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Long-term changes within macrobenthic assemblages of a temperate estuary under eutrophication

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Contents

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Summary

The main goals of this dissertation were a) to evaluate long-term changes in the dynamics of the macrobenthic communities in a temperate estuary under eutrophication; b) to better understand some of the processes that operate in estuarine systems (*e.g.* relationships between primary producers, the macrobenthic community and abiotic factors) and c) to assess the success of the mitigation plan implemented in the system by evaluating the response of the biological communities (primary producers and macrofauna) to the management measures. In recent years, techniques to measure and detect environmental impacts have advanced considerably, but, few of them have been incorporated into studies of restoration. In the present work, it was possible to compare the utility of three different methodologies (univariate indices, the MDS ordination method and PRC analysis) in measuring the effectiveness of, and understanding restoration.

Chapter 1 describes the ecological changes occurring over relatively short temporal and small spatial scales in a well-documented seagrass habitat experiencing eutrophication. From 1993 to 1995 distinct changes in the structure of the macrobenthic communities were observed, specifically a decrease in species diversity along the eutrophication gradient and over time. Other changes included an increase in detritivores and a decline in herbivores together with a significant increase in small deposit-feeding polychaetes.

In Chapter 2, the effects of two morphologically different species of algae, the red alga *Gracilaria verrucosa* and the green *Enteromorpha intestinalis*, on the intertidal community were compared through a field experiment in two contrasting areas, an undisturbed seagrass bed and a eutrophic mudflat. Different effects of the macroalgae were detected at the two sites, being more marked in the eutrophic site. In addition, the effects of *Gracilaria* and *Enteromorpha* were significantly different, with *Enteromorpha* having a greater detrimental effect for most of the macrofauna. However, three of the most abundant species (*Hydrobia ulvae*, *Hediste diversicolor* and *Capitella capitata*) benefited from the presence of the weed. Between-site differences in overall impact were related to their previous disturbance history.

In Chapter 3 a novel approach to assess environmental quality is proposed based on PRC (Principal Response Curves) analysis. Assessing the well-being of ecological systems and instituting mitigation measures has become a priority. However, the traditional ordination methods currently used (*e.g.* PCA, MDS, etc) to describe changes in community structure produce complex diagrams for the non-ecologist and are not easily understood. The PRC method has advantages over traditional ones, since it provides a powerful statistical analysis of temporal data series along spatial gradients. It uses selected areas as reference sites and other (impacted) areas are compared to these reference sites, allowing changes in the environmental quality to be assessed over time. Moreover, individual species responses to stressor agents can be inferred from the PRC curves.

In Chapter 4 the success of a restoration project implemented in the Mondego estuary is evaluated by comparing water nutrient concentrations, the seagrass cover extent and the dynamics of *Hydrobia ulvae*, before and after implementation of the management measures. In the pre-mitigation period, following the decline of the seagrass bed a decrease in *H. ulvae* abundance, biomass and growth production was recorded. However, after the introduction of management measures, dissolved nutrients and green macroalgal blooms were much reduced, and the seagrass bed started to recover. Also, *H. ulvae* responded positively, the population becoming more structured, with higher abundance and biomass. In the most impacted area, the *H. ulvae* population continued to be dominated by small individuals, despite the improvements in water quality.

In Chapter 5 the hypothesis is explored that between-site differences observed for *Hydrobia ulvae* were related to enhanced survival (due to protection from avian or fish predators) of snails to larger size classes in the more complex habitat provided by seagrass, through a large-scale experiment employing seagrass mimics. The results revealed higher densities in *H. ulvae* adults in the artificial plots than in the controls (bare sediment). This experiment has confirmed the important role of the habitat structure provided by seagrasses on species protection against predators.

In Chapter 6 the success of the mitigation measures applied in the Mondego estuary is assessed, by comparing the dynamics of the overall macrobenthic assemblages along a eutrophication gradient before and after the implementation of the management measures. Three different methodologies (univariate methods, the MDS ordination method and PRC analysis) were used and the utility of each for measuring and understanding restoration evaluated. Before the introduction of management measures, 2 different scenarios were detected along the eutrophication gradient. In the *Z. noltii* bed, there was a general tendency to an increase in total density and biomass of the macrobenthic assemblage (without *Hydrobia ulvae*), while in the eutrophic area the opposite pattern was observed. In addition, a decline in the species diversity along the eutrophication gradient and through time was observed. After the management measures were introduced and following the recovery of seagrass, a large increase in total biomass of the community was observed. The recovery of species diversity followed but was interrupted by an additional external disturbance, heavy flooding. With respect to statistical methods available, PRC analysis was deemed to be the most suitable technique for assessing how ecosystem integrity changes over time.

Resumo

Esta dissertação teve como principais objectivos a) a avaliação das alterações a longo prazo das comunidades macrobentónicas, num estuário temperado sujeito a um processo de eutrofização; b) o conhecimento aprofundado de alguns processos que operam nos sistemas estuarinos (*e.g.* relações entre produtores primários, comunidade macrobentónica e factores abióticos) e c) a avaliação do sucesso do plano de mitigação implementado no sistema, através da análise das respostas das comunidades biológicas (banco de *Z. noltii* e macrofauna) às medidas de mitigação. Recentemente, novas metodologias têm sido desenvolvidas para avaliar e detectar impactos ambientais, no entanto, um reduzido número tem sido utilizado em estudos de recuperação de comunidades (community restoration). Assim, neste trabalho, também foi possível comparar a eficiência de três métodologias distintas (índices biológicos, método de ordenação MDS, análise PRC) na avaliação e compreensão de estudos de recuperação de comunidades.

O capítulo 1 descreve as alterações ecológicas ocorridas segundo uma escala temporal e espacial, relativamente pequenas, num habitat dominado pela macrófita *Zostera noltii*. De 1993 a 1995 foram observadas alterações relevantes na estrutura das comunidades macrobentónicas, nomeadamente um decréscimo na diversidade específica ao longo do gradiente de eutrofização e ao longo do tempo. Outras alterações tais como, o aumento de detritívoros e o declínio de herbívoros juntamente com o aumento significativo de pequenos poliquetas detritívoros foram registadas.

No capítulo 2 foram comparados os efeitos de duas espécies de algas morfologicamente diferentes, *Gracilaria verrucosa* e *Enteromorpha intestinalis* na comunidade intertidal, em duas áreas distintas, uma área não perturbada (banco de *Z. noltii*) e uma área eutrofizada. Detectaram-se efeitos distintos das macroalgas nas duas áreas de estudo, sendo mais marcantes na área eutrofizada. Adicionalmente, verificou-se que *Enteromorpha* tinha uma acção mais prejudicial para a maioria da macrofauna. Contudo, 3 das espécies mais abundantes (*Hydrobia ulvae*, *Hediste diversicolor* and *Capitella capitata*) foram beneficiadas pela presença da alga. As

diferenças observadas entre os 2 locais estão relacionadas com o seu prévio historial de perturbações.

No capítulo 3 é proposto um novo método para avaliar a qualidade ambiental, baseado na análise PRC (Principal Response Curves). Actualmente, tem-se tornado uma prioridade avaliar o bem-estar dos sistemas ecológicos e implementar medidas de mitigação. Contudo, os métodos de ordenação tradicionais geralmente usados (*e.g.* PCA, MDS, etc) criam diagramas demasiado complexos para os não ecólogos, não permitindo uma fácil compreensão das alterações ocorridas nas comunidades biológicas ao longo do tempo. A análise PRC tem vantagens sobre os métodos tradicionais, dado que fornece uma análise estatística forte relativa a uma série de dados temporais ao longo de gradientes espaciais. Utiliza determinadas áreas como locais de referência, com os quais as outras áreas serão comparadas, permitindo uma avaliação das alterações da qualidade ambiental ao longo do tempo. Para além disso, este método permite concluir acerca das respostas individuais das espécies relativamente aos factores de stress.

No capítulo 4 é avaliado o sucesso do plano de gestão implementado no estuário do Mondego. No período de pré-mitigação, seguindo o declínio da macrófita, foi observado um decréscimo na abundância, biomassa e produção somática de *H. ulvae*. No entanto, após a aplicação das medidas de gestão, os nutrientes dissolvidos e os blooms macroalgais sofreram uma redução considerável e a macrófita começou a recuperar. Também, a população de *H. ulvae* respondeu positivamente, tornando-se mais estruturada e apresentando uma maior densidade e biomassa. Na área eutrofizada, devido à ausência de produtores primários a população permaneceu dominada por indivíduos jovens.

No capítulo 5 pretendeu-se testar a hipótese de que as diferenças observadas na população de *Hydrobia ulvae* entre os 2 locais de estudo (banco de *Z. noltii* e área eutrofizada) estariam relacionadas mais provavelmente com a maior taxa de sobrevivência dos indivíduos em locais com maior complexidade do habitat como os bancos de macrófitas. Para tal, realizou-se uma experiência de campo em grande-escala utilizando unidades de macrófitas artificiais. Os resultados revelaram

diferenças em termos da densidade de adultos entre os "plots" com *Zostera* artificial e os controlos (sedimento descoberto), sendo superior nas primeiras. Apesar do aparecimento das diferenças entre elas ter sido lento, pode ser atribuído às taxas de crescimento da espécie. Esta experiência confirmou o papel importante que a complexidade e estrutura do habitat, exercida pelas macrófitas com raiz, desempenham na protecção das espécies contra potenciais predadores.

No capítulo 6 é avaliado o êxito das medidas de mitigação aplicadas ao banco de *Zostera noltii* do estuário do Mondego, através da comparação da dinâmica das comunidades macrobentónicas, antes e depois da implementação do plano de gestão. Para tal, utilizaram-se três metodologias distintas (índices biológicos, análise MDS e análise PRC) no sentido de concluir qual seria mais eficaz na avaliação e compreensão de estudos de recuperação de comunidades (community restoration). No período pré-mitigação, foram observados 2 cenários distintos ao longo do gradiente de eutrofização. No banco de *Zostera* foi observada uma tendência para um incremento da densidade e biomassa totais da comunidade macrobentónica (sem *Hydrobia*), enquanto que na área eutrofizada detectou-se um padrão contrário. Também foi observado um declínio na diversidade específica ao longo do gradiente de eutrofização e ao longo do tempo. No período pós-mitigação, seguindo a recuperação da planta, foi detectado um grande aumento na biomassa total e também na diversidade específica, apesar desta última ter sido afectada pelas fortes cheias de 2000/2001 ocorridas na região. Relativamente à eficácia dos métodos estatisticos, a análise PRC revelou ser a mais apropriada na avaliação da integridade dos ecossistemas.

General introduction

1. Environmental threats to estuaries and implications for biological communities

Among the most important environments of the coastal zone are estuaries, which constitute transition zones where freshwater from land drainage mixes with seawater, creating some of the most biologically productive areas on Earth.

Numerous anthropogenic disturbances affect estuarine environments, leading to habitat modification and changes in the structure and dynamics of biotic communities. Environmental problems encountered in these systems invariably stem from overpopulation and uncontrolled development in coastal watersheds, as well as human activities in estuarine embayments themselves (Kennish 2002). Eutrophication of coastal waters as a result of anthropogenic activities is now widely recognized as a major, worldwide pollution threat (Beukema & Cadée 1997, Valiela *et al.* 1997, Raffaelli *et al.* 1998, Lillebø *et al.* 1999, Pardal *et al.* 2000, Cloern 2001, Sfriso *et al.* 2001, Cardoso *et al.* 2002). One of the direct symptoms of enrichment in estuaries is the extreme growth of opportunistic green macroalgae, often associated with increases in nutrition load (Norkko & Bonsdorff 1996 a, b, Raffaelli *et al.* 1998, Martins *et al.* 2001, Sfriso *et al.* 2001).

In intertidal areas, these algae can form dense mats, which negatively impact on the underlying macrofaunal assemblages, although at low biomasses there may be enhanced effects (Hull 1987, Raffaelli *et al.* 1998). At low densities and for restricted periods of time, a patchy cover of algae increases habitat complexity and may facilitate local recruitment of larvae of macroinvertebrates (Norkko & Bonsdorff 1996 a, b, Raffaelli 1998). However, at high biomasses, macroalgal mats have been shown to negatively affect macrofaunal communities (Everett 1994, Norkko & Bonsdorff 1996 a, b, Norkko *et al.* 2000, Cardoso *et al.* 2004b). Changes in marine benthic vegetation as a consequence of excessive organic pollution and increases in primary production, have been documented worldwide. The mechanisms responsible for seagrass decline under eutrophication are complex and probably involve a suite of direct and indirect effects of changes in water quality, smothering by green macroalgal blooms (Den Hartog & Phillips 2000) and

competition for light and nutrients with epiphytic microalgae and with phytoplankton (Nienhuis 1996). Whatever the causes, the consequences of seagrass plants loss are well-documented and involve changes in the associated biological communities, namely a decline in species diversity (Reise *et al.* 1989, Cardoso *et al.* 2004a) and the functions, services and goods that seagrass beds provide (Duarte 2000, 2002, Jackson *et al.* 2001).

For most cases, the temporal trajectory of the eutrophication-induced ecological changes that take place in seagrass beds is not well-documented, because the early stages of the process are so gradual that they usually are not recognised till changes are well under way. However, this knowledge is essential to develop a successful restorative program for seagrass beds.

2. Restoration

In the face of global change, declines in environmental quality are of increasing concern, especially in densely populated areas. In recent years there has been an enormous upsurge in interest in restoration as a technique for reversing habitat degradation worldwide (Hobbs & Norton 1996, Jonge *et al.* 2000). The purpose of restoration projects is to return a habitat from an altered or disturbed condition to a previously existing natural condition (Kennish 2000). Although some disturbed patches of habitat are left to recover on their own (secondary succession), active restoration is an increasingly important managerial tool (Zedler 2001).

Ecological restoration is becoming an essential component of the conservation of biodiversity. Both approaches, conservation and restoration, seem necessary because while conservation is the typically more reliable and less costly means of sustaining ecosystem services, restoration is often required to respond to unexpected losses and to compensate for a long cumulative history of degradation and destabilization of natural ecosystems (Jackson *et al.* 2001, Scheffer *et al.* 2001). In contrast to conservation, which involves managing human activities to reduce their influence on nature, restoration requires explicit human intervention into degraded ecosystems to achieve a desired target. Restoration ecology depends on population, community and ecosystem ecology to provide the conceptual basis for

predicting not only direction but also magnitude of responses of ecosystems to restoration actions (Peterson & Lipcius 2003).

Restoration has two components: 1) on-the ground works which it is hoped will improve the degraded habitat, and, 2) the evaluation of the success or failure of these works with respect to improvement of the ecological structure and functioning of the restored habitat (Chapman 1999).

The success of restoration plans in turn depends on several key processes: a) identifying and understanding the processes which have driven the observed ecological changes; b) determining realistic objectives and measures of success; c) developing methods for implementing the goals and incorporating them into management, and, d) monitoring the restoration and assessing its success (Hobbs & Norton 1996, Kennish 2000, Pardal *et al.* 2004). Without quantitative evaluation of the success of different practices, the science of restoration will not progress (Chapman 1999). The success of the restoration projects is evaluated by comparing the flora and fauna of the improved community with those of the natural undisturbed community (reference sites). The probability of restoration success increases as the communities become more similar in composition and function (Kennish 2000). Evaluating a project's success is a long-term process, requiring many years, sometimes decades. Detailed and frequent sampling is required to accurately assess ecological communities on a restoration site. As a result, these programs usually entail a multidisciplinary effort conducted over a protracted period of time (Kennish 2000).

In recent years, several techniques to measure and detect environmental impacts have been developed (*e.g.* use of abundance, biomass comparisons - ABC curves, multidimensional ordination procedures and analysis of similarities of multivariate data, etc). However, few of them have been introduced in restoration studies (Chapman 1999) and their value for measuring and understanding restoration is not well understood. It is crucial for the development of the science of ecological restoration that new methodologies are created for the assessment of restoration success. Most of traditional techniques (*e.g.* redundancy analysis: RDA, principal component analysis: PCA or multidimensional scaling: MDS) produce ordination plots and diagrams which can appear illusive and obscure to non-specialists and hence difficult to interpret. This is especially true when a time factor

is present within the data, since temporal trajectories are often non-linear in such plots. One method to deal with the complexity of time dependency is the application of a recently developed technique, principal response curves (PRC) (Van den Brink & Ter Braak 1999). PRC has advantages over traditional techniques, in that it allows a powerful statistical analysis of long-term data series from spatial gradients. It uses selected areas as reference sites, which are compared with other impacted areas, allowing the assessment of environmental changes over time. PRC analysis has the additional advantage of providing an interpretation of impacts and changes at the species-level.

3. Importance of long-term studies in ecology

Many ecological investigations need long-term data for definitive testing, such as those involving successional processes, ecosystem changes associated with succession, with changes in predator-prey and, competitive interactions and ecosystem responses to atmospheric inputs (pollutants, nutrient loadings), as well as the implementation of a restoration project.

The implementation of long-term monitoring programs, as opposed to annual field campaigns, is recognized as an important issue for understanding certain ecological phenomena, such as: slow processes (*e.g.* population dynamics of long-lived organisms), rare events (*e.g.* disturbances - floods) and complex phenomena (involving many interacting factors), in which a long time is required to detect changes or trends. Long-term studies allow a better understanding of the environmental changes occurring in the ecosystems as well as allowing the interpretation of community' response to habitat change.

If long-term studies are essential to the robustness of the science of ecology, they are equally important in identifying and resolving societal issues (*e.g.* sustained productivity of agricultural lands and fisheries). Ecological issues at the regional and global levels require long-term data sets for identification, evaluation, prediction and decision making. Other issues with a significant long-term component include the effect of climate change, changes in key biogeochemical cycles, the effects of genetically engineered organisms and losses of biological diversity at the subspecies and species level (Franklin 1989).

4. The Mondego estuary – case study

The Mondego estuary is located on the Atlantic coast of Portugal (40° 08 N, 8° 50 W). It comprises two contrasting arms, northern and southern, separated by the Murraceira Island. The northern arm is deeper (4-8 m during high tide, tidal range 1-3 m) and constitutes the principal navigation channel and the location of the Figueira da Foz harbour. The southern arm is shallower (2-4 m during high tide, tidal range 1-3 m) and till 1998 was almost silted up in the upper zones, so that the freshwater outflow was mainly via the northern arm. Circulation in the southern arm was mostly dependent on the tides and on the freshwater input from the Pranto River, a small tributary. The discharge from this tributary is controlled by a sluice and is regulated according to the water needs of rice fields in the Mondego Valley.

Three distinct areas can be recognised in the southern arm: an undisturbed *Zostera noltii* bed, a eutrophic area and an intermediate area between these two. The seagrass bed is located downstream and is considered a non-eutrophic area. The intermediate area is located just upstream of the *Z. noltii* bed and has no seagrass cover although some rhizomes remain in the sediment. The eutrophic area is located upstream in the inner part of the estuary. It comprises a sandy-muddy sediment, which in the early 1980's was covered by rooted macrophytes. However, as eutrophication increased, *Zostera noltii* declined progressively and this area is now covered seasonally by green opportunistic macroalgae (*Ulva* sp.) (Cardoso *et al.* 2002, 2004a, b, Ferreira *et al.* 2004, Pardal *et al.* 2004).

In the mid-1980's several interventions were made along the river bed and Mondego valley in order to improve irrigation efficiency of the agriculture fields. They included a construction of channels, regularization of margins (to avoid flooding of fields), construction of sluices to regulate the water level inside the fields and intensification of land use (Neto 2004). All these actions together with the impact of several industrial and aquaculture activities within the estuary increased eutrophication. As consequence of this nutrient enrichment, the seagrass (*Zostera noltii*) bed has been drastically reduced in area extent and biomass in the south arm (Cardoso *et al.* 2004, Pardal *et al.* 2004). For instance, a seagrass bed that in the mid 1980's measured 15 ha was progressively reduced to 1.6 ha in 1993 and

to less than 0.03 ha in 1997. In 1998, several mitigation measures were applied in order to restore the original seagrass bed. The hydraulic regime in the south arm was improved by enlarging the connection between the two arms. The Pranto sluice-opening regime was minimised in such a way, that most of the freshwater from the Pranto River (loaded with nutrients) is discharged directly into the north arm (by another sluice located more upstream), thereby reducing the nutrient loading in the south arm. In addition, the remaining seagrass patches were protected with wooden stakes to prevent further disturbance of that area (by fishermen digging in the sediment for bait), and several stakeholder fora were convened to appraise local people of the ecological and economic importance of the seagrass bed.

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CHAPTER 1

DYNAMIC CHANGES IN SEAGRASS ASSEMBLAGES UNDER EUTROPHICATION AND IMPLICATIONS FOR RECOVERY

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Dynamic changes in seagrass assemblages under eutrophication and implications for recovery

ABSTRACT: Over the last 20 years, loss of seagrass beds, often related with increased eutrophication became a common problem worldwide. In the Mondego estuary (Portugal), eutrophication has triggered serious biological changes, which led to an overall increase in primary production and to a progressive replacement of seagrass *Zostera noltii* beds by coarser sediments and opportunistic macroalgae. The effects of this eutrophication on benthic assemblages were studied along a spatial gradient in the Mondego estuary from 1993-1995. Over these short temporal and small spatial scales, distinct changes in the structure of the macrobenthic communities were observed. One of the main structural modifications was the decrease in species diversity along the eutrophication gradient and over time, with a marked impoverishment of the most disturbed inner area. Other changes included an increase in detritivores and a decline in herbivores together with a significant increase in small deposit-feeding polychaetes.

In the long term, sustained eutrophication of this estuary is expected to lead to complete replacement of seagrass habitat by unvegetated coarser sediments, occasionally covered by green macroalgal blooms and dominated by opportunistic invertebrate taxa. Recovery from this situation may not only require reduction in nutrient loadings to the estuary, but also active seagrass restoration programmes to reverse the positive feedback processes thought to be presently taking place.

INTRODUCTION

Declines in seagrass beds have been documented for many areas of the eastern Atlantic (reviewed in Schramm & Nienhuis 1996), including much of the Iberian Peninsula (Niell *et al.* 1996, Oliveira & Cabeçadas 1996), often associated with increased eutrophication. The mechanisms responsible for seagrass decline under eutrophication are complex and probably involve a suite of direct and indirect effects of changes in water quality, smothering by green macro-algal blooms (Den Hartog & Phillips 2000) and competition for light and nutrients with epiphytic microalgae and with phytoplankton (Nienhuis 1996). Loss of the seagrass plants

leads to changes in the associated biological communities (Reise *et al.* 1989, Flindt *et al.* 1997) and the functions, services and goods that seagrass beds provide (Duarte 2000).

The temporal trajectory of the eutrophication-induced ecological changes that take place in seagrass beds with eutrophication, is not well-documented. This is partly because the early stages of the process are so gradual that they usually are not recognised until changes are well under way, and partly because, for many locations, the major changes occurred decades ago (Schramm & Nienhuis 1996). However, an appreciation of such trajectories is vital if remedial and restorative programmes for seagrass beds are to be successful (van Katwijk & Hermus 2000, Reise 2002). Hobbs & Norton (1996) provide a framework, pointing out that restoration will only be effective if all the processes which have driven the observed ecological changes are understood and addressed, and this may include additional engineered disturbance to replace an ecosystem component, as well as removing the original stressors. For aquatic systems there is much evidence that nutrient-induced changes in the growth of opportunistic macroalgae and/or phytoplankton are implicated in catastrophic shifts in aquatic communities ranging from freshwater lakes to coral reefs (Scheffer *et al.* 2001) because of non-linearities (hysteresis) in the dynamics of the systems as they change. These systems may be pushed into an alternate stable state by positive feedback processes from which recovery is very difficult to engineer (Hobbs & Norton 1996, Scheffer *et al.* 2001). Such positive feedback dynamics may explain why long-term recovery of seagrass beds throughout much of Europe and Australia has been painfully slow or non-existent (Kendrick *et al.* 2002) despite reductions in nutrient loadings. The mechanisms underlying potential hysteresis in seagrass beds are likely to be associated with the positive feedback processes leading to changes in the local physical environment following the loss of plants, *i.e.* the further loss of sediments, especially fine material, and sediment resuspension, which further accelerates bed decline (Duarte 1995, 2000, Schramm 1996, van Katwijk & Hermus 2000, van de Koppel *et al.* 2001, Reise 2002, Widdows & Brinsley 2002). Clearly, the sediment issues (ecosystem components) need to be addressed as well as the stressors (nutrients). In the present study we describe the ecological changes that have taken place over relatively short temporal and small spatial scales in a well-documented

seagrass habitat experiencing eutrophication, the Mondego estuary, Portugal, and comment on the likely form of the dynamics of this system under change.

MATERIALS AND METHODS

Study site and sampling programme

The Mondego estuary is located on the Atlantic coast of Portugal (40º 08 N, 8º 50 W). It is about 7 km long and is 2-3 km across at its widest part. Wetland habitats occupy about 1072 ha (Lopes *et al.* 2000). The estuary comprises two contrasting arms, northern and southern, separated by Murraceira Island, formed by the deposition of detrital materials transported by the river, as the river flood-plain (Fig. 1). The northern arm is deeper (4-8 m during high tide, tidal range 1-3 m) and constitutes the principal navigation channel and the location of the Figueira da Foz harbour. The southern arm is shallower (2-4 m during high tide, tidal range 1-3 m) and is almost silted up in the upper zones, so that the freshwater outflow is mainly via the northern arm. Circulation in the southern arm is mostly dependent on the tides and on the freshwater input from the Pranto River, a small tributary. The discharge from this tributary is controlled by a sluice and is regulated according to the water needs of rice fields in the Mondego Valley. The estuary supports several industrial activities and aquacultures, as well as receiving nutrients from 15000 ha of cultivated land upstream. Green macroalgal mats have appeared regularly over the last 20 years with a concurrent decline in seagrass beds (Marques *et al.* 1993a, b, 1997, Flindt *et al.* 1997, Lillebø *et al.* 1999, Pardal *et al.* 2000, Martins *et al.* 2001, Cardoso *et al.* 2002, Dolbeth *et al.* 2003).

Three different study areas were established along a spatial gradient in the southern arm of the Mondego estuary: 1) the *Z. noltii* beds, corresponding to a non-eutrophic area, 2) an intermediate area and 3) the most eutrophic area (Fig. 1).

The *Z. noltii* beds are characterised by higher salinity values (20-30 g. l^{-1}), lower total inorganic nitrogen concentrations (15-30 μmol N l^{-1}) and higher water-flow velocities (1.2-1.4 m s^{-1}) comparatively to the others areas. The mean organic-matter content of the sediment is 6.8 % ± 0.99 (± SD). (Pardal *et al.* 2000, Martins *et al.* 2001, Cardoso *et al.* 2002). In the 1980's, *Zostera noltii* beds occupied a broad expanse along the southern arm (150000 m^2) reaching the inner most parts of the

estuary. By the mid-1990's, *Z. noltii* had become restricted to a small patch (200 m²) located downstream, having been replaced elsewhere by blooming, fast-growing green macroalgae (free-floating forms) (Fig. 1). Mapping of benthic vegetation was done based on field observations, aerial photographs and GIS methodology (ArcView GIS version 8.2).

The intermediate area is located just upstream of the *Z. noltii* beds and has no seagrass cover although some rhizomes remain in the sediment. The physical-chemical conditions are otherwise similar to those of the *Z. noltii* beds.

The most eutrophic area is upstream in the inner part of the estuary. It comprises a sandy-muddy sediment characterised by the absence of seagrasses (for more than 15 years) and now covered seasonally by green macroalgae, especially *Enteromorpha* sp. (Pardal *et al.* 2000, Martins *et al.* 2001, Cardoso *et al.* 2002). In contrast to the *Z. noltii* beds, this area has lower salinities (15-25 g. l^{-1}), higher total inorganic nitrogen concentrations (30-50 µmol N l^{-1}) and lower water-flows (0.8-1.2 m s^{-1}). The mean organic-matter content of the sediment is 3.7 % ± 1.0 (± SD).

Sampling of the macrobenthic community was carried out from January 1993 to September 1995. Samples were collected fortnightly in the first 18 months and monthly during the rest of the study period. On each sampling occasion 6 to 10 cores (13.5 cm diameter) were taken to a depth of 20 cm. Samples were washed in estuarine water through a 500 µm mesh at each sampling station and the fauna retained preserved in 4 % buffered formalin. Later, animals were separated and kept in 70 % ethanol.

On each sampling occasion (monthly) and at each station, environmental parameters were measured *in situ*, in low water pools and water sampled for physical and chemical analysis (*e.g.* temperature, salinity, dissolved oxygen, ph, total inorganic nitrogenic, dissolved inorganic phosphorus).

Laboratory procedures

All macrofauna were identified to the lowest possible taxon and counted. Seagrass and macroalgal biomass was determined as ash free dry weight (AFDW) after oven drying at 60 ºC for 72 h and combustion at 450 ºC for 8 h. The same procedure was used to quantify the organic matter content of the sediment.

Macrobenthic Trophic Group Assignments

Each of the macrobenthic taxa was assigned to a trophic group based on feeding behaviour and food type.
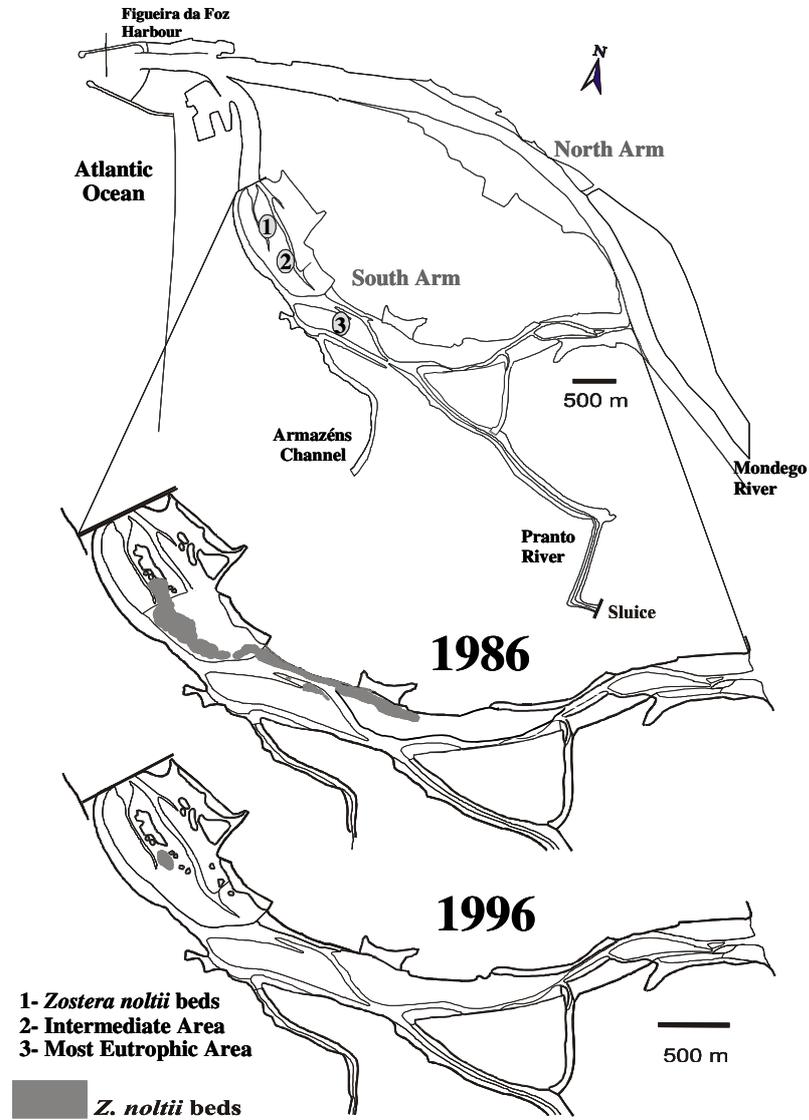
Fig. 1 – Location of the sampling areas in the south arm of the Mondego estuary and comparison of the area covered with *Z. noltii* between 1986 and 1996.

