IMPACT ASSESSMENT OF MULTIPLE STRESSORS ON THE MONDEGO ESTUARY: A MULTIDIMENSIONAL APPROACH ON THE BIVALVE *SCROBICULARIA PLANA*.

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THE IMPACT OF EXTREME FLOODING EVENTS AND ANTHROPOGENIC STRESSORS ON THE MACROBENTHIC COMMUNITIES' DYNAMICS
Cardoso PG, Raffaelli D, Lillebø AI, Verdelhos T, Pardal MA
Estuarine, Coastal and Shelf Science, 76, 553 – 565, 2008;

LONG TERM RESPONSES OF TWO INFAUNAL BIVALVE POPULATIONS (*SCROBICULARIA PLANA* AND *CERASTODERMA EDULE*) TO ANTHROPOGENIC AND NATURAL STRESSORS IN THE MONDEGO ESTUARY (PORTUGAL)
Verdelhos T, Crespo D, Cardoso PG, Dolbeth M, Pardal MA
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A VALIDATED POPULATION-DYNAMICS MODEL FOR *SCROBICULARIA PLANA* (MOLLUSCA, BIVALVIA) IN A SOUTH-WESTERN EUROPEAN ESTUARY
Anastácio PM, Verdelhos T, Marques JC, Pardal MA
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LATITUDINAL GRADIENTS ON *SCROBICULARIA PLANA* REPRODUCTION PATTERNS, POPULATION DYNAMICS, GROWTH AND SECONDARY PRODUCTION
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ABSTRACT

The main goal of the present thesis was to assess the impacts of anthropogenic and natural stressors on the Mondego estuary. In order to achieve this purpose, the ecological responses of the macrobenthic community, and particularly of the bivalve *Scrobicularia plana*, to multiple stressors and under different ecological scenarios were studied. Focusing on the dynamics and production of macrobenthic assemblages and key species of the estuary is a good evaluation method of the ecological integrity and is important to understand how the ecosystem reacts to ecological impacts and how it will respond to future changes. The thesis core is divided in three main chapters, focusing on: 1) the impacts of anthropogenic and natural stressors on the macrobenthic community and on one of its main components (bivalves); 2) the ecological behaviour of *S. plana* under different environmental scenarios and by the development of a population dynamics model, simulating eutrophication and restoration conditions; 3) the ecological patterns of several *S. plana* populations along its distributional range, intending to assess different life strategies on populations of the same species.

In Chapter 1, the interactions between eutrophication and intense floods were assessed, centring on the dynamics of the macrobenthic assemblages of the Mondego estuary. Therefore, changes in density and biomass, trophic structure, diversity and spatial distribution were analysed from 1993 to 2002. The eutrophication process clearly affected the macrobenthic community (decline in species richness, decline in herbivores and increases in detritivores and small deposit feeding polychaetes), which showed strong signs of recovery after restoration. However, additional stressors (flood) had more severe effects on these assemblages then expected, stopping the recovery process. Furthermore, two of the main species of this community were studied and compared, analysing long-term changes in dynamics and production over a 13-year period on two distinct habitats on the estuary, intending to assess the influence of multiple stressors and the existence of interactions between these species. *S. plana* and *Cerastoderma edule* showed different spatial distribution patterns on the estuary and contrasting responses to eutrophication. The combined effects of multiple stressors seem to severely affect the *S. plana* population.

In Chapter 2 an ecological model to simulate the population dynamics was developed, using data from three sampling areas under different ecological scenarios – eutrophication (1993 to 1995) and restoration (1999 to 2002). The model is regulated by water temperature, salinity and population density, controlling recruitment and mortality. The occurrence of extreme values of environmental variables had the strongest effect on the model, and possibly on the real system. Results seem to corroborate the notion that system restoration was successful. In fact the model performance was highest under the restoration scenario, indicating that the system became more predictable.

Finally, in Chapter 3, the existence of latitudinal variations on the ecological patterns of a species along its distribution range was assessed on *S. plana*. An extended bibliographic research and field data from the Mondego estuary was the base of this study, focusing on reproduction patterns, population dynamics, growth and production. Areas in the middle of the distribution range of this species seem to show optimal ecological conditions, showing long reproduction periods and the highest abundance, growth rates and production values. The ecological performance of *S. plana* seemed to decrease towards both its North and South limits of distribution and different life strategies were observed along the geographic range of the species.

RESUMO

A presente dissertação tem como principal objectivo a avaliação do efeito de impactos de origem antropogénica e natural no estuário do Mondego. Desta forma, foram realizados estudos para inferir as respostas ecológicas da comunidade macrobentónica, com especial incidência no bivalve *Scrobicularia plana*, em relação a agentes de stress múltiplos e em diferentes cenários ecológicos. A produção e dinâmica das associações macrobentónicas e de espécies-chave do estuário são bons métodos de avaliação da integridade ecológica do ecossistema e importantes para prever como irá reagir a impactos e alterações futuras. Assim, a estrutura da tese é constituída por três capítulos principais incidindo em: 1) impacto de perturbações antropogénicas e naturais na comunidade macrobentónica e num dos seus grupos principais, os bivalves; 2) o comportamento ecológico de *Scrobicularia plana* em diferentes molduras ambientais, através do desenvolvimento de um modelo populacional com simulações de condições de eutrofização e de recuperação; 3) padrões ecológicos de várias populações de *S. plana* ao longo da sua distribuição latitudinal, com o intuito de determinar diferentes tipos de estratégias em populações da mesma espécie.

No capítulo 1, as interacções entre a eutrofização e cheias intensas foram determinadas, centrando-se ao nível da dinâmica das comunidades macrobentónicas do estuário do Mondego. Assim, foram analisadas alterações de densidade, biomassa, estrutura trófica, diversidade e distribuição espacial entre os anos de 1993 e 2002. Concluiu-se que o processo de eutrofização afectou claramente a comunidade macrobentónica, reflectindo-se no declínio da riqueza específica e herbívoros e no aumento de detritívoros e pequenos poliquetas, mas após a instauração do plano de gestão registaram-se sinais significativos de recuperação. No entanto, o efeito adicional de cheias causou consequências mais severas nas associações do que o inicialmente esperado, levando à interrupção do processo de recuperação. Ainda, duas das espécies mais importantes da comunidade foram estudadas e comparadas através da análise de alterações de longo prazo da sua dinâmica e produção, durante um período de 13 anos, em dois habitats estuarinos distintos, com o intuito de determinar o efeito de agentes de stress múltiplos e a existência de interacções entre as espécies. *S. plana* e *Cerastoderma edule* mostraram diferentes padrões de distribuição espacial no estuário e respostas contrárias face à eutrofização. O efeito cumulativo de agentes de stress múltiplos parece ter afectado mais intensamente a população de *S. plana*.

No capítulo 2, desenvolveu-se um modelo ecológico para simular a dinâmica de população, com dados de 3 locais de amostragem sob cenários ecológicos diferentes – eutrofização (1993-1995) e recuperação (1999-2002). O modelo é regulado pela temperatura da água, salinidade e densidade populacional que controlam o recrutamento e mortalidade. A ocorrência de valores extremos de variáveis ambientais causou o efeito mais forte no modelo e possivelmente no sistema real. Os resultados obtidos parecem sustentar o sucesso do programa de recuperação ambiental, uma vez que o desempenho do modelo foi maior neste cenário e indicando ao mesmo tempo que o sistema se tornou mais previsível.

No capítulo 3, foi determinada a existência de uma variação latitudinal nos padrões ecológicos de *S. plana* ao longo da sua área de distribuição. Este estudo teve como base uma pesquisa bibliográfica extensa e dados de campo do estuário do Mondego, tendo incidido nos padrões de reprodução, dinâmica populacional, crescimento e produção. As áreas no centro da distribuição geográfica desta espécie parecem apresentar condições ecológicas óptimas, com períodos de reprodução mais longos, elevadas abundâncias, taxas de crescimento e valores de produção. O desempenho ecológico de *S. plana* parece diminuir em direcção aos limites de distribuição, apresentando estratégias de vida diferentes ao longo do gradiente latitudinal.

INTRODUCTION

COASTAL ECOSYSTEMS

THE ROLE OF COASTAL ECOSYSTEMS TO MANKIND

Coastal ecosystems are widely distributed areas around the world, throughout an ample variety of physical, geo-morphological and climatic conditions, being highly heterogeneous concerning their biotic and socioeconomic features (Martinez et al., 2007). As a borderline between land and ocean, these areas cover complex broad scale interactions between these contrasting environments, and can be considered as "the part of the land most affected by its proximity to the ocean and the part of the ocean most affected by its proximity to the land" (Hinrichsen, 1998).

Coastal areas are extremely important to mankind, as living and subsistence habitats, as well as leisure areas. Their huge socioeconomic value has been widely recognised and estimated at US$ 15 to 20 trillion per year globally, especially through a large variety of goods (e.g. food production, salt, minerals, oil resources, construction materials) and services (e.g. shoreline protection, nutrient storage and recycling, water capture) provided (Hays et al., 2005; Harley et al., 2006; Martinez et al., 2007). Moreover, they are also highly appreciated areas either to live or for recreation and tourism (van der Meulen et al., 2004; Martinez at al., 2007).

The vast opportunities given by these areas have historically attracted the humans and highly dense populations have been settling on the proximity of the ocean and riverbeds, using them as essential navigation and transport routes and developing important urban, industrial and commercial centres. In fact, many of the major cities in the world are nowadays located on coastal areas (Martinez et al., 2007).

HUMAN IMPACTS ON COASTAL ECOSYSTEMS

Presently, the ongoing demographic growth is a major global issue, and the situation in coastal areas is even more dramatic, due to the higher population growth rates in those areas (Martinez et al., 2007). Such overpopulation raises the demand for the ecosystems goods and services and so, increased construction of infrastructures, exploitation of natural resources and waste disposal, as well as changes in land use, agricultural and industrial expansion are expected. As a result, more anthropogenic pressures will impact coastal ecosystems, through physical processes (e.g. habitat and shoreline modification), organic and chemical pollution, and over exploitation of natural resources (Mclusky and Elliott, 2004; Fleume, 2006; Valiela and Bowen, 2007; Martinez et al., 2007; Vasconcelos et al., 2007), affecting their long term integrity.

In fact, anthropogenic impact has been increasing and is likely to increase even more in the future. Therefore, studies focussing on natural and anthropogenic induced changes on coastal ecosystems are necessary and even mandatory in order to maintain our coasts and its associated ecosystems and resources, without exploiting them to exhaustion. Knowledge on land use, urban and industrial construction, waste disposal, organic and chemical pollution, resources exploitation and natural extreme events is thus essential in the decision-making process. Only with a global effort, extensive scientific knowledge and well informed decisions we will be able to achieve economic efficiency, social equity, and ultimately, ecological sustainability (Martinez et al., 2007).

ESTUARINE ECOSYSTEMS

CHARACTERISTICS AND IMPORTANCE

Estuaries are found wherever rivers meet the sea (Molles, 1999). They are semi enclosed coastal ecosystems constituting a transition area where fresh land-derived water mixes with saline ocean water (Molles, 1999; Mclusky and Elliott, 2004; Neill, 2005), or according to Pritchard (1967) an estuary is a semi-enclosed coastal body of water which has a free connection with the open sea and within which sea water is measurably diluted with freshwater derived from land drainage. As transition areas between distinct environments, estuaries are extremely ecologically challenging to its inhabitants, which are exposed to great physiological stress due to unique environmental characteristics. High daily variations on temperature, water circulation, salinity and oxygen conditions (Molles, 1999) result in a considerable lower biodiversity, when compared either to rivers or to the ocean. However, they are among the most important environments on Earth, both ecologically, ranking amongst the most productive biomes (Molles, 1999; Kennish, 2002; Mclusky and Elliott, 2004; Dolbeth et al., 2007), and socio-economically (Molles, 1999; Mclusky and Elliott, 2004; Svensson et al., 2007).

Estuaries receive frequent nutrient inputs from both freshwater and marine sources and function either as a filter for particulate matter, through recycling mechanisms that contribute to an efficient use of nutrient supply, as well as detritus traps for the abundant autochthonous and allochthonous material (Flemer and Champ, 2006; Elliott et al., 2002; Hartnett and Nash, 2004; Svensson et al., 2007). This will result in a high primary production and in abundant available food resources for the entire trophic web, through direct or indirect consumption (Elliott et al., 2002; Mclusky and Elliott, 2004).

Additionally, a wide variety of habitats is offered by these ecosystems to plant and animal communities and several species of invertebrates (Cardoso et al., 2005; Verdelhos et al., 2005; Dolbeth et al., 2007), fish (Elliott et al., 2002; Leitão et al., 2007; Martinho et al., 2007) and birds (Mclusky and Elliott, 2004; Lopes et al., 2006) live or depend on estuaries, using them as nursery grounds, migratory routes or feeding areas, favoured by good shelter, protection and food supply conditions. Moreover, they can be considered as strategic locations to human populations, which have settled many of its biggest cities on the surrounding areas, using them as food sources, natural transport routes and recreation facilities, and developing extensive fish or shellfish cultures, agriculture fields and industries, representing an important economic resource (Kennish, 2002; Mclusky and Elliott, 2004; Martinez et al., 2007; Svensson et al., 2007; Vasconcelos et al., 2007).

MAJOR THREATS TO ESTUARINE ECOSYSTEMS

THE EUTROPHICATION PROBLEM

Human activities often lead to a series of anthropogenic pressures, such as habitat loss and over exploitation of resources resulting from overpopulation, or organic and chemical pollution resulting from extensive agriculture and industry, causing severe ecological stress and endangering the ecosystem. Nowadays, eutrophication is one of the major threats that estuaries have to face (Paerl, 2006). As a result of high nutrient input derived from urban, agricultural and industrial effluents, phytoplankton and macroalgal growth is stimulated, due to the particular characteristics of these systems (shallow depth and reduced water exchange) (Kennish, 2002; Mclusky and Elliott, 2004; Lillebø et al., 2005, 2007; Dolbeth et al., 2007). In fact, one of the most frequent symptoms/consequences of eutrophication is the occurrence of macroalgal blooms (Raven and Taylor, 2003; Lillebø et al., 2005; Cardoso et al., 2008; Dolbeth et al., 2007). These events usually result in oxygen depletion, both in the water column and in the sediment, and consequent hypoxia and anoxia conditions, related to algal death and decay, having severe impacts on the system (Bolam et al., 2000; Pardal et al., 2000; Raven and Taylor, 2003; Verdelhos et al., 2005; Cardoso et al., 2008).

Declines in seagrass beds are often associated with increased eutrophication, resulting from the complex interaction of mechanisms such as changes in water and sediment quality (Bolam et al., 2000), smothering by algal mats (den Hartog and Phillips, 2000) and competition for light and nutrients (Niehuis, 1996). The replacement of rooted macrophytes by faster growing opportunistic macroalgae may occur, leading to a shift from a stable seagrass/grazing controlled system to a more dynamic detritus/mineralization system (Pardal, 1998). This may impact several key species and the entire trophic structure, resulting in an overall ecological impoverishment of the ecosystem (Raffaelli et al., 1998; Cardoso et al., 2004, 2008; Verdelhos et al., 2005). Global awareness upon these problems has increased during the last decades, focussing on the assessment and protection of the ecological status of these ecosystems. Conservation and restoration have then become a priority, in order to return a system from an altered or disturbed condition to a previously existing stable state condition (de Jonge and de Jong, 2002; Kendrick et al., 2002; Webster and Harris, 2004).

CLIMATE CHANGE

In addition to anthropogenic pressures, estuarine ecosystems face another major problem: the increased climate variability associated with global warming. Global warming is certainly one of the major environmental problems the world faces, receiving considerable attention from scientists, policy makers and general public. Climate change was defined as “a change of climate, attributed directly or indirectly to human activity, that alters the composition of the global atmosphere and which is, in addition to natural climate variability, observed over comparable time periods” (United Nations, 1994).

Several human activities, such as combustion of fossil fuels, industrial expansion or widespread deforestation are contributing to this change and are accentuating a natural warming tendency through increased atmospheric concentration of the main greenhouse gases (Short and Neckles, 1999; Simas et al., 2001; Epstein and Mills, 2005; Houghton, 2005; Harley et al., 2006). Global air and water temperature increments are then expected, along with widespread melting of snow and ice, sea level rise and increased climate related extreme events, such as floods, droughts or heat waves (Short and Neckles, 1999; Simas et al., 2001; Houghton, 2005). An alarming evidence that climate may be changing, is the occurrence of several weather-related extreme events (such as heat waves, storms, heavy precipitation episodes) in the last decade. These seemed to become more frequent, with increasing intensity, and are expect to rise in the future.

Climate is expected to affect the performance of individuals, populations and communities, with diverse geographical distributions (Short and Neckles, 1999; Simas et al., 2001; Adams, 2005; Harley et al., 2006). Possible impacts are defined as changes that may have deleterious effects on ecosystems, socioeconomic systems and on human and animal welfare (United Nations, 1994). The entire ecosystem may even be disrupted, due to climate impacts, as a consequence of differences in response times of species (IPCCWGF, 2001). It is then extremely important to understand the wide complexity of the climate change problem, its causes, the mechanisms involved and worldwide impacts. Studies on population and community level processes are thus required for a holistic and integrative view of the response of an ecosystem to global climate change.

MULTIPLE STRESSORS

Natural and anthropogenic stressors often interact with each other, producing combined effects, which can impair the health and fitness of resident biota. The combined action of these stressors may impact the biota through single, cumulative and synergistic processes, leading directly or indirectly to changes in abundance, diversity and fitness of individuals, populations and communities (Vinebrooke et al., 2004; Adams, 2005; Cardoso et al., 2005, 2008b; Dolbeth et al., 2007), lowering the overall ecological condition of the ecosystem. Thus, the understanding of the ecosystem functioning and dynamics in response to multiple stressors becomes a key issue on nowadays ecology. Further knowledge on the complex processes and interactions among causes and effects of natural and anthropogenic stressors is then essential to the assessment and evaluation of ecosystems, defining accurate study approaches and restoration techniques of damaged environments.

CASE STUDY: THE MONDEGO ESTUARY

GENERAL DESCRIPTION AND MONITORING PROGRAM

The field work of the present thesis was done in the Mondego estuary, which is located in a warm temperate region, on the Atlantic coast of Portugal (40º08'N, 8º50'E), near Figueira da Foz. It is a small estuary of 8.6 km^2, comprising two arms, North and South, separated by the Murraceira Island (Fig. 1).

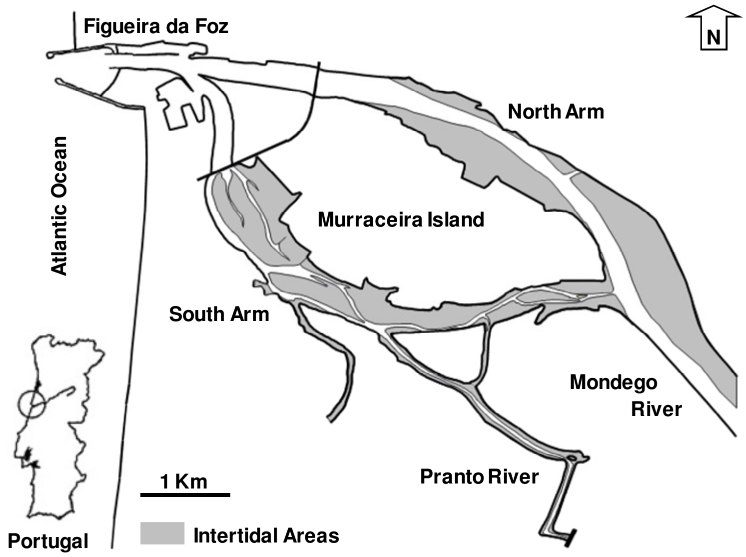

Fig. 1 – The Mondego estuary.

The North arm is deeper (4–10 m during high tide, tidal range 1–3 m), highly hydrodynamic and provides the main navigation channel and the location of the Figueira da Foz harbour. The South arm is shallower (2–4 m during high tide, tidal range 1–2 m) and is characterized by large areas of exposed intertidal flats during low tide, with extended *Spartina maritima* marshes and *Zostera noltii* beds. Until 1998, the South arm was almost silted up in the innermost areas, and the river outflow occurred mainly via the North arm. Therefore, water circulation was here mostly dependent on tides and on the freshwater input from the Pranto River, a small tributary with a flow controlled by a sluice, which was regulated according to the water level of rice fields in the Mondego Valley.

The Mondego estuary is a well documented ecosystem, with several studies on the structure and functioning of the system (from nutrient dynamics, flora and fauna structure and dynamics and ecosystem processes) over the last decades, providing a large database and wide background knowledge. The long-term monitoring program of the present thesis has been carried out since the early 1990s by an IMAR-Institute of Marine Research team, within the scope of European and national projects.

Three different sampling areas were initially chosen (Fig. 2), representing different habitats, and impact scenarios, along the South arm: (1) the seagrass bed, located downstream and composed by muddy sediments covered with *Zostera noltii*. This area is characterised by higher organic matter content on the sediment (mean 6.2% ± 1.76), and higher water-flow velocity (1.2-1.4 $m.s^{-1}$) compared to the other areas; (2) an intermediate area, adjacent to the previous, with similar physical-chemical water and sediment characteristics, but with no seagrass coverage although some roots of *Zostera noltii* are still found; (3) an eutrophic area, a bare bottom composed by muddy sand sediments, with lower organic matter content (mean 3.0% ± 1.14) and characterised by lower water flows (0.8– 1.2 $m\ s^{-1}$), which has not supported rooted macrophytes for more than 15 years and has been covered seasonally by green macroalgae.

Sampling was taken fortnightly for the first 18 months and monthly thereafter, during low tide. Ten (during the first 18 months) to six sediment cores (141 cm^2 core sectional area) were randomly taken to a depth of 25 cm, using a manual corer. Each sample was sieved through a 500 µm mesh using estuarine water and then preserved in 4% buffered formalin. At each sampling station, water temperature and salinity were measured directly *in situ* (in low water pools), and sediment was collected for further analysis.

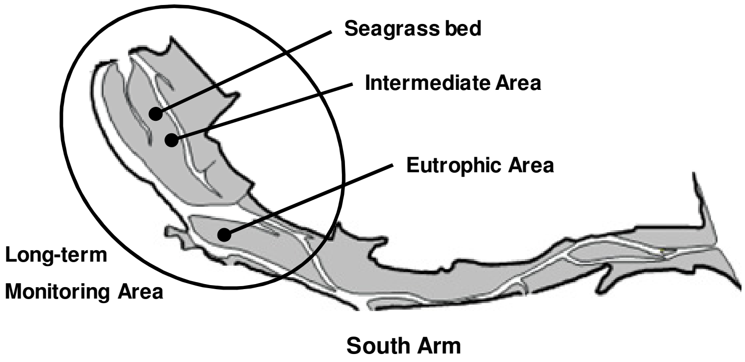

Fig. 2 – Long-term monitoring area – location of the sampling stations on the South Arm of the Mondego estuary.

ANTHROPOGENIC PRESSURES

The Mondego estuary is highly valuable for local human populations, which explore its natural resources, such as food (e.g. fishes, shellfishes) or salt. The city of Figueira da Foz has more than 60 000 habitants and is the location of an important mercantile harbour and a recreational marina (Ribeiro, 2001). Moreover, there has been a considerable expansion of industries (mostly cellulose and paper related industries), aquacultures (several old salt-ponds transformed into semi-intensive aquacultures) and agriculture (more than 15 000 ha of cultivated land in the Lower Mondego valley, upstream the estuary).

The human activities on the estuary have caused severe ecological pressures, either 1) physical: regularization of navigation channels by the construction of harbour facilities and bottom dredging, construction of channels and dams to improve industrial water supplies and agricultural irrigation efficiency, changes in land use by the construction of new urban, industrial and agricultural facilities; and 2) chemical: increased inputs of organic nutrients and pollutants from urban waste sewage, agricultural and aquaculture activities or industrial discharges. This resulted in changes on the riverbed topography and hydrodynamics, increased water turbidity and increased concentration of growth limiting nutrients. In fact, this estuary has experienced significant eutrophication over the past 20 years, which has led to a decline in the overall environmental quality of the estuary, degradation of water quality and increased turbidity.

As a consequence of eutrophication, seagrass beds declined both in extent and biomass (Cardoso et al., 2005; 2008; Verdelhos et al., 2005). In the early 1980's, the area occupied by the seagrass was 15 ha, being reduced to 0.02 ha in the mid-1990's (Fig. 3), affecting population dynamics and production of key species and of the entire community (Cardoso et al., 2005; 2008; Verdelhos et al., 2005; Dolbeth et al., 2007).

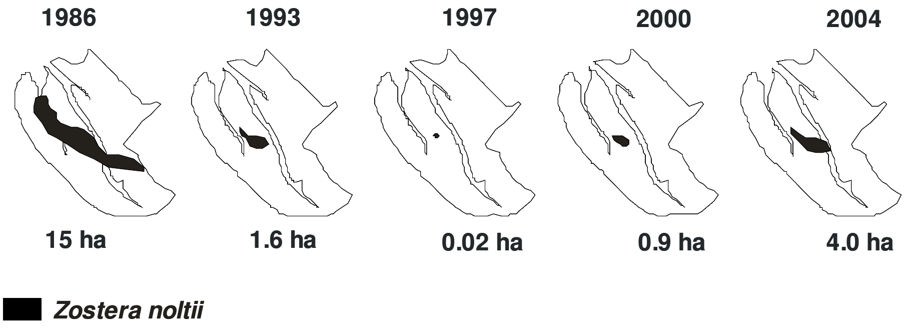

Fig. 3 – Seagrass bed evolution in the South Arm of the Mondego estuary**.**

RESTORATION: MANAGEMENT PLAN AND CONSEQUENCES

A management plan was introduced in 1998, in order to restore the original seagrass bed of the South arm by decreasing nutrient loading, improving water circulation and protection of the seagrass bed (Cardoso et al., 2005, 2007; Lillebø et al., 2005; Verdelhos et al., 2005; Dolbeth et al., 2007). The implemented measures included (Fig. 4): (1) the re-establishment of the South arm riverhead connection, improving the hydraulic regime; (2) most of the nutrient enriched Pranto freshwater was diverted to the Northern arm by another sluice located further upstream, leading to nutrient loading reduction, essentially ammonia (Lillebø et al., 2005); (3) physical seagrass bed protection, using wooden stakes to prevent further human disturbance;

and (4) public education of the ecological importance of intertidal vegetation for health and related socio-economic activities of the estuary.

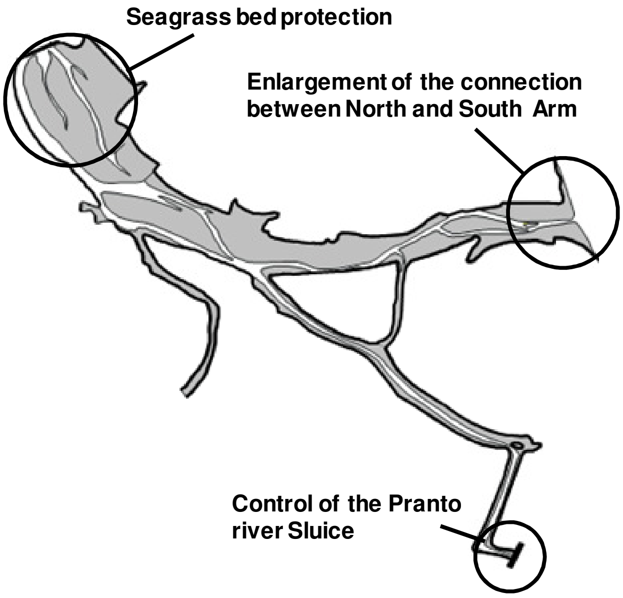

Fig. 4 – Restoration Program – Implemented measures on the Mondego estuary.

These measures seemed to have effective results on the restoration of the ecosystem (Cardoso et al., 2005, 2007; Lillebø et al. 2005; Verdelhos et al., 2005; Dolbeth et al., 2007; Leston et al., 2008), improving water circulation on the South arm (Fig. 5), reducing residence time and nutrient loading (Table 1). Consequently, no macroalgal blooms were recorded ever since and *Zostera noltii* seems to be gradually recovering, both in biomass and extent (Fig. 3), with positive impacts on several macrofaunal key species and on the entire community, showing increased biodiversity, biomass and production (Dolbeth et al. 2007).

