied areas (Fig. 3 B). The only exception was the decrease observed in the most heavily eutrophied area in early summer, which was related with the algae crash causing a drastic reduction of total biomass (Fig. 2 C).

The observed spatial variation of heterogeneity was not in agreement with the working hypothesis. This originates in the fact that the Shannon-Wiener's index integrates two components, the number of species (species richness) and their relative abundances (evenness). As expected, species richness decreased as a function of increasing eutrophication, but the extremely high concentration (dominance) of a few species in the *Zostera* community, namely *Hydrobia ulvae*, a detritic feeder and epiphytic grazer gastropod, and *Cerastoderma edule*, a filter feeder bivalve, decreased species eveness, and consequently heterogeneity values. In this case, lower values of heterogeneity must be interpreted as expressing higher biological activity of these species, probably due to the abundance of nutritional resources in the *Zostera* community, and not as a result of environmental stress (Legendre and Legendre 1984).

To what extent did exergy and biodiversity follow the same trends in space and time, as hypothesised, decreasing from non-eutrophied to eutrophied areas? Taking into account the whole year data set, the variation of exergy and specific exergy along the eutrophication gradient (non eutrophied, intermediate eutrophied, and eutrophied areas) was significantly correlated ($P \leq 0.05$) (Fig. 4 A), providing an equivalent information from the system. Values were consistently higher and more stable in the non-eutrophied area as compared to the eutrophied ones. Moreover, through the comparison of yearly exergy data series for each site (t test, $P \leq 0.05$) it was possible to distinguish between the three situations. However, for specific exergy, differences between the intermediate eutrophied and eutrophied areas were not significant, which suggests that exergy might be more sensitive to detect subtle differences.

Species richness and exergy appeared significantly correlated ($P \leq 0.05$), following a similar spatial pattern, both decreasing from non-eutrophied to eutrophied areas (Fig. 4 B). On the contrary, heterogeneity and exergy appeared to be negatively, although not significantly correlated (Fig. 4 A), providing totally diverse information of the benthic communities along the eutrophication gradient. As explained above, this resulted from the properties of the heterogeneity measure.

Different results were obtained comparing the patterns of variation of species richness, heterogeneity and specific exergy. Species richness and specific exergy appeared clearly positively correlated ($P \leq 0.05$) (Fig. 4 B), while the patterns of variation of heterogeneity and specific exergy were distinct (Fig. 4 A). Moreover, from the comparison of yearly data series (t test, $P \leq 0.05$), heterogeneity values were not significantly different between the intermediate eutrophied and eutrophied areas, and therefore did not permit to discriminate the existing differences.

