

MIGUEL ÂNGELO PARDAL  
JOÃO CARLOS MARQUES  
MANUEL AUGUSTO GRAÇA  
Scientific Editors

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



Coimbra • Imprensa da Universidade

MIGUEL ÂNGELO PARDAL  
JOÃO CARLOS MARQUES  
MANUEL AUGUSTO GRAÇA  
Scientific Editors

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



Coimbra • Imprensa da Universidade

COORDENAÇÃO EDITORIAL  
Imprensa da Universidade de Coimbra

CONCEPÇÃO GRÁFICA  
António Barros

INFOGRAFIA  
António Resende  
Estímulus [design] • Coimbra

EXECUÇÃO GRÁFICA  
GRAFIASA

ILUSTRAÇÃO DA CAPA  
P. P. Cunha e J. Dinis

ISBN  
972-8704-04-6

DEPÓSITO LEGAL  
175038/02

© JANEIRO 2002, IMPRENSA DA UNIVERSIDADE DE COIMBRA



OBRA PUBLICADA COM O PATROCÍNIO DE:  
IMAR – INSTITUTO DO MAR  
IPIMAR – INSTITUTO DE INVESTIGAÇÃO DAS PESCAS E DO MAR



TIAGO MÚRIAS <sup>1</sup>  
JOÃO ALEXANDRE CABRAL <sup>1</sup>  
RICARDO LOPES <sup>1</sup>  
JOÃO CARLOS MARQUES <sup>1</sup>

## 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 enclosure 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 widespread 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 deoxygenation 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

<sup>1</sup> IMAR - Instituto do Mar, Centro Interdisciplinar de Coimbra, Departamento de Zoologia, Universidade de Coimbra, 3004-517 Coimbra, Portugal

the populations from the uppermost trophic level organisms, mostly dominated by wading birds (Grant 1981, Masero et al. 1999, Raffaelli 1992).

The most straightforward 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 density/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 enclosure 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).