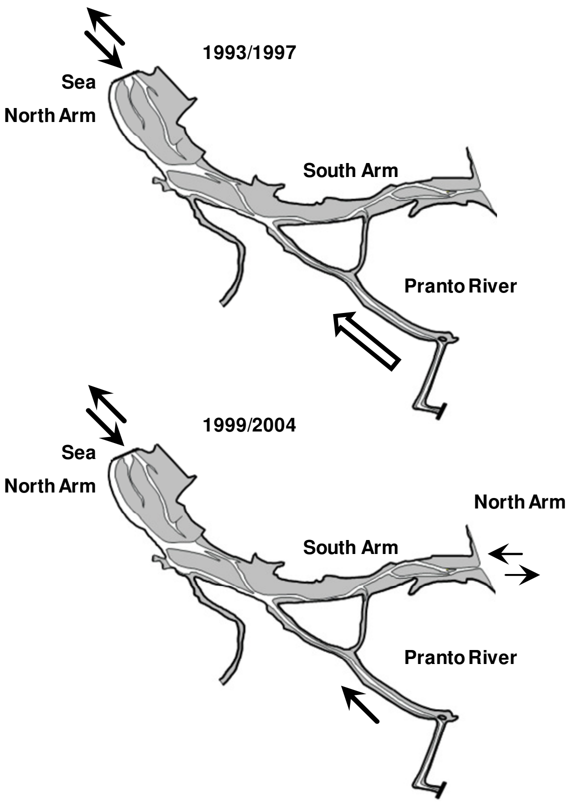

Fig. 5 – Restoration Program – Main freshwater inputs before the management (1993 – 1997) and after (1999 – 2005).

LOCAL CLIMATIC VARIABILITY

During the last decades the climate in Portugal has undergone several changes, when compared to the general climate patterns for the period 1931-1990, with the occurrence of several extreme climate events, which became more frequent and intense (Miranda et al., 2006) (INAG - Portuguese Water Institute, http://snirh.inag.pt/ and IM - Portuguese Weather Institute, http://web.meteo.pt/pt/clima/clima.jsp). Mean air temperature rose progressively (from 1931 to 2005: + 0.15°C per decade), and some of the hottest years ever were registered, with the occurrence of heat waves during the summer (e.g. 2003 and 2005) (Diaz et al., 2006; Miranda et al., 2006). Additionally, high precipitation variability was registered, with an increase in the frequency and intensity of heavy rainfall, followed by low precipitation, with prolonged drought events (Miranda et al., 2006; Cardoso et al., 2008). Along the study period, from 1993 to 2005, the winter 2000/01 reached unprecedented high values of precipitation, especially for the central Portugal (2000/01: 1802.1 mm against a mean annual value for 1940 to 1997: 1030.6 mm), causing one of the largest floods of the century. It was followed by the gradual occurrence of a drought, starting in 2004 and attaining a severe drought in 2005 (2005: 486.1 mm against the mean annual of 1030.6 mm).

Table 1 – Restoration Program – Summary characterization of the South arm of the Mondego estuary before the management (1993 – 1997) and after (1999 – 2004).

Characteristic	**1993-1997** **Before management**	**1999-2004** **After management**
Salinity range	1.9 – 33.1	0.2 – 33.7
Mean water temperature range (ºC)	8.0 – 23.7	8.1 – 22.1
Residence time	Moderate (weeks)	Short (days)
Current velocity	Low and dependent on the Pranto river sluice	Higher and not dependent on the Pranto river sluice
Turbidity	High	Lower
DIN (mean) (μmol L^{-1})	35.59	14.52
DIP (mean) (μmol L^{-1})	1.01	1.59
N/P (mean)	35.09	9.13

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MAIN GOALS AND THESIS STRUCTURE

The increasing anthropogenic and climate related problems that has affected the Mondego estuary over the last decades have triggered the need to assess its ecological status and to take management measures for the recovery of this system, that has been widely recognised as ecologically and socio-economically relevant.

The macrobenthic community is an essential component of the ecosystem as a central element on the food web and some of its species have also highly economic value to local human populations. Therefore, the study of the benthic community, focussing on the dynamics, production, interactions of key species and responses to stressors, may provide a good evaluation of the estuary ecological integrity. This sort of knowledge becomes essential to mitigate anthropogenic and climate impacts and to set possible recovery measures.

Long term studies are required to understand slow ecological processes, rare events and complex phenomena. The knowledge on population dynamics, growth and production along the distribution range of a species is essential to better understand natural trends and changes in response to stressors on a broader scale and to make predictions on global future scenarios. Moreover, in this changing world, it becomes essential to document the ecosystem before the environmental change intensifies.

This thesis is structured in three main chapters, and it aims to:

- Assess the influence of multiple stressors on the Mondego macrobenthic community;
- Understand the dynamics and responses of economically important bivalve species populations in different ecological scenarios, and interactions within the community components;
- Make future predictions based on information gathered from the system.

Consequently several questions arise:

- What is the influence of anthropogenic and natural stressors on the Mondego estuary?
- What is the response of the bivalve *Scrobicularia plana* to different scenarios?
- How do the main infaunal bivalves of the community (*Scrobicularia plana* and *Cerastoderma edule*) respond to long term changes?
- What is the response of the macrobenthic community facing multiple stressors and different ecological scenarios?
- How will these populations and communities evolve facing future changes?

CHAPTER 1

LONG-TERM CHANGES ON THE INTERTIDAL MACROBENTHIC ASSEMBLAGES OF THE MONDEGO ESTUARY

The first chapter of this thesis deals with the macrobenthic community of the intertidal flats on the South Arm of the Mondego estuary, assessing its variability and ecological changes over a long-term period, during which several anthropogenic and natural stressors affected the system.

The impacts of multiple stressors on the macrobenthic communities' dynamics are evaluated on the paper **"The impact of extreme flooding events and anthropogenic stressors on the macrobenthic communities' dynamics"**, exploring the interactions between extreme weather events (e.g. flooding) and anthropogenic stressors (e.g. eutrophication). Impacts at community-level processes are assessed, through the analysis of changes on biodiversity, density and biomass, on the trophic structure and on the spatial and temporal dynamics.

This community is clearly dominated by deposit feeder species and two of the most important are the bivalves *Scrobicularia plana* and *Cerastoderma edule*, considering population biomass, production and economic value, since these are highly exploited by local fishermen. The dynamics and production of these infaunal bivalves is analysed and compared on the paper **"Long-term responses of two infaunal bivalve populations (*Scrobicularia plana* and *Cerastoderma edule*) to anthropogenic and natural stressors in the Mondego estuary (Portugal)"**, evaluating its variation during a 13-year period in response to several environmental changes on the ecosystem. The main goal of this paper is to understand the dynamics and production changes and to evaluate the ecological responses of the two populations to stressors, under different scenarios.

CHAPTER 2

THE BIVALVE *SCROBICULARIA PLANA* UNDER DIFFERENT ECOLOGICAL SCENARIOS: A POPULATION DYNAMICS MODEL

Scrobicularia plana is a long lived deposit feeder distributed along a wide geographic range, recognised as a key species on the soft substrate assemblages in coastal areas and is one of the most important species of the Mondego estuary macrobenthic community. This chapter proposes a population dynamics model under different situations of natural and anthropogenic stress.

On the paper **"A validated population dynamics model for *Scrobicularia plana* (Mollusca, Bivalvia) in a Southwestern European estuary"** a population dynamics model is proposed for this species, which has never been modelled, simulating a pre (eutrophication) and a post management (restoration) situations. At this point, long-term data series are available, providing an excellent opportunity to test a population dynamics model, aiming to understand the dynamics of *Scrobicularia plana* allowing us to extract information and make predictions under different environmental scenarios.

CHAPTER 3

THE ROLE OF LATITUDE ON THE BIVALVE *SCROBICULARIA PLANA*

The paper **"Latitudinal gradients on *Scrobicularia plana* reproduction patterns, population dynamics, growth and secondary production"** results from a vast bibliographic research and data from the Mondego estuary. Here, *Scrobicularia plana* patterns of reproduction, dynamics, growth and production are compared and analysed, intending to assess ecological differences on populations throughout a wide range of distribution.

Latitudinal gradient studies are useful to provide increased knowledge on the ecological patterns and life strategies of a species, which may be important to fully understand different changes along its geographic range of distribution, and to make future predictions facing the increasing climate variability. Moreover, we can extract useful information to other ecological approaches.

CHAPTER 1

LONG-TERM CHANGES ON THE INTERTIDAL MACROBENTHIC ASSEMBLAGES OF THE MONDEGO ESTUARY

The impact of extreme flooding events and anthropogenic stressors on the macrobenthic communities' dynamics

Long-term responses of two infaunal bivalve populations (*Scrobicularia plana* and *Cerastoderma edule*) to anthropogenic and natural stressors in the Mondego estuary (Portugal)

THE IMPACT OF EXTREME FLOODING EVENTS AND ANTHROPOGENIC STRESSORS ON THE MACROBENTHIC COMMUNITIES' DYNAMICS

ABSTRACT Marine and coastal environments are among the most ecologically and socio-economically important habitats on Earth. However, climate change associated with a variety of anthropogenic stressors (e.g. eutrophication) may interact to produce combined impacts on biodiversity and ecosystem functioning, which in turn will have profound implications for marine ecosystems and the economic and social systems that depend upon them. Over period 1980 to 2000, the environment of the Mondego estuary, Portugal, has deteriorated through eutrophication, manifested in the replacement of seagrasses by opportunistic macroalgae, degradation of water quality and increased turbidity, and the system has also experienced extreme flood events. A restoration plan was implemented in 1998 which aimed to reverse the eutrophication effects, especially to restore the original natural seagrass (*Zostera noltii*) community. This paper explores the interactions between extreme weather events (e.g. intense floods) and anthropogenic stressors (e.g. eutrophication) on the dynamics of the macrobenthic assemblages and the socio-economic implications that follow. We found that during the previous decade, the intensification of extreme flooding events had significant effects on the structure and functioning of macrobenthic communities, specifically a decline in total biomass, a decline in species richness and a decline in suspension feeders. However, the earlier eutrophication process also strongly modified the macrobenthic community, seen as a decline in species richness, increase in detritivores and a decline in herbivores together with a significant increase in small deposit-feeding polychaetes. After the implementation of the management plan, macrobenthic assemblages seemed to be recovering from eutrophication, but it is argued here that those earlier impacts reduced system stability and the resilience of the macrobenthic assemblages, so that its ability to cope with other stressors was compromised. Thus, heavy flooding in the Mondego region during the recovery process had more severe effects on these assemblages than expected, effectively re-setting the recovery clock, with significant socio-economic impacts (e.g. high mortality of fish in fish farms, and a large decline of economically important species, such as the bivalves *Scrobicularia plana* and *Cerastoderma edule*). The frequency and magnitude of these extreme events is predicted to increase in future years (IPCC 2001) and there is a risk that impacted ecosystems will never recover fully, with far-reaching consequences for human well being.

INTRODUCTION

Among the most ecologically and socio-economically important environments on Earth are coastal zone ecosystems. Marine and coastal habitats have huge socioeconomic value, estimated at ~ US$ 15-20 trillion y^{-1} globally, through food production, nutrient recycling, recreation and gas regulation (Hays et al. 2005, Harley et al. 2006). However, in addition to the numerous anthropogenic disturbances that affect coastal environments leading to habitat modification and changes in ecosystem function, these ecosystems, along with goods and services they provide are threatened by global climate change. Changes in climate (e.g. temperature rise, sea-level rise, increased risks of floods and droughts) may increase the risk of abrupt and non-linear changes in many ecosystems, which would affect their composition, function, biodiversity and productivity. When subjected to climate change, including changes in the frequency of extreme events, ecosystems may be disrupted as a consequence of differences in response times of species (IPCC 2001). Episodic events such as extreme rain events and flooding can result in the catastrophic deposition of fine sediments with profound influences on the structure and function of macrobenthic communities (Norkko et al. 2002).

In recent years there has been an upsurge of interest in climate change impacts in marine systems, but most of the literature is focused on the effect of the temperature and most work is conducted at the level of individual organisms (Harley et al. 2006 and references therein). A few studies have focused on the impact of large-scale weather events, such as flooding, on the functioning of macrobenthic communities (e.g. Norkko et al., 2002; Salen-Picard and Arlhac, 2002; Salen-Picard et al., 2003), confirming that extreme rain events may have implications for the ecosystem functioning. According to Norkko et al. (2002), catastrophic clay deposition associated with severe flooding, can have markedly deleterious effects on estuarine macrobenthic communities. Other studies have shown an increase in the density of opportunistic species after flood events (Salen-Picard and Arlhac, 2002; Salen-Picard et al., 2003).

Studies carried out on population and community-level processes are required for a holistic and integrative view of the response of an ecosystem to global climate change, preferably over the long time scales associated with such change. However, there are relatively few long time-series of biological measurements in estuarine/marine environments (e.g. Beukema, 1991, 1992; Beukema et al., 1999).

In addition to climate change, coastal ecosystems such as estuaries are naturally subjected to a variety of anthropogenic stressors which can damage the health and fitness of the resident organisms. Multiple stressors including pollutants, excess of nutrients (e.g. eutrophication), altered habitat and hydrological regimes as well as floods and droughts can impact resources through single, cumulative or synergistic processes, lowering the overall system stability (Vinebrooke et al., 2004; Adams, 2005; Cardoso et al., 2005; Dolbeth et al., 2007). Responses of biota to these environmental stressors are the integrated result of both direct and indirect processes which can be manifested as changes in abundance, diversity and fitness of individuals, populations and communities (Adams, 2005). The accelerating rate of biological impoverishment may render ecosystems incapable of compensating for the loss of biodiversity, thereby reducing their resilience to environmental change (Vinebrooke et al., 2004). Distinguishing and integrating the effects of natural and anthropogenic stressors is an essential challenge for understanding and managing coastal biotic resources (Vinebrooke et al., 2004; Paerl, 2006).

This paper deals with the impact of multiple stressors (natural and anthropogenic) at the community-level processes in benthic ecosystems. In order to improve our understanding of benthic recovery processes following disturbance, the main goals of the present paper are to evaluate the impact of extreme events (e.g. intense flooding) on the dynamics of macrobenthic communities, using a long-term data series (10 – years), and to assess possible interactions between climate change and other anthropogenic stressors (e.g. eutrophication).

MATERIALS AND METHODS

STUDY SITE

The Mondego estuary, located on the Atlantic coast of Portugal (40º 08 N, 8º 50 W) consists of two distinct arms, Northern and Southern, separated by Murraceira Island (Fig. 1). A detailed description of the system can be found in Cardoso et al. (2004, 2005) and Verdelhos et al. (2005).

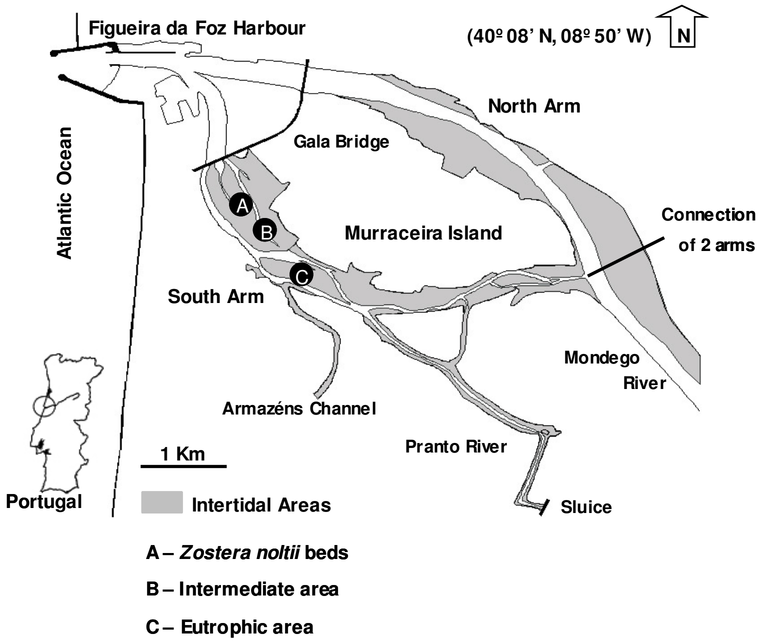

Fig. 1 – Location of the Mondego estuary and sampling stations.

Mainland Portugal has a mild Mediterranean climate. Precipitation data for Portugal for the period 1931 to 2000 shows a generalized but weak decreasing trend that becomes more pronounced after 1976. Since 1976, there is also a significant difference in precipitation trends between seasons, with a systematic reduction of spring precipitation partially compensated by less coherent changes in the other seasons (Miranda et al., 2006). Over all, the data imply a somewhat shorter rainy season. Climate data for the mainland reveals some increase in the frequency/intensity of extreme weather events in the second half of the 20th century (Miranda et al., 2006). The maximum 5-day total precipitation, which is an indicator of flood producing events, is also increasing (Santos et al., 2002). All data concerning monthly precipitation, presented in the present paper have been collected from the nearby city of Coimbra (Instituto de Meteorologia, Coimbra forecast station) since no meteorological forecast station was present in the study area.

In addition to climate change, the Mondego estuary has experienced marked eutrophication over the last 20 years, which led to a decline in the environmental quality of the estuary (including a replacement of seagrass beds by opportunistic macroalgae, increased degradation of water quality through increased turbidity and excess of nutrients, decline of species diversity and secondary production as well as a decline in herbivores and an increase in detritivores). This phenomenon has been reported fully elsewhere (Pardal et al., 2004; Cardoso et al., 2005, 2007; Verdelhos et al., 2005; Dolbeth et al., 2007). Due to the decline of seagrass beds and progressive impoverishment of the habitat, a management programme was implemented in 1998 in order to restore the original seagrass community. This programme included measures to decrease nutrient loading, physical protection of the seagrass bed and improvement of the hydraulic regime, by enlarging the connection between the two arms. The Pranto sluice-opening regime was changed so that most of the freshwater from the Pranto River was diverted to the Northern arm, reducing the nutrient loading in the Southern arm. In addition, the remaining seagrass patches were protected with wooden stakes to prevent further disturbance by fishermen digging in the sediment for bait and cockles (see in detail Cardoso et al., 2005, 2007; Lillebø et al., 2005; Verdelhos et al., 2005; Dolbeth et al., 2007).

Three distinct areas were selected as sampling sites along the Southern arm: the seagrass *Z. noltii* bed towards the marine end of the estuary, a eutrophic area further upstream and an intermediate area located in between (Fig. 1). In the 1980s, the *Z. noltii* bed occupied a broad expanse along the Southern arm (15 ha) reaching the inner most parts of the estuary (Cardoso et al., 2005). By the mid-1990s, *Z. noltii* had become restricted to a small patch (0.02 ha) located downstream, having been replaced elsewhere by blooms of fast-growing green macroalgae. The intermediate area located just upstream of the present *Z. noltii* bed has no seagrass cover, although some rhizomes remain in the sediment. The eutrophic area located upstream comprises sandy-muddy sediment, which in the early 1980s was covered by *Z. noltii*, but as eutrophication increased, *Z. noltii* declined progressively (Cardoso et al., 2005). This area has less energetic hydrodynamics than the others and is covered seasonally by green macroalgae (*Ulva* spp.) (Martins et al., 2001; Cardoso et al., 2002, 2004; Pardal et al., 2004).

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 within each area, 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 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.

DATA ANALYSIS

STATISTICAL ANALYSES

Changes in macrobenthic densities and biomasses were assessed using the non-parametric Wilcoxon two-sample test, comparing the pre- with post-restoration periods, for each study site. Comparisons of species richness of the three study areas (before and after restoration) were made using a Kruskal-Wallis test. For evenness, differences between pre-and post-restoration for the intermediate and eutrophic areas were assessed using t-tests. The effect of flooding events on macrobenthos was statistically demonstrated through a non-parametric Wilcoxon two-sample test, comparing the species richness before and after the floods of 2000/01. Temporal changes in the structure of macrobenthic communities were assessed by multidimensional scaling (MDS) ordination on the Bray-Curtis similarity index and by Principal Response Curves (PRC) analysis (Van den Brink and Ter Braak (1999)).

MACROBENTHIC TROPHIC GROUP ASSIGNMENTS

Each of the macrobenthic taxa was assigned to a trophic group based on feeding behaviour and food type. Trophic groups used in this study were detritivores (D), carnivores (C), herbivores (H) and omnivores (O). Since detritivores is the main trophic group, we decided to subdivide it into surface-deposit feeders (SDF), subsurface-deposit feeders (SsDF) and suspension feeders (SuF).

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 omitting this species, since it occasionally occurred in very high numbers and its inclusion masked changes in other species.

DIVERSITY MEASURES

The diversity of the macrobenthic assemblages in the three areas was assessed as species richness (simple count of number of species recognised), and by the Shannon-Wiener (log base 2), Simpson’s D and Pielou’s evenness measures (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 by the Principal Response Curves (PRC) method. This method is based on the redundancy analysis ordination technique, the constrained form of Principal Component Analysis. A full account of the method can be found in Van den Brink and Ter Braak (1999) and Pardal et al. (2004). The method computes differences in species composition between “treatments” (areas, 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 visually.

In the present study, “treatments” correspond to the different areas under different degrees of eutrophication. In previous studies that have used PRC analysis, an experimental “control” treatment level was used as the reference treatment (Van den Brink and Ter Braak, 1999). Here, however, and in common with Frampton et al. (2001) and Pardal et al. (2004), 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 and Similaeur, 1998). We considered the *Z. noltii* meadows in 1993 as the reference area or control. PRC analysis was performed using the CANOCO software package, version 4 (Ter Braak and 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 and Ter Braak, 1999).

The faunal samples were also analysed using non-metric Multi Dimensional Scaling (MDS), described by Clarke and Gorley (2001) and Clarke and Warwick (2001). Numbers of individuals for each species were square root transformed prior to analysis in order to scale down the effects on the ordination of highly abundant species (Clarke and 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 and Warwick, 2001).

RESULTS

CLIMATE-PRECIPITATION

Our analysis of the seasonal accumulated precipitation pattern for Portugal for the last 60 years compared with the climate normal of 1961-1990 (IM – Portuguese Weather Institute, *http://web.meteo.pt*), revealed many rainfall events exceeding 406 mm (mean winter precipitation for the period 1961-1990) (Fig. 2A). However, the frequency of flood events (precipitation in excess of 50% of the winter mean) has clearly increased during the last 30 years. Figure 2B shows that from 1940 until the mid 1960's no flood events were recorded, while since then the frequency of flooding events has increased substantially. For instance, during the winter of 2000/01 precipitation reached unprecedented high values, especially for central Portugal (2000/01: 1802.1 mm against a mean annual value for 1961 to 1990 of 1016 mm), causing a large flood (Fig. 2C).

The Mondego estuary is a warm temperate coastal system in a region with a typically Mediterranean temperate climate. It shows a clear seasonal pattern of precipitation throughout the 10-year study period, with higher rainfall periods during winter. However, comparing the Mondego scenario with the mean precipitation regime for central Portugal for the period of 1961-1990 (winter: 406 mm, spring: 257 mm, summer: 79 mm, autumn: 272 mm; IM – Portuguese Weather Institute, *http://web.meteo.pt*), three winters of above-average precipitation (1993/1994, autumn: 593 mm, 1995/1996, winter: 670 mm and 2000/2001, winter: 767 mm) are apparent. In addition, 2000/01 was even more atypical than 1993/94 and 1995/96, since it was characterized by long periods of intense flooding (Fig. 2C).

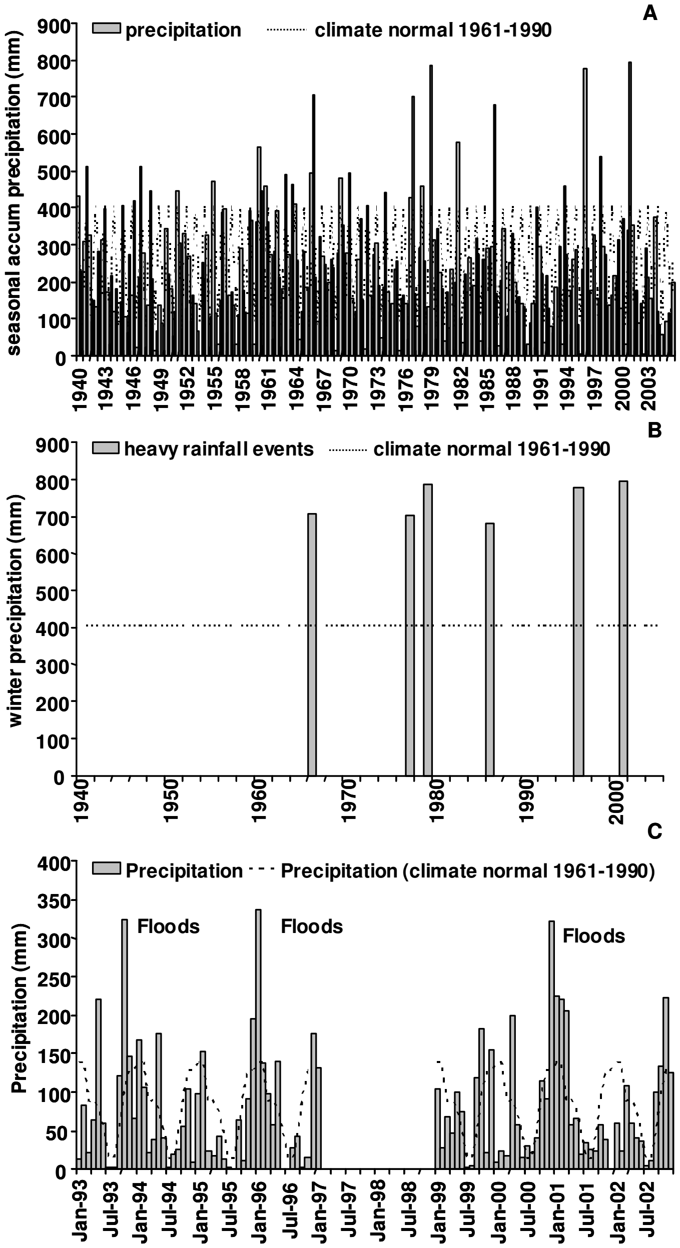

Fig. 2 – Long-term variation in precipitation. (A) Seasonal accumulated precipitation for the centre of Portugal from 1940 to 2005. (B) Frequency of flood events (assuming values in excess of 50% of the winter mean), for the centre of Portugal from 1940 to 2005. (C) Monthly precipitation compared to the climate normal 1961 – 1990 for the centre of Portugal.

MACROBENTHIC ASSEMBLAGE DYNAMICS

CHANGES IN DENSITY AND BIOMASS:

In the *Z. noltii* bed, during the pre-restoration period there was a general increase in macrobenthic biomass, whilst density showed annual cyclic oscillations (Fig. 3A). Significant differences in density and biomass were recorded between the pre- and post-restoration periods (density, Wilcoxon two sample test, W= 1377, P< 0.05; biomass, Wilcoxon two sample test, W=1438, P< 0.05).

In contrast, in the eutrophic area, both density and biomass showed seasonal fluctuations over the pre-restoration period that could be related to algal blooms which occurred during that period (see Cardoso et al., 2005 for details) (Fig. 3C). Significant differences in density and biomass were observed between the pre- and post-restoration periods (density, Wilcoxon two sample test, W= 1491, P< 0.05; biomass, Wilcoxon two sample test, W= 558, P< 0.05).

The intermediate area displayed intermediate trends and patterns (Figure 3B). In the post-restoration period, recovery of biomass was greater and faster than the recovery of density (Fig. 3 A-C). However, this recovery phase was affected by the extreme precipitation event which occurred during the winter of 2000/2001. During this period a decline in total density and biomass of the macrobenthic community was apparent, just when it seemed to be recovering.

TROPHIC GROUPS

Preliminary analyses which included *Hydrobia ulvae* were difficult to interpret, due to the masking effect of the large number of individuals of this species. Thus, the most representative groups were detritivores and herbivores, due to the dominance of H. ulvae, with other groups comprising only a small fraction of the community (Figure 4 A, I, III, V).