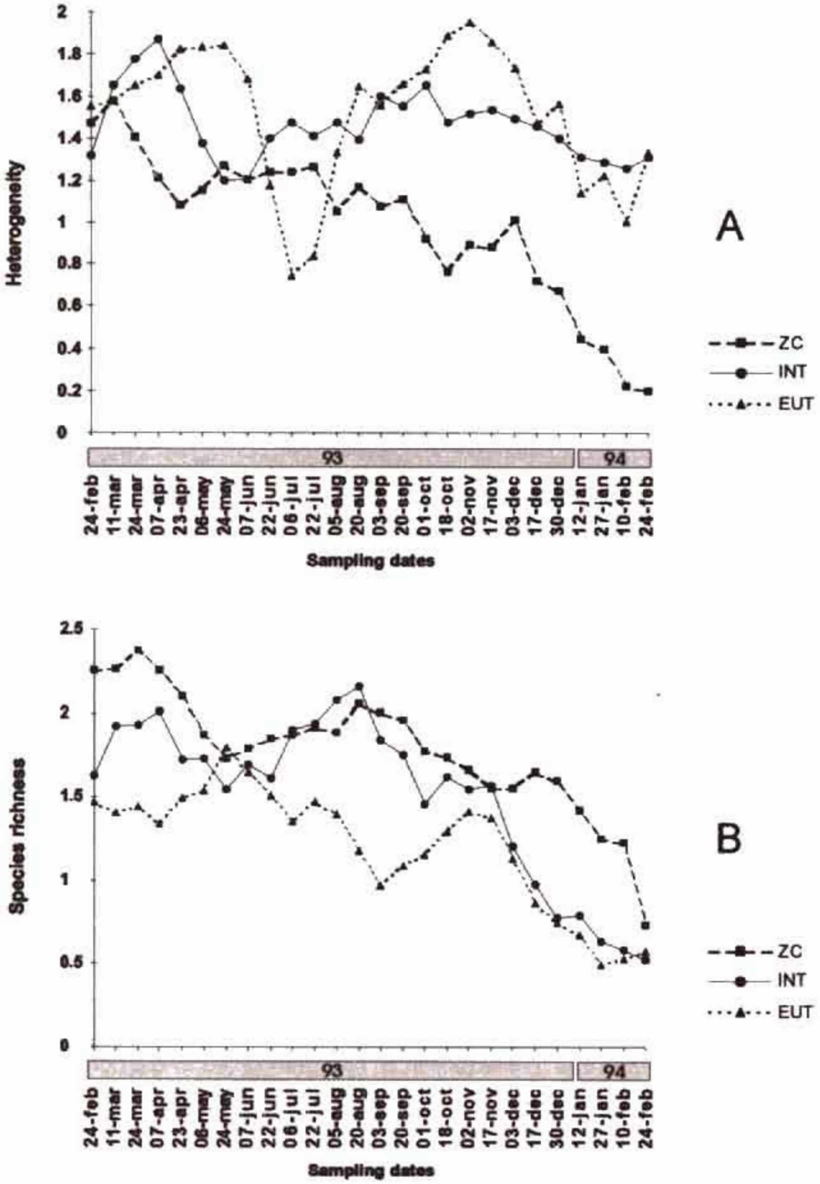

Figure 3. Spatial and temporal variation of heterogeneity (A), species richness (B) in non eutrophied (ZC), intermediate eutrophied (INC) and eutrophied (EUT) areas. Heterogeneity was computed using the Shannon-Wiener's index, and species richness using the Margalef's index.

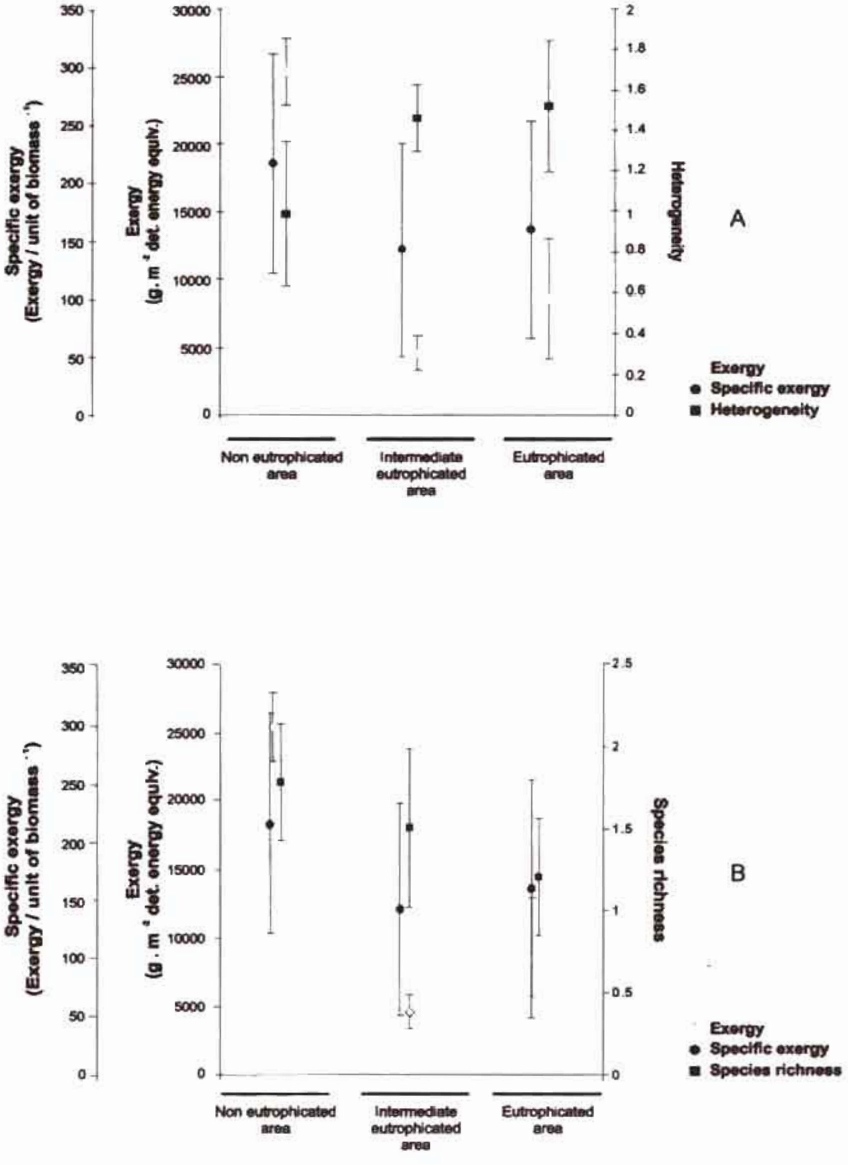

Figure 4. Variation of exergy and specific exergy in comparison with heterogeneity (A) and species richness (B) along the gradient of eutrophication gradient. For each situation, respectively non eutrophied (ZC), intermediate eutrophied (INT), and eutrophied (EUT), we indicate the average values and the standard deviation, taking into account the entire yearly data set. The spatial variation of exergy and specific exergy was significantly correlated ($r = 0.59$; $P \leq 0.05$). The spatial variation of heterogeneity was not significantly correlated neither with exergy or specific exergy ($r = -0.48$ and $r = 0.38$ respectively; $P \leq 0.05$). The spatial variation of species richness was significantly correlated with both exergy and specific exergy ($r = 0.60$ and $r = 0.90$ respectively; $P \leq 0.05$).