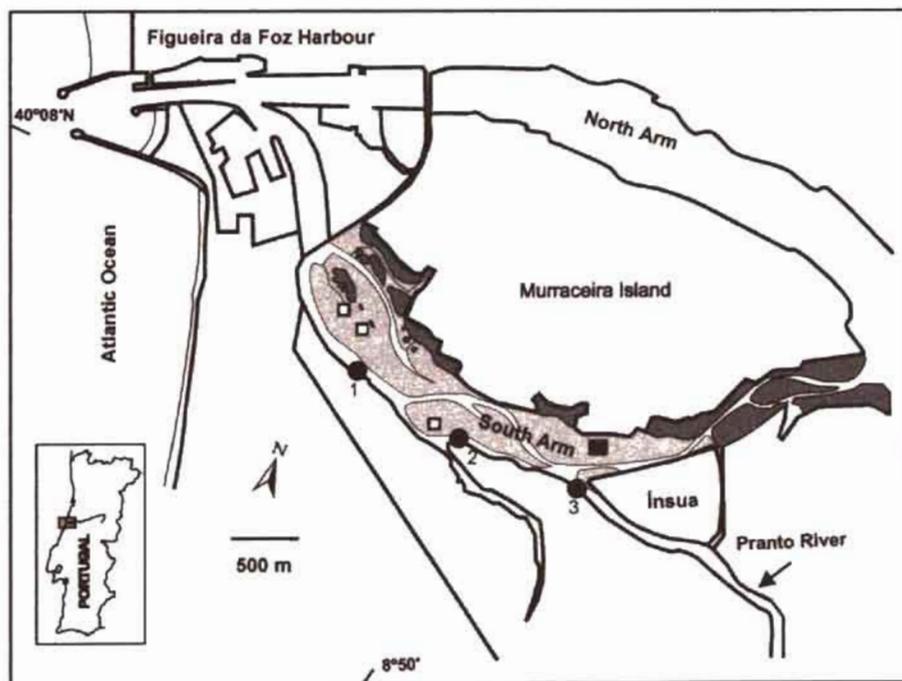


Fig. 1. Map of the study area, showing the intertidal mudflats (light-grey) and the saltmarsh (dark grey) areas of the south arm. Also shown are the positions of the three fixed stations used to census the waders in the south arm. Areas A1, A2 and A3 corresponds to the three main areas of mudflats, from 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 ( $\pm 2$  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, where 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 polychaete *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 1, 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 x 38 m was used. Treatments were interspersed in a rectangular 4 x 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<sup>2</sup> 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  $\mu\text{m}$  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<sup>-2</sup>, in 1993-94 and 50 g DW.m<sup>-2</sup> in 1994-95 reaching peak values from April to October (Figure 2). However, average monthly biomass ( $24.3 \pm 22.4$  (s.d.) g DW.m<sup>-2</sup> and  $23.1 \pm 14.0$  g DW.m<sup>-2</sup>, 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 ( $16.0\% \pm 12.4\%$  of total area in 1993-94, and  $35.5\% \pm 27.4\%$  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).

532

The hypothesis that algae deterred the birds was tested for two extreme situations: (1) when the algae was scarce ( $< 5$  g DW.m<sup>-2</sup> per area, in December/January and February/early March in both years) and (2) when it was abundant ( $> 10$  g DW.m<sup>-2</sup> 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.

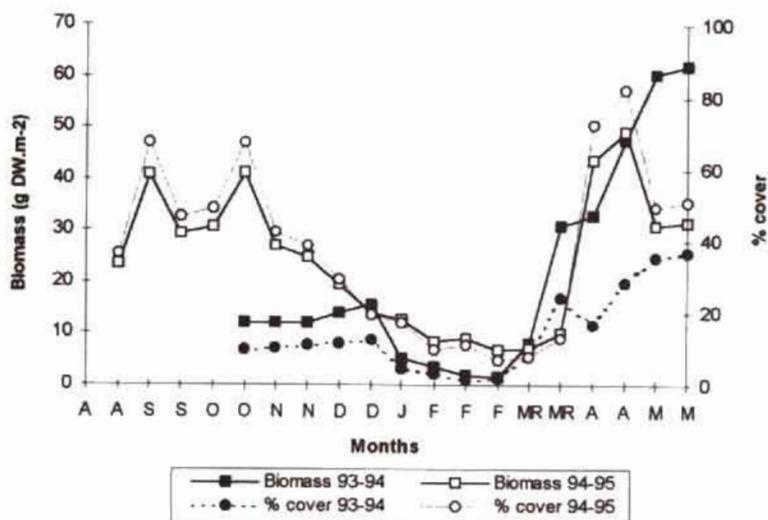


Fig. 2. Seasonal variation in the biomass and the percentage cover of algae on the sediments of the Mondego estuary, in 1993-94 and 1994-95.

Table 1. Number of birds feeding (mean  $\pm$  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 <sup>-2</sup> )				Abundant (> 10 g DW.m <sup>-2</sup> )			
	n	No algae	Algae	U	n	No algae	Algae	U
1993-94								
Kentish Plover	7	15.3 $\pm$ 4.9	0.3 $\pm$ 0.3	49.0*	10	4.5 $\pm$ 1.1	4.0 $\pm$ 2.5	69.5
Ringed Plover	5	37.4 $\pm$ 17.4	5.8 $\pm$ 5.8	22.0*	10	8.7 $\pm$ 3.9	1.9 $\pm$ 0.9	75.0
Grey Plover	10	42.1 $\pm$ 9.0	13.8 $\pm$ 5.6	71.5	10	8.1 $\pm$ 4.8	12.1 $\pm$ 5.7	67.5
Dunlin	8	207.1 $\pm$ 75.4	14.1 $\pm$ 5.9	54.0*	11	223.1 $\pm$ 162.1	14.0 $\pm$ 7.3	70.0
1994-95								
Kentish Plover	15	35.3 $\pm$ 6.3	3.3 $\pm$ 2.0	12.0***	21	21.3 $\pm$ 6.4	12.4 $\pm$ 4.3	167.0
Ringed Plover	13	35.9 $\pm$ 6.0	1.8 $\pm$ 0.9	9.0***	21	2.5 $\pm$ 1.2	9.0 $\pm$ 1.8	82.5**
Grey Plover	19	122.7 $\pm$ 20.5	9.3 $\pm$ 3.2	68.0**	12	9.6 $\pm$ 3.4	12.7 $\pm$ 2.4	46.0
Dunlin	17	188.6 $\pm$ 31.4	55.1 $\pm$ 25.2	53.0**	23	28.8 $\pm$ 24.8	77.5 $\pm$ 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<sup>-2</sup> (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 brackets. 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 <sup>a</sup>	Winter (n =6) <sup>b</sup>	Spring (n =18)	All year (n =46)	Autumn (n =15)	Winter (n =16)	Spring (n =15)
All species	-0.24	-	<b>-0.43</b>	-0.07	-0.10	-0.07	-0.03	-0.004
Kentish Plover	+0.18	-	<b>-0.46</b>	<b>+0.55</b>	-0.10	-0.11	<b>+0.53</b>	-0.11
Ringed Plover	+0.05	-	<b>-0.41</b>	+0.31	+0.12	+0.06	<b>+0.40</b>	-0.06
Grey Plover	-0.24	-	<b>-0.43</b>	-0.30	-0.14	-0.32	-0.26	<b>-0.42</b>
Dunlin	-0.11	-	-0.09	-0.03	+0.11	+0.13	+0.07	<b>+0.43</b>

