



DEPARTAMENTO DE ZOOLOGIA

FACULDADE DE CIÊNCIAS E TECNOLOGIA
UNIVERSIDADE DE COIMBRA

Resposta dos produtores primários às medidas de mitigação implementadas para reduzir os sintomas de eutrofização num estuário temperado (Mondego, Portugal)

Sara Isabel Falcão Navarro Leston Ferreira

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Dissertação apresentada à Universidade de Coimbra para cumprimento dos requisitos necessários à obtenção do grau de Mestre em Ecologia, realizada sob a orientação científica do Professor Doutor Miguel Pardal (Universidade de Coimbra)

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RESUMO

O estuário do Mondego, tal como tem vindo a acontecer em estuários e outros sistemas costeiros por todo o mundo, tem sofrido nas últimas décadas um processo de eutrofização. Este processo tem como principal consequência a formação de densas massas de macroalgas, também conhecidas como “blooms”. No estuário do Mondego estes “blooms” macroalgais (sintomas de eutrofização) ocorriam frequentemente, como resultado do enriquecimento da água por elevadas concentrações de nutrientes provenientes de descargas de água-doce do rio Pranto, um afluente do rio Mondego que drena os campos agrícolas localizados a montante. Como consequência, nas zonas mais interiores do estuário, os bancos de *Zostera noltii* foram drasticamente reduzidos, ocorrendo uma substituição progressiva de produtores primários, de fanerogâmicas marinhas para espécies de macroalgas de crescimento rápido.

Com o objectivo de reverter este cenário, recuperar e conservar os bancos de *Zostera noltii*, considerados de elevada importância devido à sua elevada biodiversidade e grande produtividade, em 1998 um conjunto de medidas de mitigação foi implementado no estuário do Mondego. De modo a diminuir o crescimento acelerado de *Ulva* spp., ocorreu uma diminuição das concentrações de compostos azotados na coluna de água e uma diminuição do tempo de residência da água.

O objectivo do presente trabalho consistiu na avaliação das respostas de quatro grupos de produtores primários (fitoplâncton, com base na concentração de clorofila *a*; macroalgas verdes do género *Ulva*, a macroalga vermelha *Gracilaria gracilis* e a fanerogâmica marinha *Zostera noltii*) às alterações ambientais causadas pelas medidas de gestão introduzidas.

Os resultados mostraram que a concentração de azoto dissolvido na coluna de água diminuiu significativamente, devido à grande diminuição da concentração de azoto na forma de amoníaco (NH_4^+) enquanto que a concentração de fósforo inorgânico dissolvido (PO_4^{3-}) aumentou significativamente. Os produtores primários avaliados apresentaram respostas diferentes no que respeita à biomassa: o fitoplâncton (concentração de Chl *a*) manteve-se relativamente constante, a biomassa de macroalgas verdes foi significativamente reduzida, enquanto que as biomassas de *G. gracilis* e de *Z. noltii* registaram um aumento significativo.

Os resultados obtidos sugerem que a redução de azoto, na forma de amoníaco (NH_4^+), foi o principal factor que levou à diminuição de macroalga verde, enquanto que a luz foi o factor mais determinante no aumento de biomassa de *Z. noltii*. Relativamente à macroalga vermelha, ambos factores parecem ter tido influência no aumento de biomassa registado, enquanto que o fitoplâncton não sofreu qualquer alteração, o que sugere a inexistência de alterações dos seus factores limitantes.

ABSTRACT

The Mondego estuary is a well-described system located on the North Atlantic Ocean, where cultural eutrophication progressed over the last decades of the 20th century. Consequently, and due to a large productivity of *Ulva* spp., *Zostera noltii* meadows were severely reduced with a concomitant decrease in environmental quality. In 1998, experimental mitigation measures were implemented, via changes in hydrology to increase circulation and diversion of nutrient-rich freshwater inflow, to reverse the process in the most affected area of the estuary – its South arm.

The objective of this study was to assess the differences in response of primary producer assemblages to the implemented measures to reduce eutrophication symptoms. Results show that the mean concentrations of DIN suffered a significant decrease due to a great reduction in the ammonium concentration in the water column while DIP increased significantly. Primary producers' assemblages showed different reactions to these changes: phytoplankton, measured as concentration of chlorophyll *a*, did not show any significant changes; green macroalgae, mostly *Ulva* spp. suffered a large reduction in biomass whereas *Gracilaria gracilis* and the macrophyte *Zostera noltii* biomasses increased greatly. The results suggest that the reduction in ammonium could have been responsible for the changes in the green macroalgae's biomass and that light was a more determinant factor in the response of seagrasses whereas red macroalgae reaction seemed to be dependent on these two factors. The concentration of chlorophyll *a* has not changed significantly throughout the study period which suggests that phytoplankton's limiting factors have not been altered.

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CHAPTER 1.

INTRODUCTION

1.1 Estuaries

Estuaries represent transitional areas between freshwater and marine ecosystems presenting unique environmental and biological features that set them among the most productive areas of the world (Valiela, 1995; Kennish, 2000). They constitute dynamic ecosystems where many species with great importance to human populations are found at least for a stage of their lives (Kennish, 2000).

Traditionally, the most industrialized and densely populated regions are located near estuaries as a result of the great importance these small areas have to the human populations. Consequently, coastal aquatic ecosystems worldwide, such as estuaries and coastal lagoons, have been subjected to anthropogenic activities that disturb their inherent natural processes. Industries, fertilization of agricultural fields, aquacultures and wastewater discharges are some of the many human activities contributing to the enrichment of water bodies with nutrients, particularly the growth limiting nutrients (nitrogen and phosphorus), a process designated as eutrophication or “cultural” eutrophication (Peckol & Rivers, 1995; Menéndez & Comín, 2000; Neill, 2005).

Although nutrients are essential, when supplied in large amounts they can be detrimental for the whole ecosystem. The progressive replacement of seagrasses and slow-growing macroalgae by opportunistic fast-growing macroalgae or by phytoplankton resulting from nutrient enrichment has been reported (e.g. Hauxwell & Valiela, 2004; Domingues et al., 2005). This high productivity can be responsible for severe anoxic conditions with hydrogen sulphide emissions that are aggravated by the collapse of opportunistic blooms (Hartnett & Nash, 2004). The overall impact on the ecosystem will depend on its resilience, that is, the maximum perturbation it can endure without causing a shift from the current state (Scheffer et al., 2001). Ultimately, the ability of species to tolerate disturbances will

determine to what point the composition and structure of the habitats will be modified (e.g. Cardoso et al., 2005). Due to the great importance of estuaries, programs aiming to restore the ecosystems' health are being implemented. To guarantee the success of these interventions, it is essential to understand the processes which led to the changes observed in the systems (Zhang et al., 2003; Cardoso et al., 2004; Webster & Harris, 2004; Lillebo et al., 2005).

1.2 Primary producers

1.2.1 Phytoplankton

Phytoplanktonic communities are a common constituent of estuarine waters that often originate blooms in nutrient enriched waters (Pedersen & Borum, 1996; Kinney & Roman, 1998). Due to their small size the demand for nutrients is high (Pedersen & Borum, 1996) and they can rapidly sequester nutrients from the water column and increase their biomass, presenting high uptake and growth rates (Havens et al., 2001).

Light is another factor controlling phytoplankton growth. High turbidity can limit growth (Domingues et al., 2005), whereas waters with low degree of mixing are favorable to their production. Phytoplankton blooms, whether temporary or persistent, can have dramatic effects on the ecosystem. Among the taxa found forming blooms there are some that produce toxins (Havens et al., 2001; Domingues et al., 2005).

1.2.2 Macroalgae

One of the symptoms of eutrophication, as mentioned above, is the accentuated increase of macroalgal biomass production. Among those species found in areas undergoing eutrophication are the genus *Chaetomorpha*, *Cladophora*, *Gracilaria* and *Ulva* (Fletcher, 1996; Raffaelli et al., 1998; Mistri et al., 2001; Fong et al., 2004). These macroalgae are ruderal strategists capable of taking advantage of the available resources in transient environments (Viaroli et al., 1996; Raven & Taylor, 2003; Cohen & Fong, 2004), being able to tolerate and exploit a broad range of conditions, mostly due to their simple morphologies (Raffaelli et al., 1998). They are most commonly filamentous, branched or sheet-like algae, possessing very high surface areas per unit volume that can be used in intercepting incident light and nutrients (Raffaelli et al., 1998; Raven & Taylor, 2003). Moreover their morphologies enable them to tolerate fluctuating salinities as each individual cell is exposed to the medium and allowed to osmoregulate individually. They also tolerate and exploit high and fluctuating temperatures, typical in areas with low depth and under tidal influence (Raffaelli et al., 1998).

High uptake rates and high maximum specific growth rates (μ_{max}), associated with a high affinity for nutrients, especially N and P allow them to greatly increase their biomass in the presence of high concentrations of nutrients in the system, sometimes forming large blooms (Raffaelli et al., 1998; Raven & Taylor, 2003).

Wherever algal mats form they prevent light from reaching the underlying areas and they also cause a separation in space and time of oxygen production and consumption (e.g. Pedersen & Borum, 1996). The increase in biomass obviously implies higher photosynthetic activity with subsequent production of oxygen during the day. At night, since there is no photosynthetic activity, respiration is not compensated and this can lead to

hipoxia or in extreme cases to anoxia in the water column and sometimes in the sediment-water interface (Desprez et al., 1992; Pedersen & Borum, 1996). This has serious implications to the species that are found in these areas, as they become deprived of light and oxygen. The extent to which they are affected depends on their tolerance and mobility (Desprez et al., 1992; Raffaelli et al., 1998; Lillebø et al., 1999; Pardal et al., 2000).

1.2.3 Seagrasses

Seagrasses are highly productive rooted angiosperms often found in shallow coastal marine ecosystems, such as lagoons and estuaries. Seagrass meadows are characterized by high productivity and biodiversity (Bocci et al., 1997), providing faunal benthic communities with habitat, shelter and food (Pardal et al., 2000; Touchette and Burkholder, 2000; Cardoso et al., 2004; Verdelhos et al., 2005). They also play an important role in stabilizing the coastal shoreline and are a source of organic material in shallow waters (Bocci et al., 1997).

The abundance and distribution of these primary producers is strongly correlated with light availability (Kraemer & Hanisak, 2000). Light is considered the primary resource that limits the growth of most seagrass species. It is critical to the survival of subterranean tissues as they depend upon the O₂ released during photosynthesis to sustain aerobic respiration in an anaerobic environment (Smith et al., 1984). Moreover, light plays an important role in regulating the uptake and assimilation of N as it controls the enzymes involved in these processes (McGlathery et al., 1997; Kraemer & Hanisak, 2000).

Nutrients are considered as secondary factors limiting growth since the sediment-pore waters can supply most nutrients (with the exception of carbon), whereas the water column acts as an additional source (Hemminga, 1998; Touchette & Burkholder, 2000).

Some species of seagrasses found in temperate, coastal systems of the northern hemisphere are *Zostera marina* (e.g. eelgrass), *Zostera noltii* (seagrass), *Posidonia oceanica* and *Ruppia maritima* (Kinney & Roman, 1998)

All over the world, there has been a significant decline in seagrass meadows associated with increases in phytoplankton and macroalgae (Hemminga, 1998; Raffaelli et al., 1998), linked to anthropogenic disturbances. Several studies on seagrass decline related to increased nitrogen supply have demonstrated three mechanisms that can induce this loss: direct nitrogen toxicity, light limitation (via phytoplankton, epiphyte and/or macroalgae) and unfavorable alterations in the surrounding environments due to macroalgal canopies (Hauxwell & Valiela, 2004).

The Mondego estuary is among the coastal environments where a decline in *Z. noltii* meadows has occurred (e.g Marques et al., 1993; Flindt et al., 1997; Pardal et al., 2000; Martins et al., 2001; Cardoso et al., 2004; 2005; Lillebo et al., 2005; 2006; Verdelhos et al., 2005).

1.3 The Mondego estuary

1.3.1 Location and system description

The Mondego River source is located at 1457 m of altitude in the Serra da Estrela (Gonçalves, 1991) presenting an extension of 227 km joining to the sea in Figueira da Foz (West Coast of Portugal), where it flows into the Atlantic Ocean. It drains a hydrographic basin with an approximate area of 6670 km², where its major tributaries are the Ribeira de Ançã and the Rivers Dão and Foja, on the North bank and the Rivers Alva, Ceira, Ega, Arunca and Pranto on the South bank (Pardal, 1998).

The Mondego estuary's location is 40°08'N; 8°50'W, presenting an area of about 1071 ha, 7 km long and 2-3 km wide, characterized by a temperate coastal climate with Mediterranean and Atlantic influences (Ribeiro, 2001). Therefore, it presents a seasonal variation of temperature and precipitation typical of temperate climates, with rainy and moderately cold winters contrasting with very hot and dry summers.