Analysing in detail the detritivore assemblage, surface-deposit feeders (SDF) were the dominant group in all the three study areas. In addition, in the *Z. noltii* bed after the flood peak (December 2000) there was a large decline in subsurface-deposit feeders (SsDF) and an increase in surface-deposit feeders (SDF) (Fig. 4A, II). In the eutrophic area, there was a greater variability in the trophic structure from 1998-2002 compared to

1993-1995 period. This was particularly evident for suspension feeders (SuF), such as *Scrobicularia plana*, which were strongly affected by the large floods of 2000/01 (Fig. 4A, VI). When *H. ulvae* are excluded from the analyses, macrofaunal communities continue to be dominated by detritivores, which together accounted for more than 90% of the total macrobenthic abundance (Fig. 4B, I, III, V). However, this analysis revealed a higher variability of the other trophic groups over time.

For the *Z. noltii* bed during 2001, there was a marked decline in the percentage of detritivores, followed by a large increase in omnivores (e.g. *Hediste diversicolor*) (Figure 4B, I). Within the detritivores, surface-deposit feeders declined from 1993-1999, following the decline of the seagrass *Z. noltii* and started to increase again in 2001/2002.

In contrast, subsurface-deposit feeders (mainly small polychaetes) showed the opposite pattern, increasing from 1993-2000, declining abruptly after the floods of 2000/2001, and starting to recover in 2002 (Fig. 4B, II).

The eutrophic area had a different trophic structure compared to the seagrass bed. There was a gradual increase in subsurface-deposit feeders over the 10-year period and a decline in surface-deposit feeders, except for 2001 where there was a slight increase. In addition, suspension feeders increased in 1999/2000, but during the floods of 2000/2001, this group suffered a marked reduction, recovering over the following year (Fig. 4B, VI).

The trophic structure of the intermediate area was much more erratic over time, but there was a clear increase in the percentage of omnivores, coincident with a decline in detritivores (Fig. 4B, III).

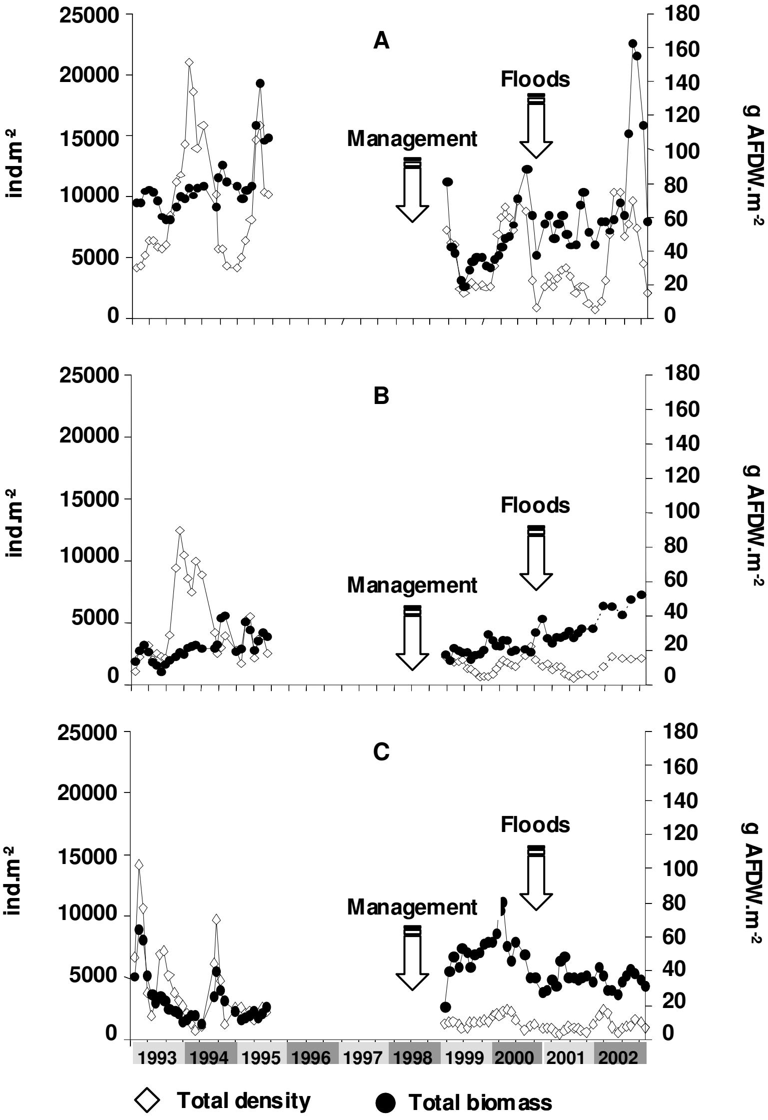

Fig. 3 – Variation of density and biomass of the total macrobenthic community from 1993 to 2002. (A) *Zostera noltii* bed; (B) intermediate area; and (C) eutrophic area.

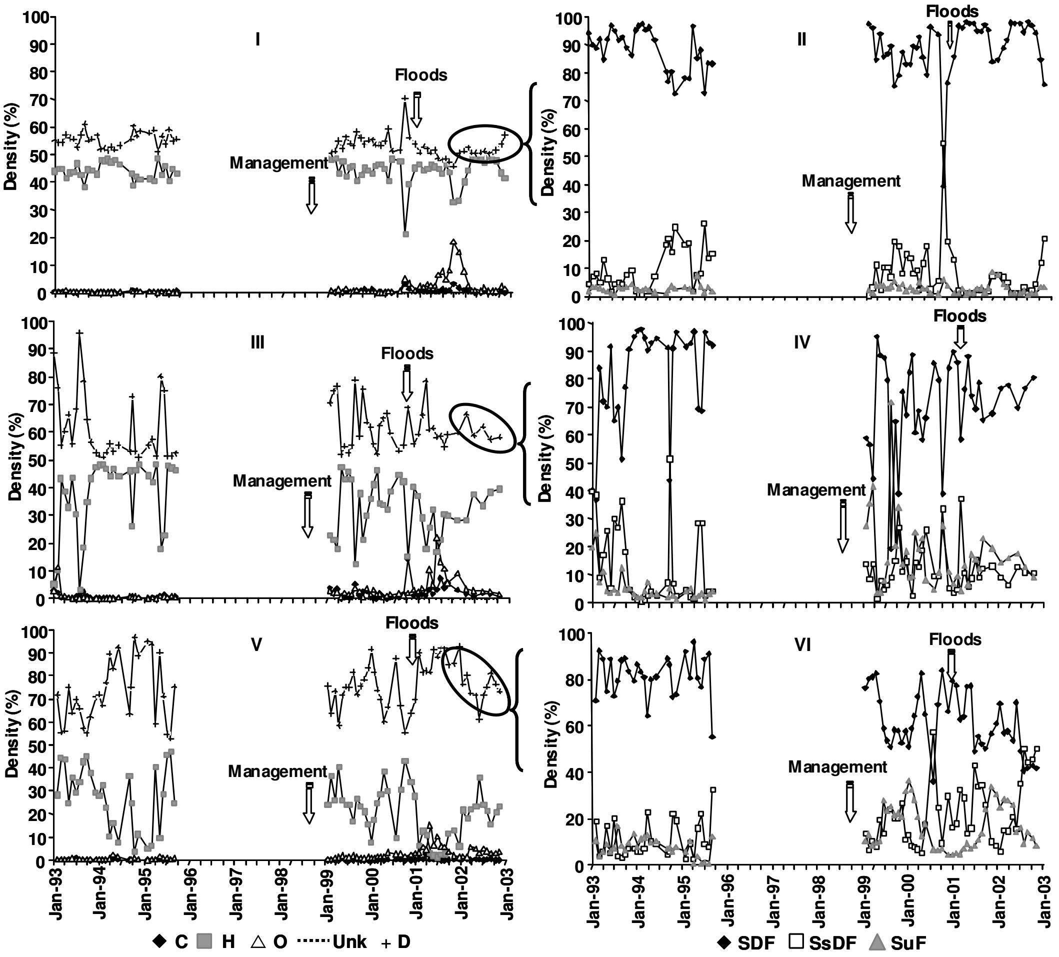

Fig. 4. - Benthic assemblages of the Mondego estuary represented by trophic groups: surface deposit feeders (SDF), subsurface deposit feeders (SsDF), suspension feeders (SuF), carnivores (C), herbivores (H) and omnivores (O). Values are percentages of total individuals. (A) In the presence of *Hydrobia ulvae*; and (B) in the absence of *Hydrobia ulvae*, I, II – *Zostera noltii* bed; III, IV – Intermediate area; V, VI – eutrophic area.

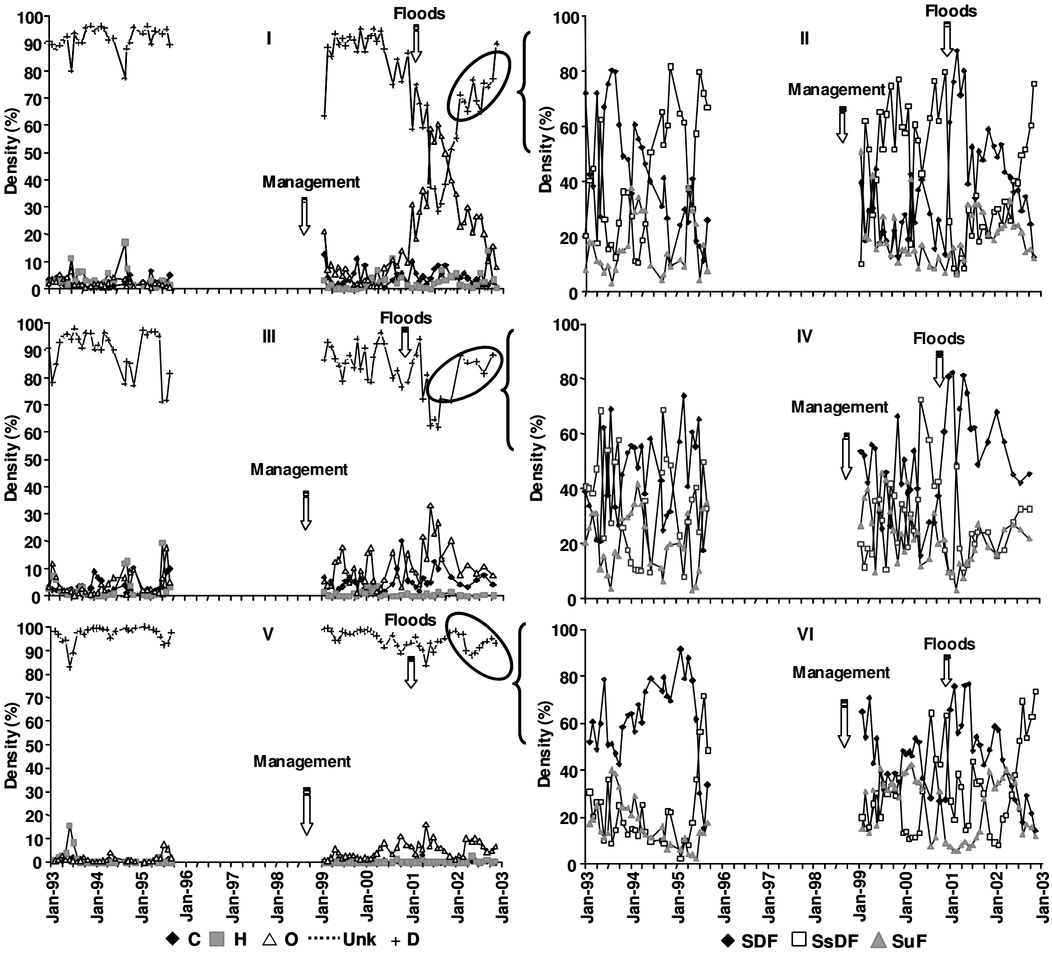

Fig. 4 (continued).

CHANGES IN DIVERSITY

During the period 1993-2002 distinct changes in the structure of the macrobenthic communities were observed. More species were present from 1993-1995 compared to after the later period, and the seagrass bed generally supported more species than the intermediate and the eutrophic areas (Kruskal-Wallis test, H = 117.67, P< 0.05) (Fig. 5). Richness declined during the first three years of study for all the three stations (Fig. 5, Table 1). Following introduction of the restoration plan in 1998, species richness of the *Z.*

noltii bed and of the intermediate area started to increase and became more similar until the winter of 2000/01. At the end of 2000/01 there was an unprecedented high rainfall in December and January that caused the largest Portuguese flood of the twentieth century, consistent with observations by the Portuguese Weather Institute (IM - http://web.meteo.pt/pt/clima/clima.jsp) that climate in the region has become more extreme in recent times compared to period 1961-1990. Significant differences in species richness were recorded between the pre- and post- 2000/01 floods (Seagrass area, Wilcoxon two sample test, W= 2427.5, P< 0.05; Intermediate area, Wilcoxon two sample test, W= 2017.5, P< 0.05; Eutrophic area, Wilcoxon two sample test, W= 2197, P< 0.05). After this extreme event, species richness only started to recover again in 2002 (Fig. 5A, Table 1).

Table 1 – Total species richness for the three study areas and mean annual biomass for the entire estuary over a 10-year period

	Nº of species (Zos)	**Nº of species (Int)**	**Nº of species (Arm)**	**Mean Biomass**	**Events**	
1993	**36**	**30**	**27**	**38.7**		Macroalgal Bloom
1994	**24**	**18**	**15**	**37.9**	**Eutrophication**	
1995	**22**	**17**	**12**	**46.6**		Some algae
1999	**16**	**16**	**12**	**32.9**		Management
2000	**18**	**17**	**13**	**43.5**	**Recovery**	**Intense floods**
2001	**12**	**14**	**10**	**40.0**		
2002	**18**	**18**	**15**	**56.3**		Recovery

Evenness was lower in the *Z. noltii* bed due to the dominance of *H. ulvae* at this site, and higher in the intermediate and eutrophic areas (Fig. 5B). Evenness increased over the 10-year period for the intermediate and eutrophic areas, showing significant differences between the pre- and post-restoration periods (Intermediate area, t-test, t70= -6.41, P< 0.05; Eutrophic area, t-test, t70= -7.21, P< 0.05).

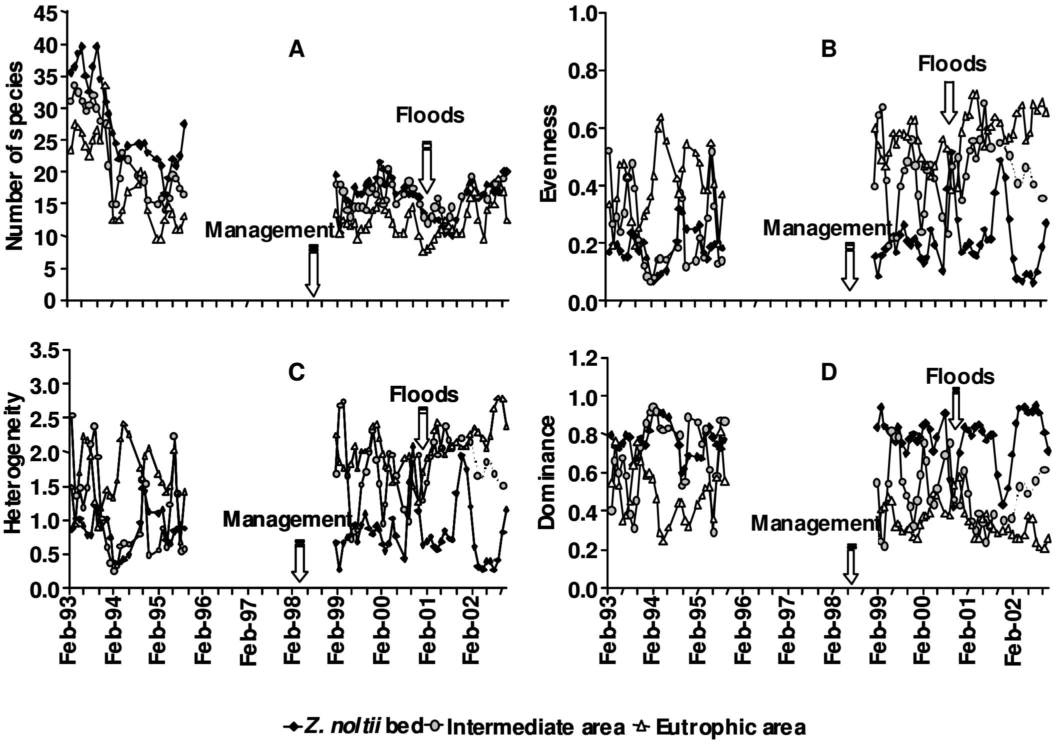

Fig. 5 – Variation of the biological indices in the three sampling stations from 1993 to 2002. (A) Number of species; (B) evenness; (C) Shannon – Wiener index; and (D) Simpson index.

The seagrass bed showed the most stable pattern over time. Diversity as measured by the Shannon-Wiener index followed a pattern similar to evenness, with Simpson's D (dominance) showing the opposite, as expected (Figures 5C and D). For the intermediate and eutrophic areas dominance tended to decrease over the 10-year period.

PRC ANALYSIS

For the pre-restoration period, PRC analysis revealed a clear spatial gradient over time 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). In the post-restoration period, the seagrass bed and the intermediate area converged to the reference until the end of 2000 (Fig. 6). The effect of the intense floods on the macrofaunal community are clearly seen in the PRC analysis, with two tentative recovery periods following the introduction of management measures, the first at the beginning of 2000 and the second in 2002 after the occurrence of the 2000/2001 floods. 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) and only 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 ($P < 0.05$) 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) (Figure 6), consistent with the premise that small deposit-feeding polychaetes increase in eutrophic conditions (Pearson and 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 the 1993 samples from the *Z. noltii* bed are separated from those of the subsequent years. The communities of the seagrass bed and intermediate area from 1999 to 2001 are closer than at the beginning of the study period, indicating a higher faunal similarity between them at this time. In addition, samples from the eutrophic area (2001) are quite isolated from the others, probably because the floods caused a strong impact on the community, specifically on suspension feeders. Samples from the intermediate and eutrophic areas showed the greatest scatter in the MDS plot, indicating more heterogeneity in time and space, perhaps reflecting less stability 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 ($R= 0.677$, $P= 0.001$).

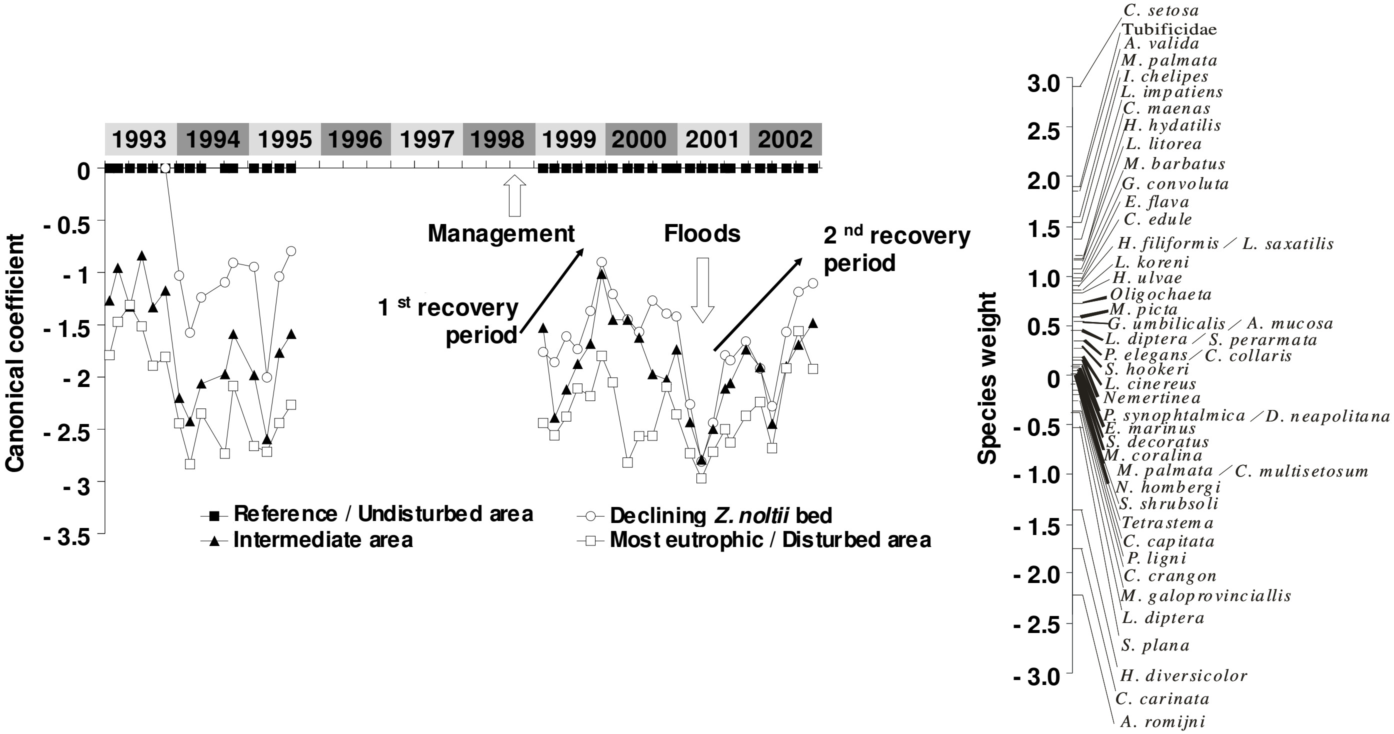

Fig. 6. – Principal Response Curves (PRC) diagram showing the response of macrobenthic communities to different degrees of organic pollution/disturbance with species weights indicating the relative contribution of individuals' species to the community response.

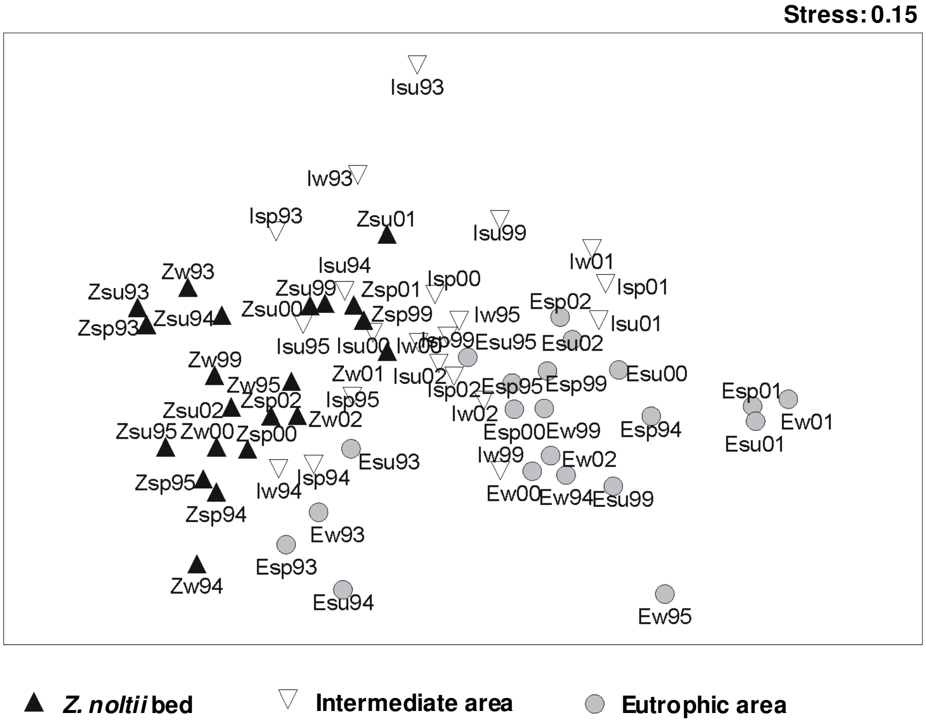

Fig. 7 – Two-dimensional MDS ordination plot of macrobenthic communities. (Z) – *Z. noltii* beds; (I) – intermediate area; (E) – most eutrophic area; (w) – winter; (sp) – spring; (su) – summer.

DISCUSSION

The environmental changes that occurred in the Mondego estuary during the last 20 years are reflected in the macrofaunal assemblages of the estuary which showed signs of recovery after the implementation of restoration measures. However, the recovery process after the management was not linear. The compounding (interacting) effect of the 2 major stressors (eutrophication and flooding) seems to have had a significant negative impact on the recovery ability (resilience) of the macrofauna. On the basis of the analyses reported here, we postulate that the loss of species and reduced performance of individuals following the first stressor (eutrophication) may have contributed to a decline of resilience of the macrobenthic assemblages to a second stressor (flooding), thereby slowing the recovery process. As the interactions between multiple stressors and the severity of the individual stressors may increase in the future due to climate change, marine systems are likely to become increasingly less resilient to their effects.

The temporal and spatial trends and patterns seen in these data are consistent with trends and patterns reported elsewhere (Savage et al., 2002). For example, over the period leading up to the introduction of the restoration plan, the assemblage in the area least affected by eutrophication, the seagrass bed, increased in biomass, coupled with an increase in opportunistic taxa, such as small deposit feeding polychaetes (mainly *Alkmaria romijni* and *Capitella capitata*) (see Cardoso et al., 2007 for more detail). In contrast, in the most eutrophic area organic enrichment from algal blooms (in 1993 and 1995), led to a greater instability of the habitat and consequently to cyclical oscillations in biomass and density of the macrobenthic assemblages.

During the post-restoration phase, recovery in biomass was greater than the recovery in density for all the three study sites, since there was an increase in longer-lived, large bodied taxa (e.g. *Hediste diversicolor* and *Scrobicularia plana*) which contributed significantly to biomass (Table 1). Furthermore, analysis of the macrobenthic assemblages revealed much less variation during this period due to the absence of algal blooms in the estuary since 1995. However, the recovery phase was significantly affected by the extreme flooding events, slowing the system’s return to its previous state. The macrobenthic assemblage at the eutrophic site appears less resilient than that in the seagrass bed, indicated by the longer return time to pre- flood event structure and composition (Fig. 3). The assemblage in the seagrass site also appears similarly less resilient to flood events following the earlier eutrophication period.

With respect to trophic groups, macroinvertebrate assemblages were dominated (90%) by deposit-feeding species, indicative of the major role of detritus at the three sites. In addition, the seagrass bed supported a higher percentage of carnivores, herbivores and omnivores than the eutrophic area, which makes the latter less functionally rich. The 2000/2001 floods had a major impact on this community, especially on the subsurface-deposit feeders and suspension feeders (e.g. *S. plana* and *C. edule*), probably through the clogging of the feeding structures of these suspension feeders by the high turbidity (Norkko et al., 2002). Both these bivalves are economically important for the region, especially for the local fishermen who depend on estuarine resources directly. Furthermore, there is a suggestion of replacement of trophic groups (detritivores by omnivores) in the *Zostera* bed and intermediate area.

Comparing our study with other similar works in which was evaluated the effect of flood events on the macrobenthic communities (e.g. Norkko et al., 2002, Salen Picard and Arlhac, 2002 and Salen Picard et al., 2003) we can conclude that different communities and habitats may respond differently to flooding events, depending on the ecology and feeding habits of the species. Some benthic communities may suffer deleterious effects due to catastrophic terrigenous clay deposition, which lead to anoxic conditions (Norkko et al., 2002) while other communities dominated by opportunistic species may be beneficiated with floods (Salen-Picard and Arlhac (2002) and Salen-Picard et al., (2003)). The floods could act on the different components of the food web as pulses of organic matter leading to an increase of surface- and subsurface-deposit feeders' assemblages.

In addition to changes in the relative abundance of individual taxa, consistent with patterns and trends seen elsewhere (Valiela, 1995), community-level attributes also responded to management. Following the decline in species richness during the eutrophication period (also observed in other systems: Lardicci et al., 2001; Hyland et al., 2005), the affected areas started to increase in diversity following the introduction of the management regime, mainly through changes in evenness and dominance (Fig. 5), and to a more limited extent in species richness. In comparison with the Orbetello lagoon, Tyrrhenian coast, Italy, (Lardicci et al., 2001) the time scale of these responses were slower in the Mondego, and more similar to that observed in Alewife

Cove, Connecticut, US, by Zajac and Whitlatch (2001). However, the latter study also revealed a decline in the abundance of organic enrichment indicator species (e.g. *Capitella capitata*) and an increase in species richness after implementation of the restoration programme.