<sup>a</sup> Only two observations for October of 1993 were available, therefore this periods was not analysed

<sup>b</sup> 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	<b>-0.89</b>	<b>+0.46</b>	-0.65*	+0.05	+0.38	+0.00
Kentish Plover	-	<b>+0.74</b>	-0.29	+0.07	-	-0.06	<b>-0.57</b>	-0.39
Ringed Plover	-	+0.14	-0.14	<b>-0.92</b>	-	<b>+0.40</b>	-0.07	-0.23
Grey Plover	-	-0.26	-0.37	-0.25	-	<b>-0.49</b>	+0.03	+0.06
Dunlin	-	<b>+0.67</b>	<b>-0.84</b>	+0.32	-	<b>+0.46</b>	<b>+0.47</b>	+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 <sup>1</sup>	Constant	$r$	$F_{(4,34)}$
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

<sup>1</sup> 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 ( $R^2$ ), 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*; HULV = *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	df.
<i>Calidris alpina</i> D = 0.192 - 0.006 (GUL) + 0.004 (TOT) - 0.010 (ALG) + 0.102 (NER)	0.64	19.51***	47
<i>Charadrius alexandrinus</i> D = 0.373 - 0.003 (GUL) + 0.002 (TOT) - 0.006 (HULV) PEK = 12.232 + 0.089 (ALG) - 3.002 (NER) + 0.521 (SPLA) + 171.655 (DIP) MOV = 32.257 - 0.98 (AIR) - 0.117 (HULV)	0.40 0.11 0.16	9.88** 4.47** 13.56***	47 143 143
<i>Charadrius hiaticula</i> D = 0.3 - 0.005 (GUL) + 0.003 (TOT) - 0.005 (ALG) PEK = 5.015 + 0.28 (AIR) + 0.342 (SPLA) MOV = 37.635 - 0.022 (TOT) + 0.186 (GUL) - 0.743 (AIR) - 1.688 (EDU)	0.40 0.11 0.15	9.90*** 8.62*** 6.56***	47 148 148
<i>Pluvialis squatarola</i> D = 0.339 + 0.001 (TOT) - 0.008 (ALG) - 0.305 (POL) - 0.49 (CAR) + 6.669 (DIP) PEK = 9.973 - 0.343 (AIR) - 12.62 (POL) + 0.574 (SPLA) + 95.372 (DIP) MOV = 42.261 - 1.084 (AIR) - 4.51 (NER) - 23.213 (AMP)	0.37 0.40 0.12	4.89** 21.07*** 6.02***	47 132 132

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 shrubsolei*, *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 enclosure 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 ( $\log_{10}(n+1)$ ) 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).