The estuary comprises two arms, North and South, separated by an alluvium-formed island (Murraceira Island) that join again 1 km from the mouth. This physical separation causes the two arms to possess different hydrologic characteristics (e.g. Martins et al., 2001). The North arm is the main navigation channel and the location of the commercial harbor of Figueira da Foz. It is subjected to dredging related to these activities, which makes it deeper (4-8 m during high tide, tidal range 1-3 m). The South arm is shallower (2-4 m during high tide, tidal range 1-3 m) and in the last years it has become largely silted up, especially in the upstream areas, which caused freshwater to flow mainly through the North arm (e.g. Martins et al., 2001, Lillebø et al., 2005). As a consequence, water circulation became more dependent on tides and freshwater discharges (controlled by a sluice) from a small tributary, the Pranto River (Fig. 1). Since the tidal penetration is faster in the North arm, the daily variations in salinity are higher here, whereas in the South arm temperature shows the highest daily variations, mainly due to its lower depth (e.g. Marques et al., 1993). Due to these differences in the hydraulic regime of the arms, their communities are different.

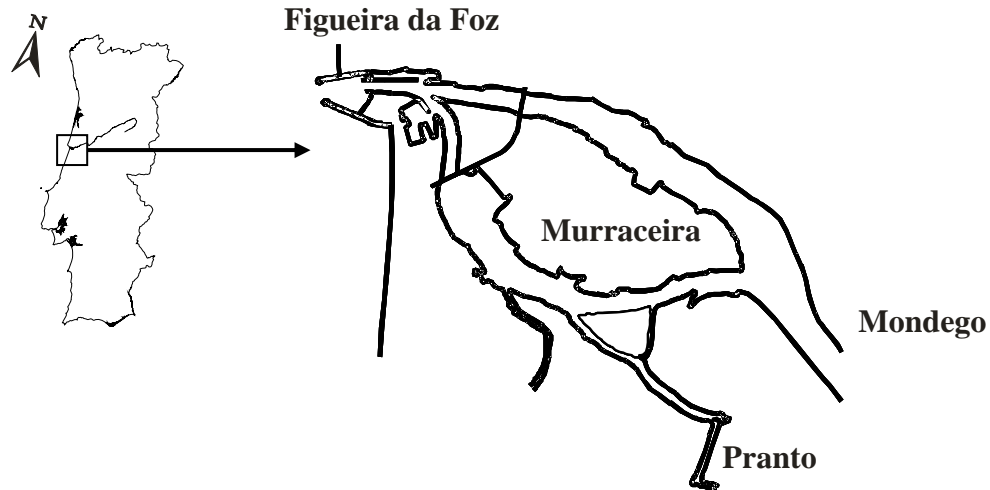


Fig. 1 – The Mondego estuary.

1.3.2 Eutrophication

The discharges from the Pranto River are regulated in accordance with the hydric needs of the rice fields located upstream. They constitute an important source of nutrients to the estuary, mainly to the South arm, since this tributary receives nutrient-rich run-offs from the agricultural lands upstream (Lillebo et al., 2005). This nutrient enrichment associated with weak hydrodynamic circulation was responsible for the eutrophication related changes occurring in the estuary for the last decades (e.g. Cardoso et al., 2004, Lillebo et al., 2005; 2006; Martins et al., 1999; 2001).

Hypernutrification of the system led to the accelerated increase of fast-growing macroalgal production, more specifically of *Ulva* spp. and the drastic decrease of the rooted macrophyte *Zostera noltii*. The seagrass meadows were dramatically reduced (from approximately 150 000 m² in 1986, to 200 m² in 1997 (Fig. 2). This habitat loss led to the impoverishment of the benthic community affecting the whole ecosystem (Dolbeth et al.,

2003; Cardoso et al., 2004; 2005; 2006; Ferreira et al., 2005; Pardal et al., 2004; Verdelhos et al., 2005).

1.3.3 Restoration program

In order to reduce the eutrophication symptoms and to protect and recover the seagrass meadows, a mitigation plan was introduced in 1998. To reduce the loadings of nutrients into the South arm from the Pranto River the sluice aperture is less frequent and most of the freshwater flow from this tributary was diverted to the North arm by another sluice located upstream. To improve water circulation the connection between both arms was enlarged allowing water to flow from the North arms during high tide. The remainder of the seagrass patches were delimited by wooden stakes to prevent physical disturbance and awareness meetings were held to inform the population of the importance of these areas.

These restoration measures led to the recovery of the seagrass meadows, presenting in 2004 an area of approximately 4 ha (Fig. 2).

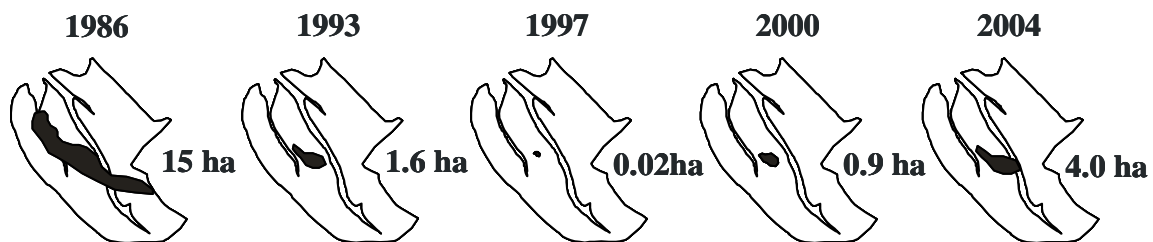


Fig. 2 - The evolution of the *Z. noltii* beds (ha) from 1986 to 2004, showing the drastic reduction from 1993 to 1997 and the recovery after the management.

1.4 Objectives of the present study

The Mondego estuary is an area of environmental and economic importance, being the subject of several studies in the last decade, mainly focusing on the benthonic and planktonic communities, but also on the primary producers. Symptoms of eutrophication at the system's South arm were one major problem affecting the estuary and they were addressed with mitigation measures to revert them. To possess the knowledge of how one action will influence the constituents of a system is essential to assure the success of any management plan. With the implementation of the mitigation program and the changes it brought to the system's South arm it became important to understand how primary producers responded to it.

The present work aimed to study these responses and for that purpose 4 groups of primary producers were selected: phytoplankton, measured as chlorophyll a, green macroalgae of the genus *Ulva*, the red macroalgae *Gracilaria gracilis* and the rooted macrophyte *Zostera noltii*. The main objectives for the present work are:

- to describe for each group possible alterations in biomass, seasonal growth patterns and tissue nutrients concentrations;
- to assess the overall responses of primary producers assemblages to the management actions;
- to determine which factors have more influence in the responses.

CHAPTER 2.

MATERIAL AND METHODS

2.1 Study Site

This study was conducted between February 1993 and December 2004 in the South arm of the Mondego estuary, as a part of a long-term monitoring program. Three sampling sites (A, B and C) were selected based on macroalgal abundance following a gradient of eutrophication, increasing from downstream to upstream (Fig. 3).

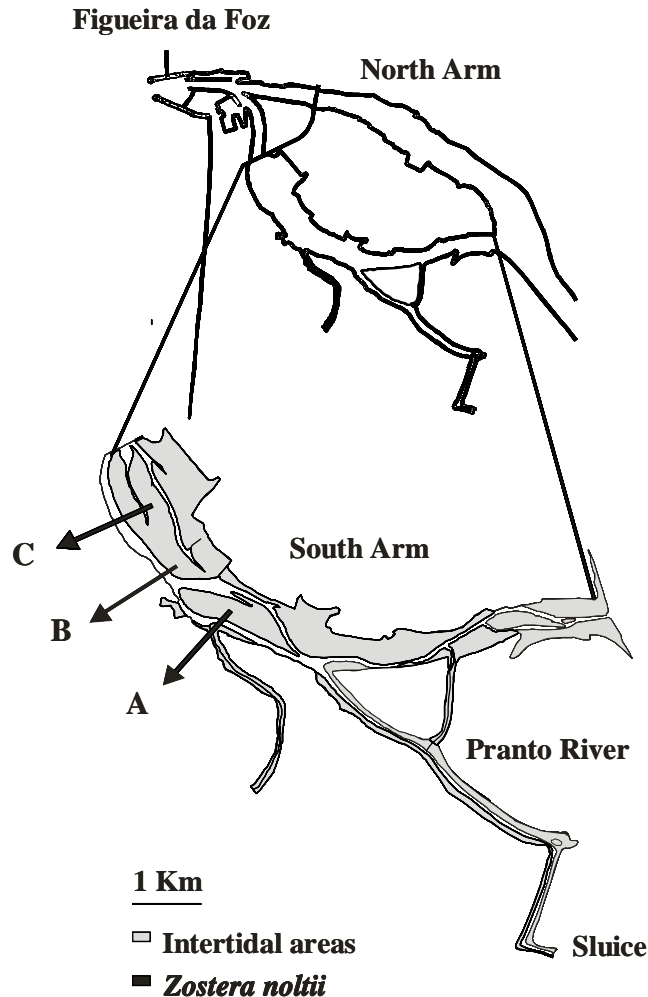


Figure 3 - The Mondego estuary, with the location of the long term monitoring area and the areas where management measures were implemented. A, B and C represent a gradient of eutrophication where A is the most eutrophicated area.

Site A is located upstream, where seagrass meadows were once present but as eutrophication progressed the meadows were completely replaced with macroalgae presenting until 1995 spring blooms. Site B is frequently covered with macroalgae, especially *Ulva* spp. and *Gracilaria gracilis* that like in site A, led to the progressive reduction of the seagrasses. Site C is located downstream and corresponds to the *Z. noltii* beds, presenting a high productivity and diversity, sheltering an important macrofaunal community.

Sampling was carried out bimonthly from February 1993 until June 1994 and monthly thereafter. From January 1997 to December 1998 no sampling was performed.

2.2. Water - Dissolved nutrients analysis

On each sampling occasion approximately 250 mL of water were collected and transported in coolers to the laboratory, where they were filtered using Whatmann GF/C glass fibre filters and then frozen at - 18°C for later analysis on dissolved nutrients. Water temperature and salinity were measured *in situ*.

The dissolved inorganic nitrogen forms determined in the water samples were ammonium (NH₄-N), nitrate (NO₃-N) and nitrite (NO₂-N), whereas dissolved inorganic phosphorus was determined as phosphate (PO₄-P). Nitrate (NO₃-N) and nitrite (NO₂-N) were analysed according with standard methods described in Strickland and Parsons (1972) and ammonium (NH₄-N) and phosphate (PO₄-P) analysis followed the Limnologisk Metodik (1992) methodology.

2.3 Phytoplankton

The phytoplankton chlorophyll *a* (Chl*a*) determinations were performed by filtering 0.5-1.0 L of water through Whatman GF/C glass-fibre filters followed by extraction according to Parsons et al. (1985). In the field and during transportation to the lab, samples were stored on ice and light protected.

2.4 Biomass assesement

Plant sampling for biomass assessment was performed randomly with a core (minimum 6 cores, with 143 cm² section), during low tide at the three sites. After rinsed, plants were sorted by species.

Macroalgae used in this study were green macroalgae of the genus *Ulva* (mainly *Ulva compressa* (L.) Greville and *Ulva intestinalis* (L.) Link according to revision by Hayden et al, 2003), and the red macroalgae *Gracilaria gracilis*. *Zostera noltii* samples were separated into leaves, roots and rhizomes. Plants were dried for 48 hours at 60°C until constant weight and then weighted to determine dry weight (DW). After this, they were combusted in a muffle furnace at 450°C for 8 hours. After cooling, the samples were weighted again to determine the ash free dry weight (AFDW).

2.5 Internal Nutrients Determination

Macroalgae and seagrass for internal nutrient content analysis were collected at site C, during low tide and transported in plastic bags to the laboratory where they were rinsed, dried for 48h at 60°C until constant weight and stored dry until further analysis. Determination of total nitrogen and total phosphorus followed the procedures described in the Limnologisk Metodik (1992).

2.5.1 Nitrogen

Dry samples were weighted and grounded to a powder for homogeneization before boiling in an acid solution with concentrated sulphuric acid and copper sulphate. After cooling and the pH neutralized with concentrated sodium hydroxide, samples were diluted with demineralized water the procedure for ammonium determination in the water was followed.

2.5.2 Phosphorus

Dry plant samples were weighted and combusted in a muffle furnace at 500° C for 3 hours to eliminate the organic matter and after cooling weighted again. The samples were boiled in an acid solution with concentrated hydrochloric acid (HCl). After cooling, they were diluted with demineralized water and filtered with Whatmann GF/F filters. The procedure for phosphate determination in water was then followed.

CHAPTER 3.