Trophic groups used in this study were surface-deposit feeders (SDF), subsurface-deposit feeders (SsDF), suspension feeders (SF), carnivores (C), herbivores (H) and omnivores (O) (*sensu* Gaston 1987, Gaston & Nasci 1988, Gaston *et al.* 1995, Gaston *et al.* 1997, Brown *et al.* 2000). Some species could not be confidently classified using the available schemes and these were entered as "unknown". Preliminary analysis included the snail *Hydrobia ulvae*, but it was also decided to analyse trophic structure ommitting this species, since it occasionally occurred in very high numbers and its inclusion masked changes in other species.

Data analysis
Macrofaunal Diversity

The diversity of the macrobenthic communities in the three areas was assessed and represented using rank-abundance curves (Molles 1999) where the total number of ranks corresponds to species richness and evenness is reflected in the slope of the curve.

Multivariate analysis of the macrofaunal assemblages

The faunal samples were analysed using the non-metric Multi Dimensional Scaling (MDS) ordination method (Clarke & Gorley 2001, Clarke & Warwick 2001). Firstly, raw data were square root transformed in order to scale down the scores of the abundant species (Clarke & Warwick 2001).

RESULTS

Seagrass and macroalgal biomass

In the *Z. noltii* beds, total seagrass biomass decreased gradually throughout the study period (Fig. 2), but there was a clear seasonal pattern. During spring and summer, biomass increased substantially due to leaf growth and in autumn and winter, below ground biomass (rhizomes and roots) represented about 50 % of the total biomass. Green macroalgae biomass was low in this area while red macroalgae (*Gracilaria* sp.) were more abundant (Fig. 3A).

In the intermediate area only small biomasses of green and red macroalgae were recorded all over the three-year period (Fig. 3B).

In the most eutrophic area there was a typical spring algal bloom (maximum of 452 g AFDW m^{-2}, April 93) during the first six months, followed by its sudden disappearance (crash) in late June (Fig. 3C). In contrast, in 1994, the biomass of green macroalgae was minimal but in 1995, biomass was higher than the previous year (111 g AFDW m^{-2}, April 95).

Diversity of the macrofauna

The rank-abundance curves clearly show that the *Z. noltii* beds, always, had a greater species richness than the most eutrophic area, with the intermediate zone having intermediate diversity (Fig. 4). Evenness increased from the *Z. noltii* beds to the most eutrophic area, due to the dominance of *Hydrobia ulvae* in the *Z. noltii* beds (see also Cardoso *et al.* 2002), reflected in the much steeper rank-abundance curve for the *Z. noltii* beds. If *H. ulvae* is omitted from the analysis, evenness appears higher in the *Z. noltii* beds.
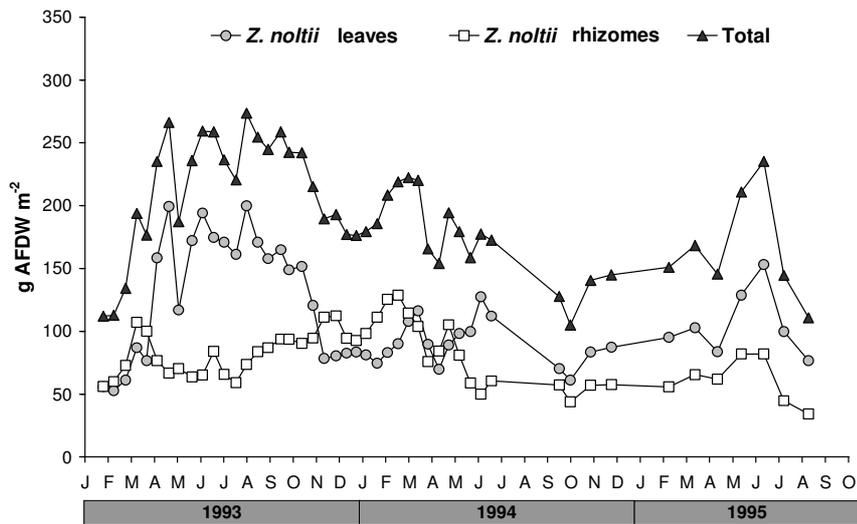
Fig.2 – Variation of *Z. noltii* biomass from January 1993 to September 1995.
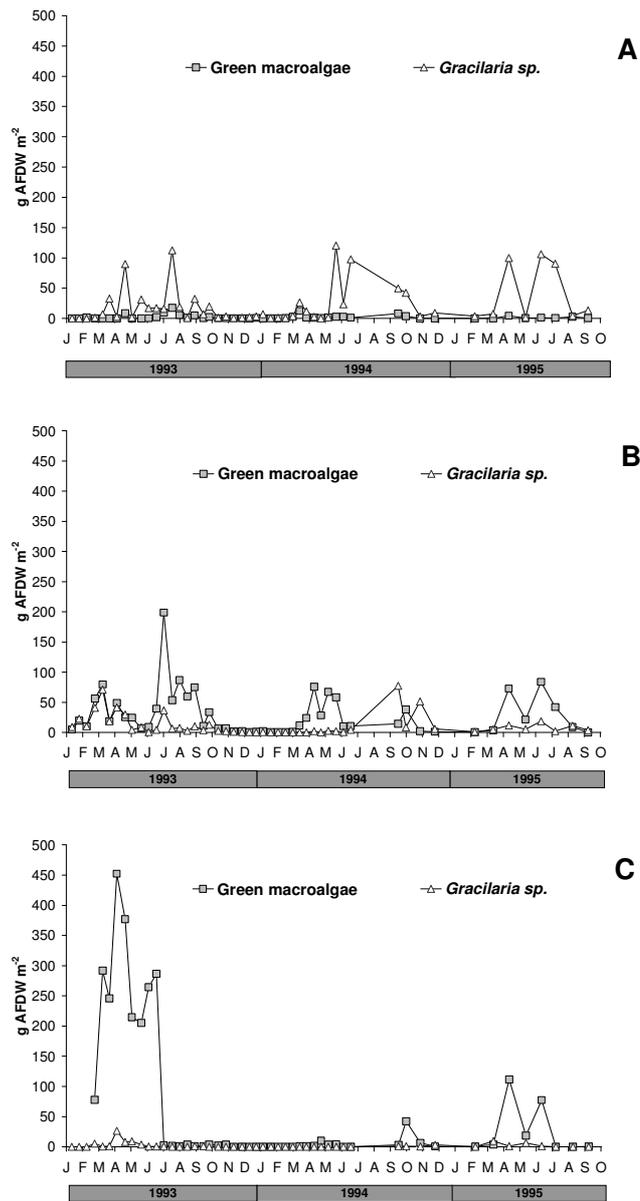
Fig. 3 - Variation of algal biomass from January 1993 to September 1995. (A) - *Zostera noltii* beds; (B) - Intermediate area; (C) - Most eutrophic area.
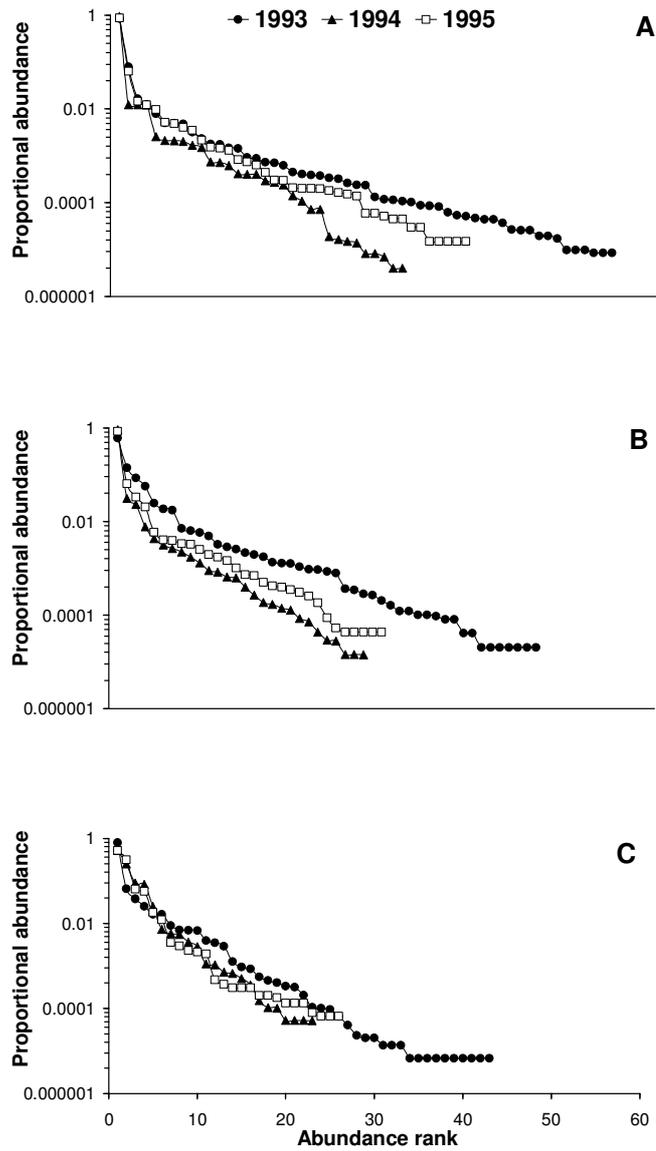
Fig. 4 - Rank-abundance curves for three macrozoobenthic communities in the Mondego estuary. (A) - *Zostera noltii* beds; (B) - Intermediate area; (C) - Most eutrophic area.

Species diversity declined throughout the 3 years of study in all three areas (Fig. 4). At the end of the study period, species richness in the *Z. noltii* beds (39 species) was similar to that observed in the most eutrophic area during the algal bloom (1993) (43 species), indicating a rapid deterioration of the seagrass habitat.

Multivariate analysis of the macrofaunal assemblages

The macrobenthic communities of the three study areas are spatially separated in the MDS plot, with the *Z. noltii* samples separated from those in the most eutrophic area by samples from the intermediate area (Fig. 5). Closer inspection shows that in the autumn of 1994 and the following winter (1995), the community of the *Z. noltii* beds was similar to that of the most eutrophic area (1993), consistent with the contention that the *Z. noltii* community is in decline. In addition, samples from the intermediate and most eutrophic areas showed the greatest scatter in the MDS plot, reflecting perhaps a greater instability of the environmental conditions of these areas.

Significant differences were detected by ANOSIM between the three sites. *Z. noltii* beds were significantly different from the intermediate area and from the most eutrophic area at the 5 % level (R = 0.387; P = 0.001; R = 0.273; P = 0.001, respectively). ANOSIM was also used to detect any differences between the three years. Significant differences were obtained between 1993 and 1994 (R = 0.192; P = 0.02) and also between 1993 and 1995 (R = 0.206; P = 0.016), but not between 1994 and 1995 (R = -0.03, P = 0.567).

Trophic groups

In the preliminary analysis, which included *Hydrobia ulvae*, we were unable to detect any significant modifications in community trophic structure, for either the spatial or temporal gradient.

The most representative groups are the surface-deposit feeders and herbivores due to the dominance of *H. ulvae*, with other groups comprising a small fraction of the community (Fig. 6A).
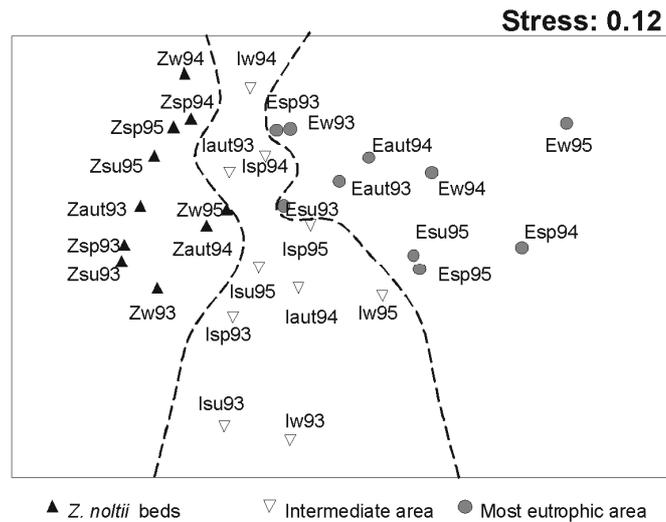
Stress: 0.12

▲ *Z. noltii* beds   ▽ Intermediate area   ● Most eutrophic area

Fig. 5 - Two-dimensional MDS ordination plot of macrobenthic communities. (Z) - *Z. noltii* beds; (I) - Intermediate area; (E) - Most eutrophic area; (sp) - spring; (su) - summer; (aut) - autumn; (w) - winter.

Omitting *H. ulvae*, revealed that the percentage of different trophic groups at the three sites, changed considerably along the eutrophication gradient and also over the study period (Fig. 6B). Macrofaunal communities were dominated numerically by detritivores (surface-deposit feeders, subsurface-deposit feeders and suspension feeders), which together accounted for more than 90 % of total macrobenthic abundance (Fig. 6). There was evidence of a gradual decline in the percentage of herbivores and subsurface-deposit feeders from the *Z. noltii* beds to the most eutrophic area, whilst the surface-deposit feeders and suspension feeders were more abundant in the upstream areas. Suspension feeders were more abundant in the intermediate area.

The trophic structure of the *Z. noltii* beds also changed over the three-year period.

Herbivores and surface-deposit feeders declined, followed by an increase in subsurface-deposit feeders. Carnivores increased over the three years whereas omnivores declined (Fig. 6B).

In the most eutrophic area, trophic structure showed greater variability over time probably due to the temporary occurrence of algal cover. Given that the presence of macroalgae can have a dramatic effect on macrofaunal trophic structure (Pearson & Rosenberg 1978, Norkko & Bonsdorff 1996a, b) a separate analysis was carried out for the years 1993 and 1995 (both of which experienced blooms) for periods with and without algal cover. This analysis indicated that the trophic structure of the most eutrophic area is more impoverished compared to that of the *Z. noltii* beds. Surface-deposit feeders represented the dominant group in all years, except in 1995 during the period without algae. Suspension feeders, which fluctuated widely with macroalgal events, were more abundant during the periods without macroalgae, when the available suspended organic matter was presumably higher.

The trophic structure of the intermediate area was much more erratic over time. Surface-deposit feeders were the only consistent group throughout the study. Carnivores and omnivores were more consistent over time in this area.

DISCUSSION

Over the last 20 years, eutrophication of the south arm of the Mondego estuary has promoted major biological changes. Seagrass beds declined rapidly throughout the 1980-1990 period, macroalgal blooms increased and there have been marked changes in the macroinvertebrate assemblages, principally a progressive impoverishment of the most impacted, inner area (Marques *et al.* 1997, Lillebø *et al.* 1999, Lopes *et al.* 2000, Martins *et al.* 2001, Pardal *et al.* 2000, Cardoso *et al.* 2002). These estuary-scale, temporal trends are mirrored in the present, relatively small-scale (1-2 km) eutrophication gradient documented here over a three year period.

With respect to trophic groups, the invertebrate assemblages were dominated (90 %) by deposit-feeding species, indicative of the major role of detritus thoughout the three sites. However, seagrass beds had more carnivores, herbivores and omnivores (8 %) than the most eutrophic area (4 %), and the percentage of herbivores and subsurface-deposit feeders decreased from seagrass beds to the most eutrophic area.

A

Zostera noltii beds　　**Intermediate Area**　　**Most Eutrophic Area**

1993

Zostera noltii beds:
O (0.43), H (43.22), SDF (50.76), C (0.27), SuF (1.51), SsDF (3.71)

Intermediate Area:
O (1.86), H (27.59), SDF (42.5), C (0.99), SuF (8.17), SsDF (18.84)

Most Eutrophic Area — With algae:
O (0.32), H (36.26), SDF (33.05), C (0.15), SuF (7.23), SsDF (6.30)

Most Eutrophic Area — Without algae:
O (0.19), H (35.35), SDF (49.18), C (0.41), SuF (9.59), SsDF (5.47)

1994

Zostera noltii beds:
O (0.2), H (47.15), SDF (48.73), C (0.1), SuF (1.68), SsDF (2.21)

Intermediate Area:
O (0.3), H (45.88), SDF (48.77), C (0.2), SuF (3.51), SsDF (1.34)

Most Eutrophic Area — Without algae:
O (0.91), H (18.63), SDF (57.11), SuF (14.80), SsDF (9.33)

1995

Zostera noltii beds:
O (0.2), H (43.45), SDF (45.68), C (0.35), SuF (2.40), SsDF (7.86)

Intermediate Area:
O (0.51), H (3.64), SDF (49.54), C (0.56), SuF (3.04), SsDF (6.71)

Most Eutrophic Area — With algae:
O (0.59), H (33.05), C (0.05), SuF (6.83), SDF (61.73), SsDF (10.05)

Most Eutrophic Area — Without algae:
O (0.51), H (30.53), SDF (52.48), SuF (7.91), SsDF (9.02)

Fig. 6 – Benthic assemblages of the Mondego estuary represented by trophic groups: surface-deposit feeders (SDF), subsurface–deposit feeders (SsDF), suspension feeders (SuF), carnivores (C), herbivores (H) and omnivores (O). Values are percentage of total individuals. (A) In the presence of _H. ulvae_; (B) In the absence of _H. ulvae_.

B

Zostera noltii beds   **Intermediate Area**   **Most Eutrophic Area**

1993

Zostera noltii beds 1993:
H (4.27), O (3.57), C (2.02), Unk (0.83), SuF (11.55), SDF (50.79), SsDF (26.97)

Intermediate Area 1993:
H (2.7), O (3.56), C (2.0), Unk (0.08), SuF (21.7), SDF (31.6), SsDF (38.37)

With algae   **Without algae**

Most Eutrophic Area — With algae 1993:
H (5.55), O (1.25), C (0.44), SuF (26.18), SDF (46.05), SsDF (20.53)

Most Eutrophic Area — Without algae 1993:
H (3.35), O (0.45), C (1.16), SuF (37.34), SDF (39.55), SsDF (18.15)

1994

Zostera noltii beds 1994:
H (4.12), O (2.54), C (0.89), Unk (0.02), SuF (37.82), SDF (30.63), SsDF (23.99)

Intermediate Area 1994:
H (1.31), O (4.15), C (3.42), SuF (48.02), SDF (28.67), SsDF (14.42)

Without algae

Most Eutrophic Area — Without algae 1994:
C (0.16), O (1.38), SuF (28.21), SDF (55.9), SsDF (14.35)

1995

Zostera noltii beds 1995:
H (1.68), O (1.41), C (3.05), Unk (0.45), SuF (22.32), SDF (16.34), SsDF (54.75)

Intermediate Area 1995:
H (3.21), O (4.77), C (3.65), Unk (0.07), SuF (28.81), SDF (33.32), SsDF (26.26)

With algae   **Without algae**

Most Eutrophic Area — With algae 1995:
C (0.12), H (0.66), SuF (10.01), O (0.99), SsDF (17.5), SDF (70.73)

Most Eutrophic Area — Without algae 1995:
C (0.35), O (3.74), SuF (22.93), SDF (30.74), SsDF (42.24)

Fig. 6 (cont.)

In contrast, surface-deposit feeders and suspension feeders increased from seagrass beds to the inner eutrophic area. Interpreting these patterns is not straightforward. Seagrass beds provide a heterogeneous and complex habitat and thus support a higher diversity of both species and trophic groups, as reflected in our data. But macroalgal blooms also create structural heterogeneity (Norkko & Bonsdorff 1996a, b, Raffaelli *et al.* 1998) and may function as an enriched trophic resource, which can result in invertebrate assemblages of eutrophic areas being similar in structure to those of seagrass beds, as seems to have occurred in 1995. However, the benefits algae bring to the benthic fauna (habitat enhancement, food, refuge), are only temporary, because algal mats can shift position on the flats and they are highly seasonal. Also, macroalgal blooms have well-documented effects on the sediment physico-chemical environment and hence on invertebrate assemblages, but these may not be dose-dependent or monotonic (Raffaelli *et al.* 1998). A further complication is that over the three year period, there was a general decline in species diversity, especially in the seagrass beds.

Notwithstanding the above, there was a consistently higher proportion of subsurface deposit-feeders in finer sediment habitats of the seagrass bed, whilst surface deposit-feeders were more abundant in the more erosive sandy sediments of the eutrophic area. This coarse sediment area occasionally accumulated superficial detritus, but periodic scouring by currents removed this fine material (Gaston 1987). Suspension feeders tended to be more abundant at the intermediate and most eutrophic areas, where, in the absence of vegetation cover, sediment resuspension and bedload transport are significant. Our results are broadly consistent with those of Bachelet *et al.* (2000) for French lagoons, although the trophic structure of the Mondego assemblages was much more dynamic through time. This probably reflects the progressive disappearance of seagrass at our site, which was relatively constant in the French study.

Our data are consistent with other, general eutrophication scenarios, *viz.* a replacement of one type of primary producer (seagrass) by another (macroalgae), declines in species diversity, and increase in detritivores and decline in herbivores and large increases in small deposit-feeding polychaetes (mainly *Alkmaria romijni, Capitella capitata*). Continued eutrophication is likely to lead to complete replacement of seagrass habitat in the Mondego by coarser sediments, mostly

unvegetated except for occasional macro-algal blooms, and a variable invertebrate assemblage characterised mainly by opportunistic species. A central question, which arises from this study, is whether these changes are easily reversed. There is no shortage of policy instruments from the EC or nationally for addressing eutrophication in coastal areas and, although these are challenging to enact for socio-economic reasons, it would be technically possible to reduce nutrient enrichment in the Mondego. But would the seagrass beds and their associated assemblages return? From the compelling evidence to date for seagrass beds in Australia (Kendrick *et al.* 2002) and northern Europe (*e.g.* Den Hartog 1996, van Katwijk & Hermus 2000, Reise 2002) we are not optimistic. The loss of seagrass from the eutrophic area of the Mondego and much of the intermediate area is associated with marked changes in the sediments of those areas, which have become coarser and more mobile, making re-colonisation by *Zostera noltii* very difficult. The dynamics of this system may well contain the hysteresis described by Scheffer *et al.* (2001) for a range of ecological systems and by van de Koppel *et al.* (2001) for intertidal flats. Re-establishment of seagrasses under such conditions may require additional active restoration initiatives, such as coastal engineering to modify the sediment regime and transplantation of seagrasses (Jonge *et al.* 2000, van Katwijk & Hermus 2000), rather than relying on natural re-colonisation. In other words by replacing lost ecosystem components as well as removing the key stressors (Hobbs & Norton 1996).

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CHAPTER 2

MACROINVERTEBRATE RESPONSE TO DIFFERENT SPECIES OF MACROALGAL MATS AND THE ROLE OF DISTURBANCE HISTORY

Macroinvertebrate response to different species of macroalgal mats and the role of disturbance history

ABSTRACT: Over the last 20 years, the Mondego estuary, Portugal, has experienced excessive growth of macroalgae especially in the inner parts of the system, with several algal species implicated. In this study, we compare the effects of morphologically different species, the red alga *Gracilaria verrucosa* and the green macroalga *Enteromorpha intestinalis* on macrobenthic assemblages, by a field experiment whereby the biomass of algae was manipulated and the resultant changes in macrofauna abundance evaluated. The experiments were carried out in different areas (a relatively undisturbed seagrass bed and an upstream eutrophic area) experiencing different degrees of overall enrichment.

Measurements of sediment redox potential revealed a rapid anoxia with a significant increase in algal biomass after four weeks. The effects of macroalgae were different at the two sites, being more marked in the eutrophic area. In addition, the effects of *Gracilaria* and *Enteromorpha* were significantly different, with *Enteromorpha* having a greater detrimental effect for most of the macrofauna, in particular *Cyathura carinata*, *Scrobicularia plana*, *Cerastoderma edule* and *Alkmaria romijni*. However, three of the most abundant invertebrates (*Hydrobia ulvae*, *Hediste diversicolor* and *Capitella capitata*) showed significant increases in abundance in weed affected compared to weed-free plots. *Gracilaria* had less of an impact on macrobenthic assemblages leading to a more enriched community. Between-site differences in overall impact were related to their previous disturbance history.

INTRODUCTION

Eutrophication of coastal waters as a result of anthropogenic activities is now widely recognized as a major, worldwide pollution threat (Vadas & Beal 1987, Beukema 1991, Fletcher 1996, Norkko & Bonsdorff 1996a, b, Beukema & Cadée 1997, Valiela *et al.* 1997, Raffaelli *et al.* 1998, Lillebø *et al.* 1999, Pardal *et al.* 2000, Cloern 2001, Sfriso *et al.* 2001, Cardoso *et al.* 2002). One of the direct symptoms of enrichment in estuaries is the extreme growth of opportunistic green macroalgae,

often associated with increases in nutrition load (Raffaelli *et al.* 1989, 1998, Norkko & Bonsdorff 1996a, b, Martins *et al.* 2001, Sfriso *et al.* 2001).

In intertidal areas, these algae can form dense mats, which negatively impact on the underlying macrofaunal assemblages, although at low biomasses there may be enhanced effects (Hull 1987, Raffaelli *et al.* 1998). At low densities and for restricted periods of time, a patchy cover of algae increases habitat complexity and may facilitate local recruitment (Norkko & Bonsdorff 1996a, b, Raffaelli *et al.* 1998). However, at high biomasses, macroalgal mats have been shown to negatively affect macrofaunal communities (Soulsby *et al.* 1982, Hull 1987, Everett 1994, Norkko & Bonsdorff 1996a, b, Norkko *et al.* 2000). The majority of studies on the effects of macroalgal mats on intertidal macrofauna has focused only on green algae, mainly *Enteromorpha* (Raffaelli *et al.* 1998, Bolam *et al.* 2000) while few, if any, studies have concentrated on the effects of red macroalgal species.

In the Mondego estuary, Portugal, which has undergone significant eutrophication over the last two decades (Marques *et al.* 1997, Lillebø *et al.* 1999, Lopes *et al.* 2000, Pardal *et al.* 2000, Martins *et al.* 2001, Cardoso *et al.* 2002, Dolbeth *et al.* 2003), seasonal macroalgal blooms have been observed, particularly in the inner areas of the southern arm of the estuary from late winter to early summer when algae often collapse. These blooms are characterised by two quite different macroalgal taxa, the red *Gracilaria verrucosa* and the green *Enteromorpha intestinalis*, which have different morphological and ecological characteristics (Cabioc'h *et al.* 1992), and distinctly different macrofaunal responses might be expected (Raffaelli 2000, Bolam & Fernandes 2002). Here, we compare the effects of these two algae on the intertidal community in two different areas, an undisturbed seagrass bed and a eutrophic mudflat – to explore the effect of disturbance history on the outcome of such experiments.

MATERIALS AND METHODS

Study site and experimental design

The Mondego estuary is a warm temperate system located on the Atlantic coast of Portugal (40° 08 N, 8° 50 W). It comprises two arms, northern and southern, separated by an alluvium-formed island (Murraceira Island) (Fig. 1).
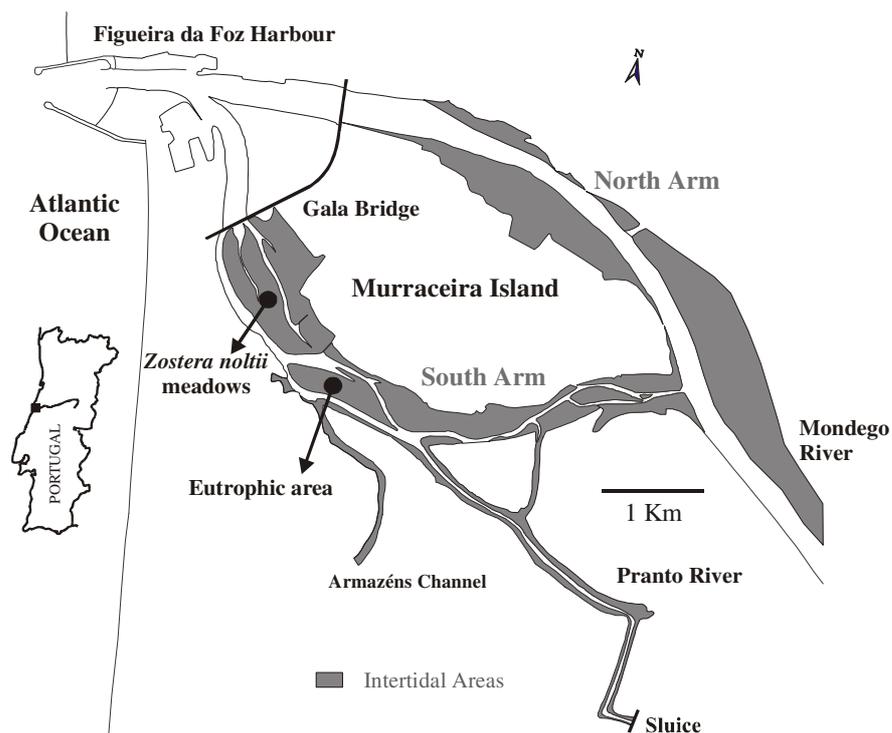
Fig. 1 - Location of the Mondego estuary and experimental areas (*Z. noltii* meadows and eutrophic area).

The northern arm is deeper (4-8 m during high tide, tidal range 1-3 m) than the southern arm (2-4 m during high tide, tidal range 1-3 m) and is almost silted up in the upper zones, resulting in the freshwater outflow being mainly via the northern arm. Circulation in the southern arm is mostly dependent on the tides and on the freshwater input from the Pranto River, a small tributary. The discharge from this tributary is controlled by a sluice and is regulated according to the water needs of rice fields in the Mondego Valley (Flindt *et al.* 1997, Lillebø *et al.* 1999).