Date	Species	Algae		Predation		Algae x predation		Quadrat (A x P)	
		F	p	F	p	F	p	F	p
28 April	<i>Hydrobia ulvae</i>	0.10	0.758	0.84	0.374	2.40	0.141	0.86	0.620
	<i>Scrobicularia plana</i>	0.12	0.739	0.01	0.925	1.17	0.296	0.83	0.648
	<i>Hediste diversicolor</i>	7.61	<b>0.014</b>	0.03	0.866	0.74	0.402	0.69	0.795
	<i>Amage adpersa</i>	16.45	<b>0.0009</b>	3.56	0.078	0.25	0.621	1.05	0.420
	<i>Streblospio shrubsolii</i>	12.24	<b>0.003</b>	1.45	0.246	0.30	0.589	1.59	0.092
	<i>Capitella capitata</i>	28.63	<b>0.0001</b>	0.32	0.577	4.69	0.046	1.84	<b>0.04</b>
	<i>Oligochaeta</i> spp.	0.65	0.431	0.17	0.686	0.17	0.685	1.59	0.091
26 May	<i>Hydrobia ulvae</i>	0.25	0.624	0.07	0.793	8.71	<b>0.009</b>	0.82	0.662
	<i>Scrobicularia plana</i>	3.24	0.091	1.92	0.185	0.75	0.400	0.79	0.691
	<i>Hediste diversicolor</i>	6.27	<b>0.024</b>	0.14	0.717	1.43	0.250	1.25	0.249
	<i>Amage adpersa</i>	6.03	<b>0.026</b>	0.78	0.389	0.17	0.682	4.50	<b>0.000</b>
	<i>Streblospio shrubsolii</i>	6.95	<b>0.018</b>	0.37	0.552	0.00	0.971	3.62	<b>0.0001</b>
	<i>Capitella capitata</i>	18.15	<b>0.006</b>	0.01	0.910	3.24	0.091	1.72	0.059
	<i>Oligochaeta</i> 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 Soulsby 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.

### Acknowledgements

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.

### References

- Azeteiro U.M.M. and Marques, J.C. 1999. Temporal and spatial structure in the suprabenthic community of a shallow estuary (Western Portugal: Mondego river estuary). *Acta Oecol.* 20: 333-342.
- Cabral J.A., Pardal, M.A., Lopes, R.J., Múrias, T. and Marques J.C. 1999. The impact of macroalgae blooms on the use of the intertidal area and feeding behaviour of waders (Charadrii) in the Mondego estuary (West Portugal). *Acta Oecol.* 20: 417-427.
- Cramp S. and K. Simmons (eds.) 1983. *The Birds of Western Palearctic*, vol III Waders to gulls. Oxford Univ. Press. London.
- Desprez R., Rybarczyk H., Wilson J.G., Ducrottoy P., Sueur F., Olivesi R. and Elkaim B. 1992. Biological impact of eutrophication in the Bay of Somme and the induction and impact of anoxia. *Neth. Sea Res.* 30: 149-159.
- Dijk G.M., Liere L., Admiraal W., Bannink B.A. and Cappon J.J. 1994. Present state of the water quality of European rivers and implications for management. *Sci. Total Environm.* 145: 187-195.
- Everett R.A. 1994. Macroalgae in marine soft-sediment communities: effects on benthic faunal assemblages. *J. Exp. Mar. Biol. Ecol.* 175: 253-274.
- Goss-Custard J.D. 1977. Predator responses and prey mortality in the Redshank *Tringa totanus*. *Ibis* 111: 338-356.
- Grant J. 1981. A bioenergetic model of shorebird predation on infaunal amphipods *Oikos* 37: 53-62.
- Hardy F.G., Evans S.M. and Tremayne M.A. 1993. Long-term changes in the marine macroalgae of three polluted estuaries in north-east England. *J. Exp. Mar. Biol. Ecol.* 172: 81-92.
- Hickel W., Mangelsdorf P. and Berg J. 1993. The human impact in the German Bight: eutrophication during three decades (1962-1991). *Helgo. Wiss. Meeresunters.* 47: 243-263.
- Hodgekun E.P. and Hamilton B.H. 1993. Fertilizers and eutrophication in southwestern Australia: setting the scene. *Fert. Res.* 36: 95-103.
- Kalejšta B. 1993. Intense predation cannot always be detected experimentally: a case study of shorebird predation on nereid polychaetes in South Africa. *Neth. J. Sea Res.* 31: 385-393.