RESULTS

3.1 Water

3.1.1 Physical parameters

Water temperature followed a seasonal pattern throughout the years, with temperatures rising in spring, reaching maximum values in the summer and gradually decreasing through winter, when the lowest values were registered (Fig. 4 A).

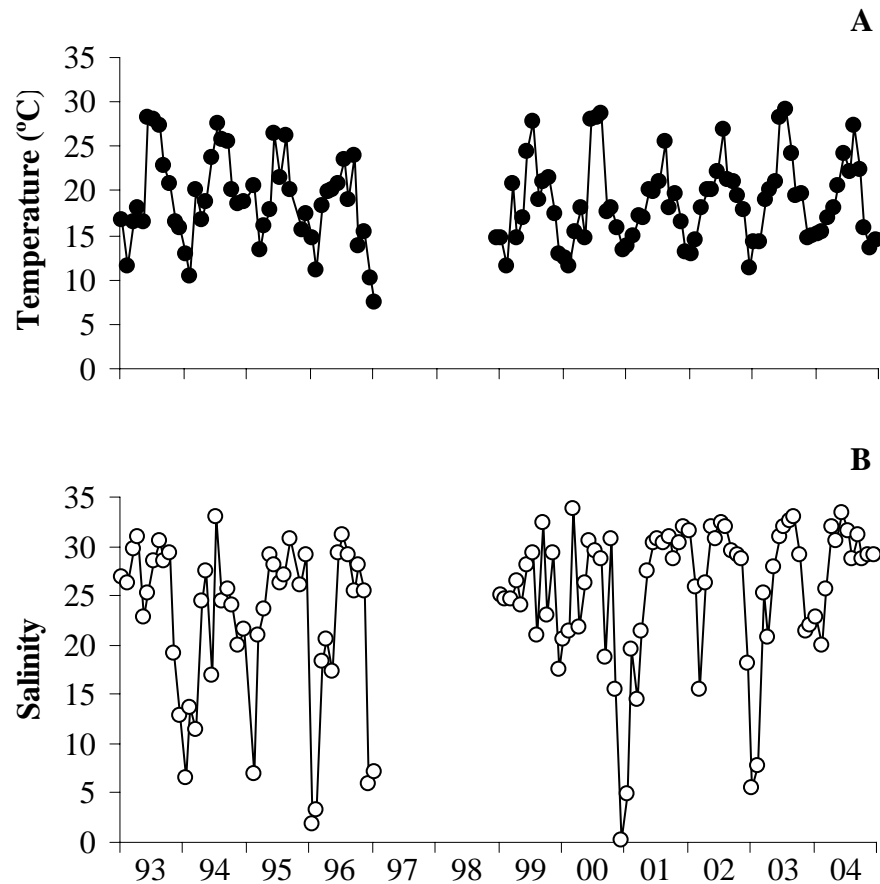


Figure 4 – Mean water temperature (A) and salinity (B) variation from January 1993 to December 2004.

Salinity also presented seasonal variation. Higher values were registered in the summer and the lowest in the cold months, for both pre and post management periods (Fig. 3 B). It was demonstrated in a previous study that there is a significant negative linear

relationship between salinity and precipitation (Lillebø et al., 2005). With the improvement in hydrodynamics in 1998, salinity values have become more regular with less abrupt variations.

Water flow and water flow velocity increase resulting from the management actions undertaken to restore the estuarine system led to a change in the water residence time from moderate to short. Also, the water flow velocity became independent of the discharges from Pranto River.

3.1.2 Dissolved Nutrients

This improvement also had consequences on the concentrations of dissolved inorganic nutrients in the water column.

The concentrations of nitrogen forms followed seasonal patterns with the highest values being registered in the winter months when precipitation is higher. Before 1998, the river Pranto discharges were higher during this time, which greatly contributed to the increase of DIN in the water column. After the mitigations measures were implemented, the concentration of DIN was significantly decreased (Fig. 5 A).

The ammonium concentration in the water column decreased significantly with the introduction of the mitigation measures, mainly due to the reduction of the flow from the Pranto River (Fig. 5 A). The mean value prior to 1998 was $28.15 \mu\text{mol.L}^{-1}$ dropping to $7.76 \mu\text{mol.L}^{-1}$ afterwards and the difference between both periods was statistically significant (Table I). The oxidized forms of nitrogen, nitrate and nitrite, remained constant throughout the study period (Fig. 5 A), without significant changes (Table I). Therefore, the total dissolved inorganic nitrogen (TDIN) in the water column was lowered due to the reduction

of the ammonium inputs (Table I). The ratio reduced: oxidized before the management actions was 4:1 and afterwards it became 1:1.

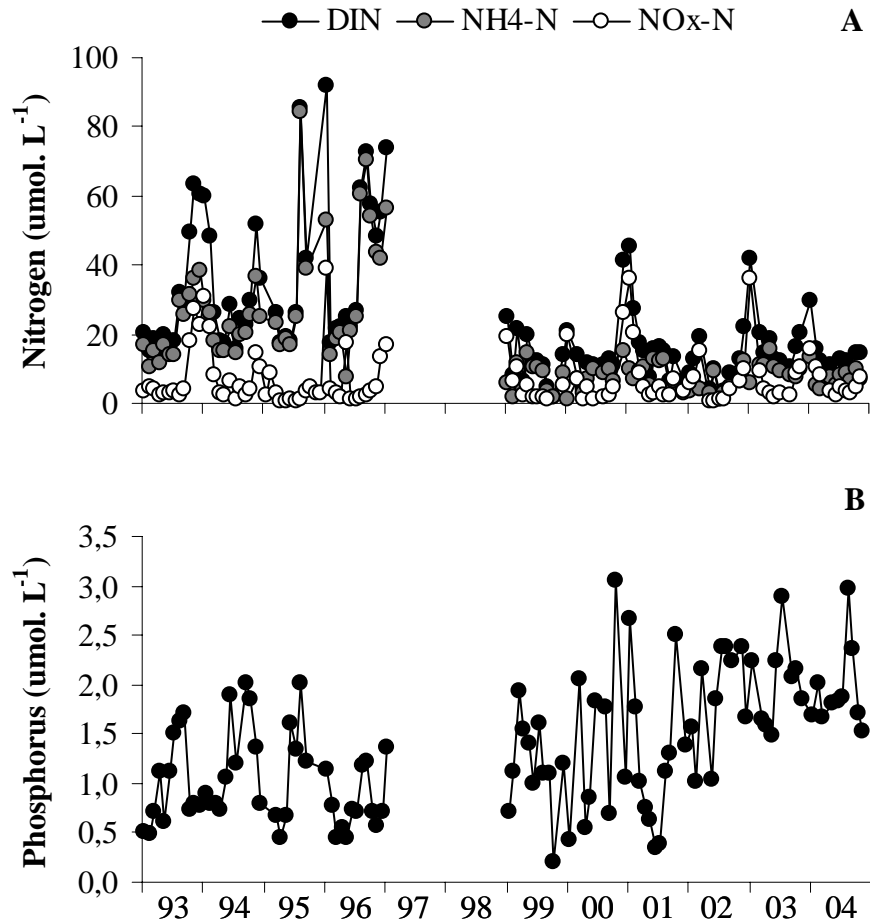


Figure 5 – A) The variation in concentration ($\mu\text{mol.L}^{-1}$) of DIN, ammonium and oxidized nitrogen forms ($\text{NO}_x\text{-N}$) and B) phosphate ($\text{PO}_4\text{-P}$) in the water column between January 1993 and December 2004.

The dissolved inorganic phosphorus (DIP) also presented seasonal variation throughout the years, with concentrations increasing in the hot summer months (Fig. 5 B). After 1998, it suffered a significant increase from a mean value of $1.01 \mu\text{mol.L}^{-1}$ to $1.59 \mu\text{mol.L}^{-1}$ (Table I).

Table I – The water column nutrients concentrations ($\mu\text{mol.L}^{-1}$) in the pre-management period (A) and post-management (B) and the statistical analysis (Mann-Whitney Rank Sum Test) of the differences between both periods (C). SE is the Standard Error.

		A 1993-1997				B 1998-2004				C Statistics
		min	Max	mean	SE	min	max	mean	SE	
Water Column	$\text{NO}_x\text{-N}$ ($\mu\text{mol L}^{-1}$)	0.50	38.93	7.13	0.16	0.74	36.06	6.98	0,14	n.s.:T=316,0; P=0.602
	$\text{NH}_4\text{-N}$ ($\mu\text{mol L}^{-1}$)	7.58	84.29	28.15	0.38	1.2	15.87	7.76	0.06	significant:T=44.0; P=0.002
	$\text{PO}_4\text{-P}$ ($\mu\text{mol L}^{-1}$)	0.44	2.02	1.01	0.01	0.20	3.06	1.59	0.01	significant:T=147.0; P=0.013

3.2 Primary Producers

3.2.1 Biomass

The responses of the potential opportunistic primary producers (chlorophyll *a* concentration and macroalgal biomass) were different.

Chlorophyll *a* concentrations did not differ ($W=749.0$; $p>0.05$) (Table II): before (min. 1.7 mg m^{-3} ; max. 14.7 mg m^{-3} ; mean $6.1 \text{ mg m}^{-3} \pm 0.06 \text{ SE}$) and after 1998 (min. 1.4 mg m^{-3} ; max. 12.9 mg m^{-3} ; mean $5.9 \text{ mg m}^{-3} \pm 0.4 \text{ SE}$).

Ulvaceans, before the management actions were implemented in the estuary, presented a very high biomass production (Fig. 6 A), with a maximum value of $452.53 \text{ g.m}^{-2} \text{ AFDW}$ and a mean value $34.66 \text{ g.m}^{-2} \text{ AFDW}$ (Table II). However, after 1998 green macroalgae showed a significant decrease in biomass, with the mean value dropping to 1.52 and a maximum of $12.63 \text{ g.m}^{-2} \text{ AFDW}$. On the contrary, *G. gracilis* biomass increased greatly (Fig. 6 B). The highest value reported prior to 1998 was $105.9 \text{ g.m}^{-2} \text{ AFDW}$, with a mean value of $17.12 \text{ g.m}^{-2} \text{ AFDW}$ but afterwards it increased to $195.3 \text{ g.m}^{-2} \text{ AFDW}$ and a mean of 40.76 (Table II).

Zostera noltii meadows were dramatically reduced from an approximate area of $150\,000 \text{ m}^2$ in 1986, to 200 m^2 in 1997 (Fig. 2). Biomass had a drastic reduction between 1993 and 1997, but after 1999 it has begun to recover (Fig. 6 C). However, it still has not reached the values of pre-management period presenting a maximum biomass value of $234.89 \text{ g.m}^{-2} \text{ AFDW}$ while before it was $333.06 \text{ g.m}^{-2} \text{ AFDW}$. Both *Z. noltii* leaves and roots have shown significant differences in biomass (Table II) revealing an increasing pattern (Fig. 6 C).