Two different areas were selected as experimental sites along a well-documented gradient (Marques *et al.* 1993a, b, 1997, Lillebø *et al.* 1999, Pardal *et al.* 2000, Martins *et al.* 2001, Cardoso *et al.* 2002, Dolbeth *et al.* 2003) in the southern arm of the Mondego estuary: a) seagrass (*Zostera noltii*) meadows, corresponding to

a non-eutrophic area, and b) the eutrophic area upstream (Fig. 1). The *Z. noltii* meadows are located downstream and are characterised by a mud flat covered by seagrass and where the red macroalga (*Gracilaria verrucosa*) is abundant, especially in late summer (50-100 g AFDW m^{-2}), (Lillebø *et al.* 1999, Pardal *et al.* 2000). The eutrophic area is located upstream in the inner part of the estuary. This sandy-mud area is characterised by the absence of rooted macrophytes (for more than 15 years) but covered seasonally by green macroalgae, especially *Enteromorpha intestinalis* (Pardal *et al.* 2000, Martins *et al.* 2001, Cardoso *et al.* 2002, Dolbeth *et al.* 2003). These two areas are characterised by different macrobenthic communities as a result of their distinct biological and physico-chemical features (*e.g.* presence/absence of seagrasses, pH, oxygen, salinity, total inorganic nitrogen) (Cardoso *et al.* 2002).

Within each of these experimental areas, six blocks, each consisting of eight treatment plots (plot size = 0.25 m * 0.25 m), were set up in mid-September 2002. For each of the two species of alga, three levels of algal biomass were added to the plots: 0.3 Kg Wet Weight m^{-2} (low biomass), 1.0 Kg Wet Weight m^{-2} (intermediate biomass) and 3.0 Kg Wet Weight m^{-2} (high biomass). The biomasses used span the range found at the sites (see Fig. 2 and Cardoso *et al.* 2002) and are consistent with those used by other workers (Hull 1987, Raffaelli 1999, 2000, Raffaelli *et al.* 1998).

Algae were maintained in position under sheets of wire mesh anchored with corkscrewed wire at each corner, penetrating several centimetres into the sediment. Additionally, a weed control (treatment left clear of algae but covered with wire mesh) and a mesh control (treatment clear of both algae and mesh) were established within each block. A mesh control was used to reveal any artefacts associated with the mesh whilst the weed control was used to assess weed effects on invertebrate assemblages by comparing this treatment with the other algal treatments. Thus, each block contained a plot of low, intermediate and high algal biomass for the two algal taxa, a mesh control and a weed control. The blocks were dispersed over the site to provide a randomized block design.

Algal material used in the experiment was previously collected from sites on the estuary where the species occur naturally and washed carefully to remove any associated fauna.

The experiment was set up at low tide and ran for four weeks and redox potential assessed regularly. After 4 weeks, the redox potential under the algal treatments became markedly negative and the experiment was terminated. This experimental period is somewhat shorter than that of other experiments (Hull 1987, Raffaelli 1999, 2000, Bolam & Fernandes 2002), due to higher temperatures recorded at these sites, which led to a rapid algal decomposition and anoxia. For this reason, we decided to finish the experiment before an algal crash could happen. After this period, plots were sampled for macrofauna, taking a single core (containing material at the surface) from each plot using a 13 cm diameter corer to a depth of 10 cm. Each sample was taken from the centre of the plot to avoid possible edge effects. Samples were washed in estuarine water over a 500 μm mesh and the fauna retained preserved in 4 % buffered formalin. Later, animals were separated and kept in 70 % ethanol. Macrofauna was identified to the lowest possible taxon and counted.
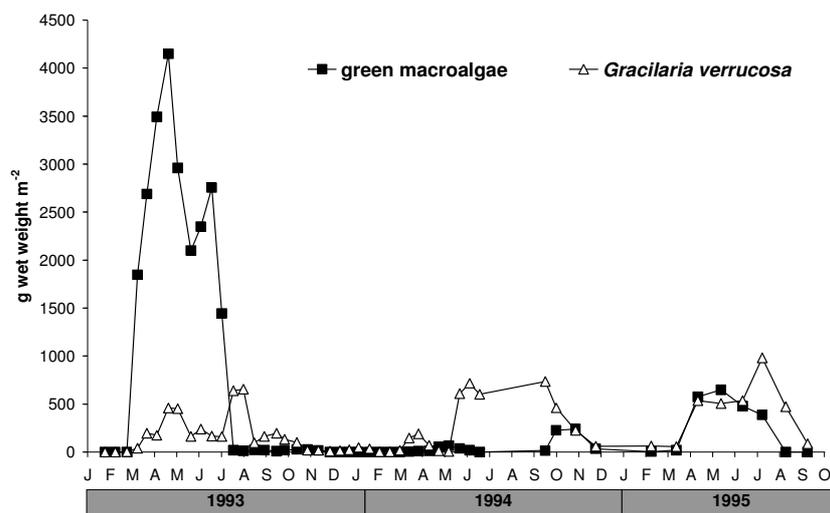
Fig. 2 - Variation of algal biomass in the Mondego estuary from January 1993 to September 1995.

Sediment redox potential values were measured at the end of the experiment using an Eh electrode (Crison pH/mv -506). Recordings were made at 4 cm depth.

Data analysis

Two-factor ANOVA (sites × algal treatments) was carried out followed by a multiple comparison test, the Tukey test, if significant, in order to determine where differences between treatments lay. All data were previously checked for normality using the Kolmogorov-Smirnov test and for homogeneity of variances using the Levene's test (Zar 1996). Data not meeting these criteria were transformed appropriately (Zar 1996) and checked again for normality and homocedasticity.

Between-treatment and between-site comparisons were also made at the community level using non-metric Multi Dimensional Scaling (MDS) ordination (Clarke & Gorley 2001, Clarke & Warwick 2001). The MDS plot was derived from the similarity matrix based species abundance data in each replicate plot for all the treatments. Raw counts were square root transformed to scale down the effects of very abundant species (Clarke & Warwick 2001). To validate our interpretation of the MDS we performed the ANOSIM test (analysis of similarities), built on a simple non-parametric permutation procedure and applied to the similarity matrix underlying the ordination of the samples (treatments) (Clarke & Warwick 2001).

RESULTS

Macrofauna

After four weeks of algal cover, there were effects of both algal species at both experimental sites. However, the effects were dependent on both the algal taxon and whether the site had a history of disturbance. The abundance of the several species suffered modifications, some of them increased while others decreased in the presence of weed (*Enteromorpha* and *Gracilaria*). Furthermore, there was no change in the total number of species: from the 18 species of the eutrophic area and 14 of the *Z. noltii* meadows, just 7 were sufficiently abundant for statistical analysis.

No significant effects of the mesh on species abundance were detected. For clarity, we review the effects of the two algal taxa at each site and then compare the sites.

Effects of algae in the disturbed area

Overall, *Enteromorpha intestinalis* had a greater impact than *Gracilaria verrucosa* for all biomass levels on most of the macroinvertebrate species, namely *Cyathura carinata*, *Scrobicularia plana*, *Cerastoderma edule*, *Hediste diversicolor*, *Alkmaria romijni* and *Capitella capitata* (Fig. 3, Table 1).
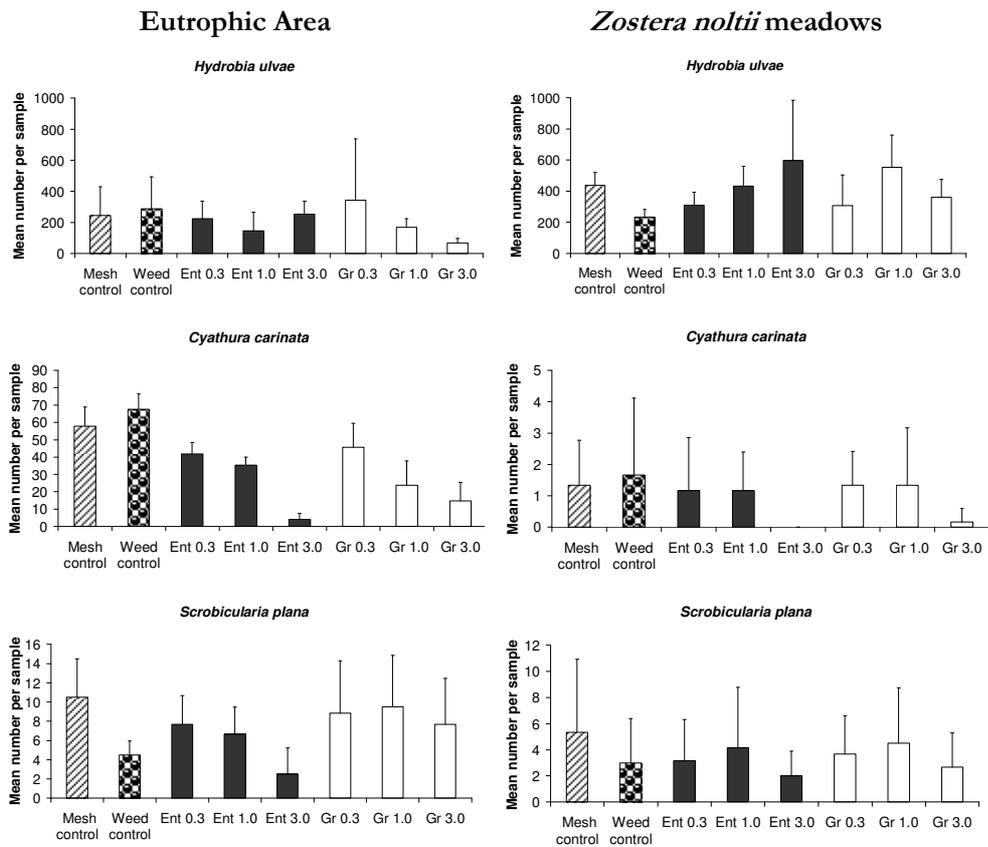
Fig. 3 – Mean species abundance (+ 95 % confidence intervals) after 4 weeks of experiment in the two experimental areas.
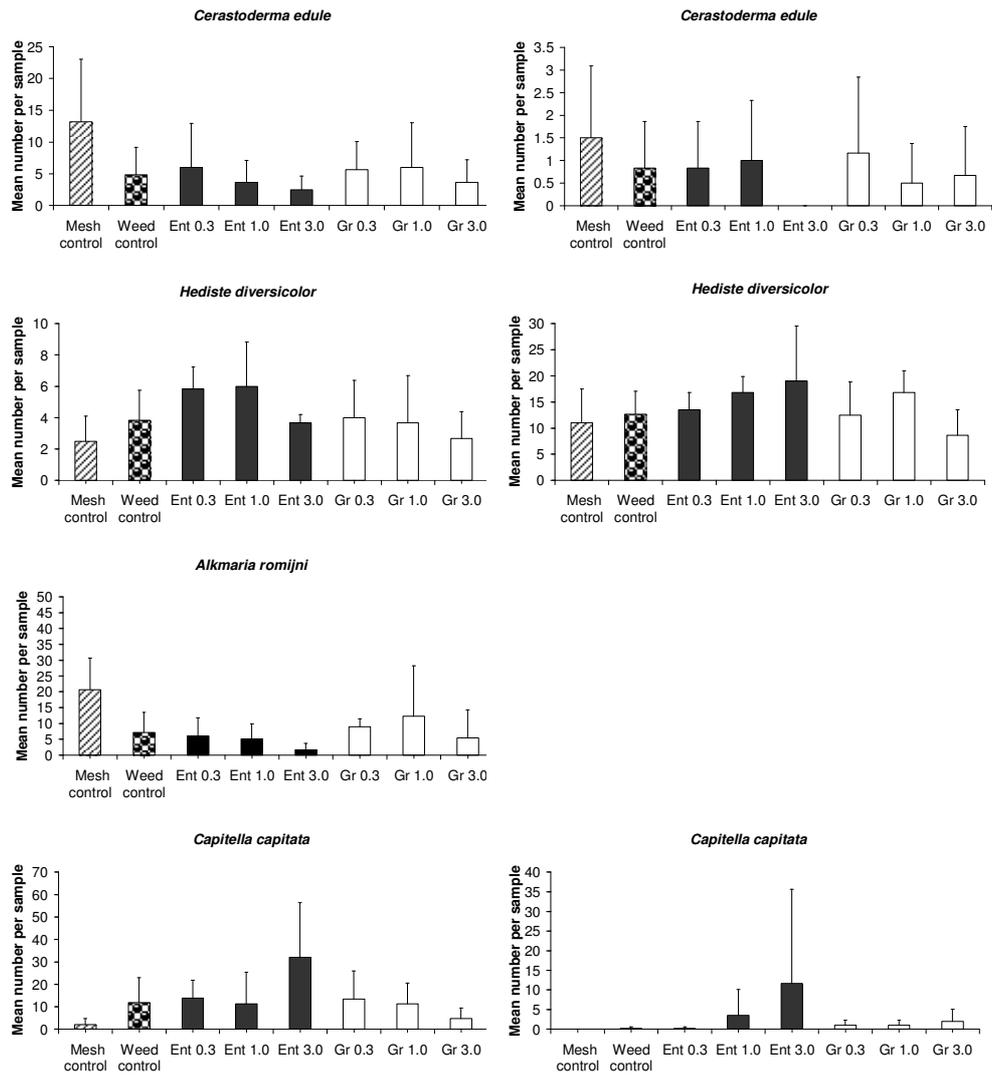
Fig. 3 (continued)

There were dose-dependent negative effects of both types of algae on *Cyathura carinata*, whilst for the bivalves, *Scrobicularia plana* and *Cerastoderma edule*, there was a slight increase in numbers in the presence of low weed biomass then a marked decline at high weed biomass.

For the polychaete *Hediste diversicolor*, there was a general trend of an increase in abundance of this species with increasing weed biomass in the *Enteromorpha* plots. *Alkmaria romijni* tended to slightly decline with increasing biomasses of *Enteromorpha* but there was no effect of *Gracilaria*. *Capitella capitata* increased at high biomasses of *Enteromorpha*, but the response was the opposite for *Gracilaria*.

Effects of algae in the seagrass meadows

In contrast to the eutrophic area, the effects of green and red macroalgae on benthic macrofauna were considerably less in the seagrass meadows except for *Hydrobia ulvae* (Fig. 3) in the presence of *Enteromorpha*. As expected, *H. ulvae* exhibited a positive response to green macroalgae, showing an increase in abundance with increasing weed biomass.

Table 1 – Statistical results of the two-way analysis of variance.

	2-way ANOVA			
	Source of variation	Degrees of freedom	Significant level	Tukey test
Hydrobia ulvae	A	1	***	
	B	7	ns	-
	A×B	7	**	
Cyathura carinata	A	1	***	
	B	7	***	-
	A×B	7	***	
Scrobicularia plana	A	1	***	E Ent3.0 < E Gr1.0
	B	7	*	
	A×B	7	ns	
Cerastoderma edule	A	1	***	
	B	7	*	-
	A×B	7	ns	
Hediste diversicolor	A	1	***	Z Ent3.0 > Z Gr3.0
	B	7	**	
	A×B	7	ns	
Capitella capitata	A	1	***	
	B	7	***	-
	A×B	7	ns	

Factor A – sites, factor B – algal treatments, A × B – interaction, E – eutrophic area, Z – *Z. noltii* meadows. *$P < 0.05$, ** = $P < 0.01$, *** = $P < 0.001$.

For most of the other species (*e.g. Cyathura carinata, Cerastoderma edule, Alkmaria romijni*) there were no effects possibly due to their low abundance in the cores. For the polychaete *Hediste diversicolor* there was slight tendency for an increase in density with increasing *Enteromorpha* biomass.

Two-way analysis of variance

There were no significant algal treatment effects for *Hydrobia ulvae* (P = 0.347), but a difference between sites (P = 0.000) and a significant interaction (P = 0.003), almost certainly due to the different direction of the algal effects at the two sites (in the seagrass meadows, *Hydrobia* increases with algal biomass, whilst at the eutrophic area, abundance decreases).

Cyathura carinata was significantly affected by site and algal treatments (P = 0.000 for both factors) and there was a significant interaction (P = 0.000) term, probably because there were effects of algal treatments at the eutrophic area while in the seagrass meadows almost no effect was recorded.
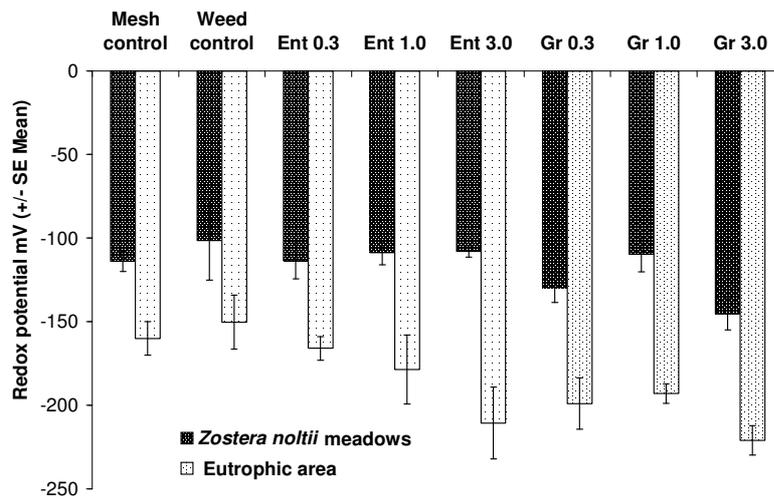
Fig. 4 – Sediment redox potential measured at 4 cm depth at the *Z. noltii* meadows and eutrophic area.

For other species (*e.g. S. plana*, *C. edule*, *H. diversicolor* and *C. capitata*), there were both between-biomass and between-site differences but no interaction between the two factors (Table 1).

Redox potential

Sediment redox potential differed consistently between the two experimental areas (Fig. 4), being significantly lower in the eutrophic area consistent with the notion that this site is more disturbed than the seagrass bed.

In the eutrophic area redox potential became more negative with increasing algal biomass and weed treatment plots were significantly more reduced than the control plots (ANOVA, $F_{23} = 3.10$, $P = 0.029$). However, Tukey tests failed to reveal any significant differences between the *Enteromorpha* and *Gracilaria* plots. In contrast, the sediments in the seagrass meadows did not show significant differences between the treatments.

Multivariate analysis of the macrofaunal assemblages

The macrobenthic communities of the two study areas are clearly separated in the MDS plot (Fig. 5).
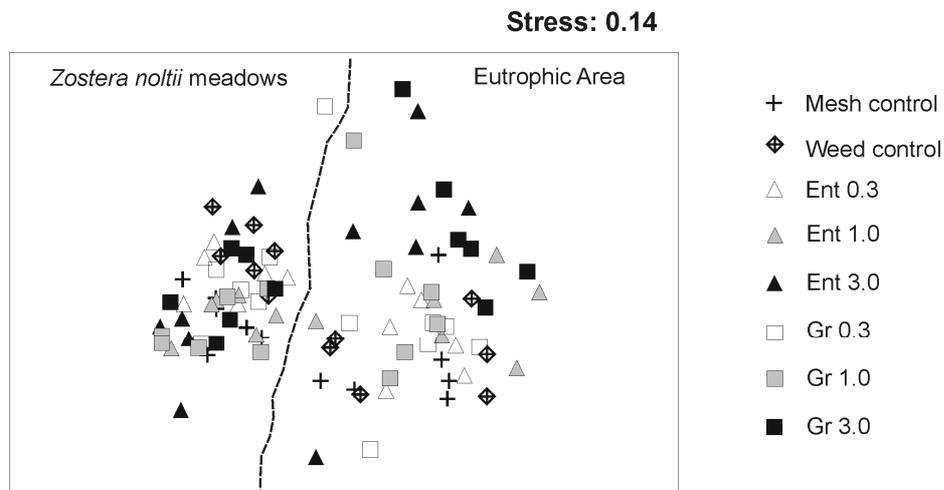
Fig. 5 - Two-dimensional MDS ordination plot of macrobenthic communities.

For the eutrophic area, macrobenthic assemblages within the weed controls are detached from the ones corresponding to the treatments with highest biomass of *Enteromorpha* and *Gracilaria* (Ent 3.0 and Gr 3.0), with the intermediate treatments occupying an intermediate position (Fig. 5). On the other hand, for the *Zostera* meadows, differences between treatments are less evident.

Significant differences were detected by ANOSIM between the two sites. *Zostera* meadows were significantly different from the eutrophic area at the 5 % level (R = 0.786; P = 0.001). ANOSIM test was also used to detect any differences between treatments in the two areas. The significant results are expressed in Table 2.

Table 2 - Statistical results of the ANOSIM test applied to the MDS analysis.

Groups	R statistic	Significant level
Z weed control*Z Ent 1.0	R = 0.439	P = 0.002
Z weed control*Z Ent 3.0	R = 0.444	P = 0.004
Z weed control*Z Gr 1.0	R = 0.506	P = 0.004
Z Ent 0.3*Z Ent 3.0	R = 0.300	P = 0.022
Z Ent 0.3*Z Gr 1.0	R = 0.267	P = 0.039
E weed control*E Ent 3.0	R = 0.696	P = 0.002
E weed control*E Gr 1.0	R = 0.243	P = 0.019
E weed control*E Gr 3.0	R = 0.600	P = 0.002
E Ent 0.3*E Ent 3.0	R = 0.515	P = 0.002
E Ent 0.3*E Gr 1.0	R = 0.269	P = 0.019
E Ent 0.3*E Gr 3.0	R = 0.474	P = 0.002
E Ent 1.0*E Ent 3.0	R = 0.476	P = 0.002
E Ent 1.0*E Gr 1.0	R = 0.196	P = 0.032
E Ent 1.0*E Gr 3.0	R = 0.207	P = 0.039
E Ent 3.0*E Gr 0.3	R = 0.615	P = 0.002
E Ent 3.0*E Gr 1.0	R = 0.281	P = 0.015
E Ent 3.0*E Gr 3.0	R = 0.344	P = 0.006
E Gr 0.3* E Gr 3.0	R = 0.357	P = 0.002
E Gr 1.0* E Gr 3.0	R = 0.241	P = 0.037

DISCUSSION

The results of the present study demonstrate that the response of the macrobenthic species to macroalgae is different in the two study areas. Previous studies have indicated that the sediment environment in the two areas is quite different (Pardal *et al.* 2000, Cardoso *et al.* 2002) and this is confirmed by our redox results. These indicate that the seagrass meadows have a less hostile redox environment than the upstream disturbed area, which is probably less resistant to additional impacts. It is perhaps not surprising that the effects of the algae are more marked in the disturbed site (characterized by bare sediments).

Within each area, the responses of the benthic macrofauna to algal mats were algal-species dependent, with some species being negatively affected and others being enhanced by the algae. On the other hand, for some species (*e.g. Alkmaria romijni*, *Hydrobia ulvae*) differences between the weed control and the mesh control were observed, however, no significant effects of the mesh on species abundance were recorded.

For *Hydrobia ulvae*, one of the dominant taxa in the Mondego estuary (see Lillebø *et al.* 1999, Cardoso *et al.* 2002), there were positive effects of the green macroalgae in the *Z. noltii* meadows. This is consistent with other studies, which show that *Hydrobia* is an opportunistic species, utilising intertidal algal mats both as food resource and refuge (Soulsby *et al.* 1982, Norkko & Bonsdorff 1996a, b, Norkko *et al.* 2000, Schanz *et al.* 2002). In contrast, the effects of this alga on *Hydrobia*, in the eutrophic area, were insignificant. This could be due to the short period of the experiment, which associated to the fragile population structure in this area (since during most of the time, only juveniles are present while adults only appear during periods with macroalgal blooms – see Cardoso *et al.* 2002) may not have allowed *Hydrobia* to respond in the same way as in the *Zostera noltii* meadows. *Gracilaria* had no detectable effects on *Hydrobia*, probably because snails do not seem to use this type of algae as a food resource because of its stiff consistency, whilst its gross morphology possibly affords *Hydrobia* less protection than *Enteromorpha*.

The isopod *Cyathura carinata* was negatively affected by both kinds of algae. The most likely explanation for this is the physical barrier created by mats of

macroalgae that will interfere negatively with its feeding mechanism at the sediment-water interface (*Cyathura* is a deposit feeder and a predator). This response is similar to that observed for the amphipod *Corophium volutator* in other estuarine systems (Hull 1987, Raffaelli 1999, 2000). In the seagrass meadows, *Cyathura* is too rare to permit the detection of such effects.

For the bivalves, *Scrobicularia plana* and *Cerastoderma edule*, despite the low abundances found, there was a positive effect at low algal biomasses and a decline at high biomasses. This was much more evident within the *Enteromorpha* plots, possibly because *Gracilaria* does not create such a rigid barrier between infauna and the oxygenated water column and hence generate such a hostile sediment environment. The effects of *Enteromorpha* are similar to those in previous surveys carried out by Everett (1994), Bolam *et al.* (2000), Bolam & Fernandes (2002) and Lewis *et al.* (2003) but dissimilar to those reported by Hull (1987) and Raffaelli (1999). In the latter studies, the positive effects were due to the presence of a large number of juveniles, which was not the case here.

Hediste diversicolor, overall, seems to benefit from green algal cover since *Enteromorpha*, gives protection and constitutes a favourable food-source. Nevertheless for this endofaunal species, occurred a small decline in the eutrophic area at the highest biomasses, probably due to a new factor of stress induced by the large amount of weed that interferes with the sediment properties on an already disturbed area. This is consistent with the findings from other studies that tested lower weed biomasses (*e.g.* Norkko & Bonsdorff 1996a). In contrast, *Alkmaria romijni* was negatively impacted by green macroalgae. This polychaete is a surface deposit feeder and the physical barrier created by macroalgae will probably interfere with its feeding behaviour.

Capitella is the only species that increased within the high biomass plots, although less so for *Gracilaria. Capitella* is an opportunistic detritivore species, usually associated with organically enriched sediments (Pearson & Rosenberg 1978, Soulsby *et al.* 1982, Raffaelli 1999, 2000, Bolam *et al.* 2000). Due to higher tissue thickness and lower surface/volume ratio, *Gracilaria* decomposition rate is much slower than the *Enteromorpha* one (sometimes only a few hours at temperatures above 25 ºC), contributing less for food resources. Therefore, this species might be

expected to do better in areas affected by green macroalgae than those covered by red macroalgae.

The present study has shown that the morphological and ecological features of the algal species involved in blooms are an important determinant of algal-invertebrate interactions. The effects of *Enteromorpha* on the macrobenthic communities are much greater than those for *Gracilaria* in the present study. In the presence of *Enteromorpha*, the community tends to be impoverished, dominated mainly by small size opportunistic species, like *Alkmaria romijni*, *Hydrobia ulvae* and *Capitella capitata*, while in the presence of *Gracilaria* the negative effects are usually less (Marques *et al.* 2003, Cardoso *et al.* 2004). In addition, the prior disturbance history of a site appears to be important in determining the extent of the impact of such blooms.

The present results and the one by Cardoso *et al.* (2004) confirmed the knowledge that, in the long-term, sustained eutrophication together with spring macroalgal blooms may lead to complete replacement of seagrass habitat by unvegetated coarser sediments. The macrofaunal assemblages tend to be dominated by opportunistic invertebrate taxa with total macrobenthic biomass and species richness tending to decrease. Recovery from this situation may not only require reduction in nutrient loadings to the estuary, but also active seagrass restoration programmes to reverse the positive feedback processes involved in the decrease of the environmental quality of eutrophic systems.

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CHAPTER 3

ASSESSING ENVIRONMENTAL QUALITY: A NOVEL APPROACH

Assessing environmental quality: a novel approach

ABSTRACT: A new approach to assess environmental integrity based on PRC analysis is proposed, tested, validated and developed. Environmental health assessment and community studies usually result in complex biological data sets. In order to find ecologically relevant patterns and tendencies from such sets of data it is necessary to reduce all the information to a summarised and simplified form, which might be more easily interpreted by ecologists, politicians, end-users and population in general. However, several multivariate ordination methods currently used (*e.g* Redundancy analysis, Principal Component Analysis, or Multi Dimensional Scaling), produce complex diagrams for the non-ecologist, which do not allow changes in biological communities over time to be easily understood. Here, we propose a recently developed method, Principal Response Curves (PRC) analysis to overcome these issues. This method has advantages over traditional ordination techniques, or any biotic index, in that it provides a powerful statistical analysis of temporal data series along spatial gradients. The PRC technique can make use of non-disturbed or unpolluted areas as reference sites with which other areas are compared, making it possible to assess changes in species composition between different areas over time. Moreover, individual species responses to stress agents can be inferred from the PRC curves. As well as providing insights into the behaviour of natural ecosystems - in particular how ecosystem integrity changes over time - this new approach can potentially provide a practical tool for monitoring and for the implementation of environmental policy instruments.

INTRODUCTION

In the face of global change, declines in environmental quality are of increasing concern, especially in densely populated areas. Assessing the well-being of ecological systems and instituting mitigation measures has become a priority. However, many of the tools and instruments presently available lack the rigour required for both the unambiguous detection of ecological impacts and the presentation of those impacts in an easily communicable way to policy makers, managers and stakeholders who are usually not biologists. For instance, within the

European Union, the implementation of the Habitats Directive, the Water Framework Directive and Integrated Coastal Zone Management has prompted the search for novel biological indicators (Borja *et al.* 2000, *in press*, Simboura & Zenetos 2002) for the assessment of environmental quality because traditional approaches are unable to capture entirely the natural variability of ecosystems. The most successful tools to date have been biological indices, which reduce the dimensionality of complex ecological data sets to a single univariate statistic, and ordination methods, which preserve more information in the data set by summarising its multi-dimensionality in a 2-D or 3-D plot within which ecological meaningful trends and patters can be seen. However, the summary ordination plots and diagrams from traditional ordination methods (*e.g.* Redundancy analysis (RDA), Principal Component Analysis (PCA), or Multi Dimensional Scaling (MDS)), can still remain illusive and obscure to non-specialists and hence are difficult to interpret (Warwick & Clarke 1991). This is especially true when a time factor is present within the data, because temporal trajectories are often non-linear in such plots.

One approach to deal with the complexity of time-dependency is the application of a recently developed method, Principal Response Curves (PRC) (Van den Brink & Ter Braak 1999). PRC analysis was originally conceived for aquatic ecotoxicology, but potentially it has much wider applications. PRC has advantages over traditional ordination techniques, in that it permits a formal and powerful statistical analysis of temporal (long-term) data series from spatial gradients. PRC analysis uses selected areas as reference sites and other areas (treated or impacted areas) are compared to these reference sites, allowing changes in the environmental quality to be assessed over time. By providing a yardstick or natural, undisturbed reference against which departures of other data can be seen, the approach is analogous to the log-normal plots described by Gray (1979) and the ordination/meta-analysis approach developed by Warwick & Clarke (1993). PRC analysis has the additional advantage of providing an interpretation of impacts and change at the species level, because individual species responses to stress agents can be inferred from the PRC curves.

One of the most common environmental problems is the increase of organic pollution in coastal waters that usually leads to a shift in primary producers (*e.g.* an

increase in green macroalgae). In common with many other estuaries, the Mondego, Portugal, has undergone significant eutrophication due to organic enrichment (Marques *et al.* 1993, 1997, Flindt *et al.* 1997, Lillebø *et al.* 1999, Lopes *et al.* 2000, Pardal *et al.* 2000, Martins *et al.* 2001, Cardoso *et al.* 2002). As a response to the increase of nutrient (phosphorus and nitrogen) concentrations, macroalgal blooms have increased substantially, leading to the decline of seagrass (*Zostera noltii*) meadows (Martins *et al.* 2001, Cardoso *et al.* 2002), accompanied by structural in macrobenthic communities. Despite interannual variations over the last 20 years, a consistent effect has been the decrease in species diversity and secondary production from the less stressed areas to the ones exhibiting stronger symptoms of eutrophication (Marques *et al.* 1997, Dolbeth *et al.* 2003, Cardoso *et al.* 2004). In 1998, a degree of management was attempted in the estuary, by protecting the small patch of the seagrass bed, by decreasing the nutrient loading to the system and increasing water transparency and velocities leading, at the present time, to the slow recovery of *Zostera* beds (Fig. 1). In the present paper, we explore and validate the use of PRC analysis in ecosystem health assessment using this well-documented system.