- Lillebø A.I., Flindt M.R., Pardal M.A. and Marques J.C. 1999a. The effect of macrofauna, meiofauna and microfauna on the degradation of *Spartina maritima* detritus from a salt marsh area. *Acta Oecol.* 20: 249-258.
- Lillebø A.I., Pardal M.A. and Marques J.C. 1999b. Population structure, dynamics and production of *Hydrobia ulvae* (Pennant) (Mollusca: Prosobranchia) along an eutrophication gradient in the Mondego estuary (Portugal). *Acta Oecol.* 20: 289-304.
- Lopes R., Pardal M.A. and Marques J.C. 2000. Impact of macroalgae blooms and wader predation on intertidal macroinvertebrates - experimental evidence from the Mondego estuary (Portugal). *J. Exp. Mar. Biology* 249:165-179.
- Lopes R. 1999. Impacto das aves limícolas sobre os macroinvertebrados nas áreas intertidais do estuário do Mondego. MSc. Thesis, University of Coimbra. Coimbra.
- Marques J.C., Rodrigues L.S.B. and Nogueira A.J.A. 1993. Intertidal macrobenthic communities structure in the Mondego estuary (Western Portugal): reference situation. *Vie Milieu* 43: 177-187.
- Masero J.A., Domínguez J. and Pérez-Cardenal D. 1999. Food supply for waders (Aves: Charadrii) in an estuarine area in the Bay of Cadiz (SW Iberian Peninsula). *Acta Oecol.* 20: 435-488.
- McComb J.A. and Davies J.A. 1993. Eutrophic waters of southwestern Australia. *Fert. Res.* 36: 105-114.
- Metzmacher K. and Reise K. 1994. Experimental effects of tidal flat epistuctures on foraging birds in the Wadden Sea. *Ophelia* 6: 217-225.
- Múrias T., Cabral J.A., Marques J.C. and Goss-Custard J.D. 1996. Short-term effects of intertidal macroalgae blooms on the microhabitat selection and feeding behaviour of wading birds in the Mondego estuary (West Portugal). *Estuar. Coast. Shelf Sci.* 43: 677-688.
- Pardal M.A. 1998. Impacto da eutrofização nas comunidades macrobentónicas do braço sul do estuário do Mondego. PhD Thesis, University of Coimbra. Coimbra.
- Pienkowski M.W. 1982. Diet and energy intake of Grey and Ringed Plovers, *Pluvialis squatarola* and *Charadrius hiaticula*, in the non-breeding season. *J. Zool. Lond.* 197: 511-549.
- Pienkowski M.W. 1983. Changes in foraging pattern of plovers in relation to environmental factors. *Anim. Behav.* 31: 244-264.
- Raffaelli D.G. 1992. Conservation of Scottish estuaries. *Proc. R. Soc. Edinb.* 100B: 55-76.
- Raffaelli D.J. and Milne H. 1987. An experimental investigation of the effects of shorebird and flatfish predation on estuarine invertebrates. *Estuar. Coast. and Shelf Sci.* 24: 1-13.
- Raffaelli D.G., Hull S.C. and Milne H. 1989. Long-term changes in nutrients, weed mats and shorebirds in an estuarine system. *Cah. Biol. Mar.* 30: 258-270.
- Raffaelli J.D., Limia J., Hull S. and Pont S. 1991. Interaction between the amphipod *Corophium volutator* and macroalgae mats on estuarine mudflats. *J. Mar. Biol. Assoc. UK* 71: 899-908.
- Schoris D. and Reise K. 1993. Germination and anchorage of *Enteromorpha* spp. in sediments of the Wadden Sea. *Helgol. Wiss. Meeresunters.* 47: 275-285.
- Sewell M.A. 1996. Detection of the impact of predation by migratory shorebirds: an experimental test in the Fraser river estuary, British Columbia (Canada). *Mar. Ecol. Prog. Ser.* 144: 23-40.
- Soulsby P.G., Lowthion D. and Houston M. 1982. Effects of macroalgae mats on the ecology of intertidal mudflats. *Mar. Pollut. Bull.* 13: 162-166.
- Tapp J.F., Shillabeer N. and Ashman C.M. 1993. Continued observations of the benthic fauna of the industrialised Tees estuary 1979-1990. *J. Exp. Mar. Biol. Ecol.* 172: 67-80.
- Trush S.F., Pridmore R.D., Hewitt J.E. and Cummings V.J. 1994. The importance of predators on a sandflat: interplay between seasonal changes in prey densities and predator effects. *Mar. Ecol. Prog. Ser.* 107: 211-222.
- Yeates M.G., Goss-Custard J.D., McGrorty S., Lahkani K.H., DiDurell S., Clarke R.T., Rispin W.E., May I., Yates T., Plant R.A. and Frost A.J. 1993. Sediment characteristics, invertebrate densities on the inner bank of the Wash. *J. Appl. Ecol.* 30: 599-614.



Série

Investigação

•

Coimbra  
Imprensa da Universidade

2002