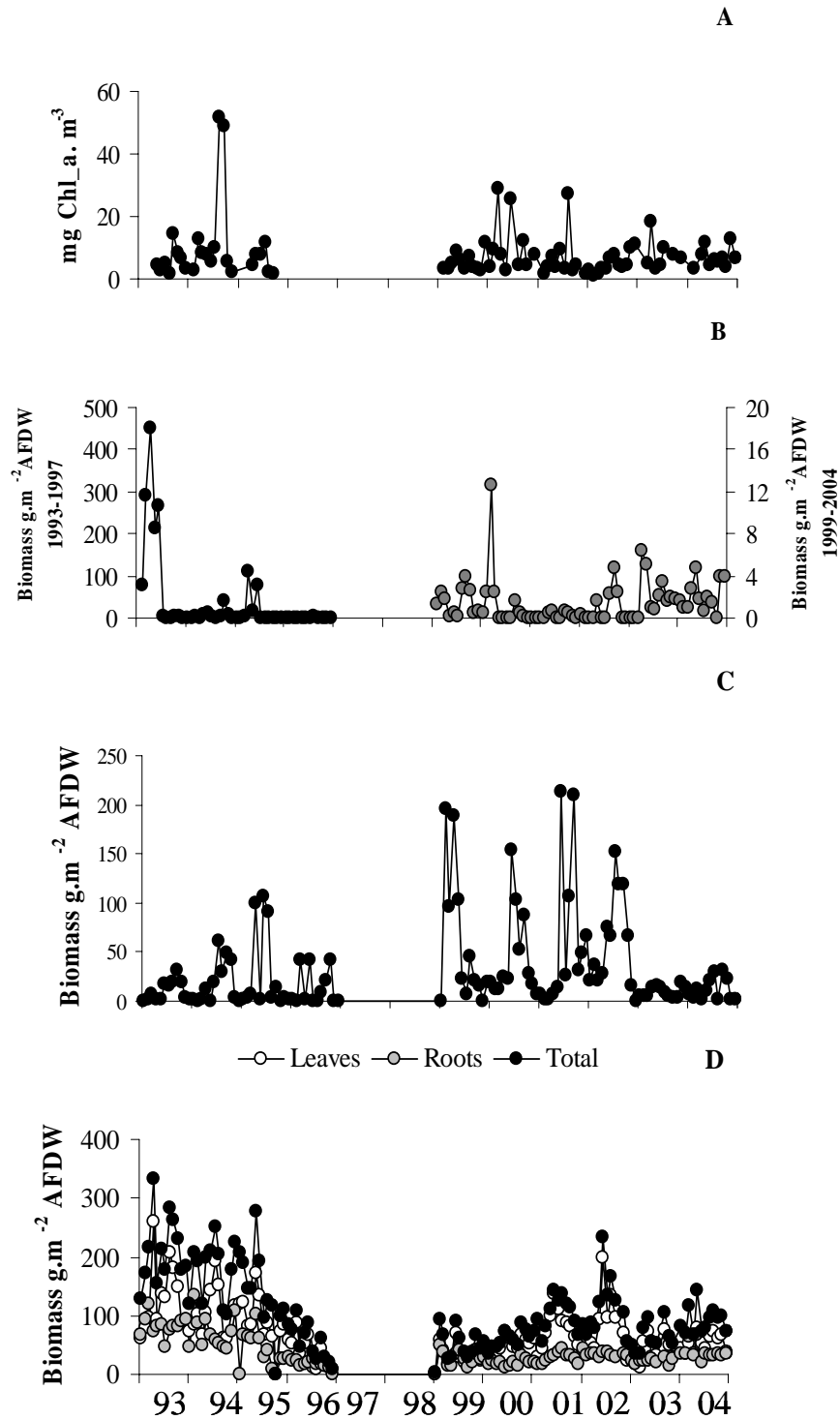


Figure 6 – The inter-annual variation of primary producers assemblages pre- and post 1998: a) phytoplankton ($\text{mg Chl } a \text{ m}^{-3}$) b) *Ulva* spp. biomass (AFDW g.m^{-2}); c) *Gracilaria gracilis* biomass (AFDW g.m^{-2}); d) *Z. noltii* biomass (AFDW g.m^{-2}).

Table II - Phytoplankton biomass (measured as Chl *a*), and the biomass and internal nutrient concentrations of *Ulva* spp., *Gracilaria gracilis* and *Z. noltii* before (A) and after the management periods (B) and the statistical differences (Mann-Whitney Rank Sum Test) between both periods (C). SE is the Standard Error and n.s. stands for not significant.

		A 1993-1997				B 1998-2004				C Statistics
		min	Max	mean	SE	min	max	mean	SE	
Chl <i>a</i>	Phytoplankton	1.7	14.7	6.1	0.06	1.4	12.9	5.9	0.4	n.s.:T=749.0; p>0.05
	<i>Ulva</i> spp.	0.00	452.23	34.66	2.12	0.00	12.63	1.52	0.03	significant: T=2754.5; P<0.05
Biomass	<i>Gracilaria gracilis</i>	0.00	105.9	17.12	0.45	0.00	195.3	40.76	0.70	significant: T=1984.5; P<0.001
	<i>Z. noltii</i> roots	0.00	119.97	53.64	0.72	7.35	66.63	27.89	0.15	significant: T=3204.0; P<0.05
	<i>Z. noltii</i> leaves	5.65	185.76	85.43	1.12	11.76	129.18	52.53	0.40	significant: T=3021.0; P<0.05
Tissue Nitrogen	<i>Ulva</i> spp.	32.88	95.61	62.02	0.40	16.55	112.89	52.83	3.15	n.s.: T=221.5; P=0.492
	<i>Gracilaria gracilis</i>	24.64	110.04	62.42	3.02	19.34	108.81	58.5	0.52	n.s.: T=289.5; P=0.844
	<i>Z. noltii</i> roots	11.56	45.56	23.92	2.85	3.37	47.42	19.14	1.91	n.s.: T=1.411; P=0.166
	<i>Z. noltii</i> leaves	19.47	64.08	37.10	3.72	6.05	45.37	29.54	1.45	significant: T=2.310; P<0.05
Tissue Phosphorus	<i>Ulva</i> spp.	1.19	5.54	2.18	7.51	0.29	3.87	2.04	0.12	n.s.: T=188.5; P=0.788
	<i>Gracilaria gracilis</i>	1.23	2.88	2.08	0.05	0.05	6.23	2.25	0.03	n.s.: T=244.0; P=0.438
	<i>Z. noltii</i> roots	1.26	6.85	3.09	0.40	0.62	8.67	4.51	0.37	significant: T=-5.126; P<0.001
	<i>Z. noltii</i> leaves	1.83	4.46	2.55	0.25	1.84	9.25	5.61	0.36	significant: T=2.343; P<0.05

3.2.2 Growth pattern

Phytoplankton presented a seasonal variation throughout the study period, with growth beginning in winter, reaching a peak of biomass in mid summer decreasing afterwards (Fig 6 A).

Green macroalgae biomass production presented two distinct annual patterns. In 1993 and 1995, when large blooms of *Ulva* spp. occurred, growth began in late winter, building up biomass that peaked in mid spring with a subsequent decay in mid summer (Fig. 7 A). In years with lower biomass production, the growth pattern often presented two peaks of biomass, one in mid spring and another in autumn (Fig. 7).

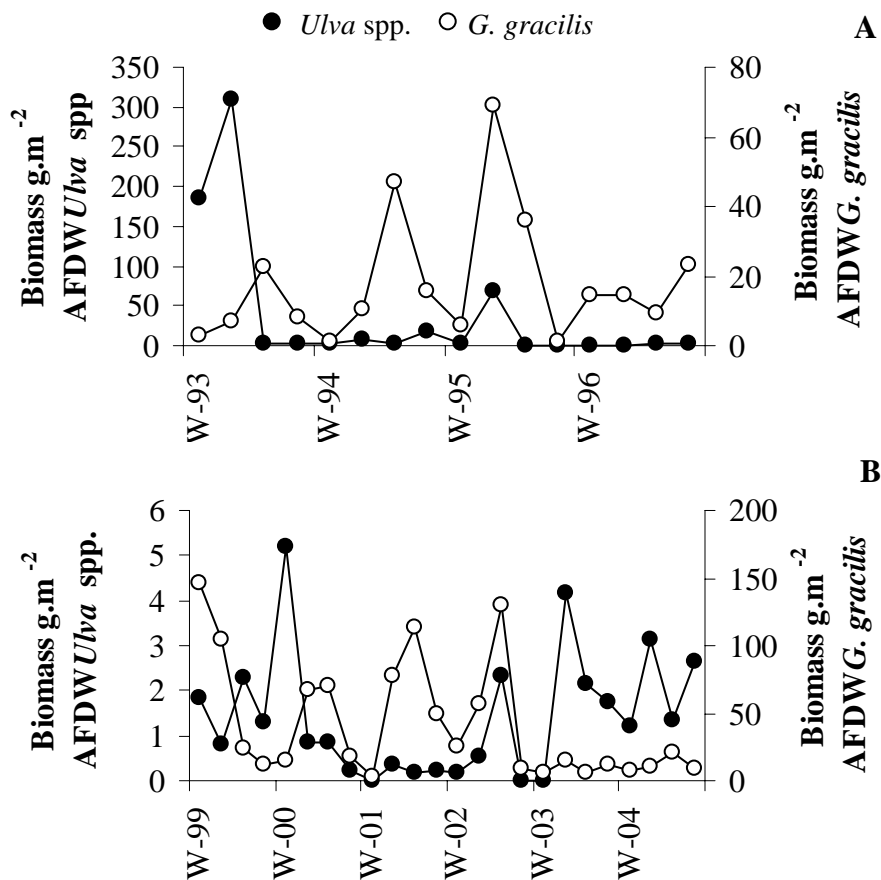


Figure 7 – Seasonal growth pattern of *Ulva* spp. and *Gracilaria gracilis* before the management (A) and afterwards (B).

Concerning *G. gracilis* biomass production started in late winter, continuing throughout spring and reaching a peak in summer (Fig. 7). In autumn biomass decreased but red macroalgae continued to be present all year round.

Z. noltii also exhibited a seasonal pattern of biomass production (Fig. 8). Leaf biomass increased mostly during the spring and summer whereas the roots/rhizome biomass increased in autumn and winter.

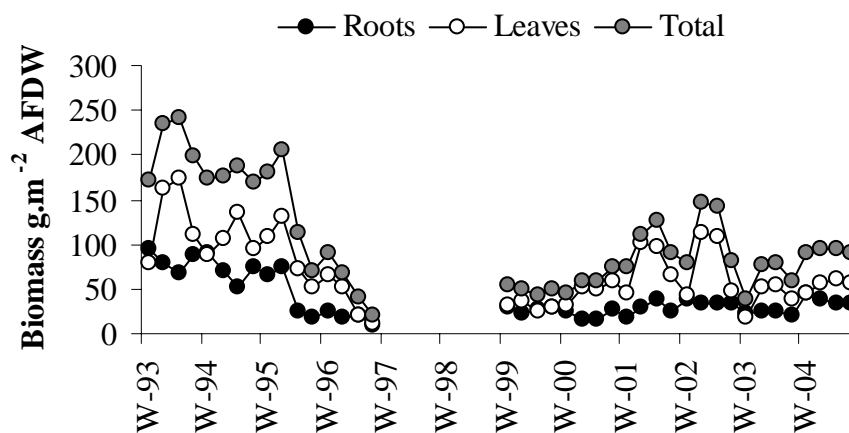


Fig. 8 – Seasonal growth patterns of *Zostera noltii* from 1993 to 2004. The values plotted correspond to seasonal means of monthly data.

3.2.3 Tissue Nutrient Concentrations

When considering the total N and total P concentrations of green macroalgae throughout the study period, no statistically significant differences were found (Table II). The same situation was observed in *G. gracilis*. All macroalgae kept the internal nutrients concentrations constant and with values within the same range (Table II).

Regarding *Z. noltii*, the nitrogen content of the roots was not significantly different between the two periods, whereas the leaves's content decreased significantly from a mean

value of 37.10 to 29.54 mg.g⁻¹ (Table II). The internal phosphorus content increased significantly both in roots and leaves (Table II).

When analysing the percentage of dry weight nutrient contents it was found that both macroalgae presented N and P values well above the critical tissue concentrations for maximum growth (1.5 % for nitrogen and 0.13 % for phosphorus according to Duarte, 1992) and thus without nutrient limitation (Fig. 9 A and B). (Fig. 9 C). Phosphorus was always above the critical level, both in leaves and roots (Fig. 9 D). The exception occurred in 2000, when both green and red macroalgae presented P values inferior to the critical level, coincident with heavy rains during that year.

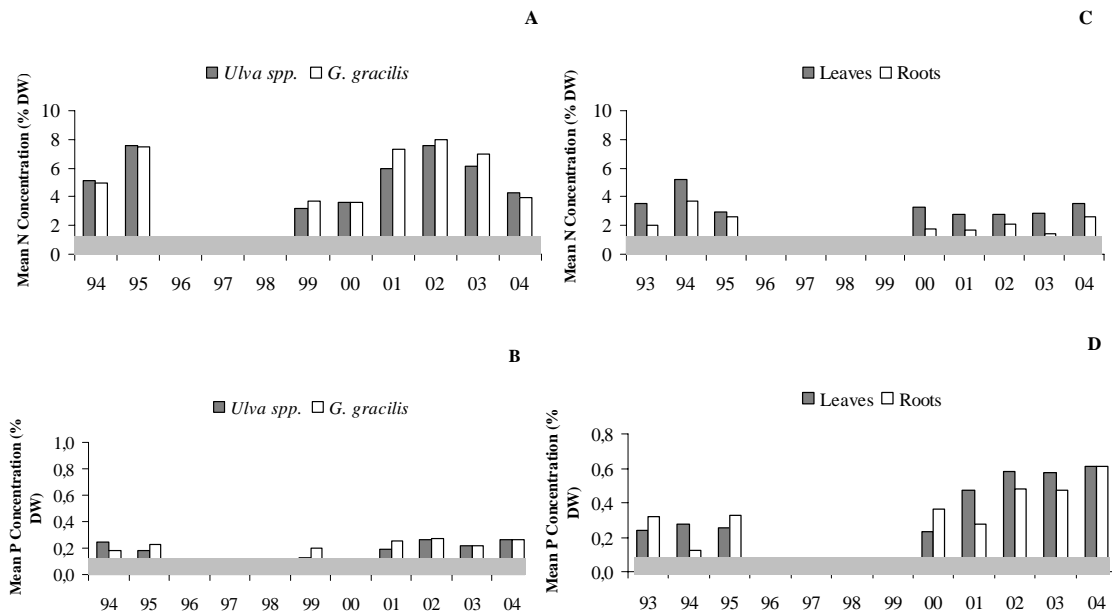


Figure 9 – The variation in the internal nutrients content in primary producers assemblages, before and after 1998 as a percentage of dry weight. A) Nitrogen in macroalgae; B) Phosphorus in macroalgae; C) Nitrogen in *Z. noltii*; D) Phosphorus in *Z. noltii*. The grey barr represents the critical concentration value for maximum growth under which there is nutrient limitation.