MATERIALS AND METHODS

The Mondego estuary, located on the western coast of Portugal, is a typical temperate small intertidal estuary. As for many other regions, this estuary shows symptoms of eutrophication which have resulted in an impoverishment of its environmental quality (Marques *et al.* 1993, 1997, Flindt *et al.* 1997, Lillebø *et al.* 1999, Pardal *et al.* 2000, Martins *et al.* 2001, Cardoso *et al.* 2002, Dolbeth *et al.* 2003). Three distinct areas within the estuary (Fig. 1) can be recognised along a spatial gradient of eutrophication: a) seagrass (*Zostera noltii*) beds, corresponding to the least eutrophic area (undisturbed/reference) and located downstream. This area is characterised by higher salinity values (20-30 g.l^{-1}), lower total inorganic nitrogen (TIN) concentrations (15-30 µmol N l^{-1}) and higher water –flows (1.2-1.4 m.s^{-1}). The mean organic-matter content is 6.8 % $\pm$ 0.99 ($\pm$ SD); b) an intermediate eutrophic area located in the middle section of the estuary corresponding to an area that supported seagrass beds five years ago but not now, although rhizomes
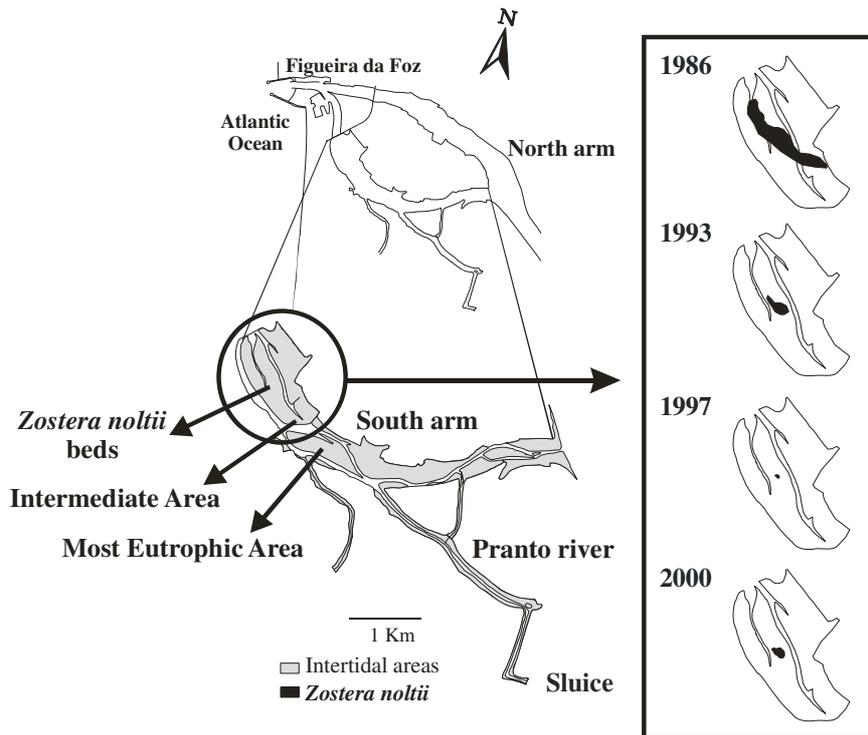
Fig. 1 - Location of the sampling areas in the south arm of the Mondego estuary. Area covered by *Zostera noltii* in 1986, 1993, 1997 and 2000.

are still present in the sediment. The physical-chemical conditions are similar to those of the previous area; c) the most eutrophic area located in the upper reaches of the estuary, from where seagrasses disappeared without trace more than 15 years ago, and where seasonal blooms of green macroalgae (*Enteromorpha* spp.) now regularly occur (Marques *et al.* 1993, 1997, Flindt *et al.* 1997, Lillebø *et al.* 1999, Pardal *et al.* 2000, Martins *et al.* 2001, Cardoso *et al.* 2002). This area is characterised by lower salinity values (15-25 g.l^{-1}), higher TIN concentrations (30-50 µmol N l^{-1}) and lower water flows (0.8-1.2 m.s^{-1}). The mean organic-matter content is 3.7 % $\pm$ 1.0 ($\pm$ SD).

The macrobenthic assemblages at these sites were sampled from February 1993 to September 1995, fortnightly for the first 18 months and monthly

thereafter. On each sampling occasion 6-10 cores (13.5 cm diameter) were taken to a depth of 20 cm, washed over a 500 μm mesh and the fauna retained identified and enumerated.

The spatial and temporal dynamics of macrobenthic communities along the eutrophication gradient were analysed by non-metric Multi Dimensional Scaling (MDS) (Clarke & Gorley 2001), following square root transformation (Clarke & Warwick 2001) and by the PRC method, which is based on the redundancy analysis ordination technique, the constrained form of Principal Component Analysis, a full account of which can be found in Van den Brink & Ter Braak (1999), Cuppen *et al.* (2000), Frampton *et al.* (2000a, b, 2001) and Van den Brink *et al.* (2000). The PRC method is a multivariate technique especially designed for data analysis from microcosm and mesocosm experiments. Due to its novelty this method was mainly applied in aquatic ecotoxicology (Van den Brink & Ter Braak 1999, Cuppen *et al.* 2000, Van den Brink *et al.* 2000), with only one incursion into soil ecology (Frampton *et al.* 2000a, b, 2001). However, this approach has potential for a wider application in community ecology and in the evaluation of ecosystem integrity. The method analyses differences in species composition between "treatments" (sites, in the present study) at each time point, similar to other ordination techniques. However, one advantage of this method is that any temporal changes in the "control" (the reference seagrass site, in the present study), are constrained in the plot to a horizontal line. Thus PRC creates a graphical display with time (sampling dates) as a horizontal line and the basic response pattern (c_{dt}) of each site d at each time t in relation to control site on the vertical axis (by definition, the control site has always a c_{dt} of zero for every time – c_{0t}). When these coefficients are plotted for each time point, a principal response curve of the community is obtained for each site in comparison with the control site (Van den Brink & Ter Braak 1999). This permits an easily understood representation of the temporal changes in the assemblages at each site in relation to the reference control site.

An additional advantage of using the PRC technique is that it allows for the detection of effects at the species level. Derived species weight (b_k) is the factor by which the basic response pattern is multiplied to attain the fitted response of species k (Van den Brink & Ter Braak 1999). Species weights thus measure the

affinity of a particular species to the community response pattern and can be used to estimate species relative abundance in each site compared to the control, using the expression exp $(b_k c_{dt})$. In practical terms, taxa with a positive species weight are expected to decrease in abundance relatively to the control in the highest treatment levels (*i.e.* the most eutrophic area in our case), whereas taxa with negative weights are expected to increase.

In PRC analysis, as described by Van den Brink & Ter Braak (1999), the statistical model for the species abundance data is:

$$Y_{d(j)tk} = Y_{0tk} + b_k c_{dt} + \epsilon_{d(j)tk}$$

where $Y_{d(j)tk}$ is the abundance of species k in replicate j of site d at sampling date t, Y_{0tk} is the mean abundance of species k on date t at the control site d_0, c_{dt} is a basic response pattern for every site d and sampling date t, b_k is the weight of each species with this basic response pattern and $\epsilon_{d(j)tk}$ is an error term with mean zero and variance σ_k^2. By definition, $c_{0t} = 0$ for every t. When the coefficients c_{dt} are plotted against sampling date t, the resulting PRC diagram displays a curve for each treatment that can be interpreted as the principal response curve of the community (Van den Brink & Ter Braak 1999). The species weight b_k indicates how closely the response of each individual taxon matches the overall community response as displayed in the PRC diagram.

In previous studies that have used PRC analysis, an experimental "control" treatment level was used as the reference treatment level $d = 0$ (Van den Brink & Ter Braak 1999). Here, however, and in common with Frampton *et al.* (2001), an obvious "control" treatment does not exist among sampling times, and the least disturbed (most natural) site is viewed as the control. Although a reference level must be specified in the PRC analysis, the choice of reference does not limit the visual and quantitative treatment contrasts that can be made using a PRC diagram (Ter Braak & Similaeur 1998).

In addition to providing a concise graphical summary of changes in community structure, PRC analysis allows an estimate of the variance in the data set that is explained by a treatment.
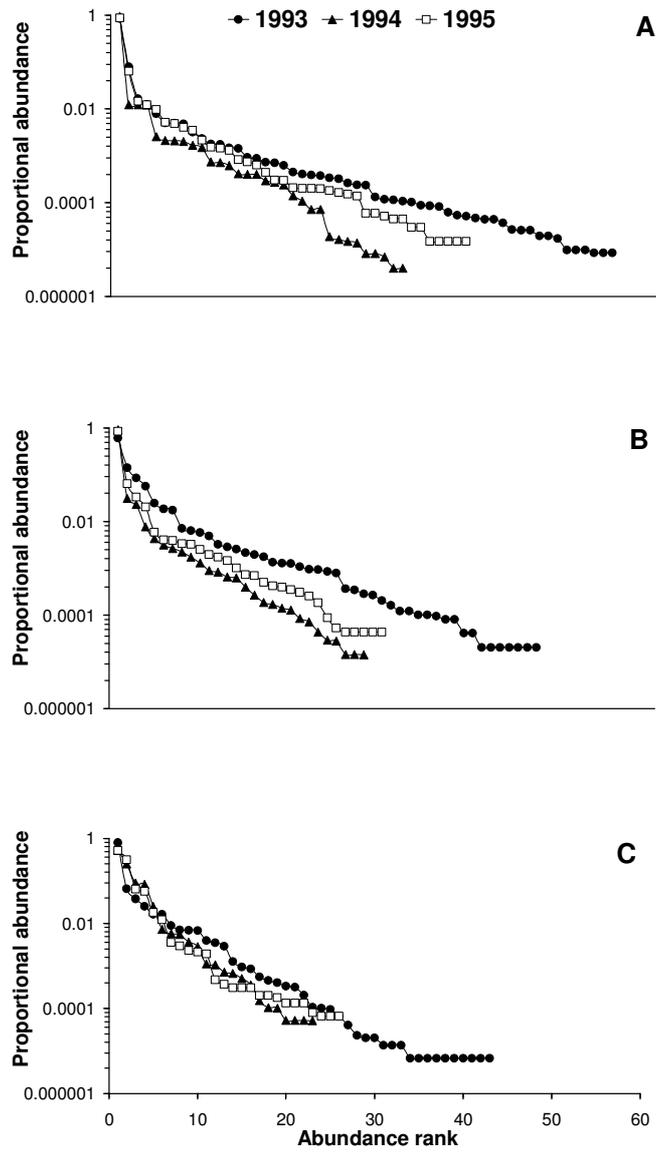
Fig. 2 - Rank-abundance curves of the three macrobenthic communities. (A) - *Z. noltii* beds; (B) - Intermediate area; (C) - Most eutrophic/disturbed area.

A PRC diagram aims to maximise the amount of variance due to treatments; the higher the proportion of the variance displayed, the more closely will the fitted relative abundance of individual taxa inferred from the diagram match the observed relative abundance.

The null hypothesis assumes that the PRC diagram does not capture the treatment variance (*i.e.* c_{dt} x $b_k = 0$ for all *t*, *d* and *k*) and can be tested using a Monte Carlo permutation. A complete description of the method is provided in Van den Brink & Ter Braak (1999).

In the present study, treatments correspond to the different macrobenthic communities under different degrees of organic pollution stress. As reference (control) we considered the *Zostera noltii* meadows in 1993. PRC analysis was performed using the CANOCO software package, version 4 (Ter Braak & Similaeur 1998). The significance of the PRC diagram was tested using a Monte Carlo permutation, by permuting the whole time series in the partial RDA from which the PRC analysis is obtained, using an F-type test statistic based on the eigenvalue of the first canonical axis (Van den Brink & Ter Braak 1999).

RESULTS

Throughout the period 1993-1995, the effects of eutrophication on benthic assemblages were studied in the Mondego estuary. Over these temporal and spatial scales, distinct changes in the structure of the macrobenthic communities were observed. The diversity of the macrobenthic communities in the three areas was assessed using rank-abundance curves (Fig. 2) (Molles 1999). These clearly show that the *Z. noltii* beds, always, had a greater species richness (indicated by the number of ranks) than the most eutrophic area, with the intermediate zone having intermediate diversity. Evenness (indicated by the slope of the curve) increased from the *Z. noltii* beds to the most eutrophic area, contrary to expectation due to the dominance of *Hydrobia ulvae* in the *Z. noltii* beds (see also Cardoso *et al.* 2002), as can be seen by comparing the first and second ranks in the three areas. If *H. ulvae* is ignored, the evenness appears higher in the *Z. noltii* beds, consistent with expectations.
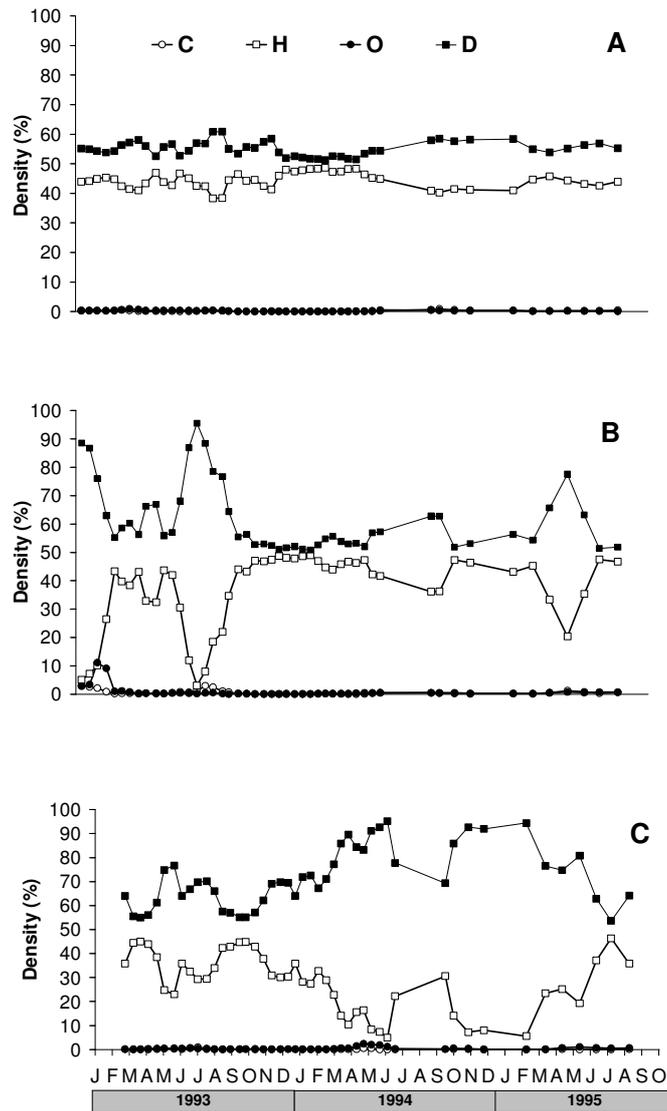
Fig. 3 - Trophic structure of the three macrobenthic communities. (A) - *Z. noltii* beds; (B) - Intermediate area; (C) - Most eutrophic/disturbed area. Trophic groups: herbivores (H), omnivores (O), detritivores (D), carnivores (C). Values are percentages of total individuals.

Over the past 10 years, there was a decline in biodiversity over time (Cardoso *et al.* 2004) with year 1993 showing higher species richness (Fig. 2). Nevertheless, some inter-annual variations in species richness can be seen and easily be understood in the scope of the intermediate disturbance hypothesis. At the end of the study period, species richness in the *Z. noltii beds* (39 species) was similar to that observed in the most eutrophic area during the algal bloom (1993) (43 species), indicating a rapid deterioration of the seagrass habitat.

With regard to trophic groups, in the *Z. noltii* meadows (Fig. 3A), detritivores and herbivores were clearly the dominant groups, taking advantage of the abundance of detritus resource provided by the decay of broken *Zostera* parts, and of grazing opportunities on epiphytes that cover *Zostera* leaves. Besides, *Zostera* leaves act as a trap for suspended sediments, and therefore for organic matter particles usable by detritivores (Valiela 1995). Omnivores and carnivores were always much less abundant. Therefore, despite seasonal variations we observed the same stable pattern throughout the study period.

In the intermediate eutrophic area (Fig. 3B) the scenario appears totally distinct. Besides the fact that detritivores are dominant most of the time and carnivores always poorly represented, it is impossible to recognize any pattern through the study period, since trophic groups alternate very much in dominance (qualitative oscillations), which appear to correspond to a very unstable situation.

Finally, in the most eutrophic area (Fig. 3C), detritivores are by far the dominant group, followed by herbivores as a function of feeding opportunities provided by the macroalgal bloom (quantitative oscillations). As in the other two sampling stations, carnivores and omnivores are poorly represented.

The MDS plot shows that samples from the *Z. noltii* beds were more different faunistically from those of the most eutrophic area, compared to those from the intermediate area (Fig. 4). Also, in the autumn of 1994 and winter of 1995, samples from the seagrass beds were closer in the ordination diagram to those from the most eutrophic area, consistent with the notion that the beds were in decline at the end of the study period. Samples from the intermediate and most eutrophic areas were the most widely dispersed in the ordination diagram (*i.e.* more variable in species composition), probably reflecting fluctuations in macroalgal biomass.

These trends were confirmed by ANOSIM. *Z. noltii* beds were significantly different from the intermediate area and from the most eutrophic area (R = 0.387; P = 0.001; R = 0.273; P = 0.001, respectively).

Stress: 0.12
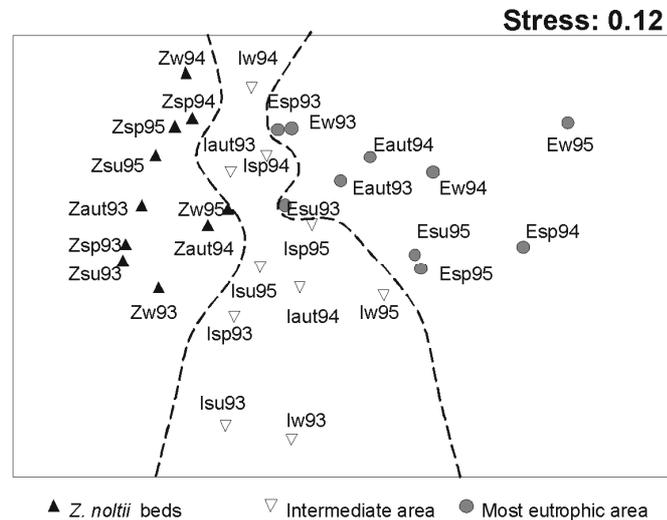
Fig. 4 - Two-dimensional MDS ordination plot of macrobenthic communities. (Z) – *Z. noltii* beds; (I) – Intermediate area; (E) – Most eutrophic/disturbed area; (sp) – spring; (su) – summer; (aut) – autmn; (w) – winter.

Significant differences were observed for all sites between 1993 and 1994 (R = 0.192; P = 0.02) and also between 1993 and 1995 (R = 0.206; P = 0.016). However, no significant differences were detected, for 1994 against 1995 (R = -0.03, P = 0.567).

The PRC analysis shows a clear spatial gradient related to eutrophication, in that the declining *Z. noltii* beds (1994-1995 data set) are closer to the 1993 *Z. noltii* reference, followed by the intermediate area and finally the most eutrophic area (Fig. 5). Furthermore, the macrobenthic communities deviate further from the reference over time. In our analysis, sampling date accounted for 26.3 % of the total variance within the data set, with 59.3 % explained by the eutrophication gradient (the remaining time * site interaction).
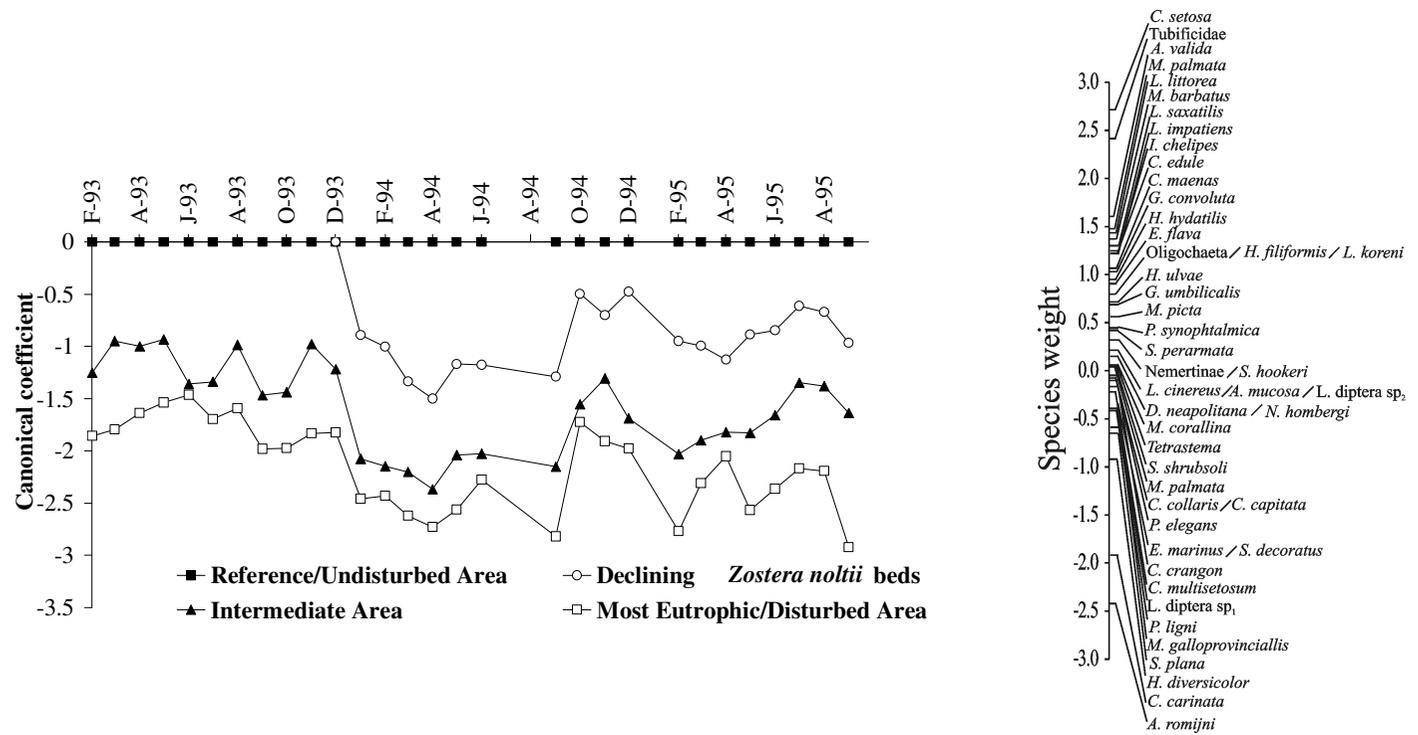
Fig. 5 - Principal Response Curves (PRC) diagram showing the response of macrobenthic communities to different degrees of organic pollution/disturbance with species weights indicating the relative contribution of individuals species to the community response.

14.4 % of the total variance can be attributed to the differences between the sample replicates. Monte Carlo permutation tests revealed that the differences between the treatments (sites) and the reference were statistically significant (P < 0.05), with the PRC diagram explaining 45.03 % of the variance in treatment (site) effects.

The most affected taxa were the polychaete *Chaetozone setosa* and the oligochaete family Tubificidae, both with positive weights, indicating a reduced abundance, compared to that in the reference site. In contrast, the taxon with the highest negative weight (*i.e.* which increased in abundance) was the polychaete *Alkmaria romijni* (Fig. 5), consistent with the premise that small deposit-feeding polychaetes increase in eutrophic conditions (Pearson & Rosenberg 1978, Simboura *et al.* 1995).

DISCUSSION

Both the MDS and PRC analyses showed significant differences between the three sites, reflecting the levels of disturbance that they experience, as well as revealing a deterioration of the seagrass beds over the study period. However, these trends are much clearer in the PRC diagram.

Mensurative or manipulative experiments in community ecology usually result in large data sets on treatment effects and how they change through time. Traditional ordination methods attempt to summarise such change but diagrams can often be messy (Warwick & Clarke 1991). The PRC approach compacts the complexity of time dependent, community–level effects of stress into a simple and user-friendly diagram, facilitating both interpretation by the ecologist and the presentation of impacts to policy makers, managers and stakeholders. The elegance lies partly in the fact that PRC analysis represents the time trajectory for the control or reference site as a horizontal line, and partly because it permits the interpretation of effects at the level of the individual species. This is not achievable in an MDS analysis, because MDS works on a derived "sample by sample" similarity matrix, which makes it difficult to show the individual taxa in the final ordination diagram.
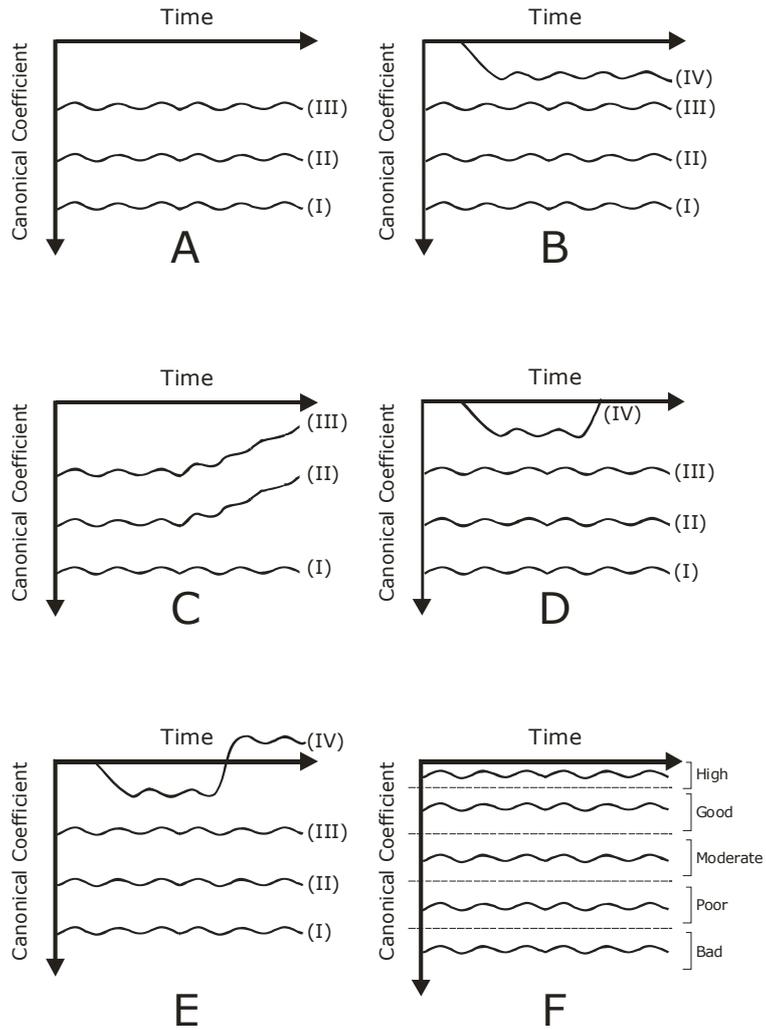
Fig 6 - Theoretical Principal Response Curves (PRC) diagrams in response to different environmental scenarios. A - typical gradient of disturbance; B - The decline of the reference area after disturbance; C - The recovery of environmental quality after management; D - The recovery of the reference area after management; E - The need to settled new reference values after management; F - The implementation of threshold values for qualitative environmental evaluation.

Principal Response Curves have advantages over other approaches used in community ecology or ecotoxicology, such as the BACI design, the use of similarity indices (*e.g.* Clarke & Warwick 1994, Heimbach & Ratte 1997, Sousa *et al.* 1997) or the use of biotic or diversity indices.

These advantages lie not only with the final graphical display but also with the level of interpretation that the PRC allows. Although BACI and similar approaches may also have a user-friendly display of differences (dissimilarities) between treatments over time, allowing also the use of statistical tests to assess the significance of treatment effects, effective representation using indices is difficult to achieve. Moreover none of these methods allow an interpretation back to species level, as does PRC. With this new technique is more likely to capture subtle changes that may only occur in a few species of the assemblage. This feature could also make this tool important for early detection of assemblage-level changes. However, one advantage of MDS or BACI over PRC is that it is possible to select a specific distance metric, such as the Bray-Curtis measure, while for PRC the user is presently restricted to Euclidean distances (Ter Braak & Similaeur 1998).

Figure 6 illustrates and summarises how PRC analysis can be applied to several common environmental scenarios independently of the number of sites analysed. For example, a very common disturbance gradient where site (I) is the closest to the disturbance point source (Fig. 6A); where changes occur in the originally defined reference area (Fig. 6B); to follow recovery of the environmental quality after management or mitigation measures (Figs. 6C, D) that might lead to a better environmental quality than considered at first for the reference area (Fig. 6E); where the establishment of threshold values/levels are necessary for qualitative evaluation of ecosystem health (Fig. 6F), as will be required under the European Water Framework Directive (WFD 2000/60/EC).

In conclusion, we believe that PRC will provide a powerful tool for environmental quality assessment in the future and should be incorporated into monitoring and assessment programmes along with the existing range of univariate and multivariate tools presently used.

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CHAPTER 4

THE RESILIENCE OF *HYDROBIA ULVAE* POPULATIONS TO ANTHROPOGENIC AND NATURAL DISTURBANCES

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This chapter is *in press* as a paper in Mar Ecol Prog Ser

The resilience of *Hydrobia ulvae* populations to anthropogenic and natural disturbances

ABSTRACT: In the Mondego estuary (Portugal), several mitigation measures (nutrient loading reduction, seagrass bed protection and freshwater circulation enhancement) were implemented in 1998 to promote the recovery of the seagrass bed and the entire surrounding environment following a long period of eutrophication. In the present study we evaluate the success of this restoration project, by comparing the water nutrient concentrations, the seagrass cover extent and the dynamics of *Hydrobia ulvae*, before and after implementation of the management measures. During the period in which environmental quality declined, *H. ulvae* abundance, biomass and growth production declined, associated with the almost total disappearance of the macrophyte *Zostera noltii*. However, after the implementation of management measures, dissolved nutrients and green macroalgal blooms were much reduced, and the seagrass bed started to recover. The *H. ulvae* population also responded positively, becoming more structured (including individuals of all age classes), with higher abundance and biomass. Major flood events demonstrated that the resilience of the *H. ulvae* population may have been lowered by the original chronic stressor (eutrophication). The population structure of *H. ulvae* in the most stressed site continued to be dominated by small individuals despite the improvements in water quality, probably a result of the absence of seagrass plants at this site. Estuarine restoration programmes need to recognise the importance of understanding the resilience of populations and the interactions of multiple stressors.