Discussion and Conclusions

The hypothesis that exergy and biodiversity would follow the same trends in space and time was validated with regard to species richness, but not for heterogeneity. In fact, exergy, specific exergy, and species richness responded as hypothesised, decreasing from non-eutrophied to eutrophied areas, but heterogeneity responded in the opposite way, showing the lowest values in the non-eutrophied area. On the other hand, exergy and species richness were able to grade situations presenting relatively subtle differences, but specific exergy and heterogeneity appeared to be less sensitive. Moreover, as ecological indicator, heterogeneity (measured using the Shannon-Wiener's index) appeared to be a more subjective interpretation. Exergy and specific exergy appeared to be able to provide useful information regarding the state of the benthic communities, and might therefore constitute suitable system-oriented characteristics, and may be good indicators of ecosystem integrity. Since specific exergy may shift drastically as a function of annual dynamics, it provides spatial and temporal information, which is different from static exergy measures. Therefore, we recommend using exergy and specific exergy as complementary parameters.

The method proposed by Jørgensen et al. (1995) to estimate exergy, which takes into account both biomass and the thermodynamic information due to genes, appears to be operational, but more accurate (discrete) weighting factors to estimate exergy from organisms biomass need to be determined (Table 1). Moreover, using the number of genes to express the thermodynamic information built in the biomass is not practical because genetic mapping available in published data is very scarce. Actually, most of the organisms in biotic systems have not been characterised owing to the long time procedures and high costs involved in the molecular work of gene analysis. Therefore, it was proposed to undertake a more practical approach (Marques et al. 1997, 1998, Fonseca et al. 2000).

The dimension of the active genome is primarily a function of the required genetic information to build up an organism. Since this genetic information is contained in DNA, it is reasonable to assume that the dimension of each active genome is roughly proportional to the contents of DNA in the nucleus of diploid cells of each organism. These contents may be determined through the isolation, purification, and analysis of cell nucleus from representative organisms. Obviously, in a certain extent, data produced will not be so accurate as data provided by genetic analysis, but this will not be a problem since the aim is to generate conceptual exergistic relationships between different kinds of organisms. In this case, their complexity will be accounted as an indirect measure of the quality (thermodynamic information) of the biomass, proportional to the distance of that matter to thermodynamic equilibrium.

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APPLIED RESEARCH IN THE MONDEGO ESTUARY – EXPLORING THE USE OF DNA CONTENT OF ORGANISMS TO ESTIMATE ECOLOGICAL EXERGY

Abstract

The Mondego estuary is an extensively studied (eco)system. Studies developed in the last years provided researchers with a large compilation of data covering both the physical-chemical qualities of the system and the biology of the autochthonous fauna and flora. This database and the 'accessibility' to the estuary incite the use of this system as a 'field laboratory', allowing the study under 'natural' conditions and improved hypothesis validation. Concerning the evaluation of ecosystems state of development, we have studied the use of nuclear DNA contents of organisms (C-values) as a more practical approach to the ecological estimation of exergy (Jørgensen et al. 1995), according to the proposal by Marques et al. (1997). Methodologies for the estimation of C-values from organisms are presented. We exemplify the use of this methodology with estuarine organisms to obtain weighing factors (b) that may estimate the exergy content per unit of biomass. The applicability of this methodology in the determination of exergy estimates from organisms (biomass) is discussed, both in theoretical and practical aspects.

Introduction

The Mondego estuary has been extensively studied in the last two decades, providing a wide embracing comprehension of this system. Researchers have assessed estuarine physical and chemical characteristics and several studies were devoted to biological processes and ecological relations concerning the local estuarine fauna and flora (Flindt et al. 1999, Lillebø et al. 1999, Lopes et al. 2000, Pardal et al. 2000, Martins

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et al. 2001, Duarte et al. 2001). Present work is being developed to complement the acquired knowledge about this ecosystem, but providing insights into new questions, also (Marques et al. 1997). From this continuous process of research, has came out an conceptual framework allowing to work in this estuary as if it was a "field laboratory", where studies can be developed and theoretical hypothesis tested under 'natural' conditions. These working conditions permit to develop 'laboratorial' (field) work that, although under a reductionist practice, foresees the development of methodologies with broad application. In this scope, we have been developing a methodology to obtain estimates for the exergy associated to a (eco)system from its qualities, in view of characterising system's state of development.