CHAPTER 4.

DISCUSSION

4.1 Abiotic Parameters

The abiotic parameters have shown to be dependent on the seasonality, more particularly, on the climate conditions of the region. Also, some parameters were strongly influenced by the discharges from the Pranto River (e.g. Lillebo et al., 2005).

The mean water temperature, as expected, remained constant as its variation is essentially dependent on the climatic seasonality. Salinity also presented a seasonal variation as it is conditioned by precipitation and the subsequent increase of the freshwater flow to the estuary. As a result, higher values were registered during the summer, when precipitation and freshwater flow were lower and decreasing towards the rainy seasons. (e.g. Lillebø et al., 2005).

The dissolved inorganic nitrogen in the water column was also dependent on the sluice opening, with concentration increases in autumn and winter. This is due to the freshwater flow from the Pranto River which was rich in nitrogen compounds (mainly ammonium) drained from the surrounding agricultural fields. With the diversion of this flow to the North arm the DIN decreased significantly as a result of the decrease of ammonium.

The dissolved inorganic phosphorus followed a different pattern, with higher concentrations during the summer, decreasing in the winter. This seasonal variation is common to estuarine systems and is related with the amount of organic matter accumulated in the sediments (Sfriso et al., 1988; Valiela, 1995) and with temperature variation (Coelho et al., 2004; Lillebø et al., 2004). Higher efflux rates in temperate regions are registered at higher temperatures since the increase in temperature stimulates the efflux of phosphate from the sediment to the water column (Lillebø et al., 2004). After the improvement in the hydrodynamics DIP in the water column increased significantly.

4.2 Primary Producers

The primary producers responded differently to the changes in the system, resulting from the restoration measures implemented. Phytoplankton, measured as concentration of chlorophyll *a*, did not show any significant changes. Ulvaceans biomass was significantly reduced whereas *Z. noltii* and *G. gracilis* have increased theirs, leading to a shift in macroalgal abundance. A similar change in the benthic community has been reported in Sacca di Goro, in north-eastern Italy, where the restriction of *Ulva rigida* resulted in the establishment of an almost monospecific algal cover of *G. gracilis* (Mistri et al., 2001).

Since chlorophyll *a* concentrations did not show any significant changes throughout the pre and post-mitigation period, it means that phytoplankton is not nutrient limited. In fact, due to a comparatively higher surface area/volume relation, phytoplankton has higher affinity for nitrogen forms, with higher uptake rates than macroalgae for both ammonia and nitrate per unit of biomass (Hein et al., 1995; Collos, 1998).

The improvement in hydrodynamics led to the increase in water flow and current velocity which in turn can lead to a higher export of floating macroalgae to the ocean. (Hernández et al., 1997; Martins et al., 2001; Flindt et al., 2004).

Salinity also plays an important role in controlling macroalgae growth rate. It has been established in a previous work that biomass production of green macroalgae in the Mondego estuary increases exponentially with salinity until 30 with the optimum range between 17 and 22 (Martins et al., 1999). There is a decrease in the growth rate at salinities higher than 28, but it is not as accentuated as for lower salinities (≤ 3) (Martins et al., 1999). Since the increase in the mean value of salinity was below 28, green macroalgae could still have attained a maximum growth rate and so it is necessary to address other factors to explain the reduction in *Ulva* spp. biomass.

Most macroalgae can rapidly remove pulse nutrient inputs from the water column before they can be detected (Fong et al., 1998; Kamer et al., 2004) and algal tissue nutrient concentrations are thus used to infer limitation of either N or P (Duarte, 1992; Wheeler & Björnsäter, 1992). Growth rates are dependent on the internal nutrient content whereas the uptake rates rely upon the difference between these contents and the concentrations in the water column (Björnsäter & Wheeler, 1990). They use the majority of nutrients taken up to sustain a maximum rate during the growth season and during this time they will only increase tissue concentrations when the nutrients are supplied in sufficient amounts (Lyngby et al., 1999). In the Palmones Estuary (Spain), *Ulva* spp. have shown a decrease in biomass not accompanied by a decrease in internal content (Hernández et al., 1997). In the Mondego estuary the same situation occurred with the Ulvaceans but *G. gracilis* has increased its biomass.

Green and red macroalgae have high affinity for ammonium and nitrate at both low and high concentrations (Fujita, 1985; Pedersen & Borum, 1997, Runcie et al., 2003) and are able to take up both forms simultaneously when they are present in the water column, but with different uptake rates (Kautsky, 1982; Pedersen & Borum, 1997; Glenn et al., 1999; Jones et al., 2001; Cohen & Fong, 2004). In *Ulva* sp the uptake rate of NO_3^- is about one-fourth of the uptake rates found for NH_4^+ (Runcie et al., 2003). This difference may be due to the nitrate's negative charge, which makes the uptake energy-dependent and thus slower (Runcie et al., 2003). After the uptake, nitrate must be converted into ammonium in a rate-limiting step catalyzed by nitrate reductase (NR) before being accumulated in vacuoles (Viaroli et al., 1996; Cohen & Fong, 2004; Lartigue & Sherman, 2005). The level of NR in *U. intestinalis* appears to be determined by the presence or absence of NO_3^- in the water column conferring macroalgae the ability to use a greater proportion of this nitrogen

form. The reduction of nitrate is energetically expensive and can use as much as one-third of photosynthetic reduction capacity in phytoplankton (Syrett, 1981). This could explain the preference for ammonium because it allows macroalgae to save the energy involved in nitrate reduction (Cohen & Fong, 2004).

Ulva spp. and *G. gracilis* have reacted differently to the decrease in ammonium in the water column.

Fong and colleagues (2004) hypothesized that *Ulva* spp. have evolved mechanisms to take advantage of high concentrations of nitrogen by prioritising allocation of available energy and carbon skeletons to nutrient uptake and assimilation of N. It was found that the addition of NO_3^- resulted in increased respiration rates and use of fixed carbon to synthesize amino acids (Turpin et al., 1988). This way the energy available may not have been enough to attain maximum growth (Fong et al., 2004). It is possible that green macroalgae in the Mondego Estuary, in order to maintain their nitrogen content, have taken up more nitrate to compensate the decrease in ammonium. Oki and Fushimi (1992) found that as the concentration of NH_4^+ decreased, the uptake rate of NO_3^- increased. This difference in uptake means that they spent more energy and possibly have not been able to attain maximum growth as a result, even when all the environmental factors were favorable.

The changes in the nitrogen forms seem to have affected red macroalgae differently. Studies show that concentrations of NH_4^+ higher than 5 μmol have an inhibitory effect in *G. gracilis* suppressing the uptake of NO_3^- up to 38% (Smit, 2002; Buschmann et al., 2004; Tsai et al., 2005). Red macroalgae are especially efficient at taking up nutrients rapidly and have mechanisms to store large reserves of nutrients (Jones et al., 2001; Menéndez et al., 2001; Hernández et al., 2002). Nitrogen is stored in the red pigment phycoerithrin and in conditions of nutrient deficiency it is quickly mobilized and used to sustain growth

(Hernández et al., 2002; Nagler et al., 2003). As a result of this ability *Gracilaria* spp. are seldom nutrient-limited except after periods of low nutrient inputs to the system and can maintain high growth rates even when N levels are low (Menéndez et al., 2001). Nitrogen can be stored for long periods of time, whereas in *Ulva* spp., this storage capacity is temporary. This can constitute a competitive advantage over green macroalgae during periods of low nitrogen availability (Comín et al., 1995). Before 1998, the mean ammonium value in the water column was $28.15 \mu\text{mol. L}^{-1}$, which means that even if nitrate uptake was severely inhibited in red macroalgae, ammonium uptake would be enough to maintain the internal nitrogen content above the critical tissue concentrations for maximum growth. It seems possible that *G. gracilis* had lower biomass before the management due to shading by the green macroalgal mats lying above (Hernández et al., 1997). Peckol and Rivers (1995) found that elevated ammonium levels had a greater inhibitory effect on ammonium uptake rates in darkness and that light substantially reversed this inhibition. Every growth season red macroalgae biomass increased after green macroalgae started to decrease and in the years when there was an absence of blooms, *G. gracilis* biomass was higher, which seems to support this hypothesis (Fig. 7 A and B).

The fact that *G. gracilis* settles to the sediment may play an important role in nutrient acquisition and may be a competitive advantage over *Ulva* spp., that depend almost exclusively on the water column to take up nutrients. Macroalgal canopies located over the sediment-water interface partially intercept nutrients released from the sediment, that otherwise would enter the water column (Lavery & McComb, 1991; Menéndez et al., 2000). This way red macroalgae would have more ammonium available than green macroalgae after the management and would need to take up less oxidized forms, saving

energy to maintain a high growth rate. Also, with the reduction of green biomass, light was more accessible to red macroalgae thus enhancing ammonium uptake and growth.

Z. noltii has reacted to the management actions with an increase in biomass, indicating the beginning of its recovery. As it happens with *G. gracilis*, light is a major factor controlling biomass production making the seagrass meadows more sensible to floating macroalgal masses. Green macroalgae mats floating over the meadows kept light from the layers below, preventing photosynthesis and thus leading to anoxic conditions (Hemminga, 1998). Also the macroalgae decay during the summer worsened the problem as decomposing bacteria consume oxygen.

Seagrasses can take up nutrients directly from the water column through leaves and from the sediment, through roots (Sfriso & Marcomini, 1999; Bocci et al., 1997; Hemminga, 1998) which allows growth even with low nutrient availability (Bocci et al., 1997). Leaves can take up both NH_4^+ and NO_3^- , whereas roots take up mostly NH_4^+ since it is the prevalent form in the sediments (Bocci et al., 1997).

N and P analysis on seagrass leaves and roots showed that the internal concentrations have been altered after the management actions. After 1998, the nitrogen in the roots decreased, probably because sediment pool of NH_4^+ is not all available for uptake, since the ions can adsorb to mineral particles (Touchette & Burkholder, 2000). Nonetheless, leaves present a higher uptake affinity for NH_4^+ than roots and they seem to be more efficient in removing low levels of this reduced form (Touchette & Burkholder, 2000) which would compensate its decrease in the roots.

The increase in the internal P both in leaves and roots may be a result of the increase of PO_4^- in the water column as *Z. noltii* tends to take up this form mainly through the leaves and relies on root uptake only when it is negligible in the water column (Brix & Lyngby,

1985). However, leaves are more sensible to light availability than belowground tissues (Pérez et al., 1994) which could have made the uptake of P more dependent on the roots before 1998 since light was kept from aboveground tissues. After the management, leaves most likely increased their uptake due to higher P concentration in the water column and to higher light availability.

4.3 Final Considerations

Phytoplankton, *Ulva* spp., *Gracilaria gracilis* and *Zostera noltii* have presented different responses to the changes resulting from the mitigation measures implemented in the South arm of the Mondego estuary in view to the revert the ongoing eutrophication process.

Phytoplankton measured as chlorophyll *a* has remained constant despite the changes introduced to the system.

The frequent spring macroalgal blooms of *Ulva* spp. stopped to occur and biomass was significantly reduced after 1998, resulting from a higher sensibility to the accentuated decrease of ammonium in the water column.

Red macroalgae, on the other hand, took advantage of the higher light availability and of its location on the water-sediment interface to intercept nutrients and thus increase its biomass.

The seagrass *Z. noltii*, started to recover as a result of the physical protection of the meadows and of the absence of green blooms. Light appears to be the major factor controlling seagrass biomass production and not the nutrients present in the water column, as it can compensate by uptake from the sediments via roots, which is an advantage macroalgae do not possess.

These important changes in primary producers's assemblages indicate that the South arm of the Mondego estuary is currently in a recovery stage, reducing the symptoms of eutrophication that have prevailed for the last decades. Indeed, the management plan initiated in 1998 with the introduction of mitigation actions led to a reduction of the eutrophication effects in the estuary. The progressive improvement on the ecological conditions of the South arm is expected.

To assure the success of any actions taken to revert eutrophication and to restore estuaries, as well as other coastal aquatic ecosystems, it is necessary to understand the effects they will have on all parts of the system, since the way they will react can be different from what was initially predicted.