INTRODUCTION

Eutrophication, or nutrient enrichment, of coastal waters is now widely recognized as a major worldwide problem (Diaz & Rosenberg 1995, Norkko & Bonsdorff 1996a, b, Raffaelli *et al.* 1998). One manifestation of estuarine eutrophication is a replacement of seagrasses and slow-growing macroalgae by opportunistic macroalgae and phytoplankton (reviewed in Schramm & Nienhuis 1996, Valiela *et al.* 1997). Loss of the seagrass bed leads to changes in the

associated biological communities (Reise *et al.* 1989, Flindt *et al.* 1997, Dolbeth *et al.* 2003, Cardoso *et al.* 2004) and the functions, services and goods that seagrass bed provides (Clark 1996, Jonge *et al.* 2000, Duarte 2000, 2002, Jackson *et al.* 2001).

In recent years there has been an enormous upsurge in interest in restoration as a technique for reversing habitat degradation worldwide (Hobbs & Norton 1996, Jonge *et al.* 2000). Restoration ecology has been hailed as a new paradigm for biological conservation (Peterson & Lipcius 2003). The purpose of restoration projects is to return a habitat from an altered or disturbed condition to a previously existing natural condition (Kennish 2000). The success of restoration plans depends on several key processes: a) identifying and understanding the processes which have driven the observed ecological changes; b) determining realistic objectives and measures of success; c) developing methods for implementing the goals and incorporating them into management and d) monitoring the restoration and assessing its success (Hobbs & Norton 1996, Kennish 2000, Pardal *et al.* 2004). There is compelling evidence that the resilience of a seagrass bed is typically low (Hobbs & Norton 1996, Kendrick *et al.* 2002, Cardoso *et al.* 2004) due to the major shifts in sediment properties that occur when seagrass plants are lost, which make the sediment unsuitable for seagrass recruits. Restoration initiatives that exclusively are based on reducing nutrient inputs from further upstream are thus unlikely to be sufficient on their own to restore seagrass bed (Cardoso *et al.* 2004).

In addition to the seagrass plants themselves, a seagrass bed supports a rich invertebrate fauna and the resilience of these populations is not well understood. In the present study we evaluate the success of a restoration project implemented in a well-documented seagrass habitat experiencing eutrophication in the Mondego estuary (Marques *et al.* 1997, 2003, Lillebø *et al.* 1999, Pardal *et al.* 2000, 2004, Cardoso *et al.* 2002, 2004, Dolbeth *et al.* 2003). We focus on the dynamics of the mud snail, (*Hydrobia ulvae*), one of the most abundant species in the seagrass (*Zostera noltii*) meadows (Lillebø *et al.* 1999, Cardoso *et al.* 2002, Dolbeth *et al.* 2003), before and after the establishment of management measures. Such long-term data sets (10 years) are required in order to capture slow ecological processes (*e.g.* population dynamics of long-lived organisms), rare events (*e.g.* floods) and complex phenomena, in which a long time is required to detect changes or trends (Franklin 1989).

MATERIALS AND METHODS

Study site

The Mondego estuary, located on the Atlantic coast of Portugal (40º 08 N, 8º 50 W) comprises two different arms, northern and southern, separated by an alluvium - formed island (Fig. 1). The northern arm is deeper (4-8 m during high tide, tidal range about 1-3 m) and constitutes the principal navigation channel and the location of the Figueira da Foz harbour. The southern arm is shallower (2-4 m during high tide, tidal range 1-3 m) and is almost silted up in the upper zones, constituting a kind of coastal lagoon in which the water circulation is mostly dependent on the tides and on the freshwater input from the Pranto River (Marques *et al.* 1993, Flindt *et al.* 1997, Marques *et al.* 1997, Lillebø *et al.* 1999, Pardal *et al.* 2000). Discharge from the Pranto River is controlled by a sluice (Flindt *et al.* 1997, Lopes *et al.* 2000, Pardal *et al.* 2000) and is regulated according to the irrigation needs of the Mondego Valley rice fields (Martins *et al.* 2001).

Since the 1980's, eutrophication of the estuary has taken place as a result of excessive nutrient release into coastal waters and the seagrass (*Zostera noltii*) bed has been drastically reduced in areal extent and biomass in the south arm (Cardoso *et al.* 2004, Pardal *et al.* 2004). For instance, a seagrass bed that in the mid 1980's measured 15 ha in areal extent was progressively reduced to 1.6 ha in 1993 and to less than 300 m² in 1997 (Fig. 1). In 1998, several mitigation measures were applied. The hydraulic regime in the south arm was improved by enlarging the connection between the two arms. The Pranto sluice-opening regime was minimised in such a way, that most of the freshwater from the Pranto River (loaded with nutrients) is discharged directly into the north arm (by another sluice located more upstream), reducing the nutrient loading in the south arm. In addition, the remaining seagrass patches were protected with wooden stakes to prevent further disturbance of that area (by fishermen digging in the sediment for bait), and several forums were convened to apprise local people of the ecological and economic importance of the seagrass bed.

Two study areas were established in the southern arm of the estuary: a) the seagrass bed located downstream and b) the eutrophic area further upstream.
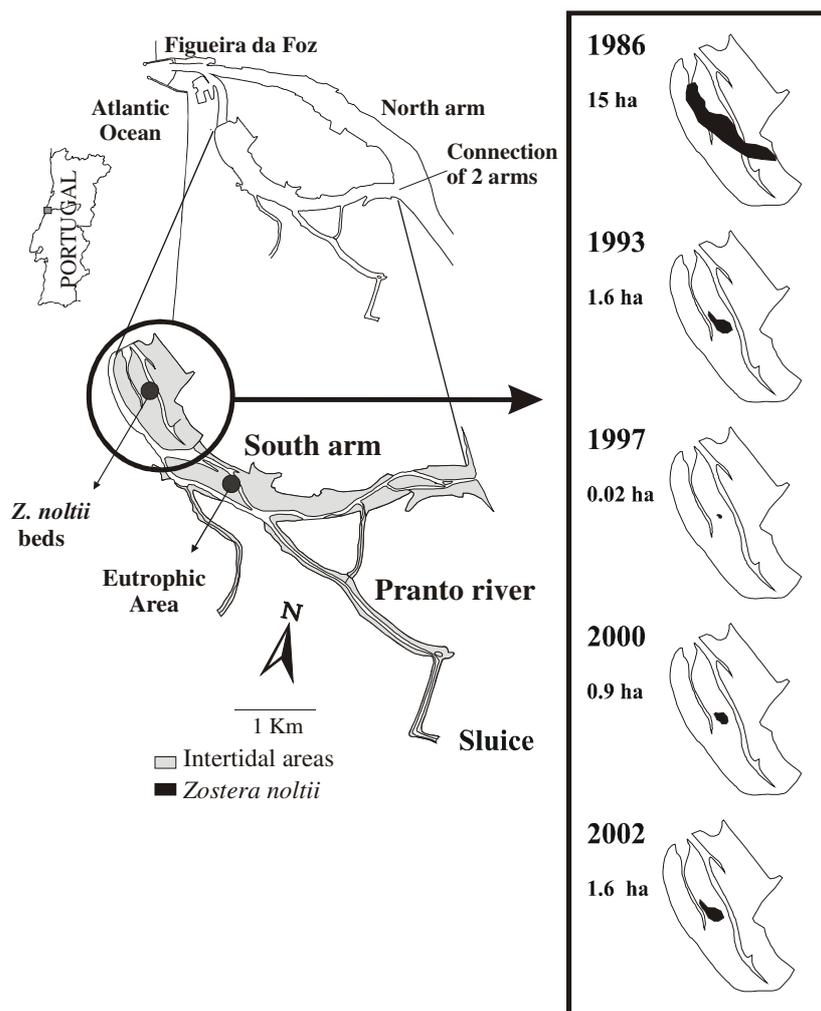
Fig. 1 - Location of the sampling stations and change in the area covered by *Zostera noltii* in the south arm of the Mondego estuary. Mapping of benthic vegetation is based on field observations, aerial photographs and GIS methodology (ArcView GIS version 8.2)

Originally, in the early 1980's, this upstream area was also covered by rooted macrophytes; however, as the eutrophication increased, *Zostera noltii* declined

progressively (Fig.1). At the time of this study, this area was characterised by the absence of seagrasses (for more than 15 years) and was covered seasonally by spring green macroalgae, especially *Enteromorpha* sp. (actually *Ulva* sp., according to the recent revision of Hayden *et al.* 2003). For a detailed description of these areas see Pardal *et al.* (2000), Cardoso *et al.* (2002, 2004) or Marques *et al.* (2003).

Field survey programme

In the eutrophic area, the *Hydrobia ulvae* population was monitored from January 1993 to January 1997 and again from January 1999 to December 2001. In the seagrass bed, the population was monitored from January 1993 to September 1995 and again from January 1999 to December 2002. From 1997 to 1998 the areas were not sampled to aid recovery of the seagrass bed.

Samples were collected fortnightly for the first 18 months of study and monthly for the rest of the study period. On each occasion 6 to 10 sediment cores (141 cm^2 surface area) were taken to a depth of 15 cm and washed in estuarine water through a 500 μm mesh sieve. The remainder (sediment, rooted macrophytes, algae and fauna) was preserved in 4 % buffered formalin.

On each occasion, during the morning, temperature and salinity were measured *in situ* during low tide. Water samples were collected for analysis of dissolved inorganic nitrogen (DIN) and dissolved inorganic phosphorus (DIP). Data on monthly precipitation derive from the nearby city of Coimbra (Instituto de Meteorologia, Coimbra forecast station).

Seagrass and macroalgal analysis

In the laboratory, plant material was sorted and separated into Chlorophycea, Rhodophycea and *Zostera noltii* (leaves and rhizomes). After that, the material was dried (for 48 h at 60º C) and the ash free dry weight (AFDW) was assessed after combustion of samples for 8 h at 450º C.

Hydrobia ulvae

Hydrobia ulvae were counted and two shell measurements were obtained: total shell length (TSL) and maximum width (MW). The last one was most suitable, since a great number of snails had damaged shells. In the present paper we used

the conversion equation (MW = 0.4369*TSL + 0.2091, n = 339, r = 0.97; Lillebø *et al.* 1999). Length-weight relationships were determined for production estimates. Preliminary ANOVA of length x ash free dry weight relationships indicated no significant seasonal differences and an overall regression equation was used (AFDW = 0.0564* $TSL^{2.2381}$, n = 191, r = 0.98; Lillebø *et al.* 1999). Biomass was calculated as ash free dry weight (AFDW) (loss after 8 h of incineration at 450ºC of specimens previously dried at 60ºC for 72 hours). The same procedure was used to quantify organic matter content of the sediment.

Growth rates were estimated by tracking recognisable cohorts in size frequency distributions over successive sample dates using the ANAMOD software package (Nogueira 1992).

Annual net production estimates (P) based on cohort recognition, were estimated as described in Dauvin (1986).

Total values of P for the population are expressed as:

$$P = \sum_{n=1}^{N} P_{cn}$$

P_{cn} is the growth production (biomass assimilated by a constant number of individuals in a certain period of time) of cohort n. $\overline{B}$ (annual mean population biomass) is expressed as:

$$\overline{B} = (1/T) \sum_{n=1}^{N} (\overline{B}_n t)$$

T is the period of study (annual cycles); N is the number of successive cohorts in the period T (year); $\overline{B}_n$ is the mean biomass of cohort n; t is the duration of the cohort n [(in our case, 16-20 months according to Cardoso *et al.* (2002)].

RESULTS

Precipitation and salinity

The Mondego estuary is a warm temperate coastal system in a region with a basically Mediterranean temperate climate. In addition to the clear annual pattern of rainfall over the 10-year period, three winters of above-average precipitation

(93/94, 96/97 and 00/01) are apparent (Fig. 2A). During periods of intense rainfall, salinity declined dramatically, occasionally reaching < 5 g l^{-1} (Fig. 2B).

Nutrient concentrations

DIN concentrations were significantly different before and after the introduction of mitigation measures for both sites (Kruskal-Wallis test, H = 83.14,
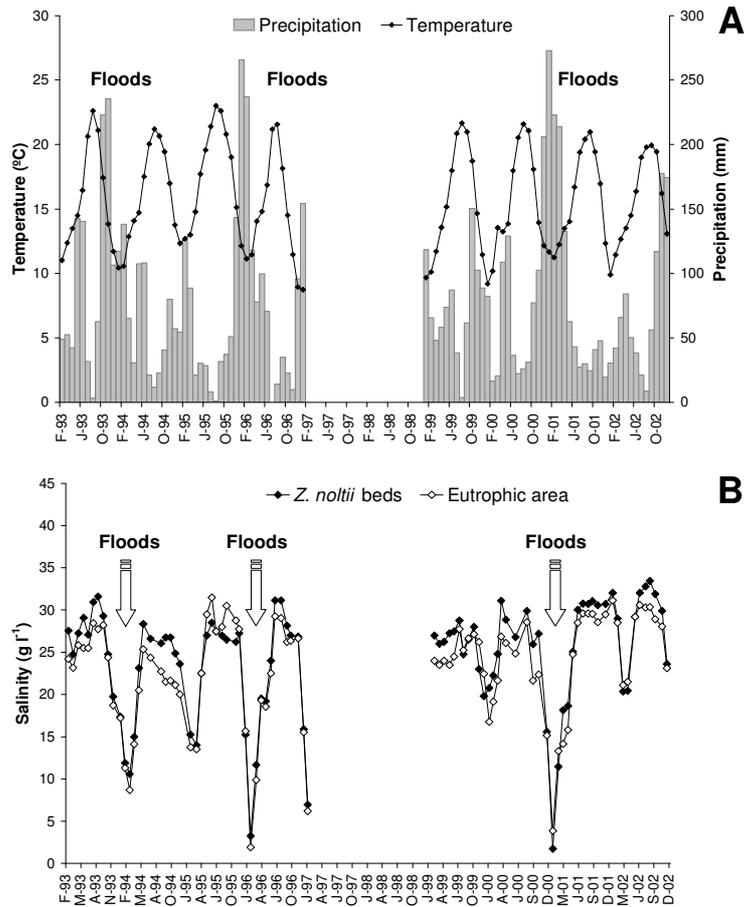
Fig.2 - Long-term variation in temperature and rainfall (A), and salinity (B).
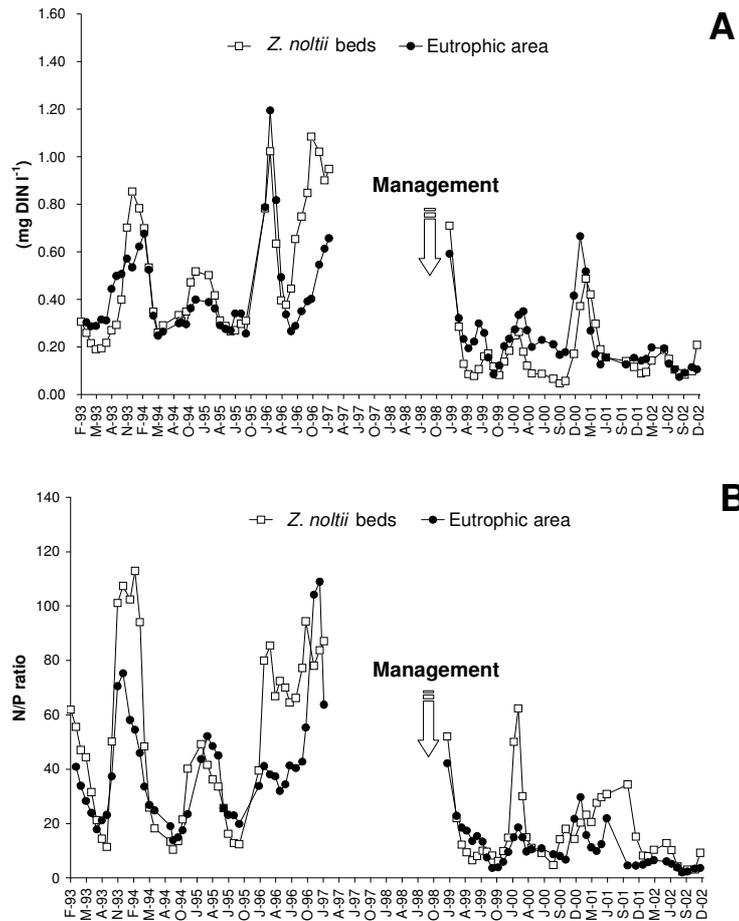
Fig. 3 - Long-term variation in DIN concentrations (A) and N/P ratio (B).

P < 0.05), being markedly reduced in the post-mitigation period for both areas (Fig. 3A). The same pattern was also observed for N/P ratios (Fig. 3B).

Seagrass and macroalgal biomass

Regarding total seagrass biomass and comparing the slope of the pre-mitigation period with the one of the post-mitigation period, significant differences were observed between them (t-test, $t_{107} = 6.12$, P < 0.05). From 1993 to 1997, the

total biomass of *Zostera noltii* declined sharply and in early 1998 the lowest biomass (5 g AFDW m⁻²) was recorded.
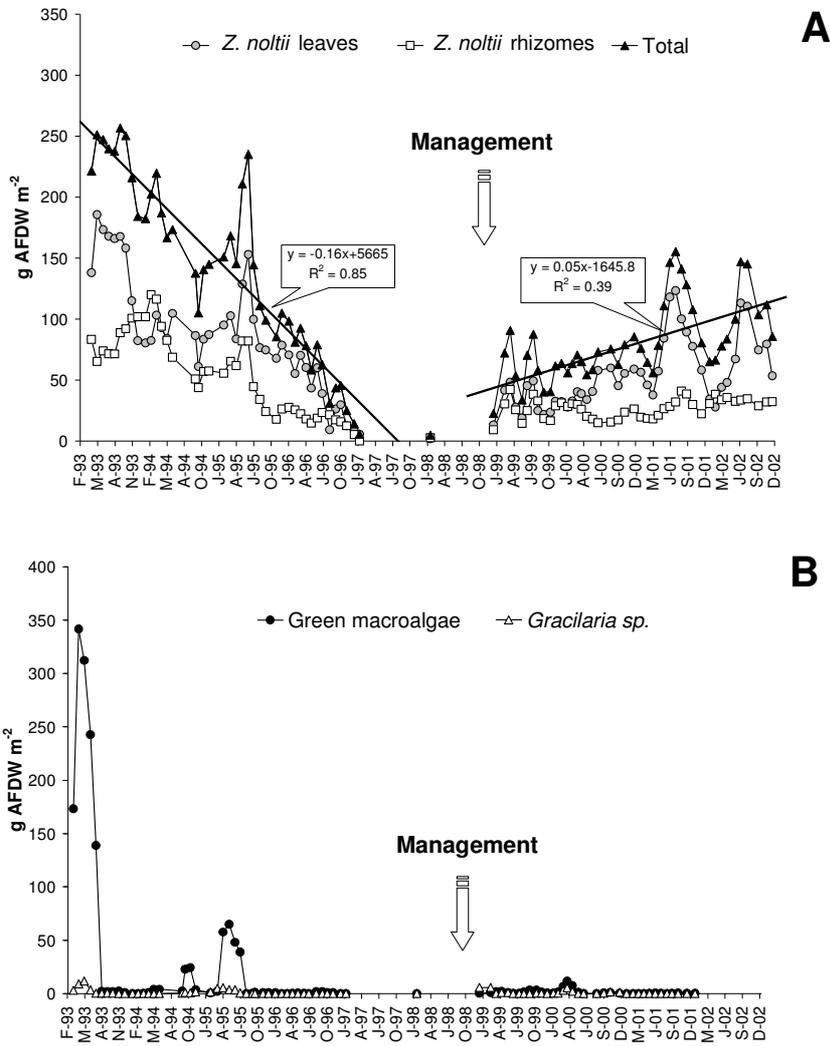
Fig. 4 - Long-term variation of plant biomass. (A) – Seagrass in the *Z. noltii* bed; (B) – Macroalgae in the Eutrophic area.

After the introduction of mitigation measures in 1998 there was a gradual recovery of the seagrass bed (Figs. 1, 4A). In contrast, in the eutrophic area, there were significant differences in green macroalgal biomass between the pre- and post-mitigation periods (Wilcoxon two- sample test, W = 2015.5, P < 0.05).

In 1993 and 1995 algal blooms were common but were never present after post-mitigation measures (Fig. 4B).

Population density

Hydrobia ulvae abundance and biomass changed markedly during the study period. In the seagrass bed from 1993 to 1995, *H. ulvae* biomass was relatively constant (range 50-100 g m^{-2}). Nevertheless, following the decline of *Zostera noltii*, biomass of *H. ulvae* was reduced by approximately an order of magnitude (Fig. 5A). In the *Z. noltii* bed, significant differences in *H. ulvae* biomass were observed between the pre- and post-mitigation periods (t-test, t_{93} = 5.54, P < 0.05). *H. ulvae* appeared to start to recover until the fall of 2000 when a period of prolonged and heavy rainfall (Fig. 2A) was associated with a decline in both density and biomass. However, the population seemed to begin recovery again in 2002 (Fig. 5A).

In the eutrophic area, the pattern of change was completely different. Density and biomass of *H. ulvae* declined significantly throughout the study period with no obvious indication of recovery (Fig. 5B). Significant differences were observed in *H. ulvae* biomass between the pre- and post-mitigation periods (t-test, t_{95} = 2.53, P < 0.05). It should be noted that the recovery of the seagrass bed *H. ulvae* population after flood events in 2001 appears slower than that in 1994. Regarding *H. ulvae* biomass, and comparing the slope in 1994 (since March 1994 till December 1994) with the one in 2001 (since March 2001 till December 2001), significant differences were observed between them (*H. ulvae* biomass, t-test, t_{14} = 6.28, P < 0.05). Despite the huge increase in *H. ulvae* biomass in 2002, the population took a longer time (almost one year) to achieve the same previous values, after the second flood event (2001), comparatively to the first flood event (1994), in which the recovery was much more rapid.

Population structure and growth

Marked differences in the population structure were observed at the two sites (Fig. 6). In the seagrass bed from 1993 to 1995, several modes are apparent in the population representing individuals of all age classes (juveniles < 1 mm; young individuals 1-1.5 mm; adults > 1.5 mm) (Anderson 1971, Planas & Moras 1987, Cardoso *et al.* 2002).
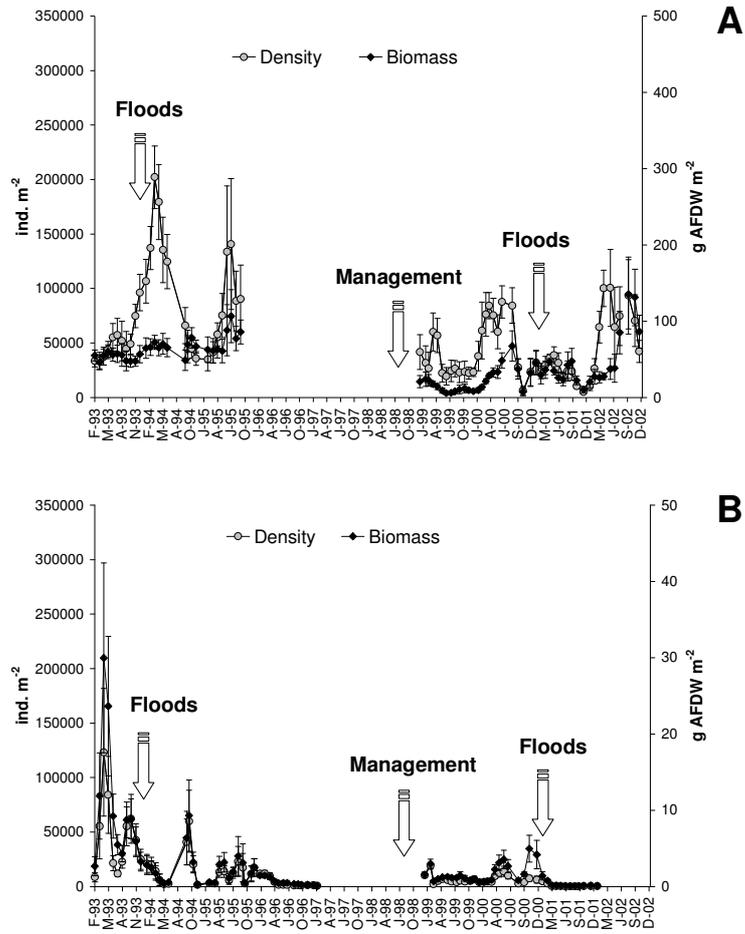
Fig. 5 - Variation of *H. ulvae* density and biomass at the two study areas. (A) - *Z. noltii* bed; (B) - Eutrophic area.
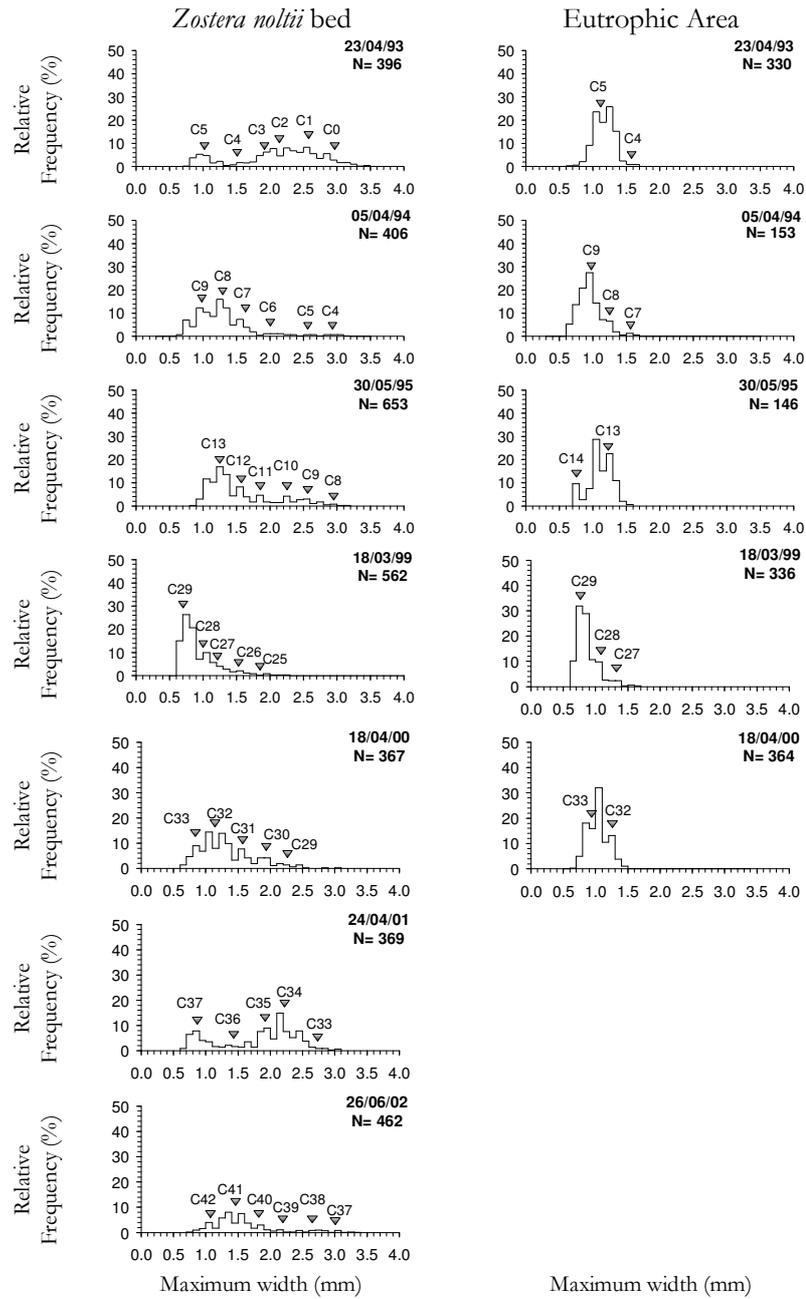
Fig. 6 - *H. ulvae*. Size-frequency distribution at *Z. noltii* bed and at the eutrophic area. The cohorts (C) and the number of individuals are represented in the figure.

In 1999, the population at this site was dominated by small individuals, being significantly different from 1994 (χ^2 test, $P < 0.05$) and only by the spring of 2001 did larger snails reappear in the size frequency distributions. Significant differences were also observed between the population in 1999 and 2001 (χ^2 test, $P < 0.05$). In contrast, the eutrophic area was dominated by small individuals of *H. ulvae*, over the entire study period, with few adults (> 1.5 mm) present (Fig. 6).

Production

Growth production (P) and mean population biomass ($\overline{B}$) of *H. ulvae* were significantly higher at the *Zostera noltii* bed than at the eutrophic site (growth production - t-test, $t_8 = 3.34$, $P < 0.05$; mean population biomass - t-test, $t_8 = 3.28$, $P < 0.05$) (Table 1). The highest values were recorded in 1994 in the seagrass bed. After the introduction of mitigation measures, the values of growth production and mean biomass in the seagrass site tended to increase.

Table 1 - Growth production estimations of *H. ulvae* for both areas. Legend: (-) – no data available.

	Z. noltii bed			Eutrophic Area		
	P (g AFDW m^{-2}y^{-1})	$\overline{B}$ (g AFDW m^{-2})	P/$\overline{B}$	P (g AFDW m^{-2}y^{-1})	$\overline{B}$ (g AFDW m^{-2})	P/$\overline{B}$
1993	125.08	59.24	2.11	45.73	9.37	4.87
1994	202.53	74.14	2.73	10.86	2.38	4.56
1995	-	-	-	8.88	3.30	2.68
1996	-	-	-	2.80	0.95	2.94
1999	26.77	11.11	2.40	3.21	1.09	2.94
2000	95.40	29.33	3.25	8.19	2.50	3.27
2001	61.97	29.94	2.07	1.30	0.36	3.66
2002	167.11	58.31	2.86	-	-	-

In 2001, the populations at both sites were clearly impacted by floods, but by 2002, the production values in the seagrass bed were similar to those observed in the beginning of the study (Table 1). In the eutrophic area, there was a general, gradual decline in P values. On the other hand, the P/$\overline{B}$ ratios were always higher in the eutrophic area.