Ecological exergy is a concept derived from thermodynamics as a function expressing the built-in measure of quality for energy, and a potential ecological indicator of ecosystem state of development (Jørgensen and Mejer 1977, 1979, 1981, Jørgensen 1992a, Jørgensen et al. 1995). Exergy is interpreted as an estimate of the maximum capacity of energy to perform useful work as the system proceeds to equilibrium with its surroundings (Brzustowski and Golem 1978, Ahern 1980. Quoted from: Schneider and Kay 1995). The ecological exergy does not correspond exactly to the thermomechanical availability/exergy functions - the work potential of a system at a certain state relatively to the state of equilibrium with the environment (dead state). Instead, it is an operative system interpretation reflecting the quality corresponding to a certain quantity of energy, and can be understood as a measure of contrast or distance from the system to the thermodynamic equilibrium (Schneider and Kay 1994, Jørgensen and Nielsen 1998a,b).

As ecosystems evolve in response to external changes (forcing functions), alterations occur as structural qualitative changes and at the energy flows through the system. As result alterations in the energy and matter conversion processes are verified (Bendoricchio and Jørgensen 1997, Marques et al. 1997, Zhou et al. 1996). Ecosystems are highly complex dynamic systems that may be seen as self-organising 'Prigoginean' systems (Prigogine 1980). In this context, several ecoenergetic considerations may be drawn, regarding its development: (a) ecosystems use high quality energy as 'fuel' in its metabolic processes to convert matter and energy (Schrödinger 1944); (b) the energy flows through the system are used to maintain its functioning and to build up new structures; while (c) systems deviate from thermodynamic equilibrium, (d) low quality energy is returned by the system, and (e) entropy in the surroundings increase (Wall 1996, Jørgensen and Nielsen 1998a). Therefore, as ecosystems develop it may be considered that (see: Bass1998): (a) its living components are selected according to the pressure imposed by evolutionary competitions ('newdarwinean' processes) and energetic imperatives, (b) it's moved away from thermodynamic equilibrium by the work done on it. Hence, its departure from the thermodynamic equilibrium can be associated to its state of development, in terms of 'potential-work'. Furthermore, energetic assessments of certain ecosystems pointed out an increasing energy 'degradation' with more mature or less perturbed ecosystems, while a decreased ability to dissipate incoming energy of stressed ecosystems (Schneider and Kay 1995). Thus, ecosystems are expected to evolve towards a state of 'optimal exergy configuration'

(Jørgensen 1992b,c) and to improve its ability to withdraw the exergy content of energy (Schneider and Kay 1994a,b). Therefore, it has been suggested that changes of exergy may express alterations in ecosystems structure or functioning, and may be applied in studies as a suitable system-oriented indicator of ecosystem states of development and health (Jørgensen and Mejer 1981, Jørgensen 1988, 1992a,b,c, Jørgensen et al. 1995, Nielsen 1990, 1994, 1995, Fuliu 1997, Marques et al. 1997, 1998, Müller 1997, Jørgensen and Nielsen 1998a,b, Patten 1998).

With reasonable approximations exergy can be computed as (Jørgensen et al. 1998a):

$$Ex/_{R \cdot T} \approx \sum \beta_i \cdot c_i \qquad \text{(Equation 1)}$$

where ci is the biomass concentration of the i-*th* species and ßi is a parameter weighing the relative amount of exergy per unit of biomass, expressing the 'quantity of information' embedded in the biomass. This approximate calculation of exergy is processed in terms of a global sum over all components of an ecosystem, where for each term it is considered the relative concentration of the corresponding component and its 'distance' from a common reference state - detritus or organic dead matter. Since detritus is assumed as a reference level (ß = 1) the different weighing factors are determined in terms of the probability, for each component, of producing organic matter (detritus) and the probability of 'selecting' its 'genetic information', regarding the ß parameter as a discriminator of the organisational level of organisms to that reference level (see: Jørgensen et al. 1995). These ('probabilistic') considerations take into account organisms gene number in order to determine the different conversion factors (ß). Since these data is not available for most species, for many cases rough estimates will result from grossly approximate figures for the number of genes. Therefore, Marques et al. (1997) suggested the use of nuclear DNA content of organisms (C-values) in the determination of the ß parameters, in view of a more efficient approach for the estimation of ecological exergy from organism biomass.

In this work we report C-values for several organisms from different groups, and present putative values of ß calculated from these data. Moreover, the use of nuclei DNA contents of organisms as an approach to estimate ecological exergy is discussed in theoretical and operative aspects, regarding its eventual practice in ecological 'exergetic' studies.