The European Water Framework Directive (WFD) constitutes a legislation with well-defined objectives that has the overall goal of attaining a 'good water status' by 2015 for all water types considered. To determine the ecological quality status (EcoQ) of each type of water several quality elements were specified and they include biological, hydromorphological and physico-chemical parameters (Borja, 2004; 2005; WFD; 2000/60/EC). Estuaries are also considered in the legislation being referred to as transitional waters. The biological elements selected to determine the ecological quality status of the estuaries are the benthonic invertebrates, fish fauna, phytoplankton, macroalgae and angiosperms. The physico-chemical elements are also very important and include many variables such as dissolved oxygen and nutrients.

Also the importance of including physico-chemical and biological processes, and hydromorphological elements to understand the ecological status of the South arm of the Mondego estuary before and after 1998 is reflected in this work. If only the changes in

physical-chemical elements were considered, the modifications underwent on the other elements would be neglected and the system's status could be wrongfully determined.

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APPENDIX

The response of primary producers' assemblages to mitigation measures to reduce eutrophication symptoms in a temperate estuary

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Abstract

The Mondego estuary is a well-described system located on the North Atlantic Ocean, where cultural eutrophication progressed over the last decades of the 20th century. Consequently, and due to a large productivity of *Ulva* spp. *Zostera noltii* meadows were severely reduced with a concomitant decrease in environmental quality. In 1998, experimental mitigation measures were implemented, via changes in hydrology to

increase circulation and diversion of nutrient-rich freshwater inflow, to reverse the process in the most affected area of the estuary – its South arm.

The objective of this study was to assess the differences in response of primary producer assemblages to the implemented measures to reduce eutrophication symptoms. Results show that the mean concentrations of DIN suffered a notorious decrease due to a significant reduction in the ammonium concentration in the water column, while DIP increased significantly. Primary producers' assemblages showed different responses to these changes: phytoplankton, measured as concentration of chlorophyll *a*, did not show any significant changes; green macroalgae, mostly *Ulva* spp. suffered a large reduction in biomass whereas *Gracilaria gracilis* and the macrophyte *Zostera noltii* biomasses increased greatly. Results show that phytoplankton was never nutrient limited, and suggest that the reduction in ammonium could have been responsible for the changes in the green macroalgae's biomass and that light was a more determinant factor in the response of seagrasses whereas red macroalgae reaction seemed to be dependent on these two factors.

1. Introduction

Anthropogenic activities have enhanced the enrichment of water bodies with nutrients, particularly nitrogen and phosphorus, in the last decades. Agricultural run offs, waste discharges from industries and fish farms among others, are responsible for nutrient inputs into aquatic systems (Menéndez & Comín, 2000; Nedwell et al., 2002; Hernández et al., 2004).

Some primary producers (phytoplankton and macroalgae) can take advantage of hypernutrification. They are usually ephemeral, r-selected species that present simple morphology i.e. filamentous, branched or sheet-like algae, with high uptake rates (Raven & Taylor, 2003) and as ruderal strategists they are capable of taking advantage of the available resources in transient environments (Viaroli et al., 1996; Raven & Taylor, 2003; Cohen & Fong, 2004). Among the macroalgal species found in areas undergoing eutrophication are the genus *Chaetomorpha*, *Cladophora*, *Gracilaria* and *Ulva* (Raffaelli et al., 1998; Mistri et al., 2001; Fong et al., 2004). The proliferation of these macroalgae under these conditions usually has severe consequences. They often out-compete other species, usually late-successional, long-lived species like perennial macroalgae (e.g. *Fucus*) and seagrasses (e.g. *Zostera*) (Peckol & Rivers, 1996; Menéndez & Comín, 2000). Their high surface-area to volume ratio and high affinity for nutrients, especially N and P, favors a rapid nutrient uptake and high growth and production rates leading to very large biomass values (Rosenberg & Ramus, 1984; Hernández et al., 1997; Raffaelli et al., 1998; Raven & Taylor, 2003), that often shade the underlying areas. Seagrasses are important primary producers in estuarine systems and their abundance and distribution is strongly correlated with light availability (Kraemer & Hanisak, 2000).

Due to the unique importance of seagrass meadows in the ecosystems, it is necessary to take measures to minimize and revert the effects of eutrophication, bringing the systems into the previous stable state (e.g. Webster & Harris, 2004). However, to guarantee that the restoration programmes are successful, it is important to understand the mechanisms that have led to the ecological changes (Zhang et al., 2003). In the case of macroalgal blooms, the knowledge of their responses to changes in their driving variables (e.g. nutrient loadings, hydrodynamics) is essential to understand the way the system will react and thus assuring its recovery (e.g. Webster & Harris, 2004;).

The aim for the present study was to assess the response of potential opportunistic primary producers like phytoplankton (accessed as concentration of Chlorophyll *a*), the macroalgae *Ulva* spp. and *Gracilaria gracilis*, as well as the seagrass *Zostera noltii* (Hornem) to the mitigation measures implemented in the Mondego estuary to reduce the eutrophication symptoms.

2. Material and Methods

2.1 Study area

The Mondego estuary is a warm temperate coastal system located on the Western Coast of Portugal (40°08'N; 8°50'W), with an approximate area of 1072 ha and 7 km long, characterized by a temperate coastal climate with Mediterranean and Atlantic influences. It comprises two arms, North and South, separated by an alluvium-formed island (Murraceira Island) and joined again near the mouth. The north arm is deeper (4-8 m during high tide, tidal range 1-3 m), while the South arm is shallower (2-4 m during high tide, tidal range 1-3 m) and until 1998 it was largely silted up in the upstream areas, which caused freshwater to flow mainly through the north arm. As a consequence, water circulation was dependent on tides and freshwater discharges

(which constituted an important input of nutrients) from a small tributary, the Pranto River (Fig. 1).

In 1998, a restoration program was implemented to reverse the process of eutrophication in the most affected area of the estuary – the South arm (Fig. 1). The measures undertaken comprised the reduction of nutrient loadings from agricultural runoff, the improvement in hydrodynamics by enlarging the connection between both arms and the physical protection of seagrass meadows (for more detailed description see Lillebø et al., 2005). The summary of the main characteristics of the South arm of the estuary are presented in Table I.

2.2 Field program and laboratory procedures

The study was conducted between February 1993 and December 2004 in the South arm of the Mondego estuary, as a part of a long-term monitoring program. Three sites (A, B and C – Fig. 1 A) were selected based on macroalgal abundance following a gradient increasing from downstream to upstream. Currently, seagrass is restricted to site A.

Sampling was carried out bimonthly from February 1993 until December 2000 and monthly thereafter. From January 1997 to December 1998 no sampling was performed.

On each sampling occasion, water temperature and salinity were recorded *in situ*, water samples were collected (approximately 250 ml), transported to the laboratory where they were filtered using Whatmann GF/C glass fibre filters and then frozen at - 18°C for later analysis on dissolved inorganic nitrogen and phosphorus. Nitrate (NO₃-N) and nitrite (NO₂-N) were analysed according with standard methods described in Strickland and Parsons (1972) and ammonium (NH₄-N) and phosphate (PO₄-P) analysis followed the Limnologisk Metodik (1992) methodology. The phytoplankton chlorophyll *a* (Chl*a*) determinations were performed by filtering 0.5-1.0 L of water through Whatman GF/C

glass-fibre filters followed by extraction according to Parsons et al. (1985). In the field and during transportation to the lab, samples were stored on ice and light protected. Samples from the three monitoring areas (A, B and C) were collected and analysed separately. Due to the small distance between the sites, data were put together and presented/related as mean values (\pm SE) for the South arm of the estuary.

Plant sampling for biomass assessment was performed randomly with a corer (minimum 6 cores, with 143 cm² section) and biomass determined as AFDW after oven drying at 60°C for 72h and combustion at 450°C for 8h.

Macroalgae and seagrass for internal nutrient content were collected at site A, during low tide and transported in plastic bags to the laboratory where they were rinsed, dried for 48h at 60°C until constant weight and stored dry until analysis for internal N and P, in accordance to Limnologisk Metodik (1992). For phosphorus analysis, samples were combusted in a muffle furnace at 500 °C for 3h.

The *Ulva* species identified were *Ulva compressa* (L.) Greville and *Ulva intestinalis* (L.) Link (according to revision by Hayden et al, 2003).

3. Results

Nutrients concentration

The total dissolved inorganic nitrogen (TDIN) in the water column was lowered mostly due to the reduction of ammonium inputs, since the concentration of the oxidized forms (NO₃-N and NO₂-N) remained constant throughout the years (Table II). The reduced form presented a mean value of 28.9 $\mu\text{mol.L}^{-1}$ prior to 1998 which dropped to 7.76 $\mu\text{mol.L}^{-1}$ afterwards (Table II). Before the management actions, ammonium was the predominant form in the estuary about four times more abundant than the oxidized forms, but afterwards the ratio reduced: oxidized was 1:1.

The dissolved inorganic phosphorus (DIP) increased significantly from a mean value of $1.01 \mu\text{mol.L}^{-1}$ to $1.59 \mu\text{mol.L}^{-1}$ (Mann-Whitney Rank Sum Test: $W=888.5$, $p<0.001$) (Table II).

Primary producers' biomass

The responses of the potential opportunistic primary producers (chlorophyll *a* concentration and macroalgal biomass) for the two distinctive periods were different. Chlorophyll *a* concentrations did not differ ($W=749.0$; $p>0.05$) (Table III): before (min. 1.7 mg m^{-3} ; max. 14.7 mg m^{-3} ; mean $6.1 \text{ mg m}^{-3} \pm 0.06 \text{ SE}$) and after 1998 (min. 1.4 mg m^{-3} ; max. 12.9 mg m^{-3} ; mean $5.9 \text{ mg m}^{-3} \pm 0.4 \text{ SE}$). Within macroalgae, Ulvaceans presented a very high biomass production (Fig. 2 B), with a maximum value of 452.23 g.m^{-2} AFDW and a mean value 34.66 g.m^{-2} AFDW (Table III), before the management actions were implemented in the estuary. However, after 1998 green macroalgae showed a significant decrease in biomass, with the mean value dropping to 1.52 and a maximum of 12.63 g.m^{-2} AFDW (Table III). On the contrary, *G. gracilis* biomass increased greatly (Fig. 2 C). The highest value reported prior to 1998 was 105.9 g.m^{-2} AFDW, with a mean value of 17.12 g.m^{-2} AFDW but afterwards it increased to 195.3 g.m^{-2} AFDW and a mean of 40.76 (Table III). *Zostera noltii* meadows were dramatically reduced from an approximate area of $150\,000 \text{ m}^2$ in 1986, to 200 m^2 in 1997 (Fig. 1). Biomass had a drastic reduction between 1993 and 1997, but after 1999 it began to recover (Fig. 2 D). However, it still has not reached the values of pre-management period presenting a maximum biomass value of 234.89 g.m^2 AFDW while before it was 333.06 g.m^2 AFDW. Both *Z. noltii* leaves and roots have shown significant differences in biomass (Table III) revealing an increasing tendency.

Seasonal growth pattern

Phytoplankton presented a seasonal variation throughout the study period, with growth beginning in winter, reaching a peak of biomass in mid summer decreasing afterwards (Fig. 2 A).

Green macroalgae biomass production presented two distinct annual patterns. In 1993 and 1995, when large blooms of *Ulva* spp. occurred, growth began in late winter, building up biomass that peaked in mid spring with a subsequent decay in mid summer (Fig. 2 B). In years with lower biomass production, the pattern of growth of *Ulva* spp. often presented two peaks of biomass, one in mid spring and another in autumn (Fig. 2 B). *G. gracilis* exhibited only one pattern of growth prevailing during the study period. Biomass production started in late winter, continuing throughout spring and reaching a peak in summer (Fig. 2 C). In autumn biomass decreased but red macroalgae continued to be present all year round. *Z. noltii* also exhibited a seasonal pattern of biomass production (Fig. 2 D). Leaf biomass increased mostly during the spring and summer whereas the roots/rhizome biomass increased in autumn and winter.

Tissue Nutrient Concentrations

When considering the total N and total P of green macroalgae throughout the study period, no statistically significant differences were found (Table III). The same situation was observed in *G. gracilis*. Macroalgae kept the internal nutrients concentrations constant and with values within the same range (Table III). Regarding *Z. noltii*, the nitrogen content of the roots was not significantly different between the two periods, whereas the leaves' content decreased significantly from a mean value of 37.1 to 29.5 mg.g⁻¹ (Table III). The internal phosphorus content increased significantly both in roots and leaves (Fig. 3).

When analysing the percentage of dry weight nutrient contents, it was found that both macroalgae presented N values well above the critical tissue concentrations for maximum growth and thus without nutrient limitation (Fig. 3).