DISCUSSION

There were clear differences in the dynamics and structure of the *Hydrobia ulvae* population before and after the introduction of mitigation measures. Density and biomass were generally declining during the pre-mitigation period, which we attribute to a eutrophication effect possibly associated with the concomitant decline in seagrass habitat. Seagrass showed signs of post-mitigation recovery and there is some evidence of recovery of *H. ulvae* in this period, both in abundance (density and biomass) and population structure, with an increasing proportion of adults represented. In the eutrophic area, density and biomass were also lower in the post-mitigation period but there was little sign of recovery post-mitigation, for either abundance or population structure. These patterns of change are complex but consistent with the first successful steps of restoration of the *Z. noltii* bed. After 1998, the controlled use of fertilisers, the opening of the upstream connection (residence time declined from 5-7 days to just 1 day) between both arms, the reduction of the number of fishermen digging for bait and the improved management of sluice openings led to an improvement of the environmental quality. All these actions promoted lower turbidity (from average particulate organic matter values of 0.02 mg.l^{-1} before mitigation to 0.003 mg.l^{-1} after mitigation) (unpublished data), a consequent increase in freshwater circulation that favoured nutrient dilution and decreased residence time, which improved the environment for seagrass bed and reduced the risk of macroalgal blooms. We are confident therefore that the adverse environmental conditions caused by eutrophication are now abating.

Nevertheless, two questions remain. First, if eutrophication of the entire estuary has lessened, why has the biology of upstream eutrophic site not returned to its original state? And, second, why has the recovery of the *Hydrobia ulvae*

population following major flood events been slower than was the case during the first period of the study? Although the recovery after floods was studied for only two events, we suggest that the answer to both questions involves the relative resilience of the seagrass bed and the eutrophic site, and the compounding effects of the two stressors, enrichment and flood events. We have argued elsewhere (Cardoso *et al.* 2004) that the intertidal flats of the Mondego manifest two alternate states. The first is characterised by vigorous seagrass bed, which because of their above and below-ground structural complexity reduce critical erosion velocities and create a more benign near-bed environment for deposit feeders, as well as secondary habitat in the form of leaves and roots, and fine particle sediment. The second state is one lacking rooted macrophytes with coarser sediment particles and an impoverished biota. The shift from seagrass to coarse sediment and *vice-versa* is probably best described by hysteresis dynamics (Hobbs & Norton 1996, Scheffer & Carpenter 2003), where moving between the two alternate states requires a major perturbation (eutrophication or large-scale sediment stabilisation). Thus, even if eutrophication is completely reduced at the eutrophic site, recovery to the original state (covered with rooted macrophytes), probably will not take place in the absence of further restoration, such as extensive physical engineering of the bed (Cardoso *et al.* 2004).

The failure of *Hydrobia ulvae* to completely recover to a structured population, including all age classes at the eutrophic site is consistent with this view of system stability. It is also interesting that the *H. ulvae* population at the eutrophic site seems less resilient than that in the seagrass bed, as indicated by the longer time to achieve the same previous values following major flood events (Fig. 5). Also, the population in the seagrass site seems similarly less resilient to flood events following the earlier eutrophication period. If so, this would add weight to the concerns expressed by other workers, that multiple stressors (here, eutrophication and flood disturbance) operating concurrently or consecutively can act synergistically, to lower overall system stability (Kennish *et al.* 2004). In this case, the eutrophication process, which leads to a decline of the seagrasses will promote indirectly the disappearance of the majority of the reproductive adults. In the presence of a new stressor (*e.g.* floods) we can admit that the recovery of the population is much slower. This fact was more evident at the eutrophic site, in

which, after the second flood event were not observed any signs of population recovery. This pattern reinforces the notion that both stressors acting synergistically can have an important role in the stability of the population. Such stability concepts need to be acknowledged in estuarine restoration programmes if the latter are to be successful.
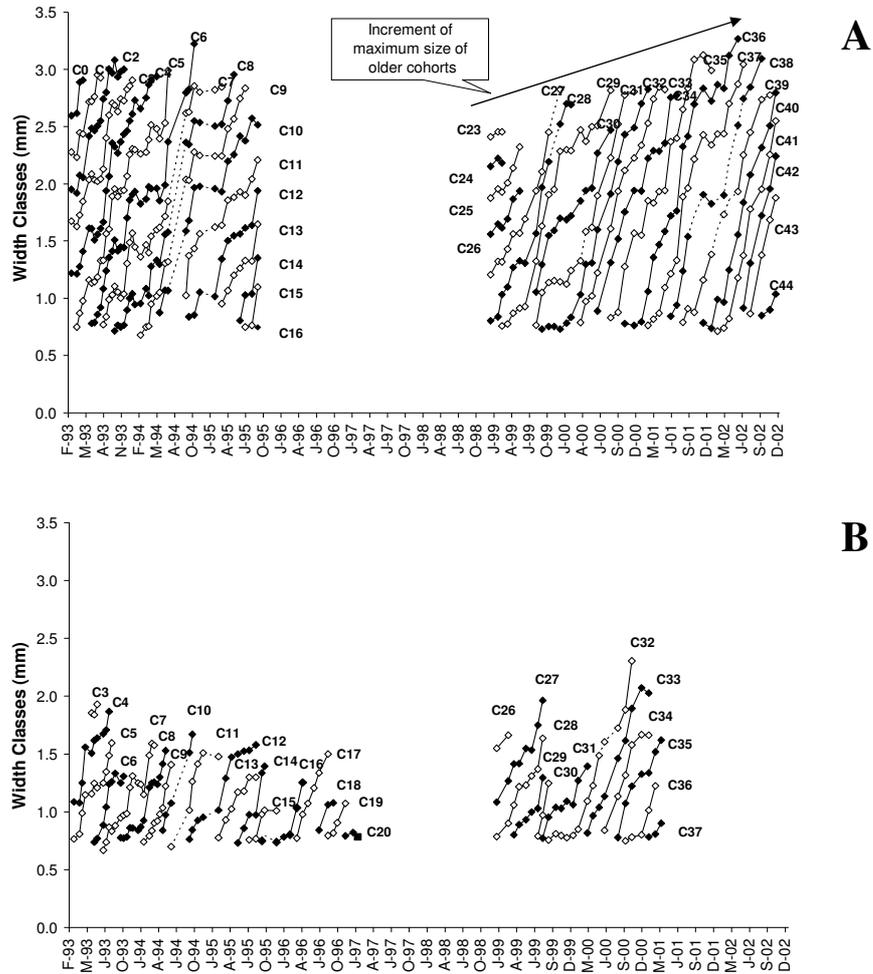
Fig. 7 - Estimated growth of *H. ulvae* cohorts. (A) - *Z. noltii* bed; (B) – Eutrophic area.

One of the most dramatic differences in *Hydrobia ulvae* populations at the two sites is the virtual absence of adult (large size - > 1.5 mm) cohorts at the eutrophic site. There are several possible explanations for this. First, growth rates at the eutrophic site may be lower than those in seagrass bed such that the populations are essentially similar in their age structure. This seems unlikely on the basis of the results of the cohort analyses and field growth rates obtained (Fig. 7). Second, trematode parasites are known to induce gigantism in *H. ulvae* and alter population structures accordingly (Huxham *et al.* 1995). However, infection rates by trematodes in the Mondego are very low (< 2 %) and cannot account for the higher abundance of larger snails (Ferreira *et al.* unpublished data). Third, size selective removal of larger individuals by shorebird predation could be responsible for the observed between-site differences (Cabral *et al.* 1999). This seems the most likely explanation, given the obvious difference in refuges between the two sites. Recognising that predation is the responsible mechanism for the removal of larger snails, this explains the reduction in growth production, as well as the deficiency of large snails observed at the eutrophic area (characterised by bare sediments) and also at the *Z. noltii* bed in 1999 (when the biomass of seagrass was lower). This mechanism associated to the reduction of food resources could have had a synergistic effect leading to a more fragile and less resilient population in the post-mitigation period than in the beginning of the study.

In conclusion, it seems that the nutrient mitigation strategy for the restoration of the estuary has been partially successful. There is evidence that the seagrass is recovering at the outermost site and that the *Hydrobia ulvae* population has the potential for recovery, although its resilience to further impacts (such as floods) may have been reduced. For the eutrophic site, there was some minor recovery of *H. ulvae* post-mitigation, but the resilience of the population at this site to a flood disturbance appears very low. Understanding the behaviour of biological populations following restoration initiatives requires acknowledgement that some changes may not be easily reversible and that the existence of multiple stressors may lower the resilience of populations.

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CHAPTER 5

SEAGRASS BEDS AND INTERTIDAL INVERTEBRATES: AN EXPERIMENTAL TEST OF THE ROLE OF HABITAT STRUCTURE

Cardoso PG, Raffaelli D, Marques JC, Pardal MA

Seagrass beds and intertidal invertebrates: an experimental test of the role of habitat structure

ABSTRACT: The majority of field experiments in the literature have been carried out on relatively small spatial and short temporal scales, but some of the most interesting ecological processes operate at much larger scales. However, large-scale experiments appropriate to the landscape features have the constraint of replication be necessarily sacrificed at the expense of realism.

Previous studies carried out on the Mondego estuary, Portugal, have shown that the seagrass bed suffered a dramatically reduction over the last two decades, especially in the inner parts of the estuary. Consequently, different macrofaunal assemblages were observed in the seagrass bed and in the areas where the macrophyte has disappeared, and the most abundant species, *Hydrobia ulvae*, has also distinct population structures in those sites. Considering that between-site differences on *H. ulvae* population are related more likely with enhanced survival (due to protection from avian or fish predators) of snails to larger size class in the more complex habitat provided by seagrass, we tried to test this hypothesis with a large-scale experiment of seagrass mimics over a 12 month period.

The results demonstrated differences in terms of *Hydrobia ulvae* adults' density between the artificial plots and the controls, being higher in the first ones. Despite the emergence of differences between them has been slow, this could be justified based on the growth rates of the species. Thus, this experiment showed that the habitat structure provided by the seagrasses has an important role on the species protection against predators. Concerning the overall macrobenthic community, no similarities were found between the artificial plots and the natural seagrass bed, however, the seagrass mimic samples were always separate from their control samples, indicating different assemblage structures.

INTRODUCTION

Dominant taxa such as fields of tube worms, mounds of lugworms and beds of mussels and seagrasses generate landscape-scale complexity in many intertidal areas. The invertebrate assemblages associated with such landscapes are often

qualitatively and quantitatively different from adjacent areas lacking these dominants due to differences in the hydrodynamic, sediment and/or predation climate (Committo & Boncavage 1989, Committo & Dankers 2001, Dittmann 1990, Flach 1992, Flach & Tamaki 2001, Raffaelli et al. 1998, Cardoso et al. 2004). Evidence of the over-ridding role of the dominant has been most compelling when mimics of the habitat structure normally created by such taxa have been used in controlled manipulative field experiments for tube fields and mussel beds (e.g. Ragnarson & Raffaelli 1999, 2001). However, such experimental set-ups are necessarily on a relatively small spatial scale, due to the need to replicate treatment plots as well as the enormous effort required to assemble the mimic structures. The effects of spatial scale (extent) on the interpretation of the results from such experiments are not well understood because few experiments of this type can accommodate a wide range of spatial scales. Where manipulative experiments have been carried out at the larger scales appropriate to the landscape features of interest, replication has been necessarily sacrificed at the expense of realism (e.g. Paine's early experiments on starfish removal in Washington, USA and limpet removals on the Isle of Man, UK) and the results remain convincing (reviewed in Raffaelli & Moller 2000).

Here, we adopt a large-scale, un-replicated approach to the role of a dominant taxon on many intertidal and shallow sublittoral areas around the European coast, the seagrass *Zostera noltii*. At a well-documented site, the Mondego estuary, Portugal, previous research has shown that seagrass has become confined in recent years to the outer reach of the estuary due to extensive eutrophication in the inner parts (Cardoso et al. 2002, 2004, Marques et al. 2003, Pardal et al. 2004). The general macrofaunal assemblage in seagrass beds and in those areas from seagrass has disappeared are distinctly different (Dolbeth et al. 2003, Marques et al. 2003, Cardoso et al. 2004, Pardal et al. 2004) and the most abundant species, *Hydrobia ulvae*, has different population structures in seagrass and non-seagrass habitats (Lillebø et al. 1999, Cardoso et al. 2002, in press). Specifically, larger sized *Hydrobia* do not occur at non-seagrass sites. The between–site differences in population structure of *Hydrobia* do not appear to be due to differences in the incidence of parasites that induce gigantism (cf Huxham, Raffaelli & Pike) and are more likely related to enhanced survival (due to protection from avian or fish predators) of

snails to larger size class in the more complex habitat provided by seagrass (Cardoso *et al.* 2002).

MATERIALS AND METHODS

The Mondego estuary is located on the Atlantic coast of Portugal (40º 08 N, 8º 50 W) and comprises two contrasting arms, northern and southern, separated by Murraceira island, formed by the deposition of detrital materials transported by the river, as the river flood-plain (Fig. 1).
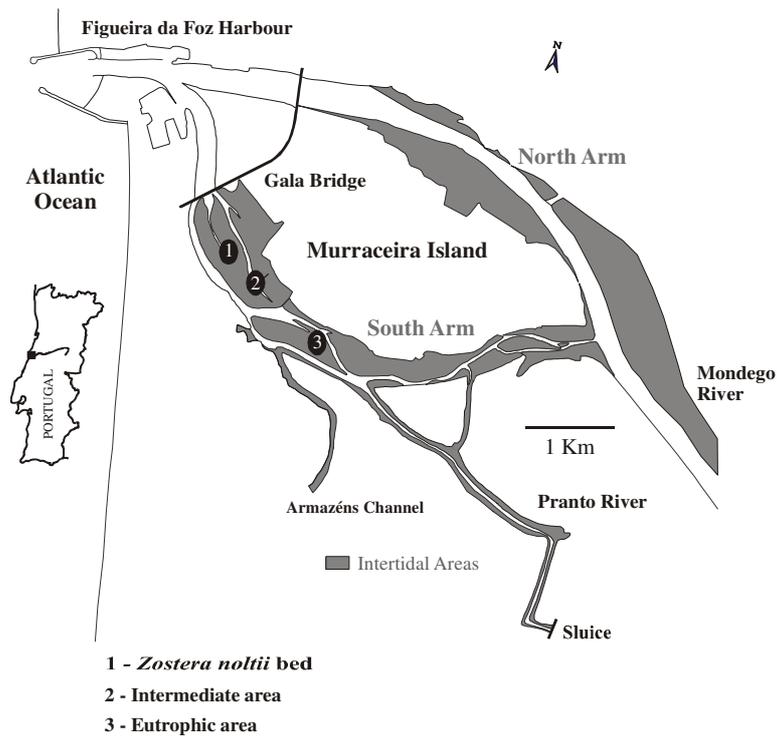
Fig. 1 - Location of the sampling areas in the south arm of the Mondego estuary.

In order to explore these hypotheses, large-scale plots of seagrass mimics were set up in non-seagrass areas and their assemblages and the population structure of *Hydrobia* compared with adjacent non-mimic plots over a 12 month period.

During the last two decades, this estuary has experienced a significant eutrophication process leading to an overall increase in primary production and to a progressive replacement of seagrass *Zostera noltii* by opportunistic green macroalgae (Lillebø *et al.* 1999, Pardal *et al.* 2000, 2004, Martins *et al.* 2001, Cardoso *et al.* 2002, 2004, Dolbeth *et al.* 2003, Marques *et al.* 2003).

Three distinct zones can be recognised in the southern arm: an undisturbed *Zostera noltii* seagrass bed, a eutrophic area (Arm) and an intermediate area (Gala) between these two. The eutrophic and intermediate zones have no seagrass and in each of these areas, a single artificial seagrass unit (ASU) (plot size = 2 m × 2 m) was set up in late September 2003. Each ASU comprised 3000 artificial shoots (~ 750 shoots m^{-2}, equivalent to those found in natural density *Z. noltii* bed). Each "seagrass" shoot consisted of five strips of 3-4 mm wide and 20 cm green plastic ribbon similar to *Z. noltii* leaves. The shoots were anchored to the sediment with corkscrewed wire, penetrating several centimetres into the sediment and mimicking a natural seagrass habitat. Additionally, in each experimental area, a control (treatment clear of artificial seagrass) was also established. Overall, there were 4 treatments (Gala artificial Zos, Gala control, Arm artificial Zos and Arm control) and a reference site (undisturbed *Zostera* bed). By virtue of their large size, each ASU required several hours/person to construct, so that replication of ASU's of this plot size was not feasible. The experiment was set up at low tide and ran for one year. During this period, the plots were sampled on 4 occasions (winter, spring, summer and fall). On each occasion and in each experimental area, three replicate cores were taken from each treatment plot and also in the natural seagrass bed, using a 13-cm diameter corer to a depth of 15 cm. Additionally, we measured the sediment height within the artificial seagrass plots relative to the adjacent sand and took sediment samples (n = 3) for organic matter estimations. All samples were washed in estuarine water through a 500 μm mesh and the fauna retained preserved in 4 % buffered formalin. Later, animals were separated and kept in 70 % ethanol.
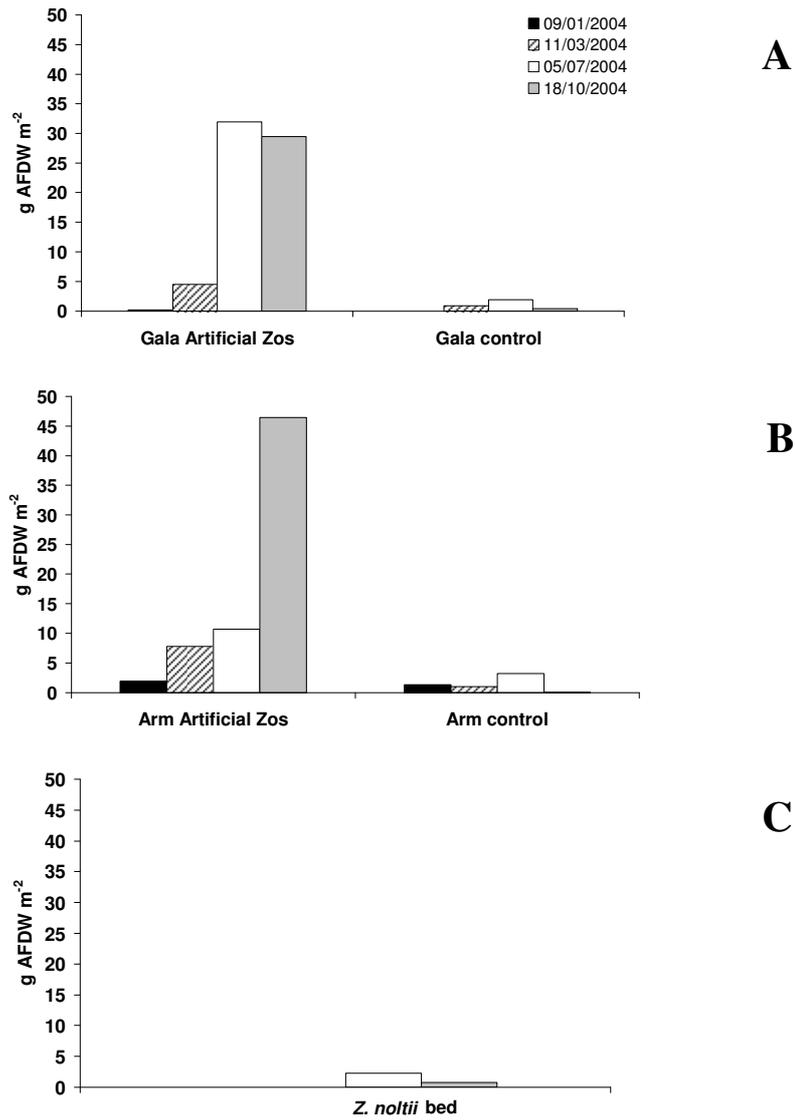
Fig. 2 - Variation of green macroalgal biomass in the 3 sampling areas. (A) – Intermediate area, (B) – Eutrophic area, (C) – *Zostera noltii* bed.

Macroalgae included in the sediment cores were picked up for posterior determination of biomass. All macrofauna were identified to the lowest possible

taxon and counted. Macroalgal biomass was determined as ash free dry weight (AFDW) after oven drying at 60 ºC for 72 h and combustion at 450 ºC for 8 h. The same procedure was used to quantify the organic matter content of the sediment.

For *Hydrobia ulvae*, all individuals were counted and measured (maximum width – MW) and grouped in different age classes (juveniles < 1 mm; young individuals 1-1.5 mm; adults > 1.5 mm) (Anderson 1971, Planas & Moras 1987, Cardoso *et al.* 2002).

Multivariate analysis of the macrofaunal assemblages

Faunal assemblages were compared using non-metric Multi Dimensional Scaling (MDS) on square root transformed data (Clarke & Gorley 2001, Clarke & Warwick 2001). Because *Hydrobia ulvae* population dominated all samples it was excluded from the MDS analysis. It should be noted that because there is no replication of plots within a site, any between–plot differences observed reflect just that: plot differences. This is a necessary feature of many large scale experiments (Raffaelli & Moller 2000).

RESULTS

Macroalgal biomass

At both experimental sites, there was greater biomass of green macroalgae in the artificial plots comparatively to the controls, with slightly more in the Arm artificial Zos than in Gala artificial Zos plots (Fig. 2). Biomass increased gradually all over the sampling periods reaching the highest values in July for Gala artificial Zos and October for Arm artificial Zos. In the natural seagrass bed, the biomass of green macroalgae was insignificant.

Organic matter content and deposition of sediment

The percentage of organic matter was always greater in the artificial plots than in the controls (Fig. 3). This difference was more pronounced at the eutrophic site (Arm), especially for March and July.
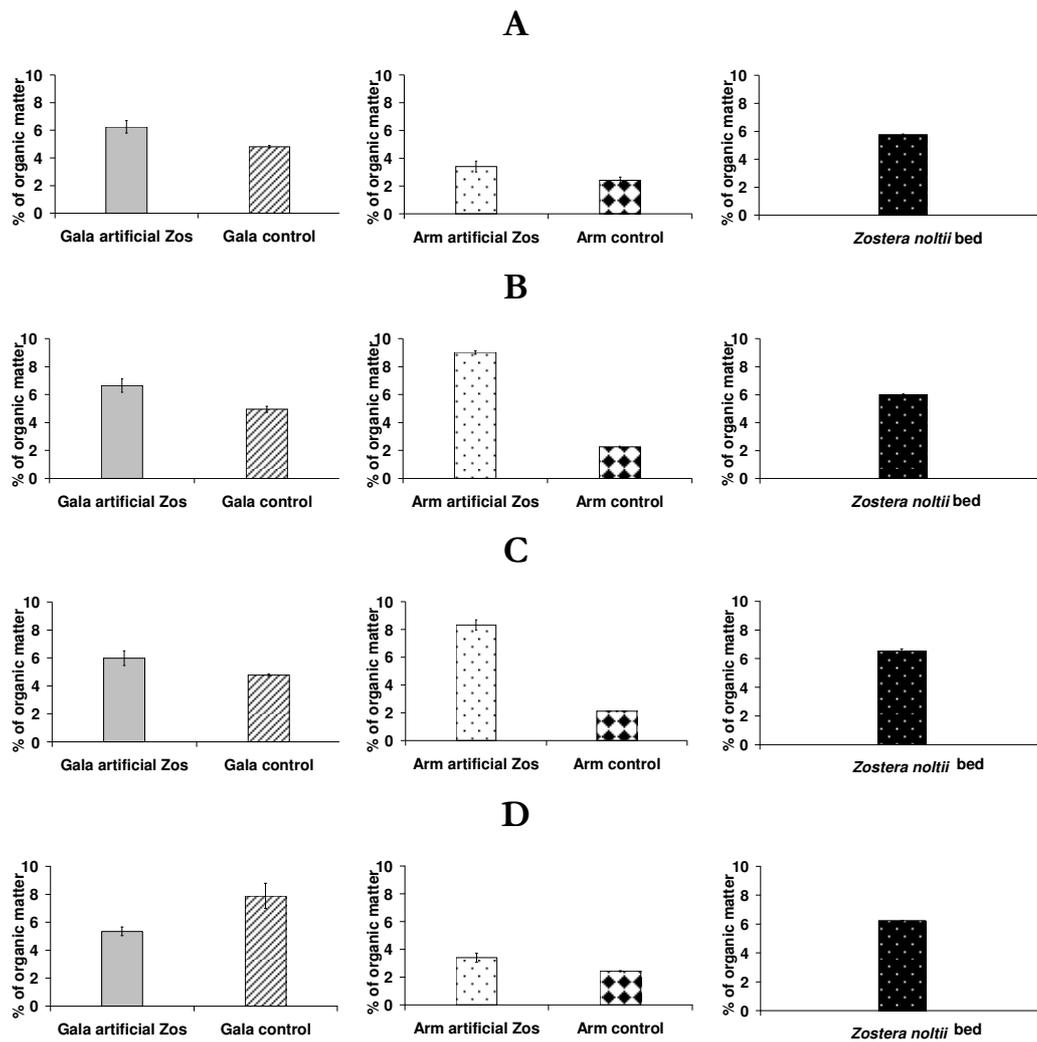
Fig. 3 - Variation of the organic matter content of the sediment (± standard error (SE)) in the 3 sampling areas. (A) – January, (B) – March, (C) – July, (D) – October.

In October, we recorded a marked reduction in organic matter content in the Arm artificial plot, probably due to high discharge occurred in that area, which

washed out most of sediment. The organic matter content in the natural seagrass bed was similar to that in the Gala artificial plot (Fig. 3).

Sediment accumulated dramatically in the artificial plots, being slightly higher in the Gala artificial plot (Table 1).

Table 1 - Sediment deposition on the artificial seagrass plots in both experimental areas all over the time.

	Gala artificial Zos	Arm artificial Zos
March	6 cm	4 cm
July	7 cm	5 cm
October	8 cm	7-10 cm

Hydrobia ulvae population density and structure

At the Gala area, densities of *H. ulvae* were always higher in the artificial plot than in the control, but declined progressively over time (Fig. 4). In the Arm area, there were no differences between the artificial plots and control in January and March, but in July the difference was significant (t-test, $t_4 = 11.84$, $P < 0.05$), being higher in the artificial plot, whilst in October, the pattern was the opposite. In the *Zostera* bed, *H. ulvae* density remained almost constant throughout the study period (Fig. 4).

At both experimental areas, the number of large individuals (> 1.5 mm MW) in artificial seagrass and control plots was similar in January and March, with relatively larger numbers of adults appearing in artificial seagrass plots by July and October (Fig. 5). In the eutrophic site, in July were observed statistical differences in terms of adults density between the artificial plot and the control (t-test, $t_4 = 3.52$, $P < 0.05$). The relatively slow emergence of differences between artificial and control plots is explained by growth rates of *Hydrobia* at these sites (see below).

At the Arm site, in October, large snails were reduced dramatically when the plot suffered mechanical disturbance thought to be due to a high discharge.

Multivariate analysis of the macrofaunal assemblages

Ordination plots (MDS) for each sampling occasion show that neither the Gala nor the Arm artificial seagrass plots samples ever overlap with samples from the natural seagrass bed (Fig. 6).

A

B

C

D
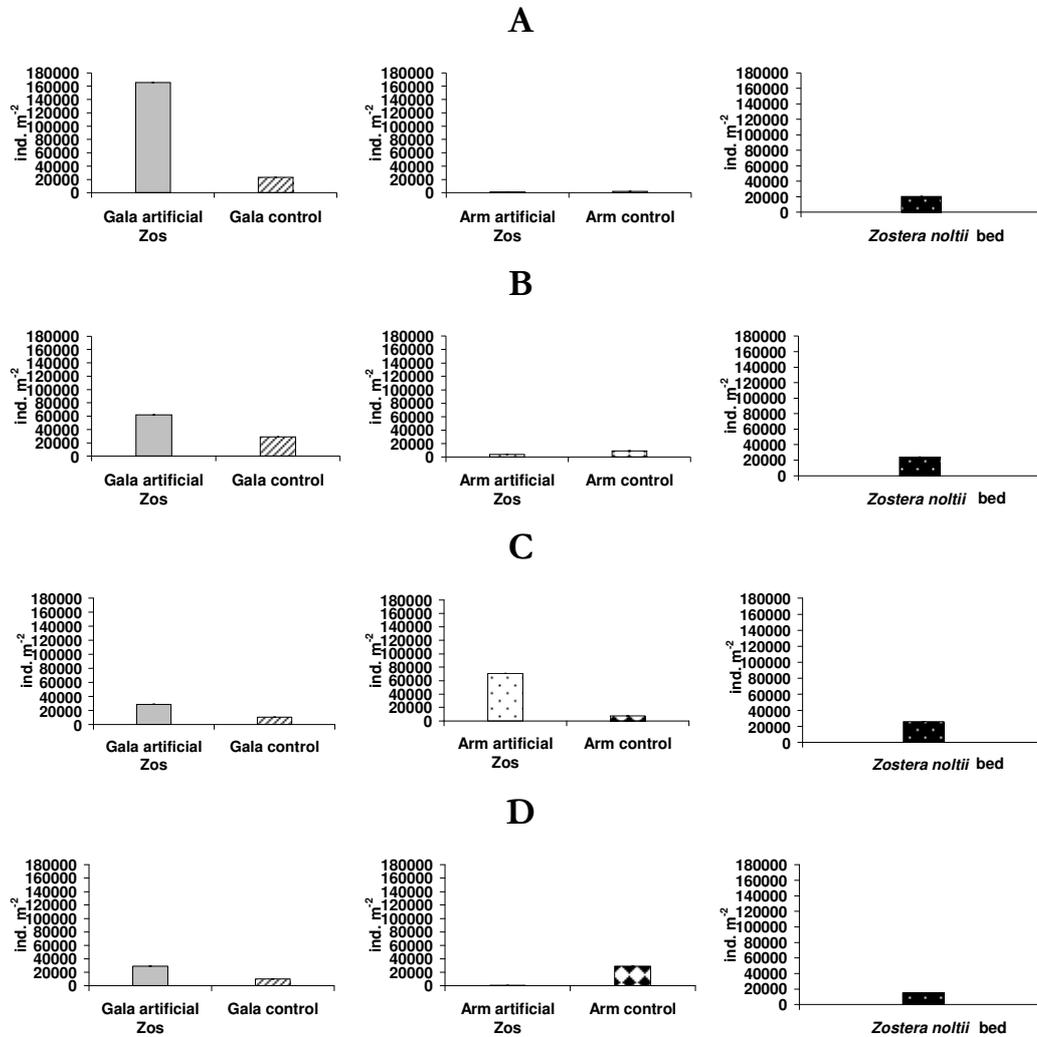
Fig. 4 - Average *Hydrobia ulvae* density ± standard error (SE) in the sampling areas. (A) – January, (B) – March, (C) – July, (D) – October.

In addition, the seagrass mimic samples were always separate from their matched control samples (Fig. 6), indicating different assemblage structures due to the primary treatment.