In the Mondego the response of the macrozoobenthic community was slower than at Alewife Cove, probably due to the combined effects of the multiple stressors described above. In summary, this study has shown that heavy flooding in the Mondego region during the process of recovery eutrophication had severe effects on these assemblages, effectively re-setting the recovery clock and slowing the overall return to the undisturbed state. This not only has implications for biodiversity conservation on the Mondego, but for the livelihoods of the people who depend on the estuary.

Thus, fish farms were directly affected due to the low salinities recorded over several consecutive months, which led to high fish mortality. Also, local fishermen that exploit the estuary mudflats directly were also affected because commercially important species such as *Scrobicularia plana*, *Cerastoderma edule* declined dramatically after the floods. Extreme weather events will become more frequent in the future and the ecosystems, and the goods and services they provide, risk never recovering fully if there resilience is been reduced by other stressors, such as pollution. Estuarine management needs to be more holistic and recognise the importance of such interactions between different stressors (Vinebrooke et al., 2004).

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LONG-TERM RESPONSES OF TWO INFAUNAL BIVALVE POPULATIONS (*SCROBICULARIA PLANA* AND *CERASTODERMA EDULE*) TO ANTHROPOGENIC AND NATURAL STRESSORS IN THE MONDEGO ESTUARY (PORTUGAL)

ABSTRACT The Mondego, as other estuaries is a highly productive ecosystem, providing essential ecological functions, services and being an important habitat to several species. Additionally, it is also very important to local human populations, which explore its economic valuable biological resources, such as the bivalves *Scrobicularia plana* and *Cerastoderma edule*. This ecosystem has been under severe ecological stress over the past 20 years, due to an anthropogenic related eutrophication problem, which has led to a decline in the overall environmental quality. Moreover, several extreme weather events (e.g. floods, drouhts, heat waves) have occurred, as a result of the ongoing global climate change. These stressors usually interact, impacting local biota through complex processes, which can lead to changes in abundance, diversity and fitness of individuals, populations and communities, being its impacts difficult to predict. On this study, population dynamics and production of the two main infaunal bivalve populations are analysed and compared, on two distinct areas – a seagrass bed and an eutrophic bare bottom, of the Mondego estuary, over a 13-year period, characterised by different ecological scenarios (e.g. eutrophication; restoration; extreme weather events). Clear differences were found between the two areas, with stable populations of both species on the seagrass bed, while on the eutrophic area, the *Scrobicularia plana* population was clearly dominant. During the eutrophication period this species declined in both areas, recovering as a result of the restoration process, while *Cerastoderma edule* shows sparse populations on both areas after the restoration process. Nevertheless with the successive occurrence of natural extreme events, *Scrobicularia plana*'s recovery trend is interrupted and this population appears to decline in the bare bottom. Instead, the *Cerastoderma edule* population appears to be favoured, showing abundance, biomass and production increase in 2004 and 2005.

INTRODUCTION

Estuaries are among the Earth's most important environments, both ecologically and socio-economically, with huge importance to several human activities. They constitute valuable resources for agriculture, fisheries, navigation routes, industry settlement and recreational purposes (Kennish, 2002; Paerl, 2006). Many of the most important industrial, commercial and highly densely populated urban centres have been established, for many centuries, near estuaries along coastlines all over the world. Its global economic value has been estimated on ~US$ 15-20 trillion y^{-1}, through food production, nutrient recycling or recreational purposes (Hays et al., 2005; Harley et al., 2006). Moreover, these natural ecosystems are usually highly productive (Kennish, 2002; Dolbeth et al., 2007; Paerl, 2006), providing essential ecological functions (decomposition, nutrient cycling and flux regulation of water, particles and pollutants) and services, such as habitat, protection, food for migratory and resident species, many of them of high economic interest (Boese, 2002; Hiddink, 2003).

As transitional areas between land and sea, estuaries are subjected to a wide variety of anthropogenic stressors, such as pollution and eutrophication, resulting from urban, agricultural and industrial effluents (Lillebø et al., 2005; Paerl, 2006) which can damage the health and fitness of the resident organisms. In addition, the ongoing climate change phenomenon and the consequent global warming impact these areas through sea-level rise, and episodes of extreme weather events including floods, droughts and heat waves (Lawrence and Soame, 2004; Beukema and Dekker, 2005; Epstein and Mills, 2005).

Global warming is considered one of the most important environmental problems the world faces, magnified by anthropogenic climate changes. In fact, over the last few centuries, human activities such as industry, combustion of fossil fuels and widespread deforestation, have caused a significant increase in the atmospheric concentration of the main greenhouse gases (Short and Neckles, 1999; Simas *et al.*, 2001; Houghton, 2005; Harley *et al.*, 2006), resulting in an accelerated warming of the Earth's surface, sea level rise and increased climate variability, with severe impacts to both mankind and natural ecosystems (Short and Neckles, 1999; Simas *et al.*, 2001; Houghton, 2005; Epstein and Mills, 2005; Harley *et al.*, 2006; Paerl, 2006).

The combined effects of all of these stressors are difficult to predict, impacting resources through single, cumulative or synergistic processes, lowering the overall system stability (Vinebrooke et al., 2004; Adams, 2005; Cardoso et al., 2005; Dolbeth et al., 2007). The consequent responses of biota are the integrated result of both direct and indirect processes which can manifest as changes in abundance, diversity and fitness of individuals, populations and communities (Adams, 2005). The accelerating rate of biological impoverishment may render ecosystems incapable of compensating for the loss of biodiversity, thereby reducing their resilience to environmental change (Vinebrooke et al., 2004). Distinguishing and integrating the effects of natural and anthropogenic stressors is an essential challenge for understanding and managing coastal biotic resources (Vinebrooke et al., 2004; Paerl, 2006).

The Mondego estuary (Southern Europe – Portugal) has been well documented over the last decades, with several studies focusing on eutrophication, restoration and more recently on extreme climate related events (Cardoso et al., 2005, 2007, 2008a,b; Lillebø et al., 2005; Verdelhos et al., 2005; Dolbeth et al., 2007). This ecosystem has experienced significant eutrophication over the past 20 years, which has led to a decline in the overall environmental quality of the estuary, degradation of water quality and increased turbidity. As a consequence, seagrass beds declined, reducing in extent from 15 ha in the early 1980's to 0.02 ha in the mid-1990's, affecting population dynamics and production of key species and of the entire community (Cardoso et al., 2005; 2008a,b; Verdelhos et al., 2005; Dolbeth et al., 2007). A management plan was introduced in 1998, which included measures to decrease nutrient loading, physical protection of the seagrass bed and improvement of water dynamics (Cardoso et al., 2005, 2007; Lillebø et al., 2005; Verdelhos et al., 2005; Dolbeth et al., 2007), with effective results on the restoration of the ecosystem (Cardoso et al., 2005; Lillebø et al. 2005; Verdelhos et al., 2005; Dolbeth et al., 2007).

Moreover, the climate in Portugal (Southern Europe) has undergone major changes during the last decades, when compared to the general climate patterns for the period 1931-1990, with the occurrence of several extreme climate events, which became more frequent and intense (Miranda et al., 2006) (INAG - Portuguese Water Institute, http://snirh.inag.pt/ and IM - Portuguese Weather Institute, http://web.meteo.pt/pt/clima/clima.jsp). Mean air temperature rose progressively (from 1931 to 2005: + 0.15℃ per decade), and some of the hottest years ever were registered, with the occurrence of heat waves during the summer, characterised by periods of several consecutive days in which the temperature is considerably higher than the monthly average temperature (Diaz et al., 2006; Miranda et al., 2006). Rainfall data for mainland Portugal show an increase in the frequency of heavy rainfall and of the maximum 5-day total precipitation – an indicator of flood producing events (Santos et al., 2002), between 1931 and 2000 and the frequency and intensity of dry years has also increased over the last 30 years (Miranda et al., 2006; Cardoso *et al*., 2008).

The Mondego is very important to the local human populations, which explore its economically valuable biological resources, such as the bivalves *Scrobicularia plana* and *Cerastoderma edule*. Bivalves are among the most productive groups of infaunal organisms (Mistri et al., 2000; Cusson and Bourget, 2005; Dolbeth et al., 2007). They play a key role on the ecosystem, as an essential link between the primary producers and epibenthic consumers, filtering organic matter, purifying the water column and influencing the food availability and energy flow on the entire community (de Montaudouin et al., 1999).

Here, we focus on the population dynamics of the two main bivalve species of the infaunal community of the estuary, in terms of its productivity and economic value. *Scrobicularia plana* is a deposit filter feeder, inhabiting intertidal and subtidal areas, burrowing on mud to muddy sand sediments to a depth of 25 cm. *Cerastoderma edule* is a suspension filter feeder living on intertidal shallow areas, burrowing just below the sediment surface (de Montaudouin and Bachelet, 1996; de Montaudouin et al., 2003).

On this study we analyse the changes on population dynamics and production of these populations on different areas of the Mondego estuary, over a 13-year period, evaluating the ecological responses to environmental changes and its long time variability under distinct scenarios: a) overall environmental quality decline, mainly caused by eutrophication as a result of large amounts of nutrients deriving from urban, agricultural and industrial effluents, causing primary producers' substitution, water quality degradation and increased turbidity (Pardal et al., 2004; Cardoso et al., 2005, 2007; Verdelhos et al., 2005; Dolbeth et al., 2007); b) ecological recovery following the management program implemented in 1998 in order to increase environmental quality by decreasing nutrient loading, protection of seagrass beds and improve water circulation (Cardoso et al., 2005, 2007; Lillebø et al., 2005; Verdelhos et al., 2005; Dolbeth et al., 2007). We intend to assess the long term impacts of extreme weather events on the dynamics and production of two of the most important species on the macrobenthic community, facing different ecological scenarios (eutrophication *vs* management).

MATERIALS AND METHODS

STUDY SITE

The Mondego estuary, is located in a warm temperate region, on the Atlantic coast of Portugal (40º08'N, 8º50'E) and is a small estuary of 8.6 km^2, comprising two arms, North and South, separated by the Murraceira island. The North arm is deeper (4–10 m during high tide, tidal range 1–3 m), highly hydrodynamic and provides the main navigation channel and the location of the Figueira da Foz harbour. The South arm is shallower (2–4 m during high tide, tidal range 1–2 m) and is characterized by large areas of exposed intertidal flats during low tide. Until 1998, the South arm was almost silted up in the innermost areas, and the river outflow occurred mainly via the Northern arm (Fig. 1).

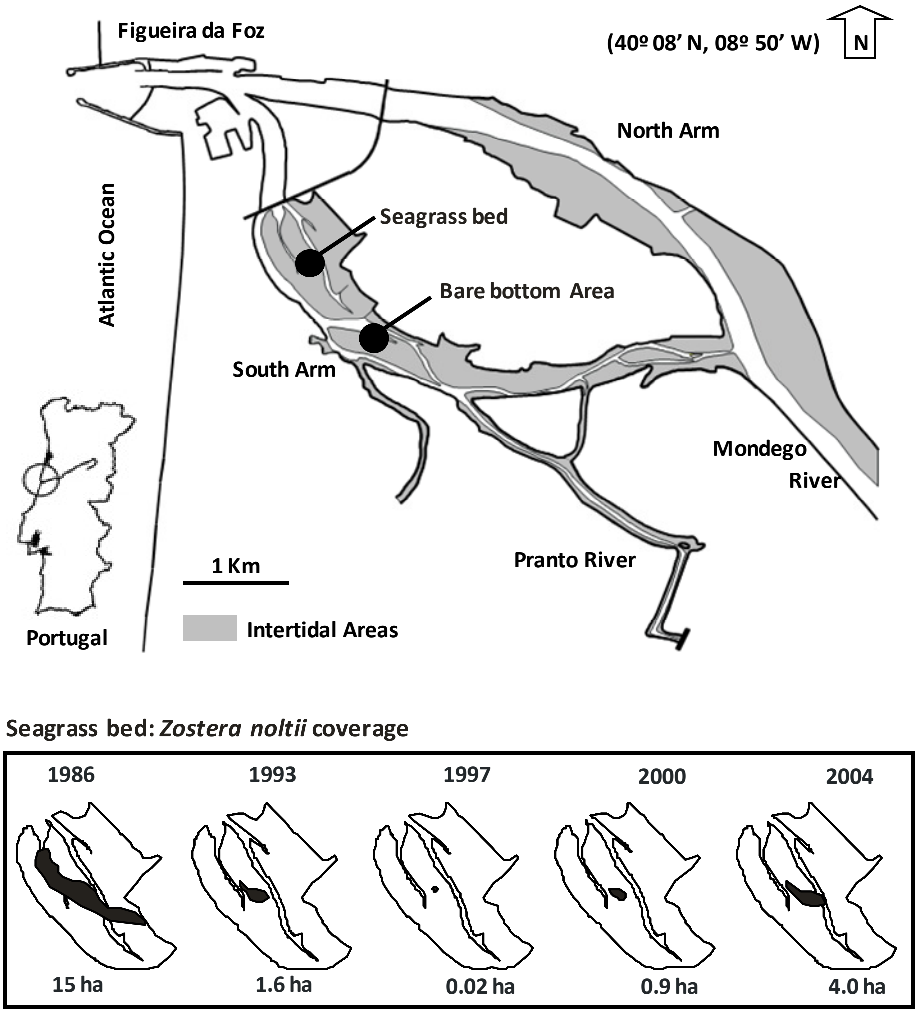

Fig. 1 – The Mondego estuary and sampling stations. The expanded area maps show the evolution of the *Zostera noltii* coverage area.

Water circulation was therefore mostly dependent on the tides and on the freshwater input from the Pranto River, a small tributary with a flow controlled by a sluice, which was regulated according to the water level of rice fields in the Mondego Valley. In the early 1980's, this sub-system showed an extended *Zostera noltii* coverage, however, as the eutrophication increased, together with human disturbance, seagrass declined progressively. In 1998 a restoration intervention improved water circulation and transparency, decreased nutrient loading, mitigate the eutrophication effects, leading to a gradual ecosystem recovery. The implemented measures included: (1) the re-establishment of the South arm riverhead connection, improving the hydraulic regime; (2) most of the nutrient enriched Pranto freshwater is diverted to the Northern arm by another sluice located further upstream, leading to nutrient loading reduction, essentially ammonia (Lillebø et al., 2005); (3) seagrass bed protection from human disturbance; and (4) public education of the ecological importance of intertidal vegetation for health and related socio-economic activities of the estuary.

In these last year's several differences in the climate of Portugal have been recorded when compared to the general climate patterns for period 1931–1990 (Miranda et al., 2006). There was a clear increase of mean air temperature (from 1931 to 2005: +0.15 ºC per decade) and a high variability in precipitation (INAG – Portuguese Water Institute, http://snirh.inag.pt/ and IM – Portuguese Weather Institute, http://web.meteo.pt). For instance, during the winter of 2000/01 precipitation reached unprecedented high values, especially for the central Portugal (2000/01: 1802.1 mm against a mean annual value for 1940 to 1997: 1030.6 mm), causing a large flood (INAG – Portuguese Water Institute, http://snirh.inag.pt/). The rainfall data were obtained monthly from the Soure forecast station (INAG – Portuguese Water Institute, http://snirh.inag.pt/) and an analysis was made from the available information on drought conditions, by constructing a drought index, based on a Decis – classification (http://www.meteo.pt/pt/clima/clima_seca3.html) (Table1), in which rainfall data are divided in 10 equal parts, delimited by 1º decil, 2º decil (…) 10º decil.

Table 1 – Drought Index: Decis Classification

Inter-decis interval	Qualitative designation
1	Extremely dry
2	Very dry
3,4	Dry
5,6	Normal
7,8	Rainy
9	Very rainy
10	Extremely rainy

SAMPLING

The long-term monitoring program in the Mondego estuary has been carried out since the early 1990s, within the scope of European and national projects. Two areas representing different environments along the South arm were sampled: (1) a seagrass bed, characterized by muddy sediments covered with *Zostera noltii*, higher organic matter content (mean 6.2% ± 1.76), and higher water-flow velocity (1.2-1.4 $m.s^{-1}$); (2) a bare bottom, composed by muddy sand sediments, lower organic matter content (mean 3.0% ± 1.14), characterised by lower water flows (0.8– 1.2 $m\ s^{-1}$), which has not supported rooted macrophytes for more than 15 years and has been covered seasonally by green macroalgae (Fig. 1). Sampling was taken in the morning, during low tide, fortnightly for the first 18 months and monthly thereafter. On each occasion 5 to 10 cores corresponding to a total area of 0.0705 m^2 to 0.1410 m^2 were randomly taken to a depth of 25 cm. Each sample was sieved through a 500 µm mesh using estuarine water and then preserved in 4% buffered formalin. At each sampling station, water temperature and salinity were measured directly *in situ* (in low water pools), and sediment was collected for further analysis.

SEDIMENT – GRANULOMETRY AND ORGANIC MATTER CONTENT

The collected sediment was dried (for 72 h at 60 ºC) and the organic matter content assessed after combustion of samples for 8 h at 450 ºC. Granulometry calculated from combusted sediment and classified according to the following nomenclature: Gravel: > 2 mm; 2.0 mm> Coarse sand> 0.5 mm; 0.5 mm> Medium sand> 0.250 mm; 0.250 mm> Fine sand> 0.063 mm; 0.063 mm> Silt> 0.038 mm; Clay< 0:038 mm.

SEAGRASS AND MACROALGAL ANALYSIS

In the laboratory, plant material was sorted and separated into Chlorophyceae, Rhodophycea and *Z. noltii* (leaves and rhizomes). The plant material was dried (for 72 h at 60 ºC) and the ash-free dry weight (AFDW) assessed after combustion of samples for 8 h at 450 ºC.

INFAUNAL BIVALVES – *SCROBICULARIA PLANA* AND *CERASTODERMA EDULE*

Scrobicularia plana and *Cerastoderma edule* individuals were counted and their total length measured. Length-weight relationships were determined for production estimates. For *Scrobicularia plana* the used regression equation was AFDW = 0.00000991 x Total length $^{2.68809}$ (r^2 = 0.97, N = 152). For *Cerastoderma edule* we used AFDW = 0.000040 x Total length $^{2.53969}$ (r^2 = 0.95, N=94). The (AFDW) of each of the individuals used for the regression equations was assessed after combustion for 8 h at 450º C.

Secondary production was calculated as following Brey (2001) method version 4-04 (worksheet provided in Brey 2001, www.awi-bremerhaven.de/Benthic/Ecosystem/ FoodWeb/Handbook/main.htm), used as an alternative empirical method for secondary production estimation (after Cusson and Bourget, 2005; Dolbeth et al., 2007). Mean biomass and $P:\overline{B}$ ratios (annual production divided by the annual mean biomass) were also computed. The $P:\overline{B}$ ratio is the turnover rate of a species' biomass, meaning the amount of time it takes to replace the biomass of its population (McLusky, 1989; Cusson and Bourget, 2005). It is closely related to the species' life span and affected by life history characteristics and potentially also by environmental factors (in a indirect way), being a clear indication of the ecological performance of a population (McLusky, 1989; Cusson and Bourget, 2005). Long lived species will have lower $P:\overline{B}$ than short lived species (McLusky, 1989).

RESULTS

CLIMATE CHANGE

Over the last years, the climate in Portugal has undergone major changes leading to an increase in climate variability from year to year, with the occurrence of several extreme temperature and precipitation events.

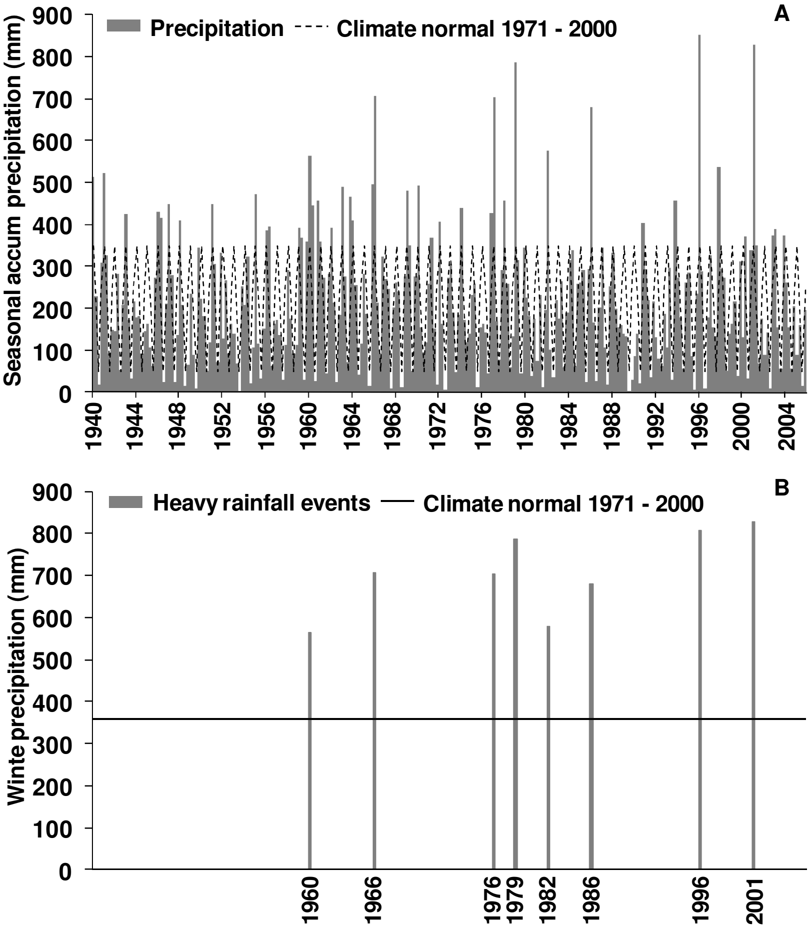

Fig. 2 (a) – Seasonal accumulated precipitation from 1940 to 2005 (A), frequency of flood events (assuming values in excess of 50% of the winter mean), for the centre of Portugal from 1940 to 2005 (B).

Many heavy rainfall events were registered in central Portugal over the last decades, (Fig. 2 A), increasing in frequency and intensity from the mid 1960s to date.

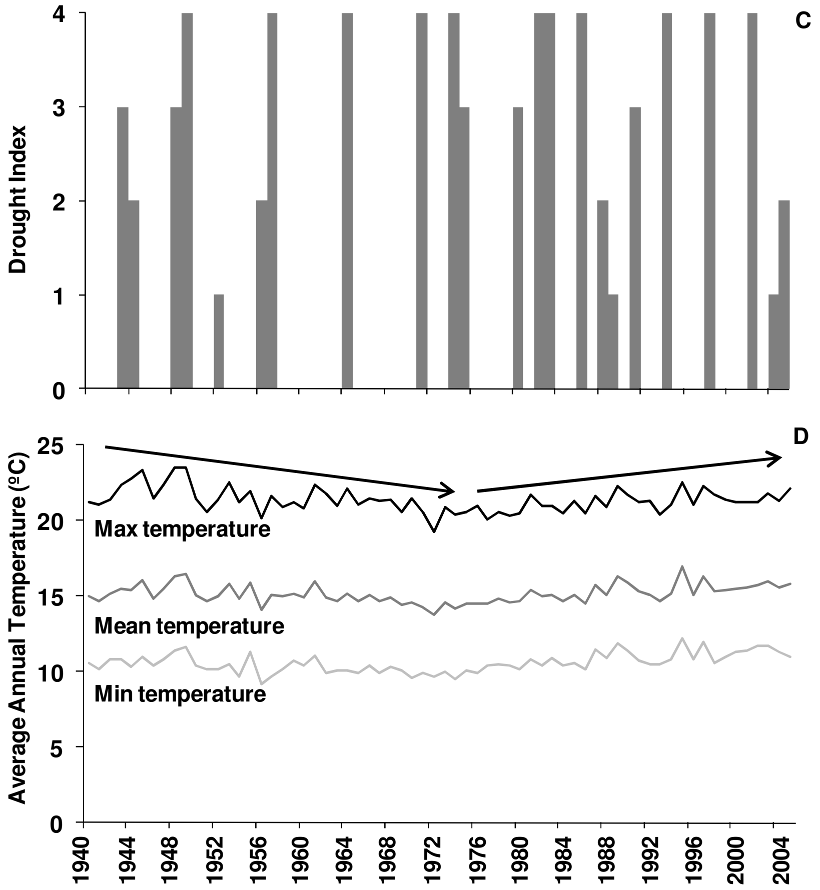

Fig. 2 (b) – Drought Index (C) based on the inter-decis interval for the centre of Portugal from 1940 to 2005 and average annual temperatures (D), for the centre of Portugal from 1940 to 2005.

In fact flood events, here defined as precipitation in excess of 50% of the mean winter precipitation (352 mm), have clearly increased over the last 30 years (Fig. 2 B), reaching unprecedented high values (796 mm) on the Winter of 2001, causing the largest flood of the 20th century (INAG - *http://snirh.inag.pt*). The frequency and intensity of dry years have also increased over the last decades, registering 2 extremely dry years, 2 very dry years and 11 dry years from 1970 to 2005, when compared to the previous 30 years (1940-1970) that showed 1 extremely dry year, 1 very dry year and 6 dry years (Fig. 2 C). Annual average temperatures followed different trends over the last 60 years (Fig. 2 D) decreasing from 1945 to 1972 and increasing from mid 1970s to date, following global warming tendency.

MONDEGO ESTUARY CLIMATE DATA

The Mondego estuary is a warm temperate coastal system showing clear seasonal patterns – cold and pluvious winters, in contrast to hot and dry summers. Nonetheless, considering the normal precipitation for central Portugal during the period of 1971-2000 (winter: 352 mm, spring: 223 mm, summer: 48 mm, autumn: 238 mm), increased variability was registered and some above-mean precipitation was evident (Fig. 3 A). Heavy rainfall events were registered in 1993/1994 (autumn: 593 mm), 2000/2001 (winter: 796 mm) and 2002/2003 (winter: 645 mm). The 2000/2001 hydrological year was particularly extreme, characterised by the occurrence of severe flooding. In addition, intense drought periods were observed in 2001/2002 (winter: 90 mm, spring: 142 mm, summer: 5 mm) and 2004/2005 (autumn: 68 mm, winter: 90 mm, summer: 13 mm). The seasonal pattern of rainfall and the flooding are consequently reflected in the seasonal and inter-annual variation of salinity in the estuary. On the one hand, during periods of intense rainfall, salinity declined dramatically (Fig. 3 B) as seen in winter 2000/2001 reaching < 5 values, while during drought episodes its values were usually high. In 2001/2002, 2003/2004 and 2004/2005 the typical salinity winter decline was lower than usual and a salinity increase trend is observed in 2004 and 2005, as a result of lower precipitation during these dry years. When comparing the two study areas, salinity in the seagrass bed, located downstream, is significantly higher (Wilcoxon two-sample test – W = 12241.5, $P < 0.05$), showing greater influence of seawater entering the estuary during high tides, then in the inner eutrophic area.

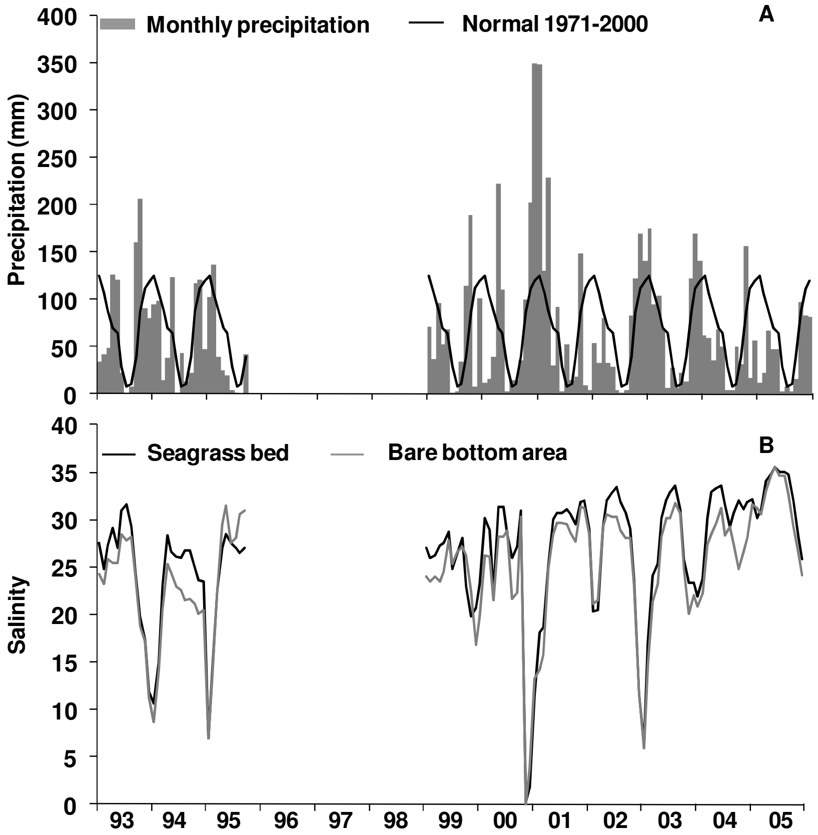

Fig. 3 (a) – Long-term variation in: A) Precipitation, compared to the climate normal; B) Water salinity on the sampling areas.