Methodologies and results

The expression used to obtain estimates of ecological exergy:

Jørgensen et al. (1995) developed probabilistic calculations for the approximate estimation of ecological exergy, for each component (Pi), in terms of the probability (P1) of producing organic matter (detritus) and the probability of 'selecting' the 'genetic information', for that component, assuming detritus as a common reference state. For detritus (i = 1), calculations were developed considering the thermodynamic expression for the chemical potential (Jørgensen et al. 1995), such as:

$$c_i^{eq} = c_1 \cdot e^{-(\mu_1 - \mu_1^{eq}) \cdot RT} \text{ and } P_1 \approx (c_1 / c_i^{eq}) \cdot e^{-(\mu_1 - \mu_1^{eq}) \cdot RT}$$

On the other hand, for the living components (i > 1) the probabilities were obtained from the number of possible permutations of 20 amino-acids relatively to a genome of g genes – note that it's assumed that organisms use an universal code of 20 amino-acids:

$$P_i = P_1 \cdot P_{i,a} \text{ and } P_{i,a} = 20^{-700 \cdot g}, \ i > 1$$

where g represents the number of genes of an organism and 700 is assumed as the mean value of amino-acids encoded per gene. Subsequently, by means of thermodynamic formulations Jørgensen et al. (1995) developed a function that permits the estimation of an ecological 'index' of exergy as:

$$Ex / R \cdot T = (\mu_1 - \mu_1^{eq}) \cdot \sum_{i=1}^{N} c_i / R \cdot T - \sum_{i=2}^{N} c_i \ln P_{i,a}$$

where R is the gas constant, T the absolute temperature ci the concentration in the ecosystem of the i-th component and 700·g stands for an average value for the number of encoded amino-acids in the genome of species i.

Determination of nuclei DNA content of organisms:

The nuclei DNA content of organisms (i. e. the quantity of DNA per cell nucleus) is a characteristic value for each species, referred to as C-value or 2C-value, regarding to the haploid or diploid genome, respectively. The determination of C-values is easily achieved following available laboratory methodologies such as flow cytometry (FCM) (Shapiro 1995, Fonseca 1999). Concerning the estimation of this quantity for a new sample, quantification is performed by reference to nuclei internal standards (usually chicken red blood cell; CRBC 2C = 2.33 pg), which permits to calibrate the data in terms of absolute DNA units (e.g. pg or base-pairs) (Rayburn 1993). Table 1 reports values obtained for the nuclei DNA content of several organisms of different groups, following FCM methodologies, assuming that: C-value ≅ (2C-value)/2

Organisms	DNA content (pg) C-value
Annelida	
Polychaeta	
Spionida	
Spionidae	
Prionospio malmgreni	0.55
Capitellidae	
Notomastus latericeus	1.32
Phyllodocida	
Phyllodocidae	
Nereiphylla paretti	2.7
Hesionidae	
Ophiodromus obscurus	1.6
Ophiodromus culveri	0.35
Kefersteinia sp.	0.22
Hesiospina sp.	0.53
Nereididae	
Platynereis dumerilii	1.0
Laeonereis culveri	0.8
Nereis succinea	2.2
Nereis diversicolor	2.3
Neanthes caudata	2.25
Nephtydae	
Nephtys incisa	7.2
Nephtys sp.	2.2
Glyceridae	
Glycera americana	3.5
Glycera lapidum	1.46
Dinophilida	
Dinophilidae	
Dinophilus gyrociliatus	0.07
Eunicida	
Onuphidae	
Onuphis eremita oculata	1.7
Onuphis sp.	2.0
Diopatra cuprea cuprea	2.0
Americonuphis magna	1.2
Lumbrineridae	
Lumbrineris tenuis	2.4
Ninoe nigripes	5.3
Terebellida	
Pectinariidae	
Pectinaria gouldii	1.3
Sabellida	
Sabellidae	
Amphiglena mediterranea	0.39
Branchiomma luctuosum	1.2
Branchiomma crispum	1.3
Myxicola infundibulum	1.6
Sabella spallanzanii	0.65

Echinodermata	
Asteroidea	
Forcipulata	
Asteridae	
Marthasterias glacialis	0.6
Echinoidea	
Diadematoida	
Arbaciidae	
Arbacia lixula	0.6
Mollusca	
Gastropoda	
Archeogastropoda	
Pattelidae	
Pattela sp.	0.8
Trochidae	
Gibbula umbilicallis	1.1
Mesogastropoda	
Hidrobiidae	
Hydrobia ulvae	0.68
Littorinidae	
Littorina littorea	1.1
Bivalvia	
Veneroida	
Cardiidae	
Cerastoderma edule	1.36
Scrobiculariidae	
Scrobicularia plana	1.6
Veneridae	
Ruditapes decussata	1.81
Venerupis pullastra	1.78
Maetridae	
Spisula solidissima	1.16
Mytiloida	
Mytilidae	
Mytilus galloprovincialis	6.92
Ostreida	
Ostreidae	
Ostrea edulis	1.16
Pterioida	
Pectinidae	
Pecten maximus	1.42
Chlamys opercularis	1.16
Arthropoda	
Crustacea	
Maxillopoda	
Thoracica	
Pollicipedidae	
Pollicipes pollicipes	0.35

Amphipoda	
Gammaridae	
Echinogammarus marinus	4.9
Isopoda	
Cyathura carinata	1.4
Spharomatidae	
Sphaeroma hookeri	3.1
Decapoda	
Crangonidae	
Crangon crangon	4.2
Portunidae	
Carcinus maena	4.5
Insecta	
Diptera	
Chironumus sp.	0.35
Pisces	
Salmo gairdneri irideus	2.5
Oncorhynchus mykiss	2.49
Cyprinus carpio	1.94
Lampreia planeri	1.73

Approximate figures are given. Sources: Cavalier-Smith 1978, Li and Graur 1991, Gambi et al. 1997, Fonseca 1999. (1 pg = 0.98 × 109 bp). CRBC (2C = 2.33 pg).