4. Discussion

Primary producer assemblages responded differently to the changes in the system resulting from the restoration measures implemented to reduce eutrophication symptoms. Phytoplankton, measured as concentration of chlorophyll *a*, did not show any significant changes. Ulvaceans biomass was significantly reduced whereas *G. gracilis* biomass increased, leading to a shift in macroalgal abundance. Simultaneously to the green macroalgae decrease in the Mondego South arm, *Z. noltii* slowly started to recover (from 0.02 ha in 1997 to 4 ha in 2004). These differences may be explained by the combination of changes in physicochemical and hydrological factors after the implementation of the mitigation measures, namely the improvement in water circulation, reducing the water residence time, and the effective reduction in nitrogen loads (Lillebø et al., 2005) specifically concerning ammonium.

Since chlorophyll *a* concentrations did not show any significant changes throughout the pre- and post-mitigation period, it means that phytoplankton is not nutrient limited. In fact, due to a comparatively higher surface area/volume relation, phytoplankton has higher affinity for nitrogen forms, with higher uptake rates than macroalgae for both ammonia and nitrate per unit of biomass (Hein et al., 1995; Collos, 1998).

Most macroalgae can rapidly remove pulse nutrient inputs from the water column before they can be detected (Fong et al., 1998; Kamer et al., 2004) and algal tissue nutrient concentrations are thus used to infer limitation of either N or P (Duarte, 1992; Wheeler & Björnsäter, 1992). Growth rates are dependent on the internal nutrient

content whereas the uptake rates rely upon the difference between these contents and the concentrations in the water column (Björnsäter & Wheeler, 1990). They use the majority of nutrients taken up to sustain a maximum rate during the growth season and during this time they will only increase tissue concentrations when the nutrients are supplied in sufficient amounts (Lyngby et al., 1999). In the Palmones Estuary (Spain), *Ulva* spp. have shown a decrease in biomass not accompanied by a decrease in internal content (Hernández et al., 1997). A similar pattern occurred with the Ulvaceans in the Mondego Estuary. In this system the improvement of water circulation led to the increase in water flow and current velocity which in turn can lead to a higher export of floating macroalgae to the ocean. (Flindt et al., 2004; Hernández et al., 1997).

Macroalgae assemblages showed different trends after 1998. Ulvaceans' biomass decreased while there was a significant increase in red macroalgae (*G. gracilis*). A similar change in the benthic community has been reported in Sacca di Goro, in North-eastern Italy, where the restriction of *Ulva rigida* resulted in the establishment of an almost monospecific algal cover of *G. gracilis* (Mistri et al., 2001).

Green and red macroalgae have high affinity for ammonium and nitrate at both low and high concentrations (Fujita, 1985; Pedersen & Borum, 1997, Runcie et al., 2003) and are able to take up both forms simultaneously when they are present in the water column, but with different uptake rates (Kautsky, 1982; Pedersen & Borum, 1997; Glenn et al., 1999; Jones et al., 2001; Cohen & Fong, 2004). In *Ulva* sp the uptake rate of the NO_3^- is about one-fourth of the uptake rates found for NH_4^+ (Runcie et al., 2003). This difference may be due to the nitrate's negative charge, which makes the uptake energy-dependent and thus slower (Runcie et al., 2003). After the uptake, nitrate must be converted into ammonium in a rate-limiting step catalyzed by nitrate reductase (NR) before being accumulated in vacuoles (Viaroli et al., 1996; Cohen & Fong, 2004;

Lartigue & Sherman, 2005). The level of NR in *U. intestinalis* appears to be determined by the presence or absence of NO_3^- in the water column conferring macroalgae the ability to use a greater proportion of this nitrogen form. The reduction of nitrate is energetically expensive and can use as much as one-third of photosynthetic reduction capacity in phytoplankton (Syrett, 1981). This could explain the preference for ammonium because it allows macroalgae to save the energy involved in nitrate reduction (Cohen & Fong, 2004).

Macroalgae assemblages responded differently to the decrease in ammonium in the water column. Fong et al. (2004) hypothesized that *Ulva* spp. have evolved mechanisms to take advantage of high concentrations of nitrogen by prioritising allocation of available energy and carbon skeletons to nutrient uptake and assimilation of N. It was found that the addition of NO_3^- resulted in increased respiration rates and use of fixed carbon to synthesize amino acids (Turpin et al., 1988). This way, the energy available may not have been enough to attain maximum growth (Fong et al., 2004). It is possible that green macroalgae in the Mondego Estuary, in order to maintain their nitrogen content, have taken up more nitrate to compensate the decrease in ammonium. Oki and Fushimi (1992) found that as the concentration of NH_4^+ decreased, the uptake rate of NO_3^- increased. This difference in uptake means that they spent more energy and possibly have not been able to attain maximum growth as a result, even when all the environmental factors were favourable. The changes in the nitrogen forms seem to have affected red macroalgae differently. Studies show that concentrations of NH_4^+ higher than $5 \mu\text{mol}$ have an inhibitory effect in *G. gracilis* suppressing the uptake of NO_3^- up to 38% (Smit, 2002; Buschmann et al., 2004; Tsai et al., 2005). Red macroalgae are especially efficient at taking up nutrients rapidly and have mechanisms to store large reserves of nutrients (Jones et al., 2001; Menéndez et al., 2001; Hernández et al., 2002).

Nitrogen is stored in the red pigment phycoerithrin and in conditions of nutrient deficiency, it is quickly mobilized and used to sustain growth (Hernández et al., 2002; Nagler et al., 2003). As a result of this ability *Gracilaria* spp are seldom nutrient-limited except after periods of low nutrient inputs to the system and can maintain high growth rates even when N levels are low (Menéndez et al., 2001). Nitrogen can be stored for long periods of time, whereas in *Ulva* spp., this storage capacity is temporary. This can constitute a competitive advantage over green macroalgae during periods of low nitrogen availability (Comín et al., 1995). Before 1998, the mean ammonium value in the water column was $28.15 \mu\text{mol. L}^{-1}$, which means that even if nitrate uptake was severely inhibited in red macroalgae, ammonium uptake would be enough to maintain the internal nitrogen content above the critical tissue concentrations for maximum growth. It seems possible that *G. gracilis* had lower biomass before the management due to shading by the green macroalgal mats lying above (Hernández et al., 1997). Peckol and Rivers (1995) found that elevated ammonium levels had a greater inhibitory effect on ammonium uptake rates in darkness and that light substantially reversed this inhibition. Every growth season red macroalgae biomass increased after green macroalgae started to decrease and in the years when there was an absence of blooms, *G. gracilis* biomass was higher, which seems to support this hypothesis. The fact that *G. gracilis* settles to the sediment may play an important role in nutrient acquisition and may be a competitive advantage over *Ulva* spp., that depend almost exclusively on the water column to take up nutrients. Macroalgal canopies located over the sediment-water interface partially intercept nutrients released from the sediment, that otherwise would enter the water column (Lavery & McComb, 1991; Menéndez et al., 2000). This way red macroalgae would have more ammonium available than green macroalgae after the management and would need to take up less oxidized forms, saving energy to maintain

a high growth rate. Also, with the reduction of green biomass, light was more accessible to red macroalgae thus enhancing ammonium uptake and growth.

Z. noltii has responded to the management actions with an increase in biomass, indicating the beginning of the seagrasses beds recovery. As it happens with *G. gracilis*, light is a major factor controlling biomass production making the seagrass meadows more sensible to floating macroalgal masses. Green macroalgae mats floating over the meadows kept light from the layers below, preventing photosynthesis and thus leading to anoxic conditions (Hemminga, 1998).

Seagrasses can take up nutrients directly from the water column through leaves and from the sediment porewater, through roots (Sfriso & Marcomini, 1999; Bocci et al., 1997; Hemminga, 1998) which allows growth even with low nutrient availability (Bocci et al., 1997). Leaves can take up both NH_4^+ and NO_3^- , whereas roots take up mostly NH_4^+ since it is the prevalent form in the sediments (Bocci et al., 1997). N and P analysis on seagrass leaves and roots showed that the internal concentrations have been altered after the management actions. After 1998, the roots seem to be N limited, probably because sediment pool of NH_4^+ is not all available for uptake, since the ions can adsorb to mineral particles (Touchette & Burkholder, 2000). Nonetheless, leaves present a higher uptake affinity for NH_4^+ than roots and they seem to be more efficient in removing low levels of this reduced form (Touchette & Burkholder, 2000). The increase in the internal P both in leaves and roots may be a result of the increase of PO_4^- in the water column as *Z. noltii* tends to take up this form mainly through the leaves and relies on root uptake only when it is negligible in the water column (Brix & Lyngby, 1985). However, leaves are more sensible to light availability than belowground tissues (Pérez et al., 1994) which made the uptake of P more dependent on the roots before 1998 since light was kept away from aboveground tissues. After the management,

leaves increased their uptake due to higher P concentration in the water column and to higher light availability. Thus, in this system light appears to be the major factor controlling seagrass biomass production.

5. Conclusion

The combined actions taken to reduce eutrophication symptoms in the South arm of the Mondego system have led to ecological changes within primary producers assemblages, as shown in table 4, namely: the beginning of *Z. noltii* beds recovery, the increase of *G. gracilis* biomass, the absence of green macroalgal blooms, whilst phytoplankton biomass did not change. Therefore, since one action can cause different responses, it is important to try to understand what changes can be expected when management plans are implemented in the ecosystems.

6. References

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Table I – Summary characterization of the South arm of the Mondego estuary

Characteristic		
Geographic location	40° 08' N, 8° 50' W	
System intertidal area (km ²)	1.75	
System subtidal area (km ²)	0.96	
System volume (10 ⁶ m ³)	5	
Mean depth (m)	2-4 high tide (shallow)	
Tidal range (m)	0.35-3.3 (meso tidal)	
Mixing characteristics	Well-mixed with irregular river discharges	
Mean substratum composition	Silt, clay and sand	
Annual insolation of PAR (400-700 nm) (mol fot. m ⁻² y ⁻¹)	3200-32000	
	1993-1997	1999-2004
	Before management	After management
Salinity range	1.9 – 33.1	0.2 – 33.7
Mean water temperature range (°C)	8.0 – 23.7	8.1 – 22.1
Residence time	Moderate (weeks)	Short (days)
Current velocity	Low and dependent on the Pranto river sluice	Higher and not dependent on the Pranto river sluice
Turbidity	High	Lower
DIN (mean) (µmol L ⁻¹)	35.59	14.52
DIP (mean) (µmol L ⁻¹)	1.01	1.59
N/P (mean)	35.09	9.13

Table II – The water column nutrients concentrations ($\mu\text{mol.L}^{-1}$) in the pre-management period (A) and post-management (B) and the statistical analysis (Mann-Whitney Rank Sum Test) of the differences between both periods (C). SE is the Standard Error.

		A 1993-1997				B 1998-2004				C Statistics
		min	max	mean	SE	min	max	mean	SE	
Water Column	$\text{NO}_x\text{-N}$ ($\mu\text{mol L}^{-1}$)	0.50	38.93	7.13	0.16	0.74	36.06	6.98	0.14	n.s.:T=316.0; P=0.602
	$\text{NH}_4\text{-N}$ ($\mu\text{mol L}^{-1}$)	7.58	84.29	28.15	0.38	1.2	15.87	7.76	0.06	significant:T=44.0; P=0.002
	$\text{PO}_4\text{-P}$ ($\mu\text{mol L}^{-1}$)	0.44	2.02	1.01	0.01	0.20	3.06	1.59	0.01	significant:T=147.0; P=0.013

Table III – Phytoplankton (Chl *a*) and macrophytes biomass and internal nutrient concentrations before (A) and after the management periods (B) and the statistical differences (Mann-Whitney Rank Sum Test) between both periods (C). SE is the Standard Error and n.s. stands for not significant.

		A 1993-1997				B 1998-2004				C Statistics
		min	max	mean	SE	min	max	mean	SE	
Chl <i>a</i>	Phytoplankton	1.7	14.7	6.1	0.06	1.4	12.9	5.9	0.4	n.s.:T=749.0; p>0.05
	<i>Ulva</i> spp.	0.00	452.23	34.66	2.12	0.00	12.63	1.52	0.03	significant: T=2754.5; P<0.05
Biomass	<i>Gracilaria gracilis</i>	0.00	105.9	17.12	0.45	0.00	195.3	40.76	0.70	significant: T=1984.5; P<0.001
	<i>Z. noltii</i> roots	0.00	119.97	53.64	0.72	7.35	66.63	27.89	0.15	significant: T=3204.0; P<0.05
	<i>Z. noltii</i> leaves	5.65	185.76	85.43	1.12	11.76	129.18	52.53	0.40	significant: T=3021.0; P<0.05
Tissue Nitrogen	<i>Ulva</i> spp.	32.88	95.61	62.02	0.40	16.55	112.89	52.83	3.15	n.s.: T=221.5; P=0.492
	<i>Gracilaria gracilis</i>	24.64	110.04	62.42	3.02	19.34	108.81	58.5	0.52	n.s.: T=289.5; P=0.844
	<i>Z. noltii</i> roots	11.56	45.56	23.92	2.85	3.37	47.42	19.14	1.91	n.s.: T=1.411; P=0.166
	<i>Z. noltii</i> leaves	19.47	64.08	37.10	3.72	6.05	45.37	29.54	1.45	significant: T=2.310; P<0.05
Tissue Phosphorus	<i>Ulva</i> spp.	1.19	5.54	2.18	7.51	0.29	3.87	2.04	0.12	n.s.: T=188.5; P=0.788
	<i>Gracilaria gracilis</i>	1.23	2.88	2.08	0.05	0.05	6.23	2.25	0.03	n.s.: T=244.0; P=0.438
	<i>Z. noltii</i> roots	1.26	6.85	3.09	0.40	0.62	8.67	4.51	0.37	significant: T=-5.126; P<0.001
	<i>Z. noltii</i> leaves	1.83	4.46	2.55	0.25	1.84	9.25	5.61	0.36	significant: T=2.343; P<0.05

Table IV – The seasonal growth pattern of the primary producers and the changes in biomass before (A) and after (B) the management actions implementation.