Temperature also followed a typical seasonal pattern throughout the study period, with lower values registered in winter and increasing towards the summer (Fig. 3 C). However, the increasing temperature tendency of the last decades led to some of the hottest years ever recorded in Portugal – summers of 2003 and 2005, characterised by episodes of considerably higher temperature values than the monthly average temperature. Such air temperature variations will certainly be reflected on the water temperature on the estuary, particularly in intertidal areas during low tides. The registered water temperature on the two studied areas, both seagrass bed and bare bottom area, shows a typical seasonal pattern (Fig. 3 D), with lower values during winter and increasing towards summer, following air temperature regime.

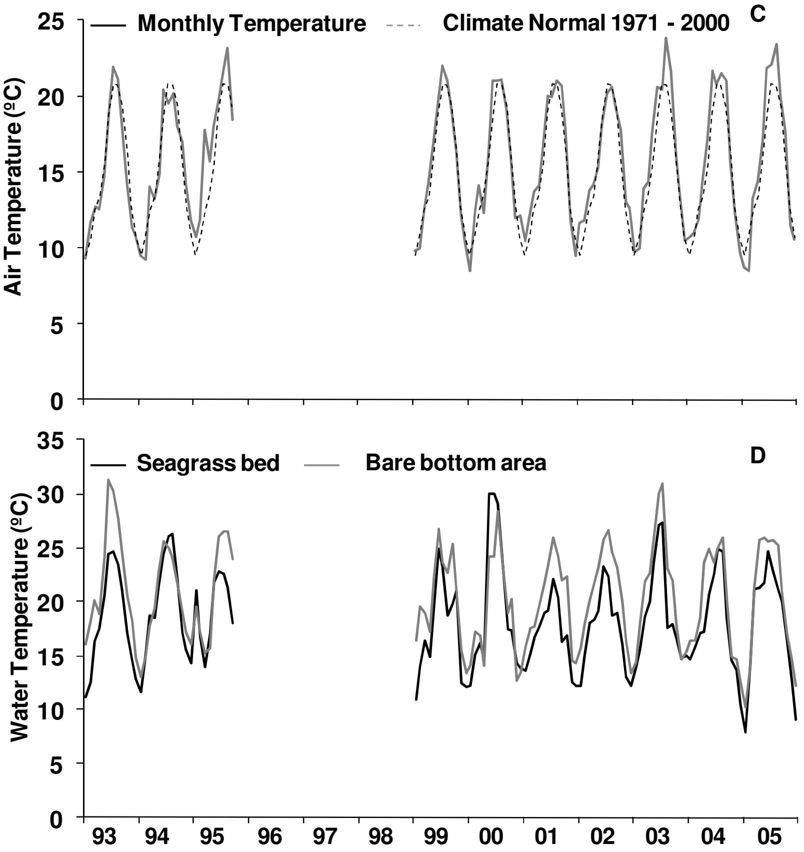

Fig. 3 (b) – Long-term variation in: C) Air temperature, compared to climate normal; D) Water temperature on the sampling areas.

SEDIMENT – GRANULOMETRY AND ORGANIC MATTER

Sediment characteristics were analysed, in terms of granulometry and organic matter content, and differences in the two areas were quite clear. Seagrass bed is characterised by fine sediments (Fig. 4 A), composed essentially by fine sand (73%) and silt (20%), while the eutrophic area (Fig. 4 B) is mainly composed by fine sand (76%) and medium sand (13%).

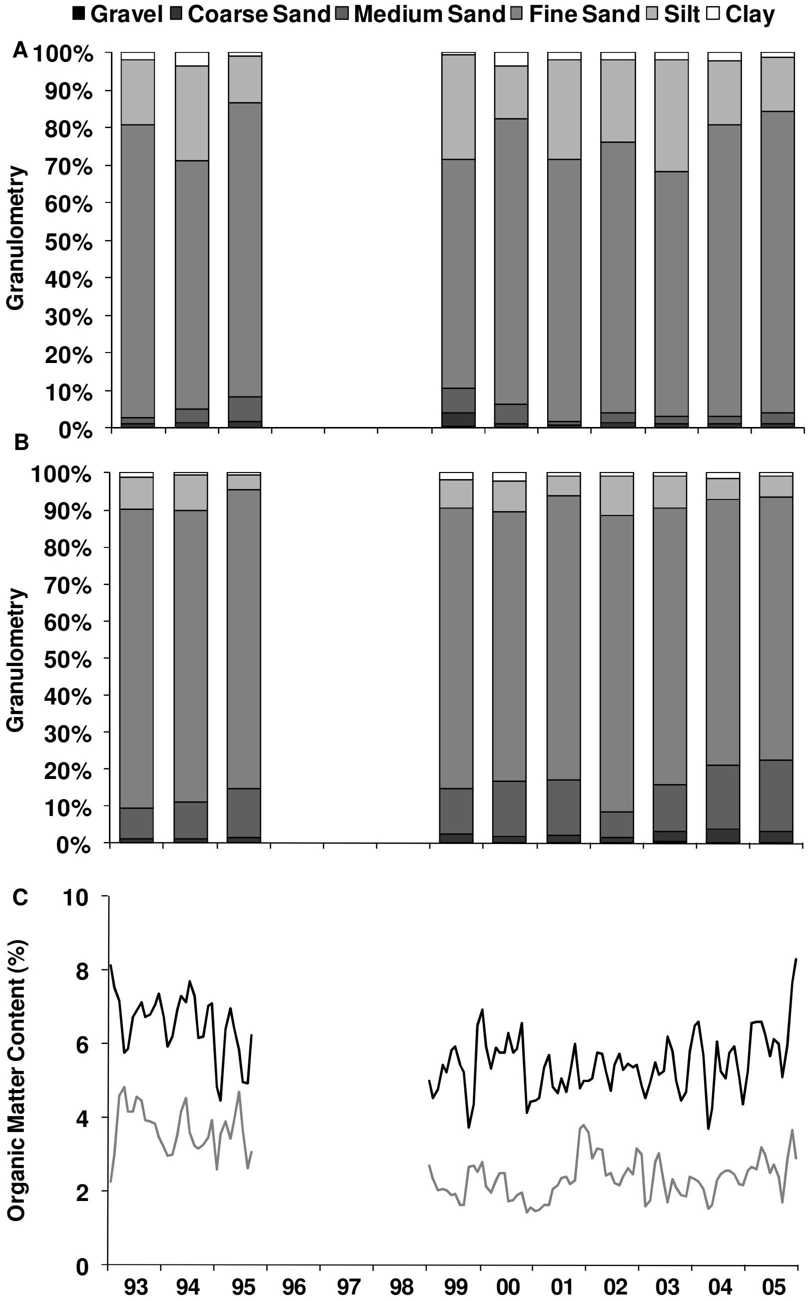

Fig. 4 – Sediment characteristics on the sampling areas. A) Granulometry on the seagrass bed; B) Granulometry on the bare bottom area; C) Organic matter content on the sampling areas.

During the study period, an increase in the proportion of coarse and medium sand is observed from 1993 to 1995 on the seagrass bed, while in the post-management period these values tend to decrease, probably related to the *Zostera noltii* decline in the area from 1993 to 1995 and its latter recovery. On the eutrophic area an increasing trend is observed from 2003 on forth, in the proportion of coarse and medium sand (up to ~22%), while the proportions of silt and clay tend to decrease. Moreover, significant differences were found in the organic matter content between the two areas (Wilcoxon two-sample test, W= 16554.0, $P< 0.05$), with ~6% organic matter on the seagrass bed, in contrast with ~3% in the eutrophic area. From 1993 to 1995 there is an organic matter reduction in the seagrass bed (Fig. 4 C), during the *Zostera noltii* decline period.

ZOSTERA NOLTII AND MACROALGAE

On the seagrass bed, the rooted macrophyte *Zostera noltii* declined sharply from 1993 to 1998, both reducing biomass (Fig. 5 A) and coverage (Fig. 1), following eutrophication. After the introduction of management measures, a gradual recovery has begun (progressive increment on biomass and coverage area). Nevertheless, the occurrence of extreme weather events seemed to affect that recovery. In fact, during the 2000/2001 winter flood a biomass reduction was registered, breaking the increment trend of the previous years. The 2003 hot summer also seems to have a negative impact on the seagrass, causing significant (Wilcoxon two-sample test, W= 40.0, $P< 0.05$) biomass loss during the summer of 2003 (54.73 g AFDW m^{-2}) when compared to the previous year (summer 02: 178.93 g AFDW m^{-2}). The seasonal presence of green macroalgae (Fig. 7 A) on this area is registered, during spring and summer, but with low biomass values (< 10 g AFDW m^{-2}).

In the bare bottom area no rooted macrophytes were found during the study period and seasonal macroalgal blooms were registered in 1993 (341.74 g AFDW m^{-2}) and 1995 (64.92 g AFDW m^{-2}). After the application of management measures, a significant green macroalgal reduction was observed (Wilcoxon two-sample test, W= 2295.5, P< 0.05) and blooms were never registered. (Fig. 5 B).

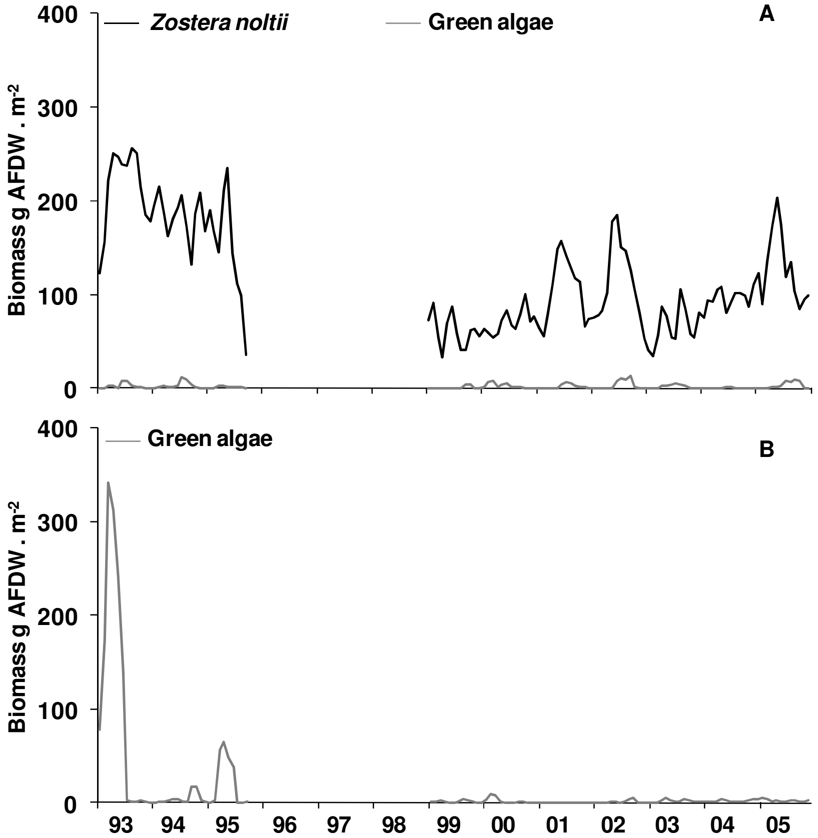

Fig. 5 – *Zostera noltii* and green macroalgae biomass on the sampling areas: A) Seagrass bed; B) Bare bottom area.

INFAUNAL BIVALVES – *SCROBICULARIA PLANA* AND *CERASTODERMA EDULE*

On the seagrass bed, both species showed stable populations at the beginning of the sampling program, considering its abundance and biomass (Fig. 6). A decrement of *S. plana*'s biomass can be observed from 1993 to 1995, despite the occurrence of high density peaks in 1994 and 1995 (Fig. 6 A). On the other hand, *C. edule* showed significantly higher biomass values during this period (Wilcoxon two-sample test, W= 643.0, P< 0.05) and a progressive biomass increment (Fig. 6 B), reaching its highest values in 1995 (31.01 g AFDW m^{-2}).

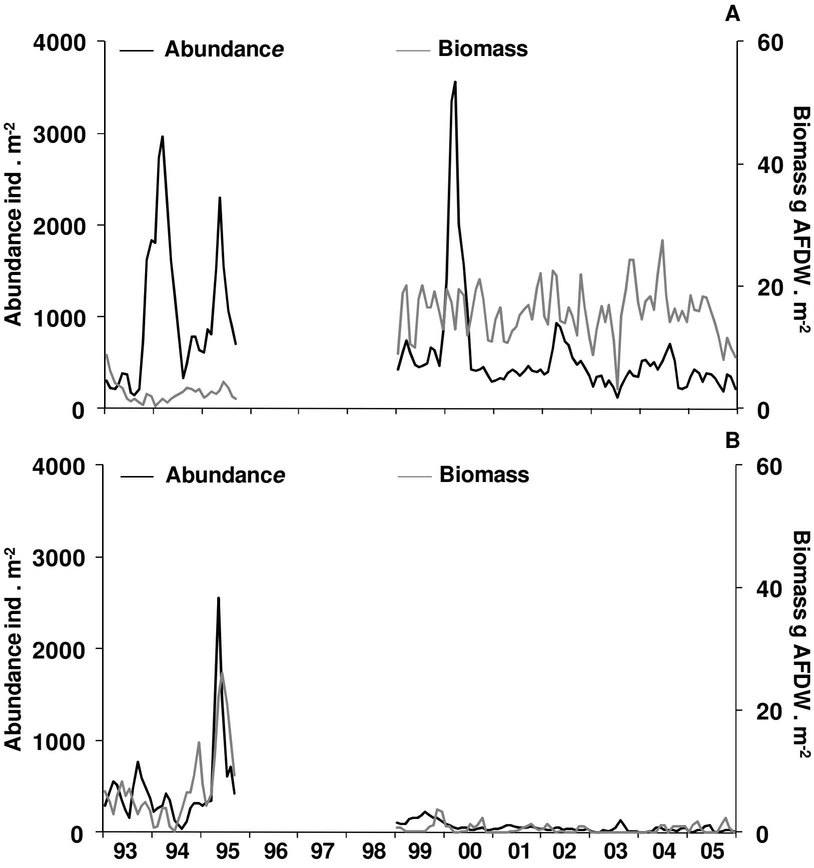

Fig. 6 – Long-term variation on the abundance and biomass of the two infaunal bivalve species on the seagrass bed: A) *Scrobicularia plana*; B) *Cerastoderma edule*.

The estimated secondary production and mean population biomass reflected the dynamics of the two populations and so, lower values were obtained for the *S. plana* population (Table 2), with *C. edule* showing considerably higher production values and mean population biomass rise during this period. After the implementation of the management plan, a total different scenario emerged, with a *S. plana*'s recovery. Significant increases in biomass (Wilcoxon two-sample test, W= 448.0, P< 0.05) were observed, reflecting in production values (Wilcoxon two-sample test, W= 6.0, P< 0.05). In contrast, a decline on the *C. edule* population was observed, with significant reduction in abundance (Wilcoxon two-sample test, W= 2619.0, P< 0.05), biomass (Wilcoxon two-sample test, W= 2538.5, P< 0.05) and production (Wilcoxon two-sample test, W= 27.0, P< 0.05). Consequently, *S. plana* became the dominant infaunal bivalve on this area, with significantly higher abundance (Wilcoxon two-sample test, W= 8896.0, P< 0.05), biomass (Wilcoxon two-sample test, W= 8929.0, P< 0.05) and production (Wilcoxon two-sample test, W= 77.0, P< 0.05).

On the bare bottom area, *S. plana* is clearly the dominant infaunal bivalve species (Fig. 7 A), with significantly higher abundance (Wilcoxon two-sample test, W=16247.0, P< 0.05), biomass (Wilcoxon two-sample test, W=16747.5, P< 0.05) and production (Wilcoxon two-sample test, W=155.0, P< 0.05) values during the whole study period, when compared to *C. edule*, which shows a scarce population characterised by low biomass (Fig. 7 B) and production values (Table 2). In the post-management period *S. plana* recovered, with biomass and production increments, while *C. edule* showed a sparse population, maintaining its low abundance, biomass and production values.

Despite these general trends, the occurrence of episodic extreme weather events seems to affect these populations. During the winter 2000/2001 flood, both species showed secondary production and mean population biomass reduction (Table 2). Later, in 2003 a similar situation occurred, with clear reductions on the *S. plana* abundance and biomass during the hot summer on the seagrass bed and on the bare bottom area, and both species' secondary production and mean population biomass diminished.

Moreover, during the extreme drought in late 2004 and 2005 *S. plana* abundance and biomass were severely affected on the bare bottom area. As a result, the lowest production and mean population biomass of the post-management period were registered. On the other hand, the *C. edule* population showed biomass and production increments on both areas.

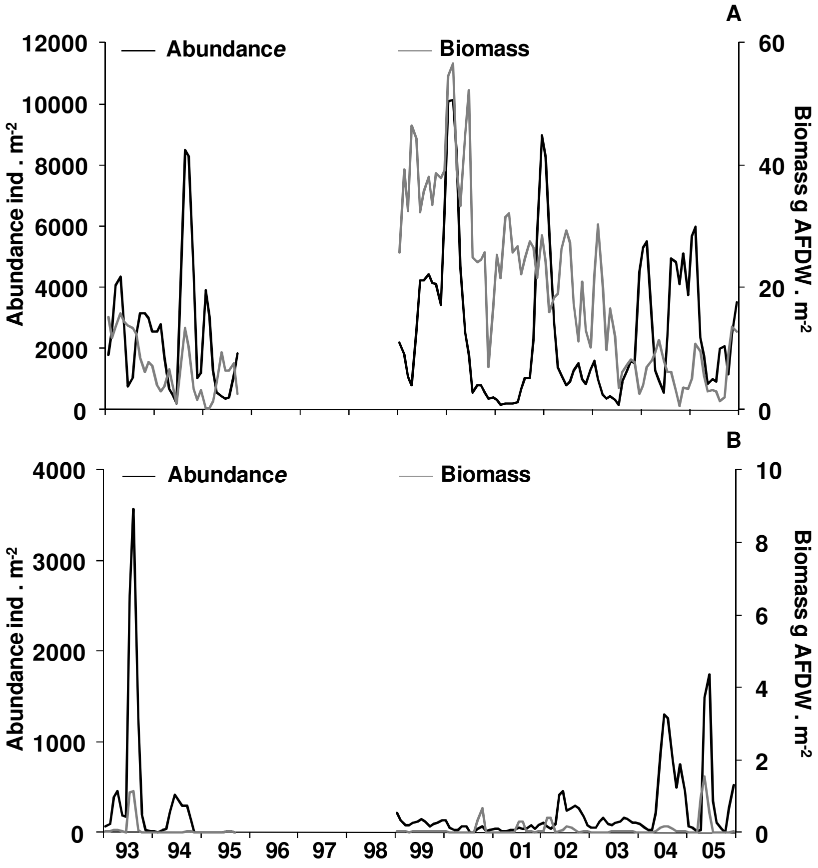

Fig. 7 – Long-term variation on the abundance and biomass of the two infaunal bivalve species on the bare bottom area. A) *Scrobicularia plana*; B) *Cerastoderma edule*.

Table 2 – Annual production (P: g AFDW. m^{-2} .y^{-1}), mean biomass ($\bar{B}$: g AFDW. m^{-2}) and P:$\bar{B}$ ratios (P:$\bar{B}$: y^{-1}) estimates for *Scrobicularia plana* and *Cerastoderma edule* on the seagrass bed and on the bare bottom area.

		Scrobicularia plana			***Cerastoderma edule***		
		P	$\bar{B}$	P:$\bar{B}$	P	$\bar{B}$	P:$\bar{B}$
Seagrass bed	**1993**	3.00	3.36	0.89	4.6	5.33	0.86
	1994	2.88	1.64	1.75	3.15	3.24	0.97
	1995	3.52	2.74	1.29	10.35	10.55	0.98
	1999	10.77	15.80	0.68	1.09	0.97	1.12
	2000	13.88	16.96	0.82	0.68	0.70	0.96
	2001	9.59	14.92	0.64	0.42	0.37	1.11
	2002	12.67	16.59	0.76	0.36	0.44	0.81
	2003	10.91	15.16	0.72	0.04	0.02	2.55
	2004	12.43	17.62	0.71	0.45	0.56	0.81
	2005	9.63	14.09	0.68	1.16	0.68	1.69
Bare bottom area	**1993**	18.42	11.60	1.59	1.16	0.20	5.86
	1994	6.67	4.20	1.59	0.05	0.01	5.58
	1995	6.59	6.30	1.05	0.04	0.01	4.41
	1999	34.09	33.60	1.01	0.10	0.03	3.91
	2000	34.83	36.80	0.95	0.14	0.07	2.15
	2001	24.59	25.48	0.97	0.24	0.06	2.56
	2002	24.75	20.87	1.19	0.27	0.12	2.24
	2003	15.54	14.22	1.09	0.08	0.02	5.28
	2004	9.74	5.97	1.63	0.31	0.06	5.15
	2005	10.24	6.02		0.47	0.26	1.79

DISCUSSION

The studied areas on the Mondego estuary, during this 13-year monitoring program can be considered two distinct existing habitats on the intertidal flats of the South arm, considering its physical and biological characteristics (Leston et al., 2008). The seagrass bed is located downstream, closer to the estuary mouth, more subjected to the marine influence, showing higher water flow velocity and it is characterised by fine muddy sediments, covered by the rooted macrophyte *Z. noltii*. The bare bottom area is characterised by a muddy sand substract, seasonally affected by green macroalgal blooms, located on an inner area of the estuary, with a less energetic hydrodynamics. Throughout the study period the seagrass bed usually showed higher salinity and higher organic matter content on the sediment, when compared to the bare bottom area. Moreover, previous studies revealed higher biodiversity, abundance, biomass and productivity on the seagrass bed (Dolbeth et al., 2007; Cardoso et al., 2008b), certainly related to the *Z. noltii* coverage. Seagrass beds provide essential processes (nutrient cycling, detrital production and export, sediment stabilization) and optimal habitat for growth, survival and reproduction of several macroinvertabrate species (Cunha et al., 2005; Polte et al., 2005), supporting higher species richness and being more productive than bare bottom habitats.

The distribution, dynamics and structure of benthic bivalves is defined by recruitment patterns and success, mortality, migration and dispersion processes, depending on the habitat characteristics, such as substrate type, vegetable coverage, hydrodynamics, food availability and interactions (e.g. predation, competition) with the associated biological communities (Hughes, 1970; Essink et al., 1991; Sola, 1997; de Montaudouin et al., 2003; Casagranda and Boudouresque, 2005; Verdelhos et al., 2005).

The two studied bivalves showed abundant populations on the seagrass bed, while on the bare bottom area we observe a clear *S. plana* dominance, with a sparse *C. edule* population. *C. edule* settlement is usually more effective on sandy substrates (de Montaudouin, 1997; de Montaudoiun et al., 2003), and in fact, the highest registered recruitment peak occurred on the eutrophic area (muddy sand bare bottom) in the summer 1993. Moreover, there is a *C. edule* bed on an intertidal sandflat situated downstream to the seagrass bed, characterised by higher cockle abundance and biomass (Crespo et al., in press). However, bivalves' burrowing behaviour is fundamental on survival as defensive strategy against predation (Hughes, 1970; Lee, 1996), and as *S. plana* usually burrows deeper on the sediment, while *C. edule* lives just below the surface (de Montaudouin and Bachelet, 1996), is expected that the latter suffers higher mortality resulting from predation impacts, in particular on a bare bottom.

From 1993 to 1995 the registered eutrophication process caused the occurrence of green macroalgal blooms, particularly on the bare bottom area – which is therefore also designated as eutrophic area in several studies (Lillebø et al., 2005; Verdelhos et al., 2005; Cardoso et al., 2008 a, b; Dolbeth et al., 2008; Leston et al., 2008), and a reduction of *Z. noltii* biomass and coverage area on the seagrass bed. And as rooted macrophytes are important on sediment stabilization, improving the fixation of fine sediments and organic matter, a decrease on the organic matter content and a slight increase in the proportion of coarse and medium sand on the sediment was observed from 1993 to 1995 on the seagrass bed. Furthermore, severe impacts on the macrobenthic community were also registered (Dolbeth et al., 2007; Cardoso et al., 2008a), reducing its biodiversity and productivity. Negative impacts were also observed in *S. plana* (Verdelhos et al., 2005) that showed biomass and production reduction on both areas during this period. Instead, *C. edule* seems to be favoured on the seagrass bed, increasing its biomass and production. This species appears to take advantage on the generated conditions, which may have favoured it; on the one hand, habitat changes related to *Z. noltii* decline, such as the increase in the proportion of coarse and medium sand on the sediment and increased water turbidity (Lillebø et al., 2005; Leston et al., 2008) may have led to higher successful settlement (de Montaudouin, 1997; de Montaudoiun et al., 2003) and food availability to this suspension filter feeder. On the other, a reduction on the number and abundance of other benthic species may result in less competition for food and space, which may have given an ecological opportunity to *C. edule*. In fact, macrofaunal benthic species co-occur naturally on these ecosystems, establishing inter and intraspecific interactions, such as competition for space or food, and long-term responses in population dynamics, growth and production are expected (Hughes, 1970; Lee, 1996; Casagranda and Boudouresque, 2005; Lefevre et al., 2009; Troost et al., 2009).

The implementation of the management plan led to changes on the ecosystem: hydrodynamism was improved by opening the upstream connection between the two arms (reducing residence time); salinity values became more regular and stable (showing lower oscillations and less drastic declines); nutrient loading was significantly reduced, especially concerning dissolved inorganic nitrogen, due to ammonium reduction (Lillebø et al., 2005); water turbidity decreased; severe algal reduction and *Z. noltii* recovery. This resulted in positive responses on the macrobenthic community (Dolbeth et al., 2007; Cardoso et al., 2008b), leading to an overall ecological improvement of the ecosystem. *S. plana* was also favoured, showing significant biomass and production increments (Verdelhos et al., 2005) in both studied areas. In contrast, the *C. edule* population seemed to be negatively affected, particularly in the seagrass bed, showing clear abundance, biomass and production reduction.

The available climate data for central Portugal reveal an increase in the frequency and intensity of extreme events of temperature and precipitation, with the occurrence of several episodes of flooding, droughts and extremely hot years, altering the system's hydrodynamics, salinity and water temperature (Cardoso et al., 2005) and consequently affecting severely dominant species of the Mondego estuary (Pardal et al. 2000, Cardoso et al. 2005, Verdelhos et al. 2005; Dolbeth et al. 2007). The registered heavy precipitation in winter 2000/2001, caused a severe flood and seem to affect these bivalves, that show abundance, biomass and production decrements. Intense flood may have flushed away a significant part of the population out of the estuary drastically affecting juvenile recruitment. Moreover, high turbidity during flooding may cause the clogging up of the feeding structures of these suspension feeders (Norkko et al., 2002), affecting its performance and survival. It was also observed that high salinity values during 2001/2002 and 2004 and 2005 drought periods, which negatively affected seagrass survival (Cardoso et al., 2008a) and extreme temperatures registered during the summers of 2003 and 2005 may have impacted the biomass and production of *S. plana*, certainly by affecting its health and survival (Guelorget and Mazoyer-Mayère, 1983; Casagranda and Boudouresque, 2005). In fact, during the 2003 summer, one of the warmest on record and considered as a prototype of future summer weather in Europe (Vautard et al., 2007), *S. plana* showed significant declines in biomass, with strong impacts on population structure and abrupt declines in production and mean population biomass.