Use of nuclei DNA content of organisms in the determination of b parameters:

To use C-values in the determination of the ßi parameter, regarding to the C-value paradox (Cavalier-Smith 1978), it is necessary to work with the minimum genome size (lowest C-value) for each group of organisms (e.g. taxonomic or functional) instead of the C-value for each specie in the group (Fonseca 1999). Subsequently, as a topmost limit the DNA data (minimum genome sizes) may be considered as an approximate (although rough) of the overall 'coding capacity' of the genome and used in the evaluation of b parameters, accordingly to both Jørgensen et al. (1995) and Marques et al. (1997) proposals. This implies that these (C-)values are used replacing the corresponding term for 'genome dimension' (700 g) of Jørgensen et al. (1995) proposal (Fonseca 1999).

As an example, the estimation of b for the biomass of the annelid can be worked as follows:

find, in Table 2, the lowest C-value for the group Annelids:

☞ 0.07 pg

☞

convert to base-pairs (bp) of nucleotides using the relation: $1 \text{ pg} = 0.98 \times 10^9$ bp

☞ $C^* = 6.86 \times 10^7$ bp

☞

Since only one polynucleotide chain is considered ('single strand DNA'), we have:

☞ $C^{**} = (C^{*} / 2) = 3.43 \times 10^{7}$ nucleotides

☞

Following the work hypothesis, the obtained number of nucleotides is considered as a topmost limit for the 'maximum coding capacity'. Since the DNA code is (indirectly) transcribed into an amino-acid code, the number of nucleotides is converted to total nucleotides triplets (thus, the topmost limit for the number of triplets):

☞ $C^{***} = (C^{**} / 3) = (3.43 \times 10^{7}) / 3 = 1.14 \times 10^{7}$ triplets

☞

This result (C^{***}) is used replacing "700 g" in the estimation of the expression 'ln P_{ia}' from Jørgensen et al. (1995) proposal:

☞ $\ln P_{ia} = \ln 20^{\,C^{***}} = 3.42 \times 10^{7}$

Subsequently, this value is used to obtain estimates from the expression for Ex/RT derived by Jørgensen et al. (1995).

Following the calculations according to the proposal of Jørgensen et al. (1995), it is assumed an average molecular weight for detritus of 100 000, and the free energy released per g of organic matter is ca. 18.5 kJ/mol. Taking T = 300 K and R ≈ 8.3 J / mol K, it is possible to estimate the corresponding 'index' of ecological exergy expressed in terms of 'g of detritus exergy equivalents', accordingly to the proposal of Jørgensen et al. (1995). This means (operatively) that the obtained value is divided by 7.43×10^{5}, assumed as the contribution of detritus in terms of g/L:

$$Ex / RT \cong \ldots c_{\mathrm{Annelids}} \times (3.42 \times 10^{7}) + \ldots + (c_{\mathrm{Detritus}} + \ldots + c_{\mathrm{Annelids}} + \ldots) \times (7.43 \times 10^{5})$$

(exergy 'equivalents', g/L)

$$Ex / RT \cong \ldots c_{\mathrm{Annelids}} \times (50) + \ldots + c_{\mathrm{Detritus}}$$

(g of detritus exergy 'equivalents', g Detritus/L)

Therefore, the contribution to the ecological exergy 'index' from an organism of the considered group (Annelids) can be calculated as:

$$Ex_{\mathrm{Annelids}} / RT \cong \ldots c_{\mathrm{Annelids}} \times (50)$$

thus, $\beta_{\mathrm{Annelids}} \cong 50$

where c_{Annelids} represents the biomass of the organism (i. e., its concentration in the ecosystem in g/L) and $\beta_{\mathrm{Annelids}}$ is the corresponding weighing factor for the group Annelids.

Table 2 lists the lowest values for the haploid DNA content in several groups of organisms, and estimates for ß, obtained according to Jørgensen et al. (1995) and based in C-values.

Table 2. Typical values for biological parameters (number of genes and cell types) and for the weighing factor (ß*) to estimate exergy from organisms biomass according to Jørgensen et al. (1995), for different groups of organisms. It is presented, also, the lowest C-values in these groups of organisms and the corresponding weighing factors (ß**) accordingly to the methodologies presented in this work.