A

B

C

D
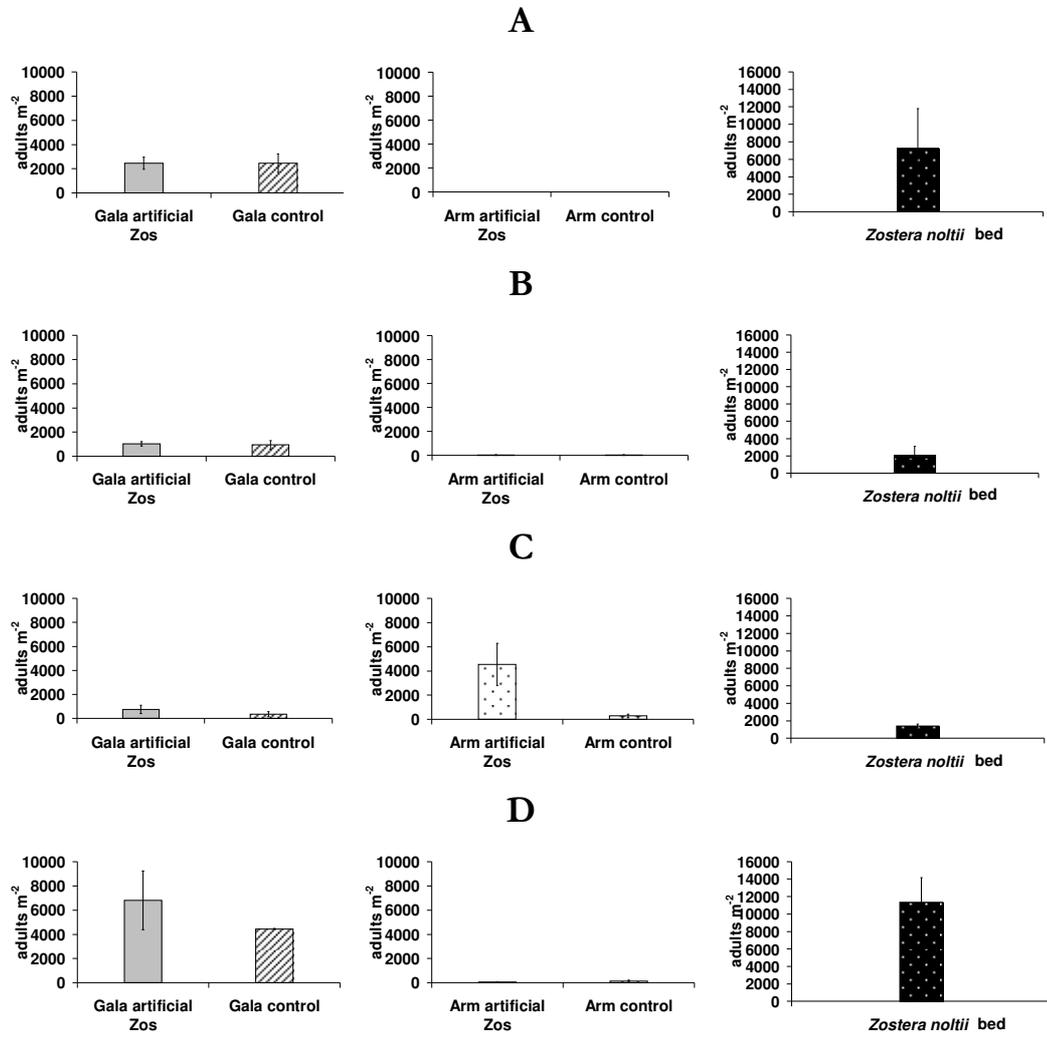
Fig. 5 - Average *Hydrobia ulvae* adults' density ± standard error (SE) in the sampling areas. (A) – January, (B) – March, (C) – July, (D) – October.
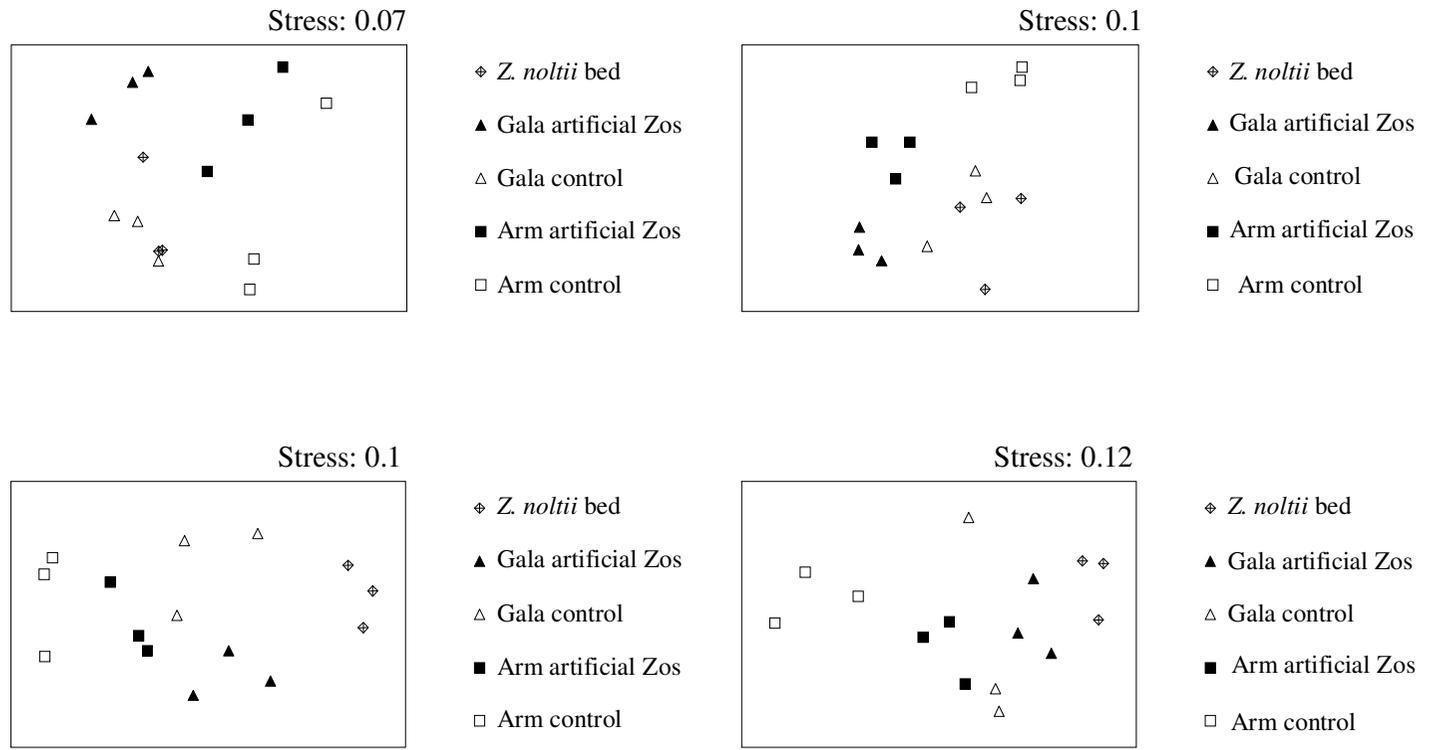
Fig. 6 - Two-dimensional MDS ordination plots of macrobenthic communities. (A) – January, (B) – March, (C) – July, (D) – October.

Inspection of the original Bray-Curtis similarity matrix shows that, on average, the artificial seagrass assemblages at the Gala site became more similar to the natural seagrass bed over time, increasing from 42 % similarity to 65 % similarity (Fig. 7). However, the control often showed even higher similarity. In addition, the artificial seagrass assemblage in the Arm area was always more similar than its control to the natural seagrass assemblage (Fig. 7).
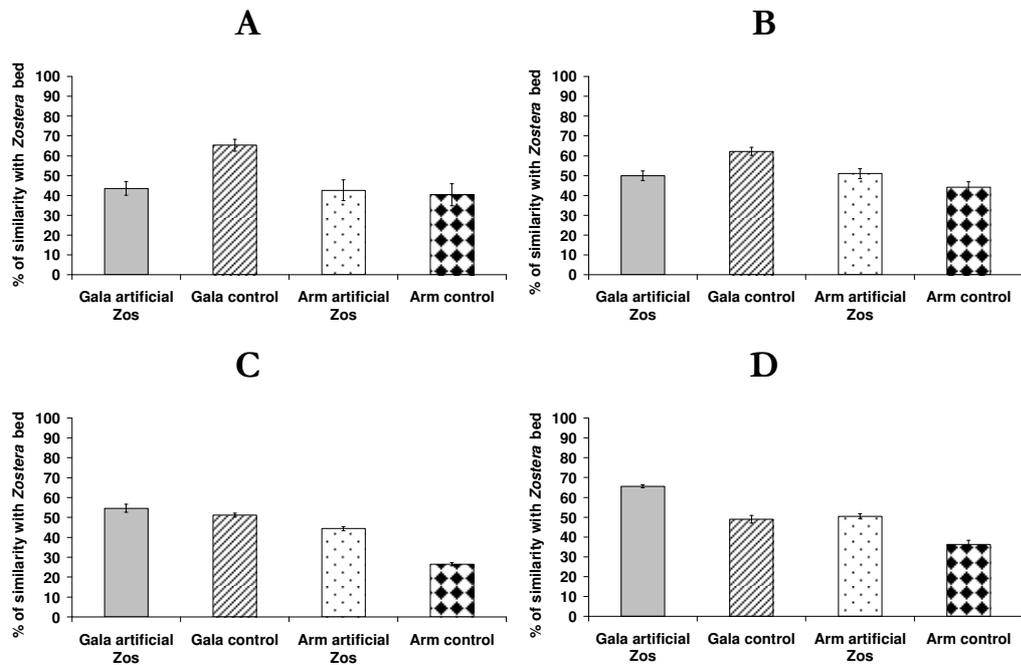
Fig. 7 - Similarities between experimental plots macrobenthic communities and *Zostera noltii* one. (A) – January, (B) – March, (C) – July, (D) – October.

DISCUSSION

Carrying out experiments at larger spatial scales often requires a trade-off with plot replication (Raffaelli & Moller 2000), reducing statistical power or as in the present case, obviating inferential statistics such as ANOVA. The persuasiveness of such experiments rests in their more realistic spatial extents (our experiments

had plots 20 times larger than those used in similar studies, Lee *et al.* 2001) and in their effect sizes (magnitude of difference between control and treatment plots).

In the present experiment, the seagrass mimics clearly changed the local sediment conditions within the experimental plots: sediment accumulated and organic matter content increased, as predicted and reflecting changes in near-bed hydrography (Orth *et al.* 1991). These results confirm the notion that seagrasses canopies influences water current flow, attenuating waves and dissipating turbulence, thereby reducing sediment ressuspension and increasing the retention of sediments within the meadows, and hence detritus accumulation rate (Duarte 2000).

Despite large-scale changes in the physical environment, there was little evidence of convergence of the invertebrate assemblages within treatment plots towards that normally recorded from natural seagrass bed at this site. There may be several reasons for this. First, not enough time had elapsed to allow recruitment, growth and relative abundance shifts in invertebrate populations. Whilst any effects on the population structure of *Hydrobia* would seem to take many months to develop (see below), insufficient time does not seem a likely explanation for the absence of typical seagrass taxa in seagrass mimic plots. An alternative explanation is that the general environmental differences, due to eutrophication, between the three main sites – seagrass bed, Gala and Arm, remain sufficiently great to permit the re-establishment of a natural seagrass assemblage if only habitat structure is restored.

Analysing the *Hydrobia ulvae* density pattern in the intermediate area (Gala), we could see that this small gastropod was always more abundant in Gala artificial Zos than in Gala control. The most probable explanation for this pattern is related to the occurrence of higher biomasses of green macroalgae in that plot. *H. ulvae* is an opportunistic species, constantly moving to fresh patches of food (Norkko & Bonsdorff 1996, Norkko *et al.* 2000, Cardoso *et al.* 2002). In January, the mean population density was much higher than in the others campaigns, which could be due to the combined effects of stronger benthic recruitments in this area and dispersion of juveniles proceeding from the *Z. noltii* meadows.

In the eutrophic area (Arm), the density pattern was completely different. The density tended to increase in the artificial seagrass plot till July but only in that time

we could observe a relevant difference between the artificial plot and the control. We can admit that only at that time the amount of algae and small epiphytes accumulated in the artificial plot was quite enough to attract *Hydrobia* individuals. In October, this pattern suffered modifications, since most of the individuals of the artificial plot disappeared. We suppose that this could be due to a strong discharge occurred in that area or due to human intervention, since the artificial seagrass shoots were removed and the sediment was very heterogeneous (personal observations).

There were no obvious effects of seagrass mimics on *Hydrobia* size within the first months of the experiment, but a greater number of larger individuals were recorded after about 9 months. We believe that this is explained by the growth rate of individual *Hydrobia* over the experimental period. Previous works at this estuary (Lillebø *et al.* 1999, Cardoso *et al.* 2002) have shown that the small *Hydrobia* present in plots at the start of the experiment (September 2003) would require at least 9 months to grow to the adult body size (> 1.5 mm width). We would not, therefore expect to see significant treatment effects till July 2004.

In the *Zostera noltii* bed, the adults' density was always higher than in the other treatments (even more than in the artificial plots). This could be explained by the food supply that natural seagrasses offer, which could be better than in the artificial seagrasses. According to Pinckney & Micheli (1998) the epiphytes biomass is greater on live than mimic blades and also the microalgae composition seem to be different. Considering that *Hydrobia ulvae* is a surface-deposit feeder, this could be very important for its growth.

Our experiment provides limited but compelling evidence that large, adult *Hydrobia* are not regularly found in the Gala and Arm areas of the estuary because of the lack of habitat structure provided by macrophytes, such as seagrasses. In the absence of such structure, large individuals are removed from these sites possibly due to their greater risk of dislodgment by water movement, but more likely to predation by epibenthic crustaceans, such as crabs and shorebirds.

The experimental plot sizes used in the present experiment are 20 times larger than those used in the nearest comparable study by Lee *et al.* (2001). Our plot sizes are at the scale of naturally occurring seagrass patches (personal observation) and thus more closely represent the appropriate scale at which to carry out such

experiments. Despite the large number of questions with no definitive answer in the present study, it remains important to attempt manipulative experiments at realistic spatial (and temporal) scales in other areas worldwide (including other scenarios) to address the environmental changes that are predictable in a near future (continuous loss of nursery grounds, loss of seagrass beds, increase of eutrophication, etc).

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CHAPTER 6

LONG-TERM CHANGES WITHIN MACROBENTHIC ASSEMBLAGES OF A TEMPERATE ESTUARY EXPERIENCING EUTROPHICATION

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Long-term changes within macrobenthic assemblages of a temperate estuary experiencing eutrophication

ABSTRACT: Over the last several decades, the ecology of the Mondego estuary (Portugal) has changed dramatically due to eutrophication. The deterioration of the estuary was recognised and management plans were implemented in the late 1990´s which aimed to restore the original natural seagrass (*Zostera noltii*) community that had shown progressive decline. This paper evaluates the success of that restoration project, by comparing the seagrass cover extent and the dynamics of the macrobenthic assemblages over that time period (before and after the management measures were implemented) and along the eutrophication gradient. Three different methodologies were used (univariate methods, MDS ordination method and PRC analysis) to infer which might be the most useful in monitoring and understanding the restoration process.

In the pre-mitigation period, two different scenarios were detected along the eutrophication gradient. In the *Z. noltii* bed (least affected by eutrophication), there was a general tendency to an increase in total density and biomass of the macrobenthic assemblage (excluding *Hydrobia ulvae*), while in the eutrophic area the reverse pattern was observed. In addition, a decline in species diversity was observed along the eutrophication gradient and through time. After the implementation of the management plan, opportunistic blooms of green macroalgae were strongly reduced, and the seagrass bed started to recover. Also, the total biomass of the community increased markedly, especially for the bivalve *Scrobicularia plana* and the polychaete *Hediste diversicolor*. Biomass recovery was stronger and faster than that of density. The recovery of species diversity followed but was interrupted by an additional (not related to eutrophication) external disturbance, heavy flooding.

In conclusion, the restoration plan implemented in the estuary seems to have been successful, both seagrass bed and macrobenthic assemblages responding positively to the management measures. With respect to the statistical tools used to explore these trends, we conclude that PRC analysis is the most suitable for evaluating ecosystem integrity changes over time.

INTRODUCTION

Human development and disturbance, coupled to population growth in watershed areas, underlie many of the ecological problems occurring in estuarine environments (Kennish 2000). Eutrophication of coastal waters as a result of man´s activities is now widely recognized as a major, worldwide pollution threat (Raffaelli *et al.* 1998, Jonge 2000, Jonge *et al.* 2000). As a consequence of this enrichment, a progressive replacement of seagrasses and slower-growing macroalgae by fast-growing macroalgae that bloom opportunistically and phytoplankton has been observed and documented for many areas worldwide (reviewed in Schramm & Nienhuis 1996). In the Mondego estuary, Portugal, the seagrass (*Zostera noltii*) bed has almost disappeared, reducing in extent from 15 ha in the early 1980´s to 0.02 ha in the mid-1990´s (Cardoso *et al.* 2004, *in press*, Verdelhos *et al. in press*). In the face of these widespread losses of production and the conservation status of the estuary (Dolbeth *et al.* 2003), large-scale ecosystem restoration became increasingly urgent. The restoration of seagrass populations is considered an important issue since they form a characteristic natural component of many ecosystems and contribute to ecosystem functioning of estuaries (Clark 1996, Jonge *et al.* 2000, Duarte 2002). The success of a restoration project depends on several key processes (see Hobbs & Norton 1996), including a deep knowledge about the physical and biological structure of the systems in which the management measures are implemented. The Mondego estuary is fortunate in this respect, there being a great deal of such data available (Pardal *et al.* 2000, 2004, Dolbeth *et al.* 2003, Cardoso *et al.* 2004, *in press*, Verdelhos *et al. in press*, and references therein).

In the present paper we evaluate the success of a restoration project implemented in the Mondego. Specifically, we compare the dynamics of the macrobenthic assemblages along the eutrophication gradient before and after the implementation of the management measures, over a 10-year period. To explore the ecological patterns and trends in these data, and to assess the performance of the mitigation plan, we will use three distinct approaches: univariate measures, MDS ordination methods and PRC analysis and evaluate which is the most efficient to measure and understand restoration.

MATERIALS AND METHODS

Study site

The Mondego estuary, located on the Atlantic coast of Portugal (40º 08 N, 8º 50 W) comprises two distinct arms, northern and southern, separated by the Murraceira island (Fig. 1). During the last two decades, a drastic reduction of the seagrass (*Zostera noltii*) bed has occurred, both in areal extent and biomass, in the south arm (Cardoso *et al.* 2004, *in press*) due to an excessive nutrient inputs into the estuary. However, since 1998, as a result of the implementation of mitigation measures (Cardoso *et al. in press*, Verdelhos *et al. in press*), a progressive recovery of the seagrass bed has been observed.

1 - *Zostera noltii* bed
2 - Intermediate area
3 - Eutrophic area
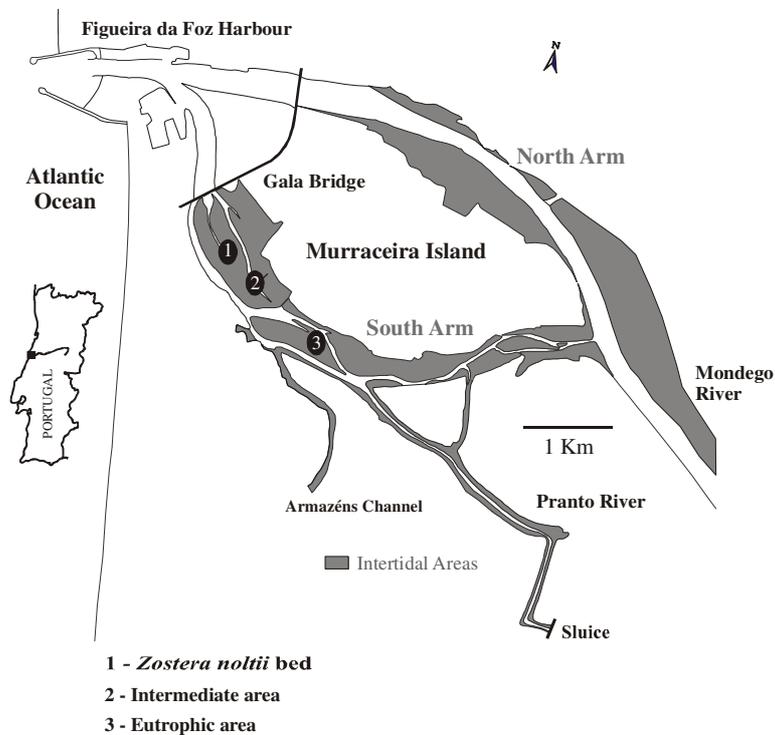
Fig. 1 - Location of the Mondego estuary and sampling stations.

Three distinct areas can be recognised in the southern arm: an undisturbed *Zostera noltii* bed towards the marine end of the estuary, a eutrophic area further upstream and an intermediate area located between these two. The intermediate area located just upstream of the *Z. noltii* bed has no seagrass cover although some rhizomes remain in the sediment. The eutrophic area located upstream comprises a sandy-muddy sediment, which in the early 1980´s was covered by rooted macrophytes, but as eutrophication increased, *Zostera noltii* declined progressively. This area is now covered seasonally by green macroalgae (*Ulva* sp.) (Cardoso *et al.* 2002, 2004, Ferreira *et al.* 2004, Pardal *et al.* 2004). For a detailed description of these areas see Pardal *et al.* (2000), Cardoso *et al.* (2002, 2004, *in press*) or Marques *et al.* (2003).

Field programme and laboratory procedures

The macrobenthic assemblages were monitored from January 1993 to September 1995 and again from February 1999 to December 2002. Samples were collected fortnightly in the first 18 months and monthly during the rest of the study period. On each sampling occasion, 6 to 10 cores (13.5 cm diameter) were taken to a depth of 20 cm. Samples were washed in estuarine water through a 500 µm mesh at each sampling station and the fauna retained preserved in 4 % buffered formalin. Later, animals were separated and transferred to 70 % ethanol, identified to the lowest possible taxon and counted. Seagrass and macroalgal biomasses were determined as ash free dry weight (AFDW) after oven drying at 60 ºC for 72 h and combustion at 450 ºC for 8 h. The same procedure was used to quantify the organic matter content of the sediment by loss-on-ignition weight difference.

Data analysis
Univariate measures

The diversity of the macrobenthic assemblages in the three areas was assessed as species richness (simple count of number of taxa recognised), and the following indices: the Shannon-Wienner (log base 2), Simpson's D and Pielou's evenness measure (Krebs 1999).

Multivariate approaches: Principal Response Curves (PRC) and Multi Dimensional Scaling (MDS)

The spatial and temporal dynamics of macrobenthic assemblages along the eutrophication gradient were analysed mainly by the PRC method. This method is based on the redundancy analysis ordination technique, the constrained form of Principal Component Analysis, a full account of which can be found in Van den Brink & Ter Braak (1999), Cuppen *et al.* (2000), Frampton *et al.* (2000a, b, 2001) and Van den Brink *et al.* (2000). The PRC method is a multivariate technique especially designed for data analysis from microcosm and mesocosm experiments. It is a relatively new method with only a few previous applications in community ecology, although it shows potential for the evaluation of the ecosystem integrity (Pardal *et al.* 2004). The method computes differences in species composition between "treatments" (sites, in the present study) at each time point, similar to other ordination techniques. However, the advantage of this particular method is that any temporal changes in the "control" (the reference seagrass site in the present study), are constrained in the plot to a horizontal line, so that deviations from the control/undisturbed condition are more readily appreciated by managers.A full description of the method is provided by Van den Brink & Ter Braak (1999) and Pardal *et al.* (2004).

In the present study, "treatments" correspond to the different macrobenthic communities under different degrees of organic pollution stress. As reference (control) we considered the *Zostera noltii* meadows in 1993. PRC analysis was performed using the CANOCO software package, version 4 (Ter Braak & Similaer 1998). The significance of the PRC diagram was tested using a Monte Carlo permutation, by permuting the whole time series in the partial RDA from which the PRC analysis is obtained, using an F-type test statistic based on the eigenvalue of the first canonical axis (Van den Brink & Ter Braak 1999).

The faunal samples were also analysed using a more traditional technique, the non-metric Multi Dimensional Scaling (MDS) ordination method described by Clarke & Gorley (2001) and Clarke & Warwick (2001). Numbers of individuals for each species were square root transformed prior to analysis in order to scale down effects on the ordination of highly abundant species (Clarke & Warwick 2001).
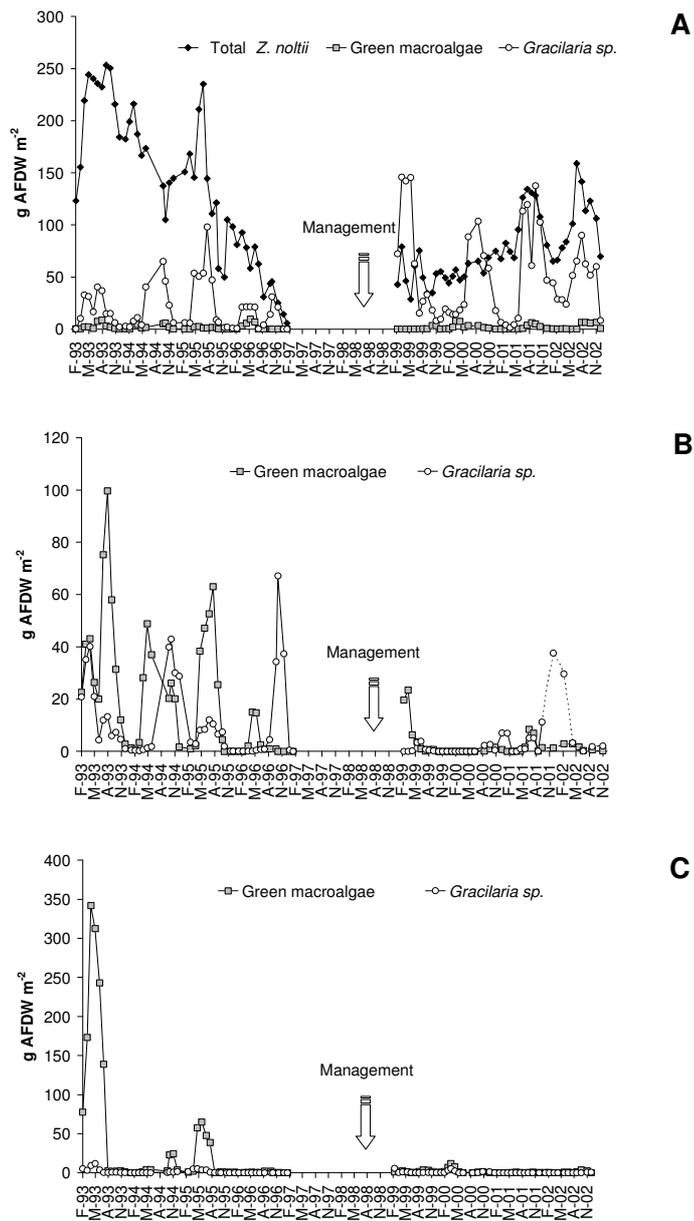
Fig. 2 - Variation of seagrass and macroalgal biomass from 1993 to 2002. (A) – *Zostera noltii* bed; (B) – Intermediate area; (C) – Eutrophic area.

RESULTS

Seagrass and macroalgal biomasses

For seagrass biomass, significantly different scenarios were observed between the pre- and post-mitigation periods (Fig. 2A) (Wilcoxon two-sample test, W = 4350, P < 0.05). From 1993 to 1997, the total biomass of *Zostera noltii*, declined sharply, but after the introduction of mitigation measures in 1998 there was a gradual recovery (Fig. 2A). Small biomasses of green macroalgae were recorded throughout the period, whilst *Gracilaria* sp. increased.

In the intermediate area there was a considerable decline of macroalgal biomass throughout the 10-year period (Fig. 2B). In the eutrophic area, there were significant differences in green macroalgal biomass between both periods (Wilcoxon two-sample test, W = 3824.5, P < 0.05). In 1993 and 1995 algal blooms were common at the eutrophic site but were never present following the implementation of mitigation measures (Fig. 2C).

Macrobenthic assemblage dynamics

Changes in density and biomass: The small gastropod *Hydrobia ulvae* dominated all the samples (Figs. 3A-C), and its inclusion in the analyses masks more interesting trends for other taxa. It was, therefore, decided to omit this species from this analysis.

In the pre-mitigation period (Figs. 3D-F), two different scenarios are apparent along the eutrophication gradient. In the *Zostera noltii* bed, there was a general increase in macrobenthic density and biomass that was more pronounced in 1995 (Fig. 3D), possibly related to the large algal biomass also present at this period. In contrast, in the eutrophic area, density and biomass declined overall despite sporadic increases in 94 and 95 (Fig. 3F). Not surprisingly, the intermediate area displayed intermediate trends and patterns (Fig. 3E).