With the successive occurrence of anthropogenic and climate related stressors, a long-term decline is then observed on the high density dominant *S. plana* population on the bare bottom area. Multiple stressors usually do not operate independently, but often interact to produce combined impacts on biodiversity and ecosystem function (Vinebrooke et al., 2004; Dolbeth et al., 2007) reducing the resilience and resistance of the populations to disturbance (Adams, 2005; Cardoso et al., 2005). The succession of these stressors (e.g. extreme weather events; eutrophication) seems to severely affected *S. plana* population, dynamics and production, compromising the ongoing recovery process in the post-management period and different scenarios emerged on the two sampling areas. In the seagrass bed, biomass and production maintained its values, despite punctual reductions when these stressors occurred, while in the bare bottom area, previously most affected by eutrophication, the population was severely affected, reverting recovery into decline. In fact, the impacts of one stressor seem to lead to a decline of the resilience of the system to additional impacts, slowing the system's return to its previous state, which seems to suggest that consecutive stressors can act synergistically to lower overall system stability.

In contrast, we can observe signals of increasing biomass and production on the *C. edule* population during these years and high density recruitment related peaks are observed on the bare bottom area, denoting higher settlement preference. In fact, from 2003 onwards, the sediment on the bare bottom area seemed to have changed, probably related to the hidrodynamics changes on the system, showing increasing coarser sediments and decreasing fine sediments trend, approaching the granulometric characteristics of the downstream *C. edule* bed, which may favour larval settlement (de Montaudouin, 1997; de Montaudoiun et al., 2003). Moreover, the existence of a high density *S. plana* population may affect the settlement and survival of *C. edule* on this area, by eating larvae and spat (Hughes, 1970; Sola, 1997; Lehane and Davenport, 2004; Casagranda and Boudouresque, 2005; Troost et al., 2009). Filter feeder bivalves do not have feeding selection mechanisms, and so they filter all particles above a certain threshold size, including larvae of others and of its own species (Troost et al., 2009) and consequently, *C. edule* may also benefit from *S. plana* decline.

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CHAPTER 2

THE BIVALVE *SCROBICULARIA PLANA* UNDER DIFFERENT ECOLOGICAL SCENARIOS: A POPULATION DYNAMICS MODEL

A validated population dynamics model for *Scrobicularia plana* (Mollusca, Bivalvia) in a Southwestern European estuary

A VALIDATED POPULATION DYNAMICS MODEL FOR *SCROBICULARIA PLANA* (MOLLUSCA, BIVALVIA) IN A SOUTHWESTERN EUROPEAN ESTUARY

ABSTRACT During a relatively recent intervention in the Mondego River estuary (Portugal), the existing connection between the North and the more eutrophic South arm was enlarged, a nutrient enriched freshwater input was diverted to the North arm and, in addition, the remaining seagrass patches were protected from human activity. System restoration did not involve disruption of the sediment and successfully reduced the eutrophic state of the estuary. This provided an excellent opportunity to test a population dynamics model of a common European estuarine bivalve, *Scrobicularia plana* under pre- and post-management periods. The model simulated the number of individuals in three different sampling stations, before and after system restoration and is regulated by water temperature, salinity and population density. Our analysis indicated that the occurrence of extreme values of the environmental variables has the strongest effect on the model response and possibly on the real system. The model was calibrated and validated with independent datasets and model performance was highest in post management conditions. This corroborates the notion that system restoration was successful and indicates that the system became more predictable after management.

INTRODUCTION

As a response to global human disturbance, in recent years there has been an enormous increase in restoration as a technique for reversing habitat degradation. The general purpose of restoration projects is to help a habitat return from an altered or disturbed condition to a previously existing natural condition (Elliott et al., 2007). In fact, a relatively recent intervention in the Mondego estuary successfully decreased its eutrophic state (Lillebø et al., 2005; Verdelhos et al., 2005), which provided an excellent opportunity to test an ecological model under different scenarios, i.e. pre- and post-management periods.

The Peppery furrow shell (*Scrobicularia plana*) is an important species in the Mondego estuary ecosystem (Marques et al., 1999) as well as in other estuaries (e.g. Hughes, 1970b; Guerreiro, 1998). This bivalve is a long-lived surface-deposit-feeding species, living in muddy to sandy sediments and is tolerant to a wide range of salinity and temperature values (Essink et al. 1991; Sola, 1997; Guerreiro, 1998). Several local predator species, namely birds, include it in the diet (Moreira, 1997; Cabral et al., 1999) but bird density is not high and it is reasonably conservative (Lopes et al., 2005). There is also some occasional recreational capture by local inhabitants.

Long-term data sets 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 span of time is needed to detect changes or trends (Franklin 1989). At this point, for this system, long data series are available and therefore it is possible to extract information and make predictions by using a population dynamics model of *S. plana*. There are some models of mussels, as shown in the review by Beadman et al. (2002), and of oysters (e.g. Hyun et al., 2001; Powell et al., 2002) but these are directed to filter-feeding food-dependent bivalve growth in an aquaculture context. In the same context, the growth of other bivalve species (e.g. Bensch et al., 1992), and oyster population dynamics have also been modelled (e.g. Dowd, 1997; Kobayashi et al., 1997; Oh et al., 2002).

This paper presents a validated population dynamics model for an estuarine bivalve species with a potential economic and social value. The purpose of the model is to provide a tool which will help to understand how *Scrobicularia plana* responds to both natural and anthropogenic environmental modifications (e.g. system restoration). This will be accomplished by the observation of model behaviour, namely changes in population processes and parameters under different environmental conditions.

MATERIALS AND METHODS

STUDY SITE

The Mondego estuary, located on the Atlantic coast of Portugal (40º 08’N, 8º 50’W) comprises a Northern and a Southern arm, separated by the alluvial Murraceira Island (Fig. 1). The Northern arm, where the Figueira da Foz harbour is located, is deeper (4–10 m during high tide, tidal range about 1–3 m) and constitutes the main navigation channel. The Southern arm is shallower (2–4 m during high tide, tidal range 1–2 m) and is almost silted up in the upper zones, constituting a kind of coastal lagoon in which the water circulation is mostly dependant on the tides and on the freshwater input from the Pranto River, a small tributary (Lillebø et al. 1999; Pardal et al. 2000). The discharge from this tributary is controlled by a sluice (Pardal et al. 2000; 2004; Cardoso et al. 2004) and is regulated according to the irrigation needs in rice fields in the Mondego Valley (Martins et al. 2001). The water in this area is highly turbid, with abundant particulate organic matter (median of 4.4 mg L^{-1}), making it unlikely that surface-deposit-feeding organisms such as *S. plana* are controlled by food quantity.

Since the 1980s, *Zostera noltii* beds in the Southern arm have been drastically reduced in areal extent and biomass (Pardal et al. 2004; Cardoso et al. 2004). For instance, an area of 15 ha was progressively reduced to 1.6 ha by 1993 and to less than 300 m^2 by 1997. In 1998, several mitigation measures were applied. The hydraulic regime in the Southern arm was improved by enlarging the connection between the two arms. The Pranto sluice opening regime was minimized in such a way that most of the nutrient’ enriched freshwater from the Pranto River is diverted to the Northern arm (by another sluice located further upstream), reducing the nutrient loading in the Southern 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 and looking for bait), and several forums were run to inform local people of the ecological and economic importance of the seagrass beds.

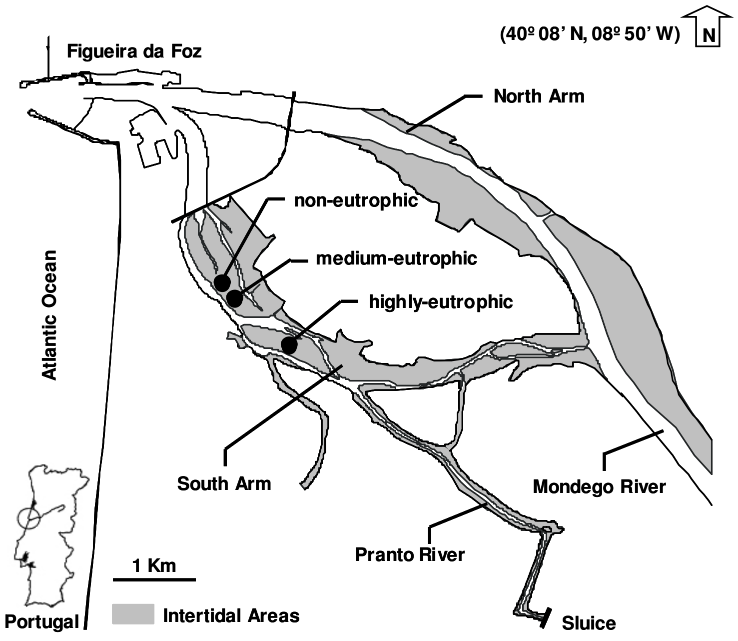

Fig. 1 – The Mondego river estuary, including the sampling areas.

Three study areas (Fig. 1) were established in the Southern arm (Lillebø et al. 2005), along an eutrophication gradient. The first one is a non-eutrophic area (*Zostera noltii* beds) located downstream, characterized by muddy sediments with high organic matter content (6.3±1.5%), higher salinity values (20–30), lower total inorganic nitrogen concentrations (15–30 µmol N.L^{-1}), and higher flow velocity (1.2–1.4 m.s^{-1}). The second is an intermediate eutrophic area, adjacent to the previous one, without seagrass cover, although some rhizomes remain in the sediment. The physico–chemical conditions are otherwise similar to those of the *Z. noltii* beds but with lower sediment organic matter content (5.8±1.3%). Finally the third area is the most eutrophic area, in the inner part of the estuary, characterized by the absence of rooted macrophytes (for more than 15 years) and covered seasonally by green macroalgae (Pardal et al. 2000; 2004; Cardoso et al. 2004). This sand flat has lower organic matter content (3.7±1.0%), lower salinities (15–25), higher total inorganic nitrogen concentrations (30–50 µmol N L^{-1}), and lower water velocities (0.8–1.2 m s^{-1}).

DATA BACKGROUND

The data for this model was obtained from a sampling program carried out from January 1993 to September 1995 and from December 1998 to December 2002 in the three areas (Verdelhos et al. 2005). Between October 1995 and January 1997, only the most eutrophic area was sampled therefore providing a longer data series (January 1993 to January 1997). These 3 areas, each with a pre- and a post-management period, provided 6 different data series that we used in the modelling process. One was used for calibration and 5 were used for validation. In the field, samples were taken fortnightly during the first 18 months and monthly thereafter. At each study area, 5–10 sediment cores of 423 cm^{-2} corresponding to a total area of 0.2115– 0.4230 m^{-2} were taken randomly to a depth of 20 cm. This is within the normal sampling effort for this species and for estuarine benthic macroinvertebrate communities (e.g. Guerreiro 1998; Silva et al. 2006). A thorough description of the methods employed for data collection can be found in Verdelhos et al. (2005). Samples were washed over a 500-µm mesh sieve, placed into plastic bottles and preserved in 4% buffered formalin. On each occasion temperature and salinity were measured *in situ*. Later, in the laboratory, animals were separated and kept in 70% ethanol. *Scrobicularia plana* individuals were counted and its total length measured.

Previously published work statistically demonstrated that after the implementation of management measures, dissolved nutrients and green macroalgal blooms were reduced, seagrass beds started to recover and the *S. plana* population became more structured, with higher biomass and growth production (Verdelhos et al. 2005). Detailed physicochemical and biological data regarding the area during the study period are available in Lillebø et al. (2005). This last study corroborates the effectiveness of the management plan, showing that maximum and mean biomass of green macroalgae was reduced by one order of magnitude, and that the seagrass-covered area and biomass of *Zostera noltii* was recovering.

MODEL STRUCTURE AND EQUATIONS

The model was built using Stella (version 8.1) which is an object oriented-modelling environment. Before giving a detailed explanation of the equations, it is necessary to introduce the reader to some of the Stella functions. The first is the “IF, THEN, ELSE” logical operators. The logic is: IF a condition is met (e.g. A > 21) THEN the function takes one value (e.g. 4) ELSE a different value is obtained (e.g. 5). The second function is MAX(, ,), and this function takes the maximum value of the ones contained between brackets and separated by commas. Third, we have the function COUNTER (minimum value, maximum value), which calculates a sequence of numbers from a minimum to a maximum value, increasing a unit at each simulated time step and restarting again when the maximum is attained. Finally we have the SWITCH (variable, limit) function. The function takes the value of 1 if the value of the variable is above the limit value if not, it becomes zero.

For the calculations performed by Stella, we used the Euler integration method and a time step of one month. The Euler integration method is the simplest of the integration methods available. It makes use of constant intervals for the successive calculations and works especially well when small intervals are used. Model components and units are listed in Table 1.

The life cycle of *Scrobicularia plana* includes a one-month larval stage, suspended in the water column (Frenkiel and Moueza 1979), followed by a benthic stage and the maximum life duration in the study area is 63 months (Verdelhos et al. 2005). The basic unit of the model is a Stella “conveyor”, which is like a moving sidewalk. In our model, each *S. plana* individual entering a conveyor takes one month to exit it and enter the next one. Fig. 2 shows two examples of these conveyors i.e. “larvae” and “controller”. These are used respectively for simulating the number of larvae and to control for the necessary minimum time lag for recovery after extreme environmental conditions.

Table 1 – Components of the model tr_x with x multiple of 12 does not exist.These are instead replaced by transf_x, indicating a transfer of individuals among age classes, i.e. submodels. When opening the submodels these transf_x appear with the names tr_'something', automatically attributed by the software

Model component	**units**
Auxiliary variables:	
all_dead	ind. * month^{-1}
controller	scalar (≥0)
deaths_larvae	ind. * month^{-1}
deaths_x (x = zero to 5)	ind. * month^{-1}
dens_regulator	scalar (0 to 1)
entry	scalar (0 to 1) * month^{-1}
maximum_regulator	scalar (0 to 1)
mortality_x (x =1 to 5)	proportion dead * month^{-1}
mortality_zero	proportion dead * month^{-1}
sal_regulator	scalar (0 to 1)
spawning	ind. * month^{-1}
temp_regulator	scalar (0 to 1)
timenew	scalar (0 to 12)
transf_x (x = 0 to 5)	ind. * month^{-1}
Deaths_x' to deaths x'''''''''''' (x= zero to 5)	ind. * month^{-1}
tr_x (x=2 to 62) [note]	ind. * month^{-1}
all_dead	ind. * month^{-1}
Calculated values:	
adults	ind. m^{-2}
total_number_of_individuals	ind. m^{-2}
Forcing functions:	
salinity	dimensionless
temperature	ºC

Model component	**units**
Graphical functions:	
spawn_reg	scalar (0 to 1)
Parameters:	
alpha	scalar (0 to 1)
beta	scalar (0 to 1)
exit (transit time of “controller”)	months (≥0)
fraction spawning	proportion
gamma	scalar (0 to 1)
k_1	scalar (≥0)
k_2	scalar (≥0)
k_3	scalar (≥0)
larvae per spawner	ind. * female^{-1}
max_sal	dimensionless
max_temp	ºC
min_sal	dimensionless
min_temp	ºC
mort_0_value	Proportion dead per month multiplied by 10000 (0-10000)
mort_larvae_value	Proportion dead per month multiplied by 100000 (0-100000)
mort_value_x (x=1 to 5)	proportion * month^{-1}
opt_sal	dimensionless
opt_temp	ºC
sex ratio	proportion
State variables:	
Larvae	ind. m^{-2}
month_x (x= 2 to 63)	ind. m^{-2}
Sub-models:	
age_x (x=0 to 5)	ind. m^{-2}

Table 1 – Continuation.

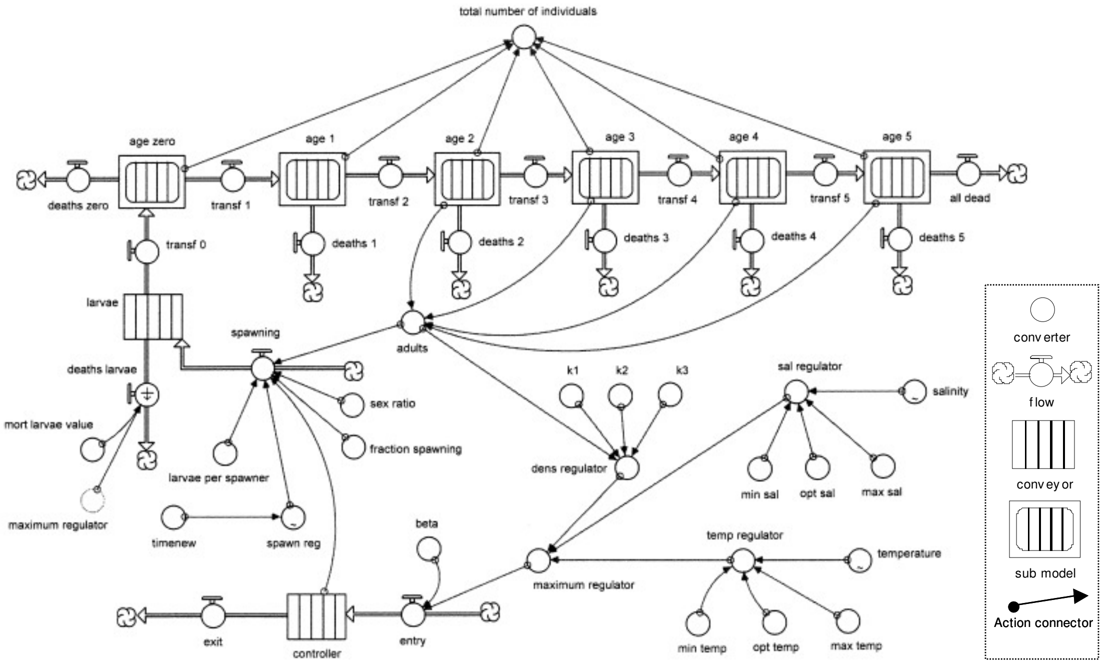

Fig. 2. Conceptual diagram of the model, built using Stella software. Converters store parameter values, make calculations or store the values of external variables (e.g. temperature). Flows transfer units (e.g. individuals) between compartments. Conveyors work in a way similar to conveyer belts. Submodels contain more detailed structures. Action connectors transport information regarding the values of model components, for use in operations elsewhere in the model.

In order to simplify the interface with the user, we decided to condense each age class into a sub-model (Fig. 2, age zero to age 5). Each age class contains a sequence of conveyors of one month: “age zero” only includes 11 conveyors because larval life lasts approximately one month (Frenkiel and Moueza, 1979) and is accounted for outside the first sub-model; “age 5” includes 3 conveyors only because these animals live 5 years and 3 months; all other sub-models, i.e. age 1 to age 4, include a full 12-unit sequence. Contents of the last conveyor inside a sub-model are transferred to the initial conveyor of the next sub-model using “transf 0”, “transf 1”… to “transf 5” (Fig. 2). The total number of individuals is the sum of all age classes i.e. zero to five and the number of individuals in each age class is the sum of all individuals inside the respective sub-model.

During the one-month transit period, each conveyor loses a certain proportion of the quantity entered. This proportion is the mortality rate and with this value we calculate the number of deaths per month within each conveyor. The sum of all the deaths inside each sub-model is called “deaths zero” to “deaths five” (Fig. 2). Mortality rates for larvae and for spat (until one year) are different from each other and different from the mortality rates of all the other age classes. These values were based on previously published work (Table 2). These mortality rates are controlled by a function called “maximum regulator” and its values increase as environmental conditions get worse (Fig. 2). If the environmental conditions are within the range of tolerated values for this species, the value of the function stays below a threshold parameter called gamma (Table 2).

Under these “normal”, i.e. not extreme, environmental conditions, the mortality rate is computed by the product of e.g. “mort_value_5” by “maximum regulator”. The “maximum regulator” function oscillates from zero to one and therefore, the harsher the environment, the closer the mortality rate is to “mort_value_5”. If the environmental conditions are outside the range of tolerated values for this species, the threshold value “gamma” is attained. Under these conditions, mass mortality occurs and mortality rate is calculated by a product of “maximum regulator” and “alpha” (Table 2). “Alpha” is a value for a “mass mortality rate”, killing approximately 50% of the animals.

Table 2 – Parameter values, their source and the results of the sensitivity analysis using only the final value of the total number of individuals or the cumulative values of total number of individuals

			Sensitivity (final value)		**Sensitivity (cumulative value)**	
Parameter	**Value**	**Source**	**+10%**	**-10%**	**+10%**	**-10%**
alpha	0.5	Calibrated	-1.530	-1.551	-0.602	-0.606
beta	0.8	Calibrated	1.280	1.208	3.895	9.559
gamma	0.95	Calibrated	11.003	6.493	-3.121	1.437
k_1	1	Corresponds to the total of a proportion	-1.398	-1.523	-4.633	-5.713
k_2	50	Calibrated	0.757	0.849	1.105	0.719
max_sal	34	(Guelorguet and Mazoyer-Mayère 1983)	2.812	6.464	1.240	4.677
min_sal	10	(Guelorguet and Mazoyer-Mayère 1983)	-1.514	-1.219	-0.569	-0.453
opt_sal	19.5	Calibration within the range 12-24 (Akberali 1978)	1.531	2.380	0.485	0.808
max_temp	27	Calibrated, but oxygen consumption drops sharply above 26ºC (Hughes 1970a) and Filtering rate drops to 5% of the maximum value at 32ºC (Hughes 1969)	7.167	9.976	1.913	7.309
min_temp	5	Calibrated, but oxygen consumption drops to 5% of the maximum value at 4ºC (Hughes 1970a)	-0.506	-0.454	-0.142	-0.133
opt_temp	24	Filtering rates maximal at 24-25ºC (Hughes 1969)	-5.116	-1.353	-0.633	0.058
fraction_spawning	0.02	Calibrated value. If we divide the number of animals (males and females) with mature gonads by the number of days of the spawning season we obtain values in the rough range of 0.005-0.01 (Sola 1997; Rodriguez-Rua et al. 2003).	0.992	0.992	0.897	0.898

			Sensitivity (final value)		Sensitivity (cumulative value)	
Parameter	**Value**	**Source**	**+10%**	**-10%**	**+10%**	**-10%**
sex_ratio	0.5	(Hughes 1971; Rodriguez-Rua *et al.* 2003)	0.992	0.992	0.897	0.898
mort_larvae_value	80000	Calibrated. In the laboratory less than 10% of the eggs hatched after fertilization (Frenkiel and Moueza 1979).	-0.717	-0.717	-1.227	-1.226
mort_0_value	9500	Calibrated, but value for newly settled juvenile invertebrates may exceed 95% (Thorson in McArthur 1998)	-2.361	-2.880	-0.664	-1.996
mort_value_1	0.019	Calibrated within the range from (Hughes 1970a)	-0.144	-0.142	-0.045	-0.044
mort_value_2	0.019	Calibrated within the range from (Hughes 1970a)	-0.117	-0.116	-0.055	-0.054
mort_value_3	0.019	Calibrated within the range from (Hughes 1970a)	-0.125	-0.124	-0.033	-0.031
mort_value_4	0.019	Calibrated within the range from (Hughes 1970a)	-0.048	-0.047	-0.008	-0.006
mort_value_5	0.019	Calibrated within the range from (Hughes 1970a)	0.000	0.000	-0.001	0.001
spawn_reg (start of zero values)	2	Calibrated within our field data. Literature range review is very variable (Sola 1997)	0.001	0.000	0.334	0.276
spawn_reg (end of zero values)	5	Calibrated within our field data. Literature range review is very variable (Sola 1997)	-0.052	-0.034	-0.732	-0.761
exit (transit time in "controller")	2	Calibrated within the 2-4 months period for gonad maturation found by Sola (1997).	-0.511	-0.440	-0.204	-0.205

Table 2 – Continuation.

Mass mortalities were recorded in field work and have been related to extreme environmental conditions (Guerreiro 1991). Conditions resulting in 50% mortalities are commonly used in biological studies (e.g. LC50). “Mortality_zero” and “deaths_larvae” calculate the mortality rate respectively for spat (age zero) and for larvae after adjustment. Once again, mortality is regulated by the “maximum regulator” function. Both equations contain a number that scales the calibrated parameter for mortality rate into the right value. This was necessary due to Stella’s constraints in accepting values with a large number of decimal places. The equation for “mortality_zero” is the product 0.0001*mort_0_value*maximum_regulator. Note that mort_0_value is a parameter equal to the mortality rate of the age class multiplied by 10000 (necessary due to Stella software constraints). The equation for “deaths_larvae” is similar: 0.00001*mort_larvae_value*maximum_regulator. Once again “mort larvae value” is a parameter equal to the mortality rate of the larvae multiplied by 100000 (necessary due to Stella software constraints).

Model regulation is accomplished by the variables: density of the adults, water salinity and water temperature respectively via the functions: “dens regulator”, “sal regulator” and “temp regulator”. The effects of these are combined in the “maximum regulator” function which computes the strongest of these effects at each instant. The regulator functions related to environmental variables such as temperature (temp_regulator) and salinity (sal_regulator) were modified from the original equation of Lehman et al. in Bowie et al. (1985). Both functions approach the value of 1 at the minimum and maximum tolerance limits of the species and the value zero at the species optimum. The functions are like an inverted and skewed bell and its calculus depends on the temperature or salinity being (or not) above the optimum value. The equation below represents the salinity regulator function:

$$\begin{aligned}&\text{sal\_regulator} = \\ &\text{IF}\left(\text{salinity} \le \text{opt\_sal}\right)\text{THEN}\left(1 - \text{EXP}\left(-2.3 \times \left(\frac{\text{salinity - opt\_sal}}{\text{min\_sal - opt\_sal}}\right)^2\right)\right) \quad (1)\\ &\text{ELSE}\left(1 - \text{EXP}\left(-2.3 \times \left(\frac{\text{salinity - opt\_sal}}{\text{max\_sal - opt\_sal}}\right)^2\right)\right)\end{aligned}$$

In this equation, min, max and opt_sal are the minimum, maximum and optimum salinities for *Scrobicularia plana*. The equation for the temperature is similar:

$$\begin{aligned}&\text{temp\_regulator} = \\ &\text{IF}\left(\text{temperature} \le \text{opt\_temp}\right)\ \text{THEN}\left(1-\text{EXP}\left(-2.3\times\left(\frac{\text{temperature - opt\_temp}}{\text{min\_temp - opt\_temp}}\right)^2\right)\right) \quad (2)\\ &\text{ELSE}\left(1-\text{EXP}\left(-2.3\times\left(\frac{\text{temperature - opt\_temp}}{\text{max\_temp - opt\_temp}}\right)^2\right)\right)\end{aligned}$$

The number of adults is calculated by the sum of all the animals over 2 years old (Bachelet 1982). This value is used in several calculations, namely the density regulatory function “dens_regulator”:

$$\text{dens\_regulator} = k_1 \times \frac{\text{adults} - k_3}{k_2 + \text{adults} - k_3} \quad (3)$$

In this equation k_1 and k_2 are parameters adjusting the shape of the equation. This function (Haefner, 1996) was adapted to our case by the use of a k_3 value of zero. This results in a simple Michaelis-Menten function starting from zero and the values of the function approach one, i.e. the value of k_1, at high densities (Table 2). The parameter k_2 is the density of adults that results in a value of “dens_regulator” equal to 50% of k_1. This function was added due to the fact that under high population densities, namely 2-3 years after successful recruitment, population density typically declined (Essink et al. 1991). Moreover, experimental work by Hughes (1970b) indicated a possible relationship between overcrowding and high mortalities.