Organisms	No. genesa	No. cell Typesa	ß* C-value	Lowest	ß**
Detritus	0	----	$1^{a,b}$	----	1
Bacteria	600	1-2	$3^{a,b}$	0.0017^{c}	2
Algae	850	6-8	$3-4^{a,b}$	0.04^{c}	25
Fungi	3000	6-7	$10^{a,b}$	0.005^{c}	3
Annelids	10500; 100000^{b}	60	35^{a}; 287^{b}	0.07^{d}	50
Arthropods	----	----	----	0.1^{c}	70
Insects	10000-15000	----	$30-46^{a}$; 144^{b}	0.1^{c}	70
Crustaceans	----	----	144^{b}	0.35^{e}	230
Molluscs	----	----	287^{b}	0.68^{e}	450
Gastropods	----	----	----	0.68^{e}	450
Bivalves	----	----	----	1.16^{e}	760
Echinoderms	----	----	144^{b}	0.54^{e}	360
Chordates	----	----	----	0.20^{c}	130
Fish	100000-120000	70	$287-370^{a,b}$	0.39^{c}	260
Amphibians	120000	----	344^{b}; 370^{a}	1.2^{c}	800
Reptiles	130000	----	344^{b}; 400^{a}	1.5^{c}	1000
Birds	120000	----	344^{a}; 390^{b}	1.7^{c}	1100
Mammals	140000	100	402^{b}; 430^{a}	3.0^{c}	2000
H. sapiens	~ 30000^{f}	----	----	2.0^{e}	1300

[a] values provided in Jørgensen et al. 1995, 1998.

[b] figures presented by Marques et al. 1997; c figures representing the lowest values in the group according to Cavalier-Smith 1978; d values from Gambi et al. 1997; e Levin, 1994. f Lander et al, 2001

Discussion

The ecological exergy is an operative interpretation of the thermomechanical availability functions (i. e., the work potential of a system at a certain state relatively to the state of equilibrium with the environment - dead state), proportional to the available energy invested by ecosystem in building up its 'structure' (information and mass). Several methodologies to estimate ecological exergy have been developed on the basis of thermodynamic principles (Jørgensen and Mejer 1979, 1981, Shieh and Fan 1982, Jørgensen et al. 1995, Zhou et al. 1996, Marques et al. 1997, 1998), stressing the relevance of these principles in the assessment of ecosystems state of development. Ecosystem structure and energy-matter balance will evolve to a state of optimal thermodynamic balance, although conditioned by the prevailing environmental parameters (Marques et al. 1998). Therefore, ecosystems are expected to evolve optimising the storage of the available energy (Jørgensen 1992b,c, Marques et al. 1998) and increasing its dissipation to maintain the acquired levels of biomass and (higher) complexity, during development (Schneider and Kay 1994a,b, 1995).

According to Jørgensen et al. (1995) the estimation of the contribution of ecological exergy from organisms (biomass) is based upon corresponding weighing factors (b) for different organisms. These authors suggested to estimate the b parameter using the number of encoded amino-acids (700·g), assuming different number of genes (g), for each organism, and that each gene codes for an average number of 700 amino-acids. These b parameters envisage weighing the contribution to the ecological exergy of the 'genetic information', but the data for the number of genes required for its determination (see Table 2), at the present, are very unreliable.

On the other hand, Marques et al. (1997) proposes the estimation of the total amount of DNA per cell nucleus (C-value) assuming this value as an approximate "estimate" for the "information content" of the genome, accounting for organisms structural 'complexity'. Table 1 illustrates two important features regarding the nature of these data: (a) C-values may vary widely in closely related species (MacGregor 1982, Gold et al. 1992, Gambi et al. 1997), and (b) some organisms may exhibit larger C-values than mammals although less morphologically "complex". Thus, for higher eukaryotes, it is evident the lack of correlation between structural "complexity" and total DNA content, at the species level (Cavalier-Smith 1978, 1985), reinforcing the concept of the "C-value paradox" (see: Futuyma 1998). This feature is essentially consequence of the repetitive (noncoding) DNA sequences in eukaryotic genome, in some organisms accounting for more than 50% of the total genome (John and Miklos 1988). Therefore, instead of calculating weighing factors, ßi, for each species from the corresponding C-values, it is preferable to use the lowest (known) C-value for different groups of organisms (Table 2).

Following this procedure the biomass of organisms from different groups is "weighed" (in terms of exergy content) according to the assumed level of complexity for each group. This way, the C-value for each group is considered as an estimate (although rough) of the overall coding capacity of the genome, and it should be understood as a topmost limit value for the "information content" of genomes from the considered group. The total DNA may affect biological events, from cell size and division, to ecological effects. Higher C-values are frequently associated with species having slower development (Bennet 1982, Rees et al. 1982, Sessions and Larson 1987), and closely related organisms may reach similar dimensions with different number of cells (MacGregor 1982). Nevertheless, although the suggested values relate to biological parameters (C-values), which were selected during the evolution processes, regarding what is focused, it should be used with great attention to the implications of the 'C-value paradox'.