Primary producers	A 1993-1997	B 1999-2004
Phytoplankton	Seasonal variation Increase chlorophyll <i>a</i> concentrations in spring/summer	Same seasonal variation No significant differences in chlorophyll <i>a</i> concentration
<i>Ulva</i> spp.	Frequent spring/summer blooms	Same seasonal variation with absence of blooms. Significant decrease in biomass
<i>Gracilaria gracilis</i>	Seasonal variation, with increase of biomass in the spring/summer	Same seasonal variation. Significant increase in biomass
<i>Zostera noltii</i>	Seasonal variation with increases in leaves biomass in spring/summer and roots in autumn/winter.	Same seasonal variation. Significant increase in biomass.

Figures

Figure 1 – A) The Mondego estuary, with the location of the long term monitoring area (A, B and C represent a gradient of eutrophication where C is the most eutrophicated area) and the areas of management measures were implemented; B) The evolution of the *Z. noltii* beds (ha) from 1986 to 2004, showing the drastic reduction from 1993 to 1997 and the recovery after the management; C) The main freshwater inputs before the management (1993-1997) and after (1999-2004).

Figure 2 – The inter-annual variation of primary producers assemblages pre-and post 1998: a) phytoplankton (mg Chl a m⁻³) b) *Ulva* spp. biomass (AFDW g.m⁻²); c) *Gracilaria gracilis* biomass (AFDW g.m⁻²); d) *Z. noltii* biomass (AFDW g.m⁻²).

Figure 3 – The mean annual variations in the internal nutrients content as percentage of dry weight, in primary producer's assemblages, before and after 1998: Nitrogen (a) and Phosphorus (b) in macroalgae; (c) and Phosphorus (d) in *Z. noltii*. The grey barr represents the critical concentration value for maximum growth under which there is nutrient limitation (1.5 % DW for nitrogen and 0.13 % DW for phosphorus).

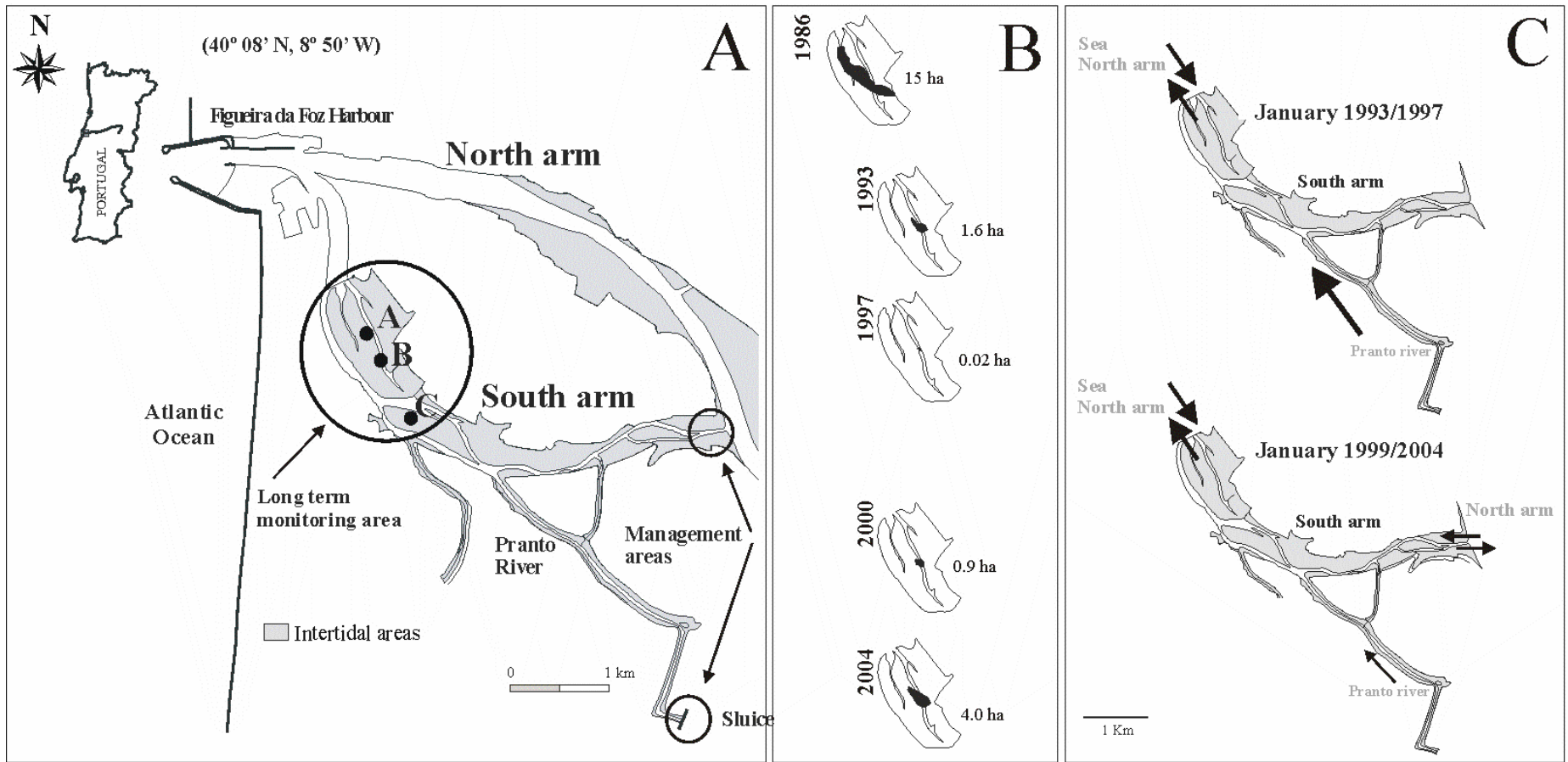


Figure 1

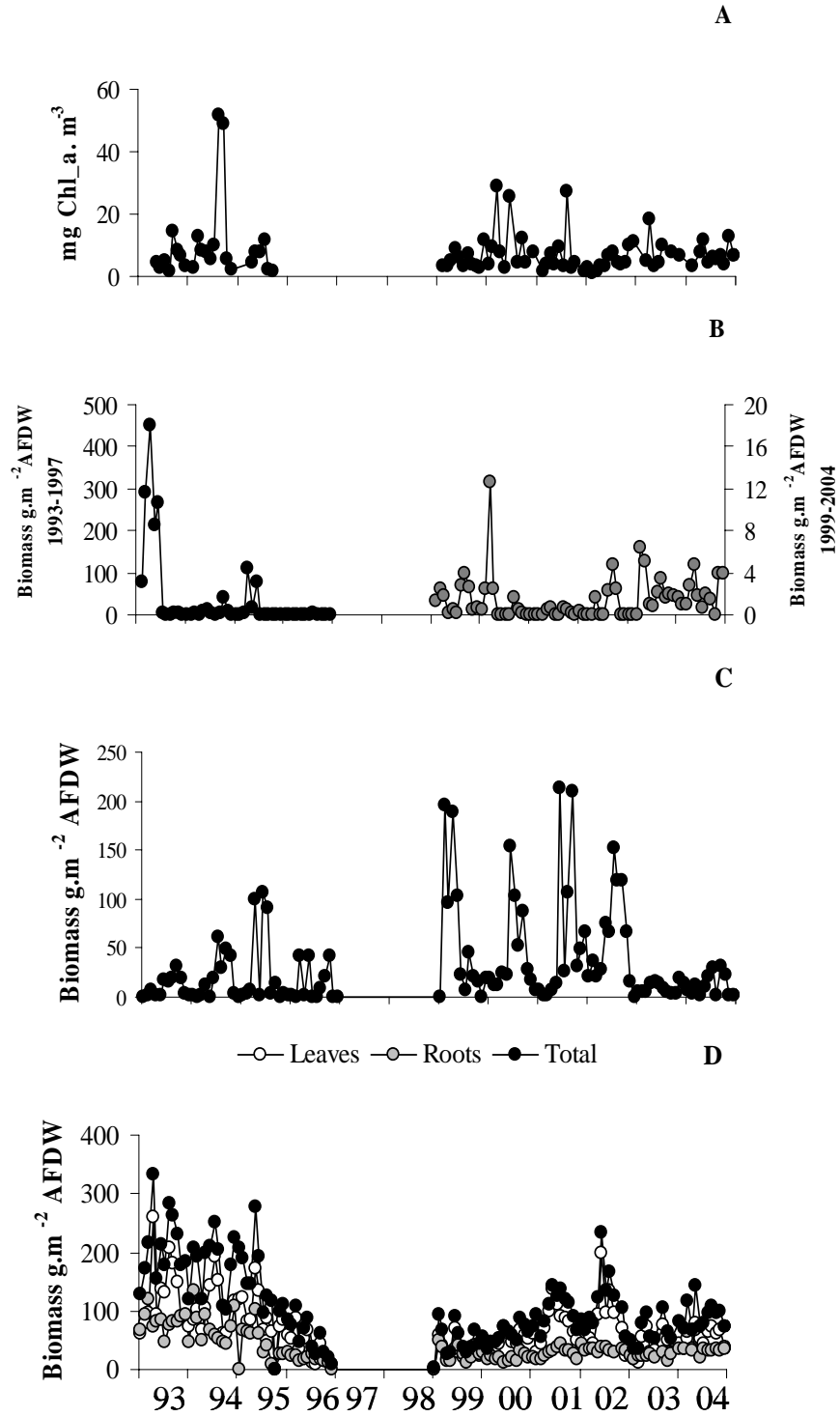


Figure 2

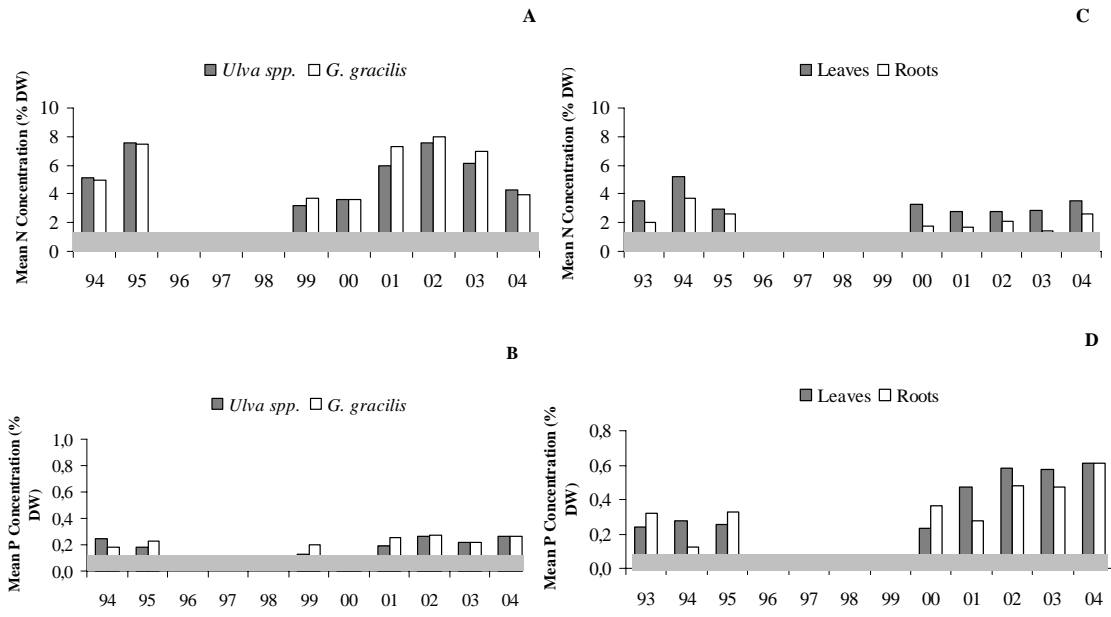


Figure 3