Several natural processes are seasonal, although this is related to the presence of “typical” environmental conditions at each month, and this forced us to calculate the month at a multi-year simulation. The variable “timenew” makes this calculation, restarting again at the end of each year. The information regarding the month of the year is used by the graphical function “spawn_reg” dictating the periods of the year when recruitment is possible. This function outputs values of one or zero respectively. Recruitment occurs only when “spawn_reg” takes the value of 1. The number of recruits added, here called “spawning” for simplicity, is limited by a minimum time gap of 2 months since the last period of extreme conditions. The value of “spawning” is calculated by the equation:

$$\text{spawning} = (1-\text{controller})*\text{sex\_ratio}*\text{fraction\_spawning}*\text{adults}*\text{larvae\_per\_spawner} \tag{4}$$

In this equation, “controller” is a control function preventing spawning after periods with extreme conditions; “sex_ratio” is calculated as the number of adult females divided by the total number of adults; “fraction_spawning” is the proportion of females spawning at each instant; “adults” is the total number of adults; and “larvae_per_spawner” is the number of larvae per female.

The 2 months time gap for recruitment since the last period of extreme conditions is in accordance with the 2-4 months found by Sola (1997) from the appearance of the first individuals with developing gonads to the appearance of the first individuals with mature gonads. The time gap is simulated by using a conveyor (Fig. 2, controller) that raises one unit each time the conditions become extreme. Anything entering the “controller” will take 2 months to exit. When the controller equals 1, spawning is absent, when the controller equals 0, spawning is at its maximum and when the controller is between 0 and 1, spawning is reduced.

The variable “entry” fills up the “controller” every time the maximum regulator takes a value above the threshold “beta” i.e. when extreme conditions are present. This is performed using a SWITCH Stella function which takes the value of 1 if the variable “maximum regulator” is above the parameter “beta”. If not, the function becomes zero. As the controller takes 2 months to empty, the result is that recruitment can only occur 2 months after each extreme event.

ANALYSIS OF MODEL PERFORMANCE

Two types of sensitivity analysis were performed, using the formula by Jørgensen (1994), both analysing the effects of ±10% variations of parameter values upon the state variable "total number of individuals" (eq. 5).

$$\text{Sensitivity} = \frac{\dfrac{SV_{\text{modified}} - SV_{\text{original}}}{SV_{\text{original}}}}{\dfrac{P_{\text{modified}} - P_{\text{original}}}{P_{\text{original}}}} \quad (5)$$

Values before and after parameter (P) manipulation are called respectively "original" and "modified". For this formula, a single value is needed for the state variable (SV) before and another one after the manipulation of the parameter. Therefore, for the first type of sensitivity analysis, we used the final value of the state variable and for the second type we used the cumulative value of the state variable i.e. the sum of the values of the state variable throughout the simulation. Due to the possibility of the magnitude of model oscillations being altered without significant modifications on the final value of the state variable, we decided to check if the two types of sensitivity measures were correlated or functionally related for each level of parameter change, i.e. plus or minus 10%. If a non-significant correlation is found and if there is no significant regression line relating the two types of sensitivity, then a choice has to be made between the two methods.

The model was both calibrated and validated (Jørgensen 1994). Calibration was performed manually using data from the highly-eutrophic area under improved estuary conditions i.e. 1999 to 2003. Model validation was performed with 5 different data series from 3 field stations and both pre- and post-management situations. The information obtained by the sensitivity analysis provided the basis for the calibration process. The model was considered calibrated if a significant r^2 was attained after a conversion to a t value (Fowler and Cohen 1996) and further calibration was not able to improve the r^2 value. Observed vs. simulated values were also plotted together with the regression equation and the appropriate x=y line. Model validation was performed with the 5 remaining data series from the 3 field stations and both pre- and post-management situations. For all data sets t–tests were used to verify if the slope of the regression equation was different from 1 and also to verify if the intercept was different from zero. Pearson correlations were calculated for each dataset and were tested for significant differences followed by Tukey-type multiple comparisons to check which pairs of correlation coefficients differ (Zar, 1984). Finally, Loague and Green's "modelling efficiency" statistics (Mayer and Butler, 1993) was computed for each dataset in order to rank them regarding model performance.

RESULTS

The values of the sensitivity analysis (Table 2) differed by -14.12 to +8.4 if we consider the final values or the cumulative values of the "total number of individuals". The rankings of the 3 highest sensitivities in absolute value were also different depending on the method for sensitivity analysis. If we use the final value and a 10% increase on the parameters, Gamma, max_temp and opt_temp are the 3 highest absolute sensitivities, but these 3 change to respectively k1, beta and gamma if we use the cumulative values. Similarly, if we use the final value and a 10% decrease on the parameters, max_temp, gamma and max_sal have the 3 highest absolute sensitivities, but these 3 change to respectively beta, max_temp and k1 if we use the cumulative values. A non-significant correlation ($r=0.06$, $p=0.781$, $n=24$) and a non-significant regression ($y = 0.0311x - 0.0592$, $F= 0.079$, $p= 0.781$) were obtained for final versus cumulative sensitivity values at 10% increases of the parameters. Nevertheless, a significant correlation ($r=0.671$, $p<0.001$, $n=24$) and a significant regression ($y = 0.6797x + 0.1204$, $F=17.972$, $p=0.0003$) were obtained for final versus cumulative sensitivity values at 10% decreases of the parameters. The 5 most important parameters regarding sensitivity are beta, k1, max_temp, max_sal and gamma.

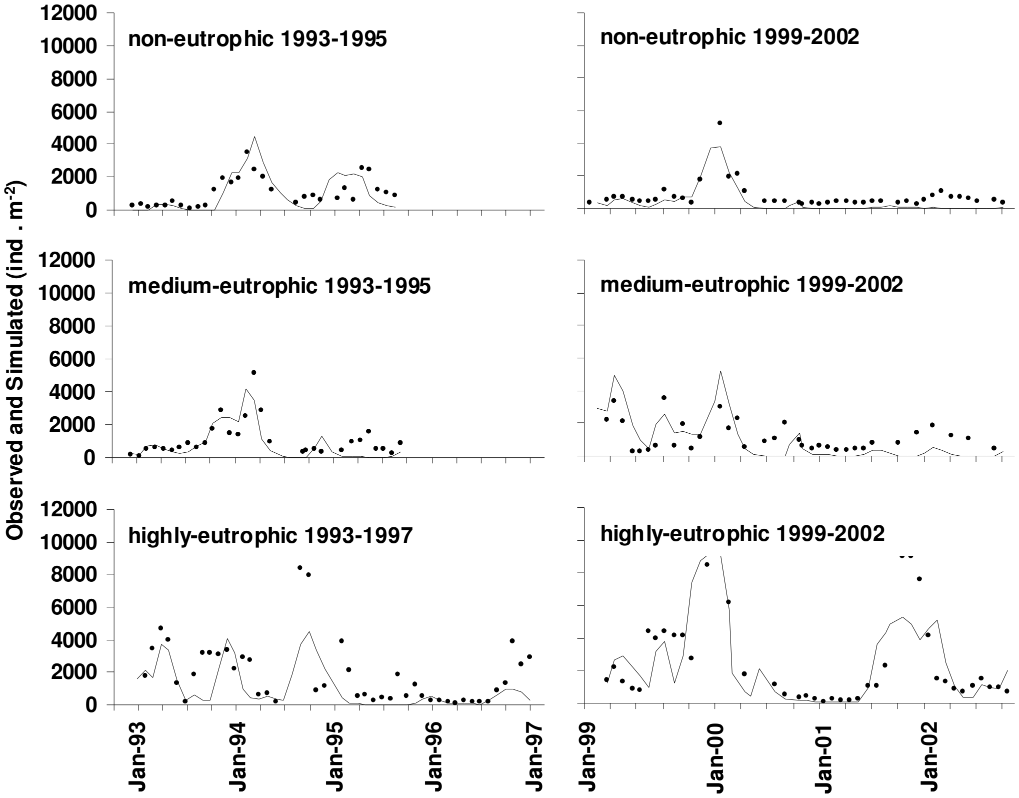

Fig. 3 –The observed (•) and simulated values (—).

This model is very sensitive to the maximum tolerated temperature (max_temp), regardless of the type of analysis or variation imposed (± 10%). It is not very sensitive to the mortality rate of the individuals from age 1 to age five (mort_value_1 to mort_value_5), but it is sensitive to the mortality rate of the larvae and of the spat ("mort_larvae_value" and mort_0_value").

Table 3 – Indices of model performance for the 6 datasets analyzed. The significance of the coefficient of determination (r^2), the departure of the regression slope from one and the departure of the regression intercept from zero, were tested with t-tests. D.F.= degrees of freedom. n.s.= not significant. F= F value for the regression ANOVA table, with the corresponding significance level.

Eutrophication	Intervention	D.F	r^2	t for r^2	F	slope	t for slope	intercept	t for intercept	modelling efficiency
Highly-eutrophic	before	43	0.478	2.074	38.256	1.003	0.016	823.52	3.084	0.28
				*	***		n.s.		***	
	after	40	0.663	4.785	78.636	0.947	0.493	317.65	0.819	0.66
				***	***		n.s.		n.s.	
Medium-eutrophic	before	28	0.585	2.816	39.534	0.748	2.121	422.23	2.571	0.48
				**	***		*		*	
	after	33	0.484	1.873	30.954	1.103	0.521	183.33	-0.631	0.48
				**	***		n.s.		n.s.	
Non-eutrophic	before	28	0.538	2.258	32.654	0.536	4.945	521.04	3.566	0.13
				*	***		**		**	
	after	40	0.845	11.464	217.931	1.041	0.580	352.27	6.194	0.64
				***			n.s.		***	

There was no need for model recalibration when validation was performed, except for the pre-management situations on the highly-eutrophic and non-eutrophic areas. The non-eutrophic area required a minimum salinity of 5 and zero values of the graphical function (spawn_reg) from month 3 to month 9. The highly-eutrophic area required an optimum temperature (opt_temp) of 27.5ºC, a maximum temperature (max_temp) of 30ºC and zero values of the graphical function (spawn_reg) from month 3 to month 4.5. All 6 data sets employed, i.e. one for calibration and 5 for validation, were correctly simulated by the model. A visual analysis of Fig. 3 shows that most of the peaks in abundance are represented, although the magnitude is not always precise.

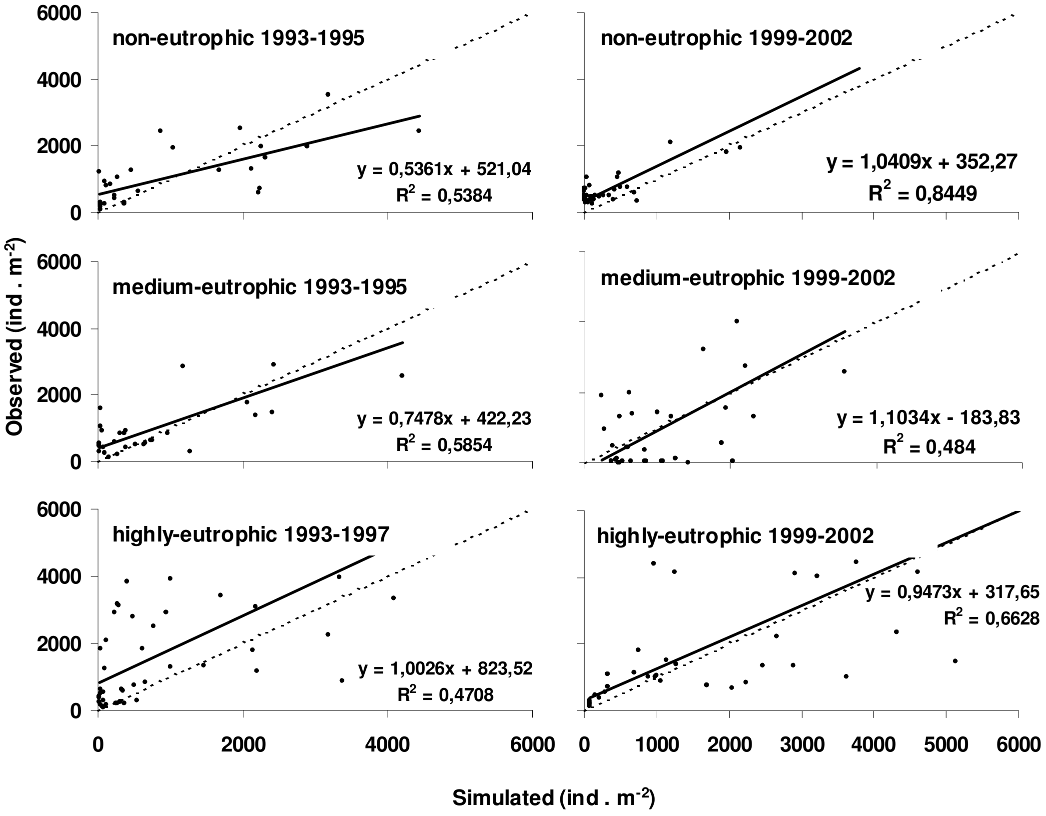

Fig. 4 – Simulated v. observed values with the respective regression equations and r^2 values. Dotted line corresponds to the ideal situation, i.e. when x=y.

The test comparing the simulated versus observed correlation coefficients (r) for these 6 datasets indicated that at least some of these coefficients differ ($X^2=14.5$, $k=5$, $p<0.05$). A Tukey-type multiple comparisons test for several correlation coefficients indicated that the non-eutrophic area after management had a significantly different correlation coefficient from all the other datasets (Table 4). In fact, this was the highest correlation coefficient ($r=0.919$) and the remaining coefficients were not significantly different from each other.

Table 4 – Tukey type multiple comparisons for correlation coefficients (Zar 1984) on 3 sampling stations, before and after system intervention. The critical value for $q(\infty,0.05,5)$ is 2.257. Significantly different values of r are indicated by an asterisk.

					q values				
					Highly eutrophic		**Medium eutrophic**		**Non-eutrophic**
		n	**r**	**z**	before	after	before	after	before
Highly eutrophic	before	45	0.691	0.851					
	after	42	0.814	1.139	1.835				
Medium eutrophic	before	30	0.765	1.008	0.905	0.738			
	after	35	0.696	0.859	0.050	1.661	0.809		
Non-eutrophic	before	30	0.734	0.937	0.494	1.143	0.372	0.422	
	after	42	0.919	1.584	*4.663	*2.777	*3.250	*4.298	*3.654

All the regressions had very highly significant values of F (i.e. $p<0.001$, Table 3), indicating that the dependent variable is explained by the independent variable. Ideally, a regression line of observed versus simulated should have a slope of one and an intercept of zero. Table 3 shows the results of the t-tests for departure of each regression slope from one and of each intercept from zero. Only the medium and the non-eutrophic datasets before management have slopes significantly different from one. For each sampling station, the regression intercepts of the data-sets obtained after management are always closer to zero, although for the non-eutrophic post management dataset it is significantly different from zero, possibly because of a much larger sample size. Considering the regression slopes and elevations (Fig. 4, Table 3), model performance increased after system management.

The Modelling Efficiency (M.E.) statistics are always above zero (Table 3), therefore indicating satisfactory model performances. Despite the fact that it is inappropriate to apply a statistical test due to a low number of values to compare before (3) and after management (3), M.E. is much higher on the highly and non-eutrophic areas after management. On the medium eutrophic area, modelling efficiency remained unchanged before and after the intervention.

DISCUSSION

COMPARING TWO METHODS FOR SENSITIVITY ANALYSIS

Although one might be tempted to think that the results of a sensitivity analysis using the final or the cumulative values are correlated, this correlation is not always present. In our data, we found correlated values of the two methods for one of the levels of parameter modification but not for the other. Moreover, the presence of a significant correlation does not mean similar rankings of sensitivity to parameters. In our model, the results of the sensitivity analysis are not the same if we consider the final values or the cumulative values of the “total number of individuals”. In fact, the parameters presenting the highest absolute sensitivity values differed depending on the method and the identification of these parameters is the most important information obtained by the sensitivity analysis. It indicates where the effects of parameter uncertainty may be most relevant and it also facilitates the process of calibration. A final value of a variable may not reflect what has been happening during the simulation, especially if large fluctuations are observed. Take as an example a population in which the simulation ends during the winter and densities are always very low during that season. This situation will provide a low sensitivity to a given parameter even if the parameter is causing massive increases in biomass during the summer. Since the two methods may present different results in theory and in practice, it is our opinion that the cumulative values of the variable describe more accurately the sensitivity to the parameters and are more useful for a correct analysis of population models' responses.

INFORMATION OBTAINED FROM THE SENSITIVITY ANALYSIS

There are two parameters with exactly the same (low) sensitivity values, the "sex ratio" and "larvae_per_spawner". In fact there is some uncertainty regarding the latter, but not regarding the sex ratio (Rodriguez-Rúa et al., 2003), even though we know that it changes throughout the year (Paes da Franca, 1956).

The reason for this similarity in sensitivity is the equation simulating the recruitment of larvae. These two parameters are multiplicative and allow the calculation of one number. For example, if we double one or the other the effect will be precisely the same. Nevertheless, the equation is a simplification of a series of mechanisms. To be precise in mechanistic terms we would have to consider that females of different size or condition have different fertilities, and that not all the eggs released are fertilised and produce larvae.

Nevertheless from the sensitivity analysis results, we conclude that the number of eggs per female is of low importance and therefore the inclusion of these processes would add unnecessary complexity to the model. This fact, together with a well known impossibility to accurately determine age from *S. plana* individuals captured on the field and an absence of weight-fertility regressions, justified a choice for an age structured instead of size structured model.

The model is most sensitive to beta, k1, max_temp, max_sal and gamma. This seems to indicate that it is the occurrence of very adverse environmental conditions that has the most importance for the model and possibly to the real system. Beta and gamma are involved in the threshold limits of "extreme" environmental conditions, respectively affecting reproduction and mortality. Maximum tolerated temperature and maximum salinity are therefore (from the analysis of sensitivity) crucial parameters because they give the model indications about the occurrence or not of extreme environmental conditions. Winter mass mortalities may occur in the North of Europe (Essink et al., 1991) and therefore this is an indication that minimum temperature is important, but further South, mass mortalities have been reported during the summer (Guelorget and Mazoyer-Mayère, 1983).

We think that in our case, since we are even further South, maximum temperatures could be more relevant. The only unexplained finding is that the model is more sensitive to changes of the maximum salinity than to changes in the minimum salinity tolerance values.

REGULATORY PROCESSES IN THE MODEL

The high sensitivity to parameters linked to responses to extreme i.e. “very high” values of temperature and salinity indicate that these variables play a fundamental role on model regulation. On the contrary, this is not so obvious in the case of the regulation by the population density. There are records of increased *S. plana* mortality at higher densities (Hughes, 1970b) and other bivalve species such as the common cockle (*Cerastoderma edule*) are known to reduce spat recruitment by cannibalism where dense populations exist (André, 1993; Montaudouin de and Bachelet, 1996). Population regulation is important for model stability and the biologically meaningful parameters regarding the population regulation mechanism were obtained by calibration, which could cause some uncertainty. Although the parameter k_1, involved in population regulation, produced one of the highest sensitivity values, this corresponds merely to the maximum value of the regulator function. There is no biological meaning for this parameter and it was never changed for calibration purposes. It is the total of a proportion i.e. 1, just like many regulator functions.

On the other hand the model is not very sensitive to k_2, a half saturation constant for the regulator function and therefore uncertainty may be reduced. This parameter (k_2) indicates the population density resulting in a value of the function of half of the maximum value (k_1).

MORTALITY AND PREDATION

Mortality was not subdivided into components such as predation, diseases, natural mortality etc. In this estuary, *Scrobicularia plana* is occasionally captured for recreational or even commercial purposes, but this has a negligible effect on the population. In other systems such as the Wadden Sea, predators like the Oystercatcher (*Haematopus ostralegus*) take an important part of the bivalve populations (Hughes, 1970b; Zwarts et al., 1997a; 1997b), but juvenile benthic bivalve predatory mortality is normally caused by flatfishes, shrimps and crabs (Zwarts et al., 1996). In the Tagus estuary 200 km further South from the Mondego estuary, *S. plana* bird predators are: *Recurvirostra avosetta*, *Pluvialis squatarola*, *Tringa totanus*, *Limosa limosa*, *Limosa lapponica*, *Larus ridibundus* and *Larus fuscus* (Guerreiro, 1991). During the winter, there is in fact a great dependence on *S. plana*, and bivalve siphon cropping was the most important item in a study addressing bird diet (Moreira, 1997). In the Mondego estuary, waders also predate *S. plana* (Cabral et al., 1999), with a 2.9 to 30% occurrence of *S. plana* in their faeces (Múrias et al., 2002). Nevertheless, bird density is not very high and it is reasonably conservative from year to year (Lopes et al., 2005).

MODEL PERFORMANCE AND THE MANAGEMENT OF THE SYSTEM

The system was, as previously described, subjected to management. This increased the area of the *Zostera noltii* meadows and decreased the eutrophic state through the increase in water circulation as demonstrated in previous papers (Marques et al., 2003; Lillebø et al., 2005). The model indicates that both the timing of the reproduction of the species and the apparent tolerance limits to environmental variables may have been affected by the management of the system. This is visible because of the need for different timings for reproduction (start and end values), minimum tolerated salinity and maximum tolerated temperature for the pre-management situations on the highly-eutrophic and non-eutrophic areas.

From the model perspective, system restoration seems to have been successful. In fact, under a pre-management scenario, simulations were less precise. This may be linked to higher system instability, with more frequent occurrence of short periods of intense environmental stress such as for example the occurrence of algal blooms, not accounted for by the present model structure.

APPLICABILITY OF THE MODEL UNDER DIFFERENT CONDITIONS

This model was built for a specific geographical area. As an example, the model makes use of 63 months of life span and this feature may differ between Northern and Southern populations (Green 1957; Bachelet 1982; Verdelhos et al. 2005). Mortality rates, carrying capacity, fertility and several other parameters are known to change, among other factors, with time and location. For the mortality rate, different authors present different values for *S. plana* (Hughes 1970b; Guerreiro 1998), and sometimes the same authors present different values (Hughes 1970b). Even some fundamental biological parameters, such as the minimum tolerated temperature, may actually vary, depending on the environmental conditions. Recalibration of parameter values may therefore account for differences in local characteristics of the populations and for differences in environmental conditions. This was the case with two of the data sets used for validation. The present type of model could be used for the same organism on similar systems after mild recalibration, but it is unsuitable for filter feeding, plankton dependent bivalves such as e.g. oysters.

CONCLUSIONS

We constructed a *Scrobicularia plana* population dynamics model based on density, temperature and salinity. These variables regulate mortality and recruitment and are the fundamental factors controlling the population in the area. With the model, we were able to compare two different sensitivity analysis procedures. Because the results of the two methods can be uncorrelated and because the parameters with the highest absolute sensitivity values are different, we consider that it is relevant to choose one or the other. In our opinion, the method making use of the cumulative values of the state variable instead of the traditionally used final value, provides more information and should be preferred for this type of models.

The *Scrobicularia plana* population dynamics model replicated the data used for its construction and was successfully validated. Sensitivity was highest for parameters linked to responses to very high values of temperature and salinity. Therefore we conclude that the occurrence of extreme environmental conditions has the most importance for the model and possibly to the real system. In fact, the most reliable model simulations were obtained for the “after system management” datasets. The model corroborates the perception that the system became more predictable after the intervention. Although the reliability in model predictions should decrease under pre-management type scenarios, the model can be used for simulations under possible future environmental change conditions, as a management tool, and as a tool for further analysis of the behaviour of this system.

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CHAPTER 3

THE ROLE OF LATITUDE ON THE BIVALVE *SCROBICULARIA PLANA*

Latitudinal gradients on *Scrobicularia plana* reproduction patterns, population dynamics, growth and secondary production

LATITUDINAL GRADIENTS ON *SCROBICULARIA PLANA* REPRODUCTION PATTERNS, POPULATION DYNAMICS, GROWTH AND SECONDARY PRODUCTION

ABSTRACT The bivalve *Scrobicularia plana* is recognised as a dominant species of the intertidal soft-substrate communities in coastal areas (e.g. estuaries, lagoons and bays) along the NE Atlantic seaboard, in terms of biomass and productivity, being an important link in the food chain, showing increasing commercial interest and high economical value, as a human food resource. Several studies suggested the existence of latitudinal variation, on the ecological patterns of the species, along its geographic distribution range. Here, we intend to analyse and compare the resulted patterns of reproduction, population dynamics, growth and secondary production, and to assess possible relations between latitude and *Scrobicularia plana* ecological patterns and strategies. Different life strategies were observed, depending on the temperature, latitudinal gradient and on local habitat conditions. Higher latitude populations (> 50º N) usually show low abundance values, shorter reproduction periods and a “slower” life style, with lower growth rates (0.1< k < 0.2), extended life span and lower productivity. Areas between 40º N and 50º N seem to show optimal ecological conditions with the highest abundance values registered, longer reproduction periods, “faster” growth (0.3< k < 0.8) and higher productivity. Further South (< 40º N), populations showed lower abundance, productivity and growth rates than the previous.

INTRODUCTION

Scrobicularia plana is recognised as a dominant species of intertidal soft-substrate in estuaries, lagoons and bays along the NE Atlantic seaboard communities (Hughes, 1970a, Bachelet, 1982; Dolbeth et al., 2005; Verdelhos et al., 2005; Casagranda and Boudouresque, 2005; Cardoso et al., 2008), from Norway (60º N) to the Mediterranean and West Africa (14º N). It is a long-living selective deposit-feeder bivalve, eurytherm and euryhaline, inhabiting intertidal soft bottoms with abundant organic matter (Hughes, 1970a; Guelorget and Mazoyer-Mayère, 1983; Essink et al., 1991; Sola, 1997; Guerreiro, 1998; Casagranda and Boudouresque, 2005; Verdelhos et al., 2005), being an important link in the food chains of estuaries and coastal lagoons, with an important role on the diet of wading birds, crabs and benthic fish (Hughes, 1969, 1970b; Bachelet, 1982; Guelorget and Mazoyer-Mayère, 1983; Casagranda and Boudouresque, 2005; Langston et al., 2007). Presently, it is a species of increasing commercial interest, as a human food resource with potential economical and social value (Rodríguez-Rúa et al., 2003; Langston et al., 2007).

Studies ranging from the United Kingdom (UK) and the Wadden Sea to Tunisia have focused on *S. plana* reproduction (Paes de França, 1956; Hughes, 1971; Worrall et al., 1983; Sola, 1997; Guerreiro, 1998; Rodríguez-Rúa, 2003; Raleigh and Keegan, 2006; Mouneyrac et al., 2008), population dynamics (Hughes, 1970a; Guelorget and Mazoyer-Mayère, 1983; Essink et al., 1991; Sola, 1997; Guerreiro, 1998; Casagranda and Boudouresque, 2005; Verdelhos et al., 2005), individual growth (Green, 1957; Hughes, 1970a; Bachelet, 1981; Guelorget and Mazoyer-Mayère, 1983; Sola, 1997; Guerreiro, 1998; Verdelhos et al., 2005) and secondary production (Hughes, 1970b; Bachelet, 1982; Guelorget and Mazoyer-Mayère, 1983; Sola, 1997; Guerreiro, 1998; Casagranda and Boudouresque, 2005; Verdelhos et al., 2005), suggesting the existence of latitudinal variations.

Latitudinal gradients on bivalves are well established, focusing either on biodiversity (Crame, 2000, 2002; Rex et al., 2000; Roy et al., 2000a) or on growth rate, body size and life span (Macdonald and Thompson, 1988; Hummel et al., 1998; Roy et al., 2000b). However, bivalve populations seem to be influenced not by latitude *per se*, but by several environmental variables – e.g. temperature, seasonality, precipitation and ecosystem energy flux. These parameters co-vary with latitude and interact with each other, influencing recruitment success, survival and growth rates, controlling primary production and consequently the food supply on the ecosystem (Macpherson, 2002; Willig et al., 2003; Angilletta and Sears, 2004; Giangrande and Licciano, 2004).

A general latitudinal trend in temperature is observed along the European coast, with temperature decreasing with increasing latitude (www.worldweather.org). The Northern latitudes show colder winter extremes, higher annual variability and shorter growing season, when compared to lower latitudes. Temperature seems to play an important role on *S. plana* population dynamics, controlling reproduction patterns (Hughes, 1971; Worral et al., 1983; Sola, 1997; Rodríguez-Rúa et al., 2003; Raleigh and Keegan, 2006; Mouneyrac et al., 2008), survival and mortality (Hughes, 1969; Guelorget and Mazoyer-Mayère, 1983; Essink et al., 1991), growth rates (Bachelet, 1981), and consequently secondary production.

Temperature related latitudinal variations may result in different life strategies along the geographic range of a species (Hughes, 1971; Bachelet, 1981, 1982; Essink et al., 1991; Sola, 1997; Clarke, 2003; Rodríguez-Rúa et al., 2003). Studying the variation of populations' dynamics and strategies along a latitudinal gradient is a good approach to increase our knowledge on a species throughout its biogeographic range. Moreover, we can extract usable information to other approaches on population studies (e.g. modelling), which are useful tools to understand the dynamics and responses of a population to both natural and anthropogenic stressors, as well as to make predictions on future scenarios (Anastácio et al 2009).

The main goals of this study are to analyse differences in (1) reproduction periods, (2) population dynamics, (3) growth rates and (4) secondary production of *Scrobicularia plana* along a wide range of its distribution, from the UK and the Wadden Sea (~ 55ºN) to Southern Europe and the Mediterranean (~36º N). By comparing patterns along the latitudinal gradient, it is possible to assess possible relations between latitude and *S. plana*'s ecological patterns and strategies. In fact, despite the general latitudinal gradient, related to temperature, certain areas may act as distinct microenvironments, where local conditions may overrule the latitudinal trend.

DATA AND METHODOLOGY

An extended bibliographic research was made and information was collected from several studies performed between 1930 and 2008 (Table 1), focusing on reproduction, population dynamics, growth and secondary production of *Scrobicularia plana* populations from the Western European and the Mediterranean Coast, along a latitudinal gradient (56º N to 36º N) (Fig. 1).

Temperature data from different geographic areas referred in this study was collected from www.worldweather.org, in order to assess the existing latitudinal gradient and to compare temperature patterns. Here, we present mean minimum and maximum monthly temperature values (Climate Normal 1971-2000) for the Wadden Sea, Ireland, the UK (Wales and Cornwall), North France (Loire region), Gulf of Biscay (Gironde region – France and San Sebastian – Spain), Portugal (Coimbra, Lisboa and Alentejo), the Mediterranean coast (Marseille – France and Tunisia) and South Spain (Cádiz).

Studies on the reproduction and gametogenic cycle of *S. plana* were published in Ireland (Raleigh and Keegan, 2006), the UK (Hughes, 1971; Worrall et al., 1983), France (Mouneyrac et al., 2008), Spain – the Gulf of Biscay (Sola, 1997); South Spain (Rodríguez-Rúa et al., 2003) and Portugal (Paes-Da-Franca, 1956; Guerreiro, 1998), focusing on gonad development, breeding cycle and recruitment. Further information concerning spawning and recruitment periods was collected from bibliography, which were used and compared on this study.

Scrobicularia plana population dynamics was studied both in long term sampling programmes and in shorter periods since the mid 20th Century, in estuaries, lagoons and bays along the Atlantic European coast and the Mediterranean Sea (Hughes, 1970; Guelorget and Mazoyer-Mayère, 1983; Essink et al., 1991; Sola, 1997; Guerreiro, 1998; Casagranda and Boudouresque, 2005; Verdelhos et al., 2005). Information on recruitment, survival and mortality, density changes and population structure was used to compare several populations along the distribution range and in different geographic areas.

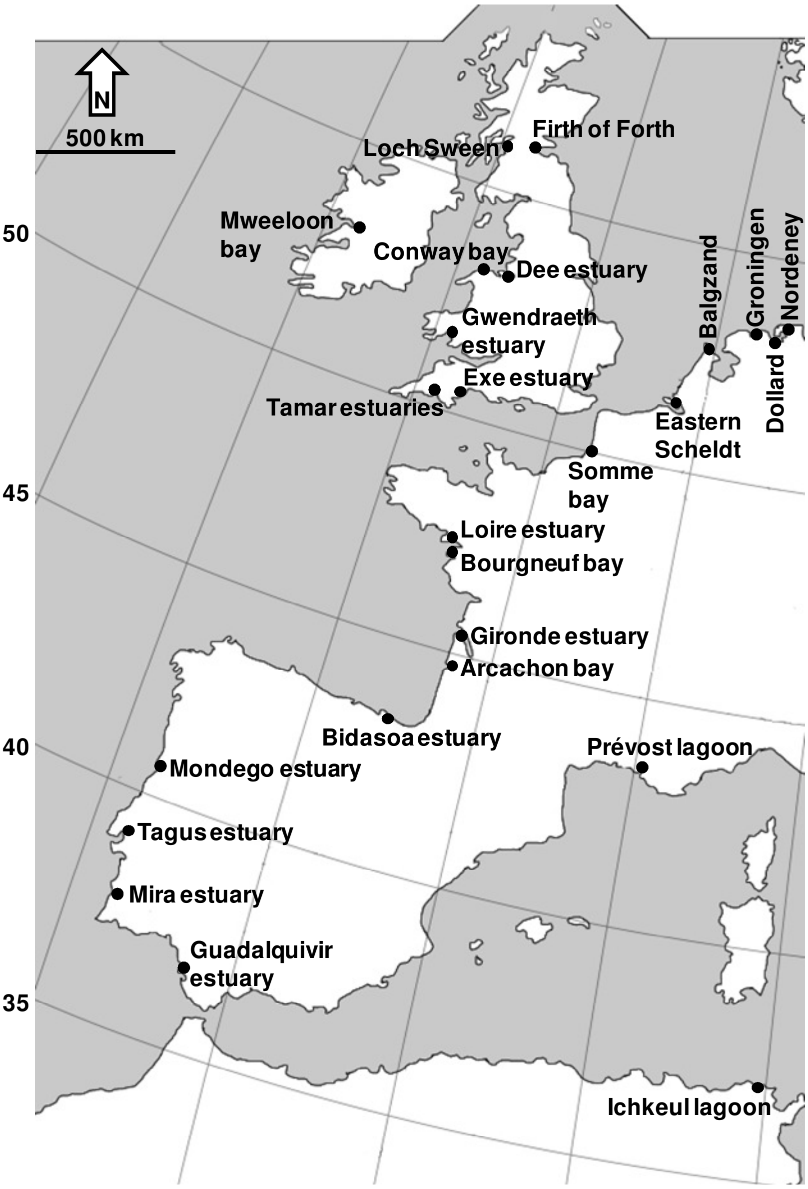

Fig. 1 – Geographical location of the *Scrobicularia plana* study sites on the Western European and Mediterranean coast.

Table 1 – Studies focusing on *Scrobicularia plana*: study sites and location, latitude, references and information on sampling methodology resulted from extended bibliographic research.

Local	Latitude	Author (Reference)	Study Focus	Sampling Methodology	
Firth of Forth (Scotland - UK)	56º 20' N	Stephen, 1930 (in Hughes, 1970a)	Macrofauna		
Loch Sween (Scotland - UK)	55º 57' N	Raymont, 1955 (in Hughes, 1970a)	Macrofauna		
Norderney (Wadden sea)	53º 42' N	Michaelis, 1987 (in Essink et al., 1991)	Population dynamics	Seasonal (1976 to 1985)	1 mm sieve
Groningen (Wadden sea)	53º 23' N	Essink et al., 1991	Population dynamics	Seasonal (1969 to 1988)	1 mm sieve
Dollard (Wadden sea)	53º 20' N	Essink et al., 1991	Population dynamics	Seasonal (from 1974 to 1988)	1 mm sieve
Dee estuary (Wales - UK)	53º 19' N	Stopford, 1951 (in Sola, 1997)	Ecological survey		
Conway bay (Wales - UK)	53º 15' N	Hughes, 1970a,b, 1971	Population dynamics; Reproduction; Growth; Production	October 1967 for survey study; Monthly (November 1966 to November 1967); Monthly (December 1966 to December 1967) for reproductive cycle study.	0.25 m^2 (depth 30 cm); 1 m^2 (depth 30 cm), to collect adults; 0.25 m^2 (depth 5 cm), sieve 1.59 mm to collect spat; Adult collection for reproductive cycle study.
Mweeloon Bay (Ireland)	53º 13' N	Raleigh and Keegan, 2006	Gametogenic cycle	Monthly (February 1996 to September 1996; January 1997 to September 1997)	Adults collected (> 22,4 mm)
Balgzand (Wadden sea)	52º 53' N	Beukema, 1989 (in Essink et al., 1991)	Population dynamics	Seasonal (1969 to 1988)	1 mm sieve
Eastern Scheldt (Wadden sea)	51º 46' N	Craeymeersch et al., 1988 (in Essink et al., 1991)	Population dynamics	Seasonal (1983 to 1985)	1 mm sieve
Gwendraeth estuary (Wales - UK)	51º 43' N	Green, 1957	Growth	Seasonal (1954 to 1955)	1 m^2 (depth 30 cm), 6 mm and 1 mm sieve.
Exe estuary (Cornwall - UK)	50º 39' N	Holme, 1949 (in Hughes, 1970a)	Macrofauna		
Tamar estuaries (Cornwall - UK)	50º 25' N	Warwick and Price, 1975 (in Sola, 1997)	Production		
		Worral et al., 1983	Physiological ecology; Gametogenic cycle	6 weeks interval (January 1977 to July 1979)	Adults collected

Local	Latitude	Author (Reference)	Study Focus	Sampling Methodology	
Somme bay (English ch - France)	50º 14' N	Ducrotoy, 1987 (in Essink et al., 1991)	Population dynamics	Seasonal (1981 to 1987)	1 mm sieve
Loire estuary (Loire - France)	47º 10' N	Robineau, 1986 (in Essink et al., 1991)	Population dynamics	Seasonal (1981 to 1983)	1 mm sieve
Bourgneuf bay (Loire - France)	47º 00' N	Mouneyrac et al., 2008	Gametogenic cycle	(April 2005 to May 2006)	Adults collected
Gironde estuary (Gironde - France)	45º 30' N	Bachelet, 1981,1982	Growth; Production	Monthly (January 1976 to February 1978)	0.25 m^2 (depth 30 cm), 1 mm sieve; 12 cm^2, 500 µm sieve;
Arcachon bay (Gironde - France)	44º 50' N	Bachelet, 1981,1982	Growth; Production	Monthly (January 1977 to January 1978)	0.25 m^2 (depth 30 cm), 1 mm sieve; 12 cm^2, 500 µm sieve;
Bidasoa estuary (S. Sebastian - Spain)	43º 50' N	Sola, 1997	Population dynamics; Reproduction; Growth; Production	Monthly (February 1987 to December 1990)	0.1 m^2 (depth 25 cm), 1 mm sieve to population dynamics; 10 cm^2 (depth 2 cm), 200 µm sieve for recruitment study; Adult collection for reproductive cycle study.
Prévost lagoon (Marseille - France)	43º 00' N	Guelorget and Mazoyer-Mayère, 1983	Population dynamics; Growth; Production	Monthly (October 1983 to December 1984)	0.1 m^2 (depth 30 cm), 1 mm sieve.
Mondego estuary (Coimbra - Portugal)	40º 07' N	Verdelhos et al., 2005	Population dynamics; Growth; Production	Monthly (January 1993 to December 2000)	5 to 10 replicates 0.14 cm^2 core (depth 25 cm), 500 µm sieve.
Tagus estuary (Lisbon - Portugal)	38º 50' N	Guerreiro, 1998	Population dynamics; Reproduction; Growth; Production	Monthly (April 1986 to October 1987)	0.25 m^2 (depth 25 cm), 1 mm sieve; Adults collected for gametogenic cycle study.
Mira estuary (Alentejo - Portugal)	37º 43' N	Guerreiro, 1998	Population dynamics; Reproduction; Growth; Production	Monthly (April 1986 to October 1987)	0.25 m^2 (depth 25 cm), 1 mm sieve; Adults collected for gametogenic cycle study.
Ichkeul lagoon (Tunisia)	37º 10' N	Casagranda and Boudouresque, 2005	Population dynamics; Production	Monthly (July 1993 to April 1994)	3 Replicates 0.18 cm^2 core (depth 20 cm), 300 µm sieve.
Guadalquivir estuary (Cádiz - Spain)	36º 30' N	Rodriguez-Rúa et al., 2003	Gametogenic cycle	Monthly/fortnighly (June 1999 to May 2000)	Adults (23 to 58 mm) were collected.

Table 1 – Continuation.

Individual growth of *S. plana* was calculated in several previous works, either based on successive growth ring distances in the shell, or by estimating and tracking cohorts in size frequency distributions over successive sampling dates, being the resulting empirical data adjusted to mathematical equations. In 1957, Green estimated growth for a *S. plana* population on the Gwendraeth estuary (Wales) based on growth rings by constructing a curve using a series of shells; provided data was then used to calculate a mathematical growth model for each location. Hughes (1970) fitted data on distances between growth rings to a Ford-Walford method, plotting the shell length at one winter ring (L_t) against the shell length at the next winter ring (L_{t+1}):

$$L_{(t+1)} = L_t \,.\, (1 - (e^{-k})) + L_\infty \,.\, (e^{-k})$$

where:

L_∞ = theoretical maximum size (asymptotic length);

k = rate at which growth rate decreases with age.

The author also plotted the data from Green (1957) by the same Ford-Walford method, obtaining similar results. In the Prévost lagoon (France), growth was estimated by applying a simple mathematical model on size frequency distributions data (Guelorget and Mazoyer-Mayère, 1983). Bachelet (1981), Sola (1997) and Guerreiro (1998) used a von Bertalanffy equation to calculate L_t, based on growth rings and size frequency distributions. This equation is one of the most frequently used methods on growth estimation of molluscs and allows us to compare growth curves calculated on different populations:

$$L_t = L_\infty \,.\, (1 - (e^{-k\,.\,(t - to)}))$$

where:

L_∞ = asymptotic length;

t_o = hypothetical age when $L_t = 0$;

k = growth constant.

In this work, we fitted our data on the Mondego estuary (Portugal) population to the same growth model, through cohort recognition using the ANAMOD software package (Nogueira, 1992). Furthermore, we adjusted the von Bertalanffy model to the results of Green (1957), Hughes (1970) and Guelorget and Mazoyer-Mayère (1983), calculating the parameters k and L_∞ from the Ford-Walford plot as in Bachelet (1981).

The estimated equation curves were analysed and parameters (k, L_∞) resulting from these studies, gathered in available bibliography, were compared on different *S. plana* populations along the latitudinal gradient.

Data from different studies on secondary production of *S. plana* were also analysed and compared. Different cohort-based methods were used to calculate secondary production: 1) removal summation method (Hughes, 1970b; Bachelet, 1982; Sola, 1997; Guerreiro, 1998; Casagranda and Boudouresque, 2005), where production is computed as the change in biomass from time *t* to time t_{+1}, over the cohort time period (sum of the standing stock gain) plus the mortality due to predation, among others (biomass eliminated), over the same period; 2) increment summation method (Bachelet, 1982; Sola, 1997; Guerreiro, 1998; Verdelhos et al., 2005) where production is computed as the change in biomass from time *t* to time t_{+1}, over the cohort time period, due to the growth increases all the members of the population; and 3) instantaneous growth method (Guerreiro, 1998), where production is also derived from the growth increments of all the members of the population, which are added for the study period, but a growth rate is computed. These different methods have been assumed to provide similar evaluations of secondary production, and are among the most accurate ones (Dolbeth et al. 2005). Results from Hughes (1970b) were converted from calories to grams and results in dry weight (DW) in Guelorget and Mazoyer-Mayère (1983) were converted in ash free dry weight (AFDW) by Bachelet (1982).

A redundancy analysis (RDA) was applied to the collected and estimated data, in order to evaluate the relationships between *S. plana* population dynamics, reproduction and growth parameters, the environmental parameters and latitude. The RDA was chosen after detecting a linear gradient with a detrended correspondence analysis (DCA), performed with the biotic data. All environmental variables were used in a first analysis and their significance was tested with the model forwards selection procedure. A second analysis was performed only with the significant environmental variables. These analyses were performed with CANOCO software (Van den Brink and Ter Braak, 1999).

RESULTS

Monthly temperatures (Climate Normal for 1971-2000) followed a clear seasonal pattern during the year, characteristic of temperate regions, with lower values during winter, increasing towards summer (Fig. 2). A general latitudinal gradient observed along the European coast and a strong relation between temperature and latitude was observed (Fig. 3). Northern regions (e.g. Wadden Sea, Ireland, UK) are usually characterized by low temperature values, severe cold winters and mild summers, while with decreasing latitude, temperature increases towards Southern regions, characterised by mild winters and hot summers, reaching ~35ºC in Tunisia and Cádiz (Spain) (Fig. 2). Nevertheless, temperature patterns are dependent not only on the latitudinal gradient, but also on local environmental conditions; for instance, on the Mediterranean region, similar temperature patterns are registered in different locations (e.g. Marseille and Tunisia).

The existence of different reproduction patterns along with latitude seems quite clear when analysing gonad development, spawning and recruitment periods. In Northern populations (> 50º N) gonad development starts during Spring and spawning periods are usually short, lasting two or three months, and occurring during summer (Fig. 4 – 1;2;3). With decreasing latitude, gonad development tends to start earlier in the year (Winter) and the same is observed with spawning periods (March/April), enlarging towards South, until lasting for ~7 months, from March to September, in the Guadalquivir estuary (Fig. 4 – 8). The extent of spawning period showed linear relationships with both latitude and temperature (Fig. 5), increasing along a North – South gradient, towards warmer climates. Benthic recruitments are also shorter in Northern populations, enlarging towards South, where populations may present one large or two separate shorter recruitment periods, originating one or two cohorts per year (Table 2).

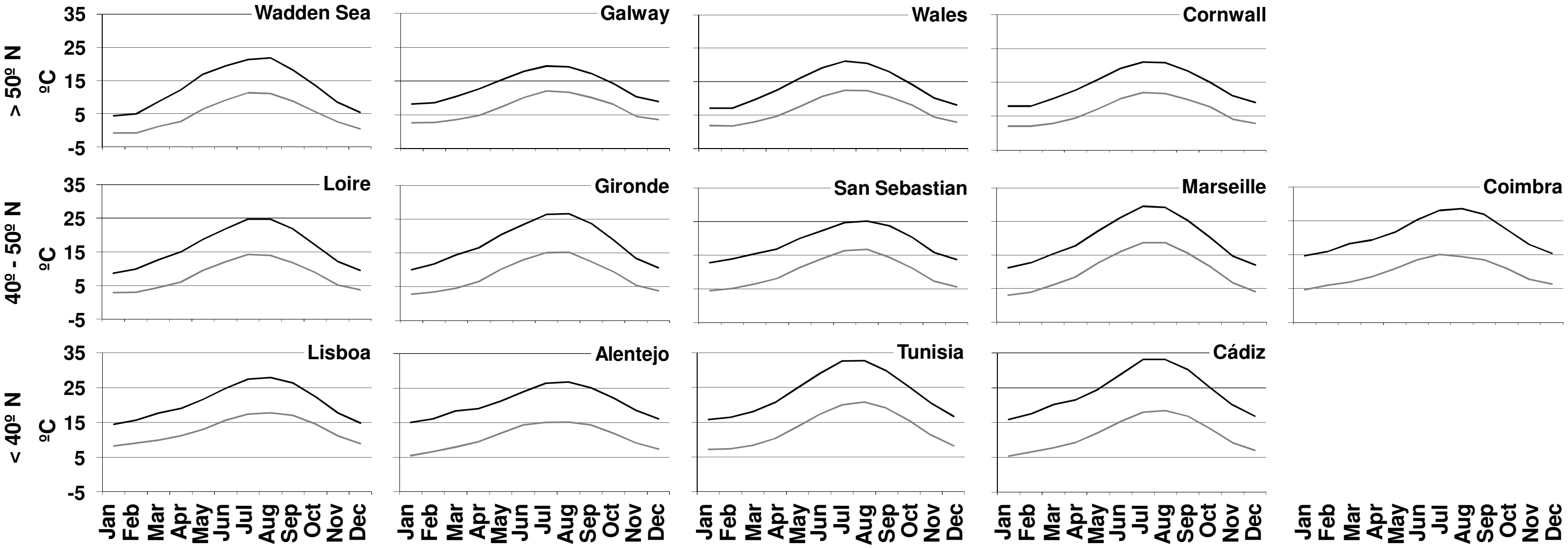

Fig. 2 – Normal climate temperature (1971-2000) on the Western European and Mediterranean coast along a latitudinal gradient. Mean maximum temperature —; Mean minimum temperature —).

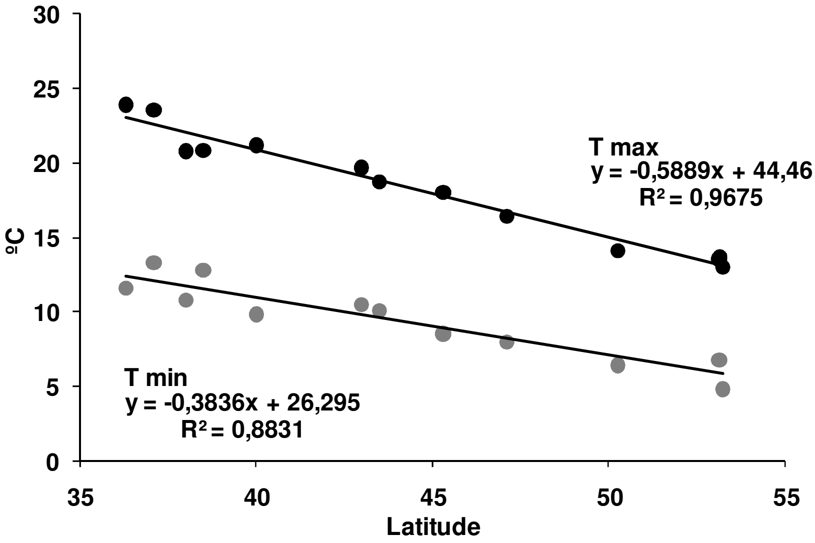

Fig. 3 – Regression equation and R^2 values relating Latitude with Temperature values.

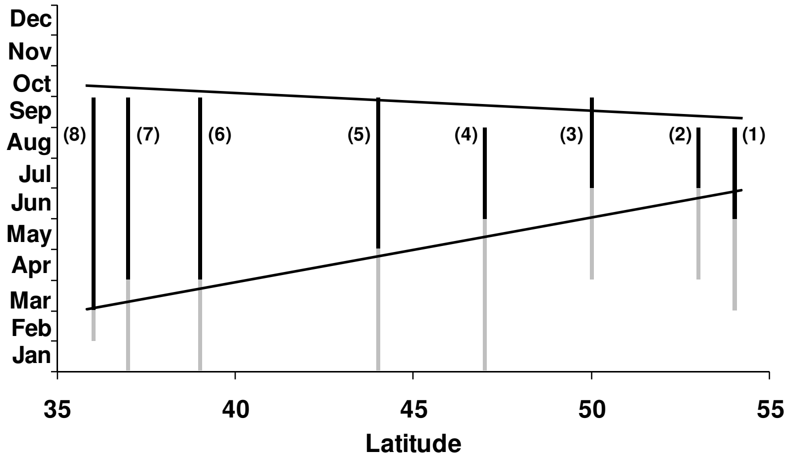

Fig. 4 – *Scrobicularia plana* spawning (—) and gonad development (—) periods at different latitudes: 1) Mweeloon bay (Ireland); 2) Conway bay (Wales - UK); 3) Tamar estuaries (Cornwall – UK); 4) Bourgneuf bay (Loire – France); 5) Bidasoa estuary (San Sebastian – Spain); 6) Tagus estuary (Lisboa – Portugal); 7) Mira estuary (Alentejo – Portugal); 8) Guadalkivir estuary (Cádiz – Spain).

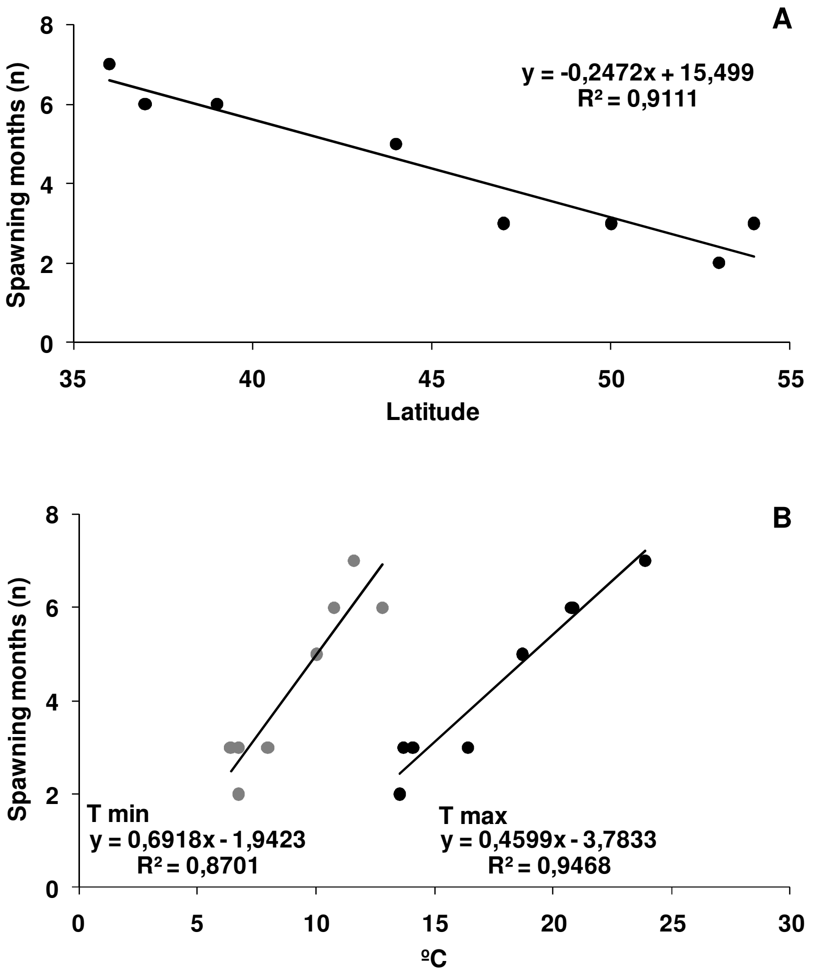

Fig. 5 – Regression equation and R^2 values relating: A) Latitude with Spawning periods; B) Temperature with Spawning periods.

In fact, two annual recruitment periods, the first occurring during spring and the second during summer, were observed in the Gironde estuary, Arcachon bay, Mondego estuary, Tagus estuary, Mira estuary, while in the Bidasoa estuary recruitment occurred during a single long period. In the studied Mediterranean populations, a post summer recruitment period was observed: in the Prévost lagoon a single short recruitment period occurs during September and October, while in the

Ichkeul lagoon (Tunisia) two recruitment periods occurred: one in March and April and the other in October and November.

Table 2 – *Scrobicularia plana* benthic recruitment periods at different latitudes.

Local	Latitude	Cohorts	Recruitment Period	Nº Months
Dee estuary (UK)	53º 19' N	1	Summer	2
Gironde estuary (Gironde – France)	45º 30' N	2	Spring Summer	5
Arcachon Bay (Gironde – France)	44º 50' N	2	Spring Summer	4
Bidasoa estuary (San Sebastian – Spain)	43º 50' N	1	Summer	4
Prévost lagoon (Marseille – France)	43º 00' N	1	Autumn	3
Mondego estuary (Coimbra – Portugal)	40º 07' N	2	Spring Summer	5
Tagus estuary (Lisboa – Portugal)	38º 50' N	2	Spring Summer	4
Mira estuary (Alentejo – Portugal)	37º 43' N	2	Spring Summer	4
Ichkeul lagoon (Tunisia)	37º 10' N	2	Spring Autumn	4

High variability on mean abundance values was observed on the studied *S. plana* populations along the Western European and Mediterranean coast (Fig. 6 A). Northern populations (> 50º N), in the Wadden Sea, Ireland, UK and French English Channel (Somme bay), registered low abundance values, often < 250 ind.m^{-2}. With decreasing latitude, abundance values tend to increase, and more abundant populations were found between 40º N and 50º N (~ 500 ind.m^{-2} in the Loire estuary; ~ 1000 ind.m^{-2} in the Gironde estuary and Arcachon bay; ~2500 ind.m^{-2} in the Bidasoa estuary; ~3000 ind.m^{-2} in the Prévost lagoon; ~1500 ind.m^{-2} in the Mondego estuary).