Different figures for the ß parameter were estimated from the Jørgensen et al. (1995) and Marques et al. (1997) proposals (Table 2). Clearly, the estimation of the "information content" of genomes of organisms may be largely biased, from both the assumed number of genes (Jørgensen et al. 1995) and the total nuclear DNA contents (Marques et al. 1997), as discussed. However, before any definite conclusions regarding the merit of these approaches, comparative studies are required, along with other ecological indicators (e.g. diversity indices, ascendancy, emergy) aiming to assess the efficiency in capturing any additional information regarding ecosystems health and integrity. As well, it is important to stress that the nonrepetitive DNA fraction of genome relates better to the complexity of organisms (see: Levin 1994). Thus, the development of methodologies to estimate the nonrepetitive DNA content of genome, such as the technique of reassociation kinetics, should be attempt in view to replace the FCM estimation of total nuclear DNA.

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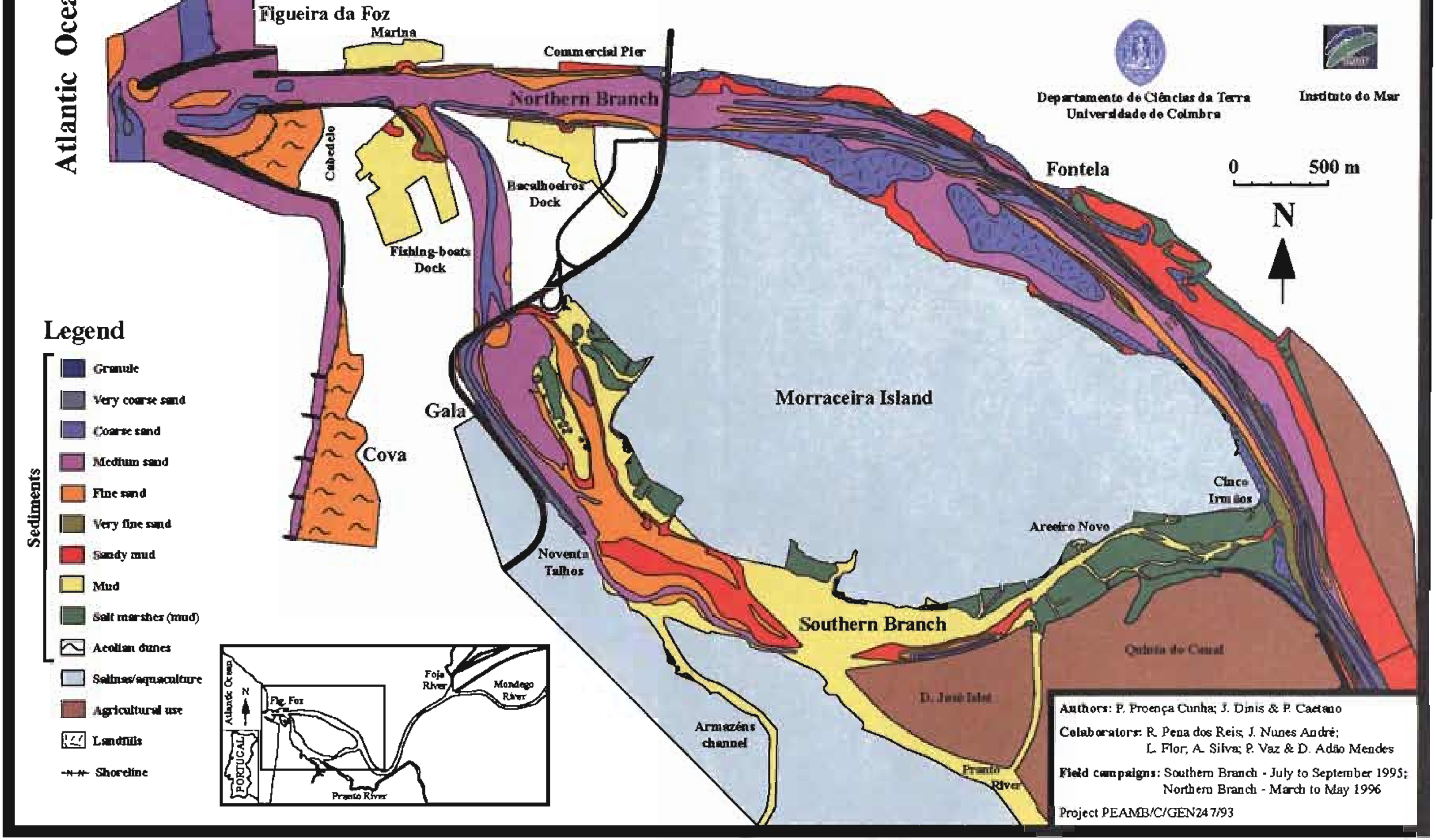
MONDEGO ESTUARY GRAIN-SIZE MAP



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Field campaigns: Southern Branch - July to September 1995;
Northern Branch - March to May 1996

Project PEAMB/C/GEN247/93

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