In the post-mitigation period, recovery of biomass was higher and faster than the recovery of density (Figs. 3D-F), possibly because many larger species could now attain adult body size.
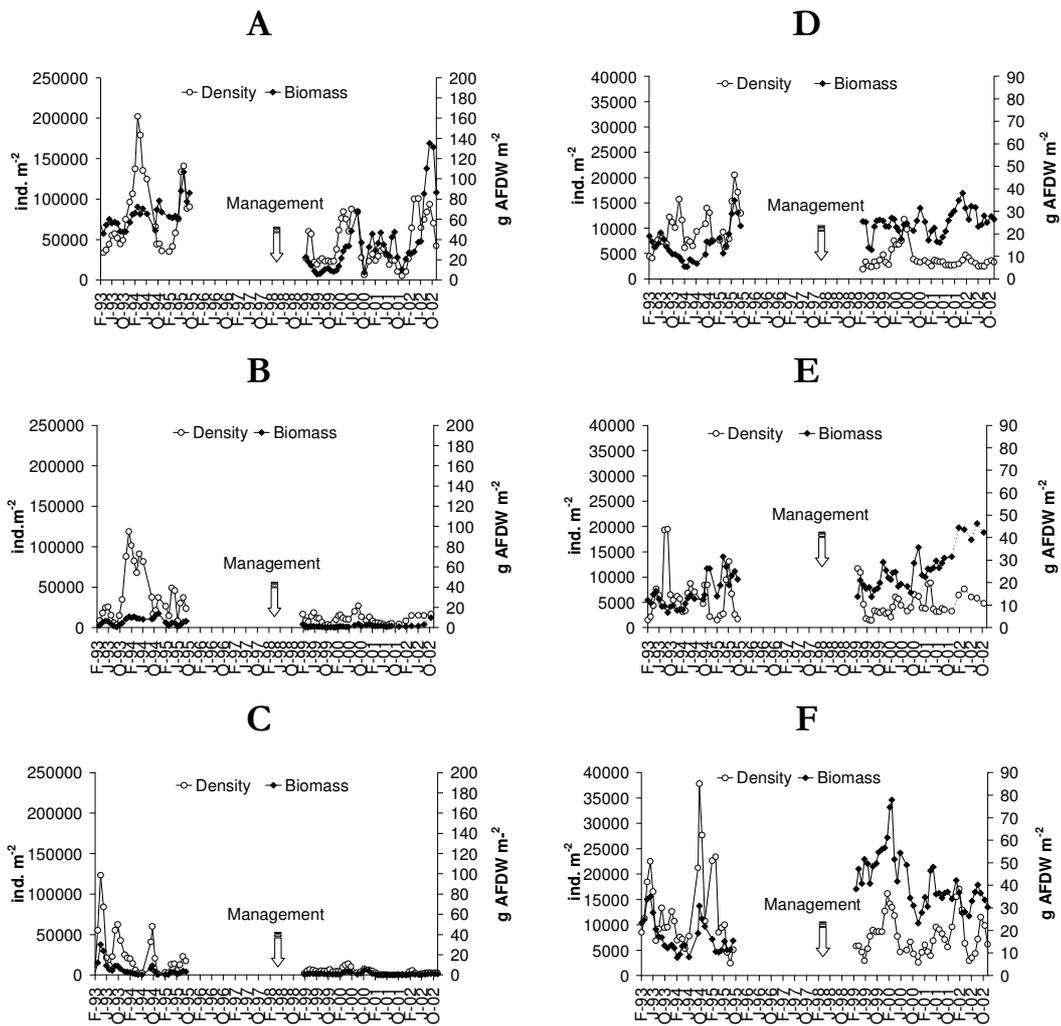
Fig. 3 - Variation of density and biomass of *Hydrobia ulvae* and of the macrobenthic assemblage (without *Hydrobia ulvae*) from 1993 to 2002. (A) – *Hydrobia Zostera noltii* bed; (B) – *Hydrobia* intermediate area; (C) – *Hydrobia* eutrophic area; (D) – assemblage *Zostera* bed; (E) – assemblage intermediate area; (F) – assemblage eutrophic area.
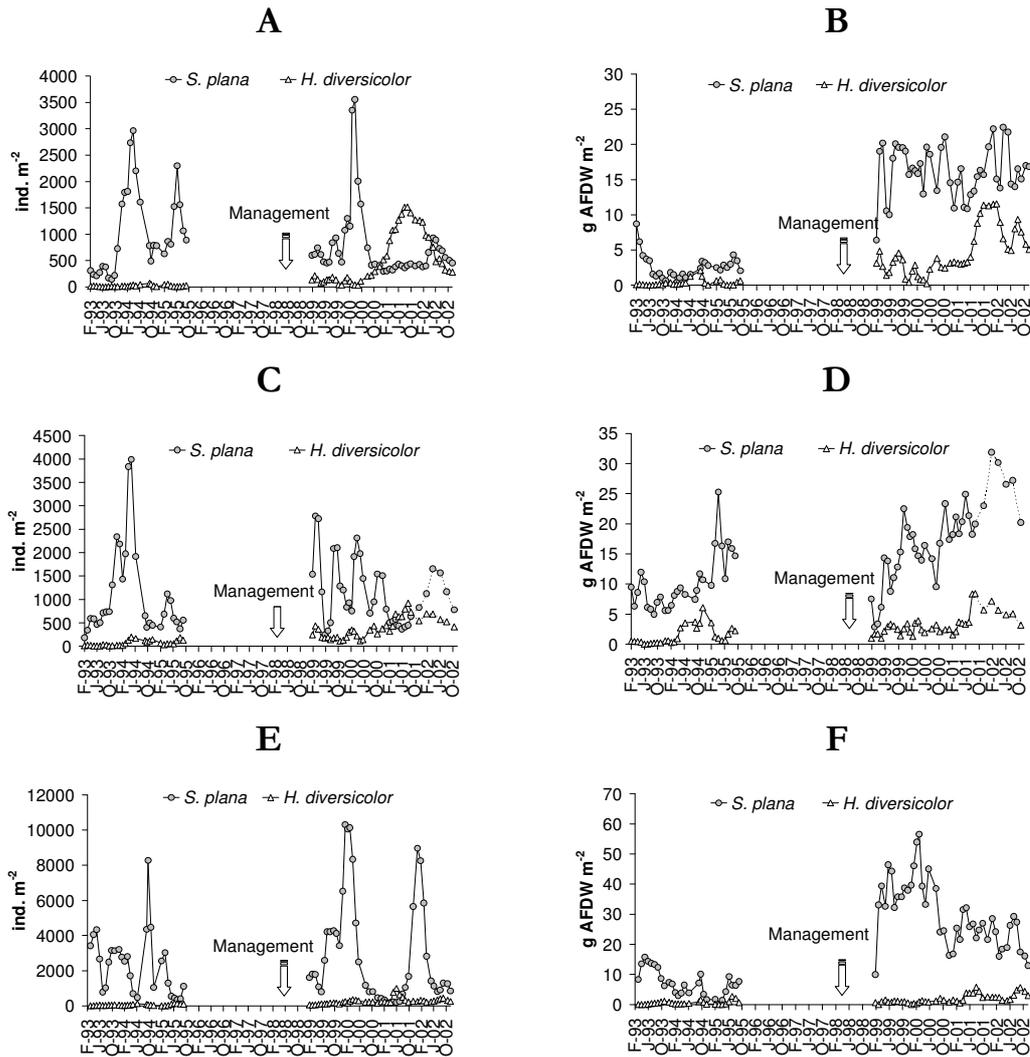
Fig. 4 - Variation of density and biomass of two main species (*S. plana*, *H. diversicolor*) from 1993 to 2002. (A) – Density of the 2 species in the *Z. noltii* bed; (B) - Biomass of the 2 species in the *Z. noltii* bed; (C) - Density of the 2 species in the Intermediate area; (D) - Biomass of the 2 species in the Intermediate area; (E) - Density of the 2 species in the Eutrophic area; (F) - Biomass of the 2 species in the Eutrophic area.

Larger body-sized species, such as the bivalve, *Scrobicularia plana* and the polychaete, *Hediste diversicolor* dominated the biomass pattern of this community following the implementation of mitigation measures (Figs. 4B, D, F). These patterns were not so evident for density (Figs. 4A, C, E).

Changes in univariate metrics: Throughout the period 1993-2002 distinct changes in the structure of the macrobenthic communities were observed. Species richness trends were significantly different before and after implementation of the management plan for the three sites (Kruskal-Wallis test, H = 117.67, P < 0.05) (Fig. 5), with more species present before compared to after and the seagrass bed always having more species than the intermediate and the eutrophic areas.
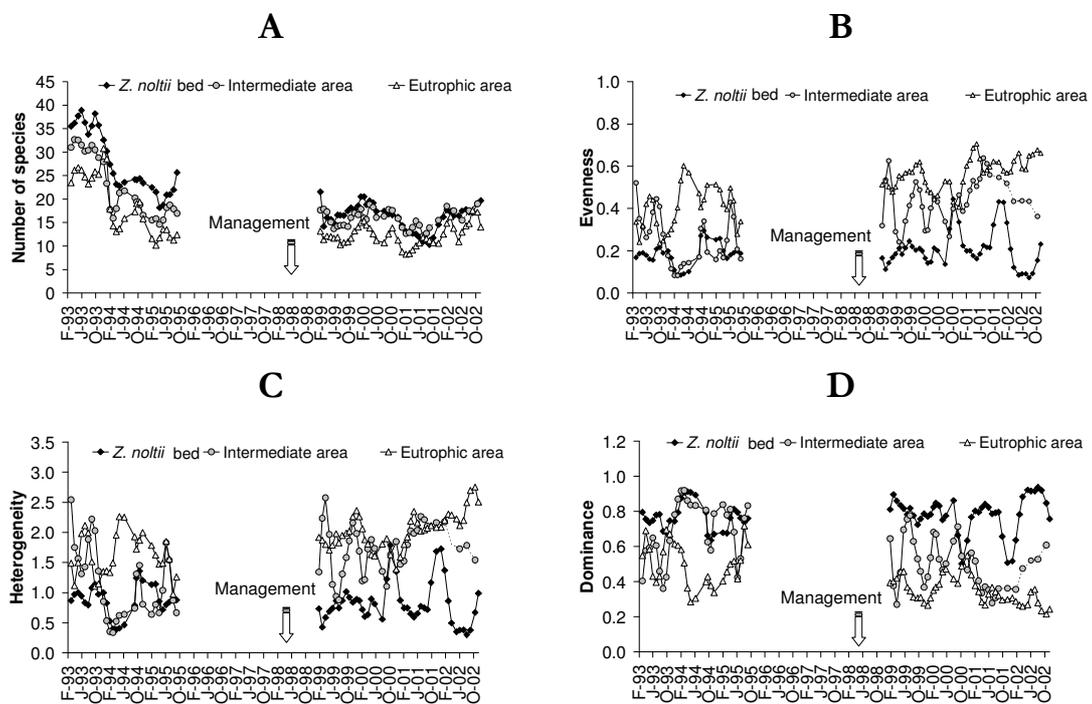
Fig. 5 - Variation of the biological indices in the three sampling stations from 1993 to 2002. (A) – Number of species; (B) – Evenness; (C) – Shannon-Wienner index; (D) – Simpson index.

Richness declined during the 3 years for all the three stations (Fig. 5).

Following introduction of the management plan in 1998, species richness of the intermediate area and *Z. noltii* bed converged in value, increased at the start of 2000, but declined during the winter of 00/01, due to disturbance from huge floods in the system (Cardoso *et al. in press*), only starting to recover in 2002 (Fig. 5A).

Evenness was lower in the *Z. noltii* bed due to the dominance of *Hydrobia ulvae* at this site, and higher in the intermediate and eutrophic areas (Fig. 5B). Evenness increased throughout the 10-year period for the intermediate and eutrophic areas, with the seagrass bed showing the most stable pattern over time.

Diversity as measure by the Shannon-Wienner index followed a pattern very similar to that of evenness, with Simpson's D (dominance) showing the opposite, as expected (Figs. 5C, D). For the intermediate and eutrophic areas dominance tended to decrease throughout the 10-year period.

PRC analysis: The PRC analysis showed a clear spatial gradient related to eutrophication, where the declining *Z. noltii* bed was closer to the 1993 *Z. noltii* reference, followed by the intermediate area and finally the eutrophic area (Fig. 6). However, in the post-mitigation period the seagrass bed and the intermediate area converged, with the macrobenthic assemblages deviated further from the reference in the pre-mitigation period (Fig. 6). The tentative recovery of the macrobenthos noted above with respect to univariate measures is also reflected in the PRC analysis. The macrobenthic assemblages started to recover at the beginning of 2000, but during the winter of 2000/2001 the assemblages suffered disturbance from floods (Cardoso *et al. in press*) and a further recovery was only apparent in 2002.

In the PRC analysis, sampling date (time) accounted for 26.3 % of the total variance within the data set, with 65.3 % explained by the eutrophication gradient (time * site interaction). 8.4 % of the total variance can be attributed to the differences between the sample replicates. Monte Carlo permutation tests revealed that the differences between the treatments and the control were statistically significant, with the PRC diagram explaining 43.8 % of the variance in treatment effects. The taxa contributing most to these effects were the polychaete *Chaetozone*

setosa and the oligochaete family Tubificidae. Both had high positive weights in the analysis, indicating a reduced abundance compared to the reference site. In contrast, the polychaete *Alkmaria romijni* had the highest negative weight (indicating an increase in abundance) (Fig. 5), consistent with the premise that small deposit-feeding polychaetes increase in eutrophic conditions (Pearson & Rosenberg 1978).

NM-MDS analysis: The macrobenthic assemblages of the three study areas occupy different regions of the MDS plot, with the *Z. noltii* samples separated from those in the eutrophic area by samples from the intermediate area (Fig. 7). Closer inspection reveals that 1993 samples from the *Z. noltii* bed are separated from those of the subsequent years. On the other hand, the communities of the seagrass bed and intermediate area from 1999 to 2001 are closer than in the beginning of the study period, indicating a higher faunal similarity between them at this time.
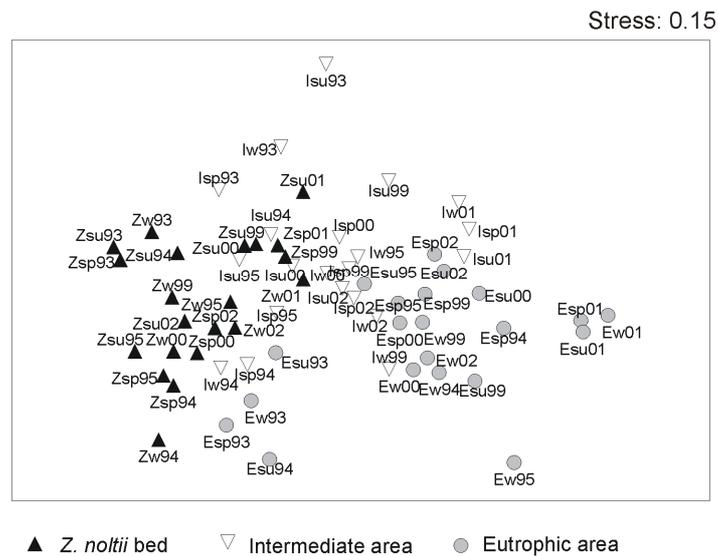
Fig. 7 - Two-dimensional MDS ordination plot of macrobenthic communities. (Z) - *Z. noltii* beds; (I) - Intermediate area; (E) - Most eutrophic area; (w) - winter; (sp) - spring; (su) - summer.

Canonical coefficient

F-93 J-93 S-93 J-94 M-94 S-94 J-95 M-95 S-95 J-96 M-96 S-96 J-97 M-97 S-97 J-98 M-98 S-98 J-99 M-99 A-99 D-99 A-00 A-00 D-00 A-01 A-01 D-01 A-02 A-02 D-02

0
-0.5
-1
-1.5
-2
-2.5
-3
-3.5

—■— Reference/Undisturbed Area
—▲— Intermediate Area
—○— Declining *Zostera noltii* bed
—□— Most Eutrophic/Disturbed Area

Species weight

3.0
2.5
2.0
1.5
1.0
0.5
0.0
-0.5
-1.0
-1.5
-2.0
-2.5
-3.0

C. setosa
Tubificidae
A. valida
M. palmata
I. chelipes
L. impatiens
C. maenas
H. hydatilis
L. litorea
M. barbatus
G. convoluta
E. flava
C. edule
H. filiformis / L. saxatilis
L. koreni
H. ulvae
Oligochaeta
M. picta
G. umbilicalis / A. mucosa
L. diptera / S. perarmata
P. elegans / C. collaris
S. hookeri
L. cinereus
Nemertinea
P. synophtalmica / D. neapolitana
E. marinus
S. decoratus
M. coralina
M. palmata / C. multisetosum
N. hombergi
S. shrubsoli
Tetrastema
C. capitata
P. ligni
C. crangon
M. galoprovinciallis
L. diptera
S. plana
H. diversicolor
C. carinata
A. romijni

Fig. 6 - Principal Response Curves (PRC) diagram showing the response of macrobenthic assemblages to different degrees of organic pollution/disturbance with species weights indicating the relative contribution of individuals' species to the assemblage response.

Table 1 – Statistical results of the ANOSIM test applied to the MDS analysis.

	R	P
1993*1994	0.282	0.004
1993*1995	0.192	0.028
1993*1999	0.539	0.001
1993*2000	0.478	0.001
1993*2001	0.818	0.001
1993*2002	0.499	0.002
1994*1999	0.286	0.011
1994*2000	0.193	0.044
1994*2001	0.45	0.001
1994*2002	0.17	0.05
1995*1999	0.168	0.035
1995*2001	0.389	0.002
1995*2002	0.162	0.044
1999*2001	0.37	0.004
2000*2001	0.424	0.003
2001*2002	0.348	0.004

Samples from the intermediate and eutrophic areas showed the greatest scatter in the MDS plot, indicating a greater heterogeneity in time and space, in turn probably a greater instability in those areas.

Significant differences between the three study sites were explored by ANOSIM. The *Z. noltii* bed samples were significantly different from those in the intermediate area (R = 0.332, P = 0.001) and from those in the eutrophic area (R = 0.677, P = 0.001). There were also significant differences between years (Table 1).

DISCUSSION

The management program drawn up for the Mondego estuary, in 1998, included the controlled use of fertilisers in the catchment and the opening of the upstream connection between both arms to improve circulation and flows. This, associated with better management of sluice openings which control the flow of water draining the agricultural hinterland, has led to marked changes in physico-chemical aspects of the estuary, specifically lower turbidity, lower suspended particulate organic matter and lower re-mineralization (Lillebø *et al.* 2004). These

features connected to an increase in freshwater circulation, favoured nutrients dilution and decreased residence time (from 5-7 days to just 1 day), contributing to the improvement of water quality (Cardoso *et al. in press*, Verdelhos *et al. in press*). Our analyses indicate that these environmental changes are reflected in the biology and ecology of the estuary to varying degrees.

Overall, the temporal and spatial trends and patterns apparent in our data are consistent with such trends and patterns reported elsewhere (Pearson & Rosenberg 1978). For instance, over the period leading up to the introduction of the management plan, the area least affected by eutrophication, the seagrass bed, saw an increase in opportunistic taxa reaching very high densities and achieving high production rates (Valiela 1995), such as small deposit feeding polychaetes (Cardoso et al 2004), whilst in the worst affected area, the eutrophic area, enrichment, especially from algal blooms, led to a marked decline in biomass and density of all macrobenthic species. In contrast, the recovery phase, post-mitigation, saw an increase in longer-lived, large bodied taxa which contribute significantly to biomass, such as the bivalve *S. plana* and the polychaete *H. diversicolor*. Verdelhos *et al.* (*in press*) has shown that *S. plana* beneficiated greatly from the implementation of the management measures, the population became more structured and included individuals of all age classes, with higher biomass and growth production. The increase in such taxa during the recovery phase is attributed to increased survival of recruits through to large adult body sizes in the absence of severe disturbance.

In addition to changes in the relative abundance of individual taxa, consistent with expectations and experience elsewhere (Valiela 1995), community-level attributes also responded to the management initiatives. Thus, diversity in the affected areas started to increase following management, mainly through changes in evenness and its converse dominance (Fig. 5) although species richness also showed signs of recovery. Heavy flooding in the region during the recovery process had catastrophic effects on these assemblages, effectively re-setting the recovery clock. Nevertheless, the trends in the dynamics of the macrobenthos indicate that many of the deteriorating ecological features of the pre-management period can be successfully reversed.

A possible exception to this is the seagrass bed, specifically the dominant structuring species *Z. noltii*. Whilst there is some evidence from our data of a

progressive recovery of *Z. noltii*, which attained biomass values in 2002 close to those recorded in 1993, the Mondego has a long way to go to full recovery in this respect: seagrasses are large and slow-growing plants and recovery can take much more than a decade to enlarge 95 % of the patch area (Duarte 1995). In addition, the sedimentary changes that followed the loss of seagrass in the intermediate and eutrophic areas many years ago, may be difficult to reverse without mechanical intervention and re-colonisation of these areas will take a long time, if at all (Cardoso *et al.* 2004).

We used several techniques to explore trends and patterns in the dynamics of the macrobenthic assemblages of the Mondego. We found the PRC method an excellent technique to assess the response of macrobenthic communities to a stress factor through time. Comparing this method with the nm-MDS and the univariate analyses, the PRC seems to have several advantages. Whilst all the methods can reveal differences between the three study areas, the PRC approach compacts the complexity of time-dependent, community-level effects of stress into a simple diagram, the elegance of which lies partly in the fact that PRC represents the time trajectory for the control as a horizontal line and because allows the interpretation of effects at the individuals species level (Pardal *et al.* 2004). Of course, it is sensible to include as many different kinds of approaches in such investigations, univariate as well as multi-variate, but the simplicity and accessibility of the outputs from PRC analysis has major advantages for communicating science to policy makers and managers.

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Final discussion

Coastal and estuarine environments are some of the most productive ecological systems on Earth and recognized to be of extremely high value to human society. However, these ecosystems are difficult to manage, involving a natural system that is ever changing and is inhabited and stressed by a growing and increasingly demanding human society. The pressures and damaging impacts of human expansion and inadequate ecological and socio-economic management are reflected in over-exploitation of fisheries resources, destruction of coastal ecosystems and deleterious effects of land-based contamination and eutrophication (Crooks & Turner 1999, McLusky & Elliott 2004).

Efforts to restore damaged coastal wetlands have been ongoing in the last 20 years (Hobbs & Norton 1996, Jonge 2000, Jonge *et al.* 2000, Katwijk *et al.* 2000, Kennish 2000, Jonge & Jong 2002, Kendrick *et al.* 2002, Peterson & Lipcius 2003). The purpose of wetlands restoration projects is to return a habitat from an altered or disturbed condition to a previously existing natural condition. The main goal of these projects is the replacement of the same type of habitat in an impacted area to compensate for adverse human effects (Kennish 2000). However, ecosystems are extremely difficult to restore in full, since they have so many components and support very complex interactions. Obviously, a full understanding of the cause-effect relationships among ecosystem components is needed (*e.g.* relationships between abiotic and biotic components and among species) and that understanding comes about only after long-term study and experimentation at multiple scales. Understanding the conditions and controlling variables at restoration sites, would allow prediction of the outcomes of restoration efforts. Unfortunately, most of the restoration projects are based on short-term and small-scale studies, which means that understanding is incomplete and restoration efforts are constrained by insufficient knowledge and by site-to-site variability. In the absence of sufficient long-term data on how restoration sites actually develop, there is little recognition of the complexity of processes that determine pathways and outcomes (Zedler 2001). In this respect, the Mondego estuary provides one of the few estuarine systems worldwide that has a considerable long-term database on its macrobenthic community' dynamics.

During the last two decades, the eutrophication of the Mondego estuary has led to a strong degradation of overall environmental quality (Cardoso *et al.* 2002, 2004a, Dolbeth *et al.* 2003, Marques *et al.* 2003, Ferreira *et al.* 2004, Lillebø *et al.* 2004, Pardal *et al.* 2004, Verdelhos *et al. in press*). The occurrence of seasonal green macroalgal blooms has promoted a rapid decline of the seagrass bed throughout the 1980-1990 period and there have been marked changes in the macroinvertebrate assemblages, mainly a progressive impoverishment of the most impacted inner area (Marques *et al.* 1997, Lillebø *et al.* 1999, Pardal *et al.* 2000, Martins *et al.* 2001). With respect to overall community, major changes were detected in species diversity and trophic level structure. In the pre-mitigation period (1993-1995), following the decline of the seagrass bed a gradual decline in species diversity was observed along the eutrophication gradient, together with considerable changes in the trophic structure, specifically a replacement of herbivores and surface-deposit feeders by sub-surface and suspension feeders in the *Zostera noltii* bed. The eutrophic area became a more impoverished site with a lower percentage of other trophic groups (*e.g.* carnivores, herbivores, omnivores) compared to the seagrass bed. These data are consistent with other general eutrophication scenarios, characterized by the replacement of macrophytic primary producers, declines in species diversity, increases in detritivores, declines in herbivores and large increases in small deposit-feeding polychaetes.

Within this overall picture of deteriorating environmental quality, the detailed responses of the macrobenthic species to macroalgae differed according to algal species and position along the eutrophication gradient. Thus, the eutrophic site had a more hostile redox environment than the seagrass bed, and it is not surprising that the effects of the algae were more marked in this most disturbed area, which is probably less resistant to additional impacts. Within each area, the responses of the macrobenthic organisms to algal mats were algal-species dependent, with some invertebrate populations being negatively affected and others being enhanced by the algae (Cardoso *et al.* 2004b). The effects of green macroalgae on the macrobenthic communities were much greater than those for red macroalgae, in such a way that in the presence of green algae, the community tended to be impoverished, dominated mainly by small size opportunistic species, while in the presence of red algae, the negative effects were generally less.

Given the importance of estuarine resources and the clear impacts of eutrophication, several mitigation measures were adopted, in order to restore the original seagrass bed (Cardoso *et al. in press*, Neto 2004, Verdelhos *et al. in press*). Since 1998, the controlled use of fertilisers, the opening of the upstream connection to improve water exchange between both arms (residence time declined from 5-7 days to just 1 day), reduction of the number of fishermen digging for bait and the improved management of sluice openings led to an improvement of the environmental quality of the estuary.

All of these actions promoted lower turbidity, a consequent increase in freshwater circulation that favoured nutrient dilution and decreased residence time, which improved the environment for seagrass bed and reduced the risk of macroalgal blooms. Parallel to the recovery of the original seagrass bed (in both spatial extent and biomass), several improvements were also observed in the invertebrate assemblages. For instance, a large increase in total biomass of the macrobenthic community, which could be an indicator of the improved conditions of the system. In the absence of macroalgal blooms and anoxic conditions, the organisms have more opportunity to grow to larger sizes, slower-growing species can be accommodated as can those with more complex morphology (Valiela 1995). In other words, k-selected species dominate to the detriment of r-selected species (Molles 1999). This probably explains a faster recovery of the biomass compared to density of invertebrates, such as the bivalve *Scrobicularia plana* and the polychaete *Hediste diversicolor*. Species diversity also increased, however, this recovery process was interrupted by the strong floods of 2000/2001, adding weight to concerns that multiple stressors (here, eutrophication and flood disturbance) operating concurrently or consecutively can act synergistically to reduce overall system resilience (Kennish *et al.* 2004).

Detailed studies of one of the most important and dominant species, *Hydrobia ulvae* in the seagrass bed, reflect these overall patterns of change (Chapter 4). Marked changes in the population structure, density and biomass, production of this species were recorded and attributed to excessive enrichment and replacement of macrophytic primary producers. Following the decline of *Zostera noltii*, the density and biomass of *H. ulvae* generally declined, in the case of biomass by an one order of magnitude. In the eutrophic area, the *H. ulvae* population, was

impoverished, characterized by small individuals, and this pattern became more evident through time (Cardoso *et al. in press*).

As the seagrass recovered progressively, there was some evidence of recovery of *H. ulvae*, both in density and biomass and also in population structure, with an increasing proportion of adults represented. In the eutrophic site, density and biomass remained lower in the post-mitigation period with no signs of recovery of either abundance or population structure.

These patterns of change are complex but consistent with the first successful steps of restoration of the *Z. noltii* bed.

Despite the clear signs of recovery of the estuary, the biology of the upstream eutrophic site did not return to its original state. This could be related to the dynamism of the intertidal flats of the Mondego, which manifest two alternate states and to the relative resilience of the areas. The shift from seagrass to coarse sediment and *vice-versa* is best described by hysteresis dynamics, where moving between the two alternate states requires a major perturbation (eutrophication or large-scale sediment stabilisation) (Scheffer & Carpenter 2003). So, even if eutrophication has lessened, the recovery of the eutrophic site to the original state would probably require further restoration, such as extensive physical engineering of the bed sediments.

One of the most dramatic differences in *Hydrobia ulvae* populations at the two sites is the absence of adults at the eutrophic site. This raises an important question as to the responsible mechanism. Through the implementation of a large-scale experiment, it was possible to test the hypothesis that the observed between-site differences were related to enhanced survival (due to protection from avian or fish predators) of snails to larger size classes in the more complex habitat provided by seagrass. This experiment concluded that large, adult *Hydrobia* are not regularly found in the eutrophic area of the estuary because of the lack of habitat structure provided by rooted macrophytes, such as seagrasses. In the absence of such structure, large individuals are removed from these sites possibly due to their greater risk of dislodgment by water movement, but more likely to predation by epibenthic crustaceans, such as crabs, and fish and shorebirds.

Another important species which makes a high contribution to the total biomass of the community, and which suffered a strong negative impact due to eutrophication is the bivalve *Srobicularia plana*. This species was negatively affected by the presence of macroalgal blooms in terms of abundance, biomass and population structure. Like *H. ulvae*, this species responded positively to the management measures, showing a higher abundance and biomass, with a significant increase in larger adult individuals, and hence a more structured and stable population (Verdelhos *et al. in press*).

In contrast to *H. ulvae* and *S. plana*, the isopod *Cyathura carinata* did not seem to be affected by eutrophication and consequent disappearance of *Z. noltii*. Even the mitigation measures applied did not seem to affect this species, since it prevails in an area characterized by bare sediments, and not yet restored (Ferreira *et al.* unpublished).

Restoration ecology is a young science and hence scientifically challenging, so that there is much to learn and develop. According to several experts on restoration there is a huge need for the implementation of methodologies to measure successful mitigation for many programmes of restoration (Chapman 1999, Chapman & Underwood 2000). In this research programme there was an opportunity to incorporate and evaluate a novel approach with high potential to detect environmental impacts, PRC analysis. This new approach was compared with traditional methods (*e.g.* univariate measures, the MDS ordination method) with respect to performance and utility. PRC analysis was shown to be the most useful for assessing human-induced changes in ecological systems, since it is the only one that produces simple diagrams more easily interpreted for non-ecologists and at the same time provides more complete information (Pardal *et al.* 2004). PRC can provide an important tool for environmental quality assessment in the future and should be incorporated into monitoring and assessment programs along with the existing range of univariate and multivariate techniques presently used.

The long-term study presented here indicates that, we can now conclude that the mitigation plan adopted for the Mondego estuary has been at least partially successful. There is evidence that the seagrass is recovering at the outermost site and that the macrobenthic communities are recovering too. However, this is a very

slow process and one that can be occasionally influenced by stochastic events (*e.g.* floods), which may reduce the resilience of certain populations.

There also remains the important question: would the eutrophic site ever return to its original state covered by rooted macrophytes? This is a pertinent question that should be answered in future works. At the present it is difficult to foresee what will happen in that area in the next few years, since so many changes have taken place at the sediment level.

Final remarks

One of the main goals of this study was to understand the dynamic changes of the macrobenthic communities under eutrophication and evaluate their response to the restoration project implemented in the system. Through a long-term study, it was possible to better understand some basic processes that operate in estuarine systems such as the direct relationships between the physical (*e.g.* nutrient loading) and biological structures (*e.g.* macrobenthic communities) of the intertidal mudflats, which together structure the system functioning.

Over a 10-year period it was possible to observe dramatic changes in estuarine ecology at multiple levels: nutrient concentrations, primary producers and macrobenthic community. As for many other estuarine systems worldwide, the Mondego estuary experienced a long phase of environmental quality decay, characterized by the degradation of water quality (excess of nutrients) which led to a progressive decline of the seagrass *Zostera noltii*. As a consequence of the replacement of primary producers, major biological changes were observed in the macroinvertebrate assemblages, namely a decline of species diversity and modifications in the trophic structure. This eutrophication process affected in different ways the macrobenthic species. While some of them were strongly affected (*e.g. H. ulvae, S. plana*), others seemed to be not affected by this process (*e.g. C. carinata*) or even others were beneficiated by this organic enrichment (*e.g. Capitela capitata* and other small size polychaetes).

Faced with the progressive degradation of the estuarine integrity, in 1998 several mitigation measures were implemented that aimed to restore the original seagrass bed. The restoration project seems to have had success, at least partially.

Nutrient concentrations suffered a strong decline comparatively to the pre-mitigation period and there is evidence that the macrophyte is recovering too. Concerning the macrofauna, most of the species negatively affected by eutrophication are also responding positively (in terms of recovering of biomass, population structure, species diversity). However, this recovery process is a slow process that can take several years to be completed.

Finally, I would like to emphasize the importance of the long-term studies for understanding the complex and slow processes that operate in dynamic systems, like estuaries. Only through such long-term programmes it is possible to understand the functioning of these systems and address to their management and conservation needs.

I hope that this thesis will have contributed in this respect to the sustainable management of estuaries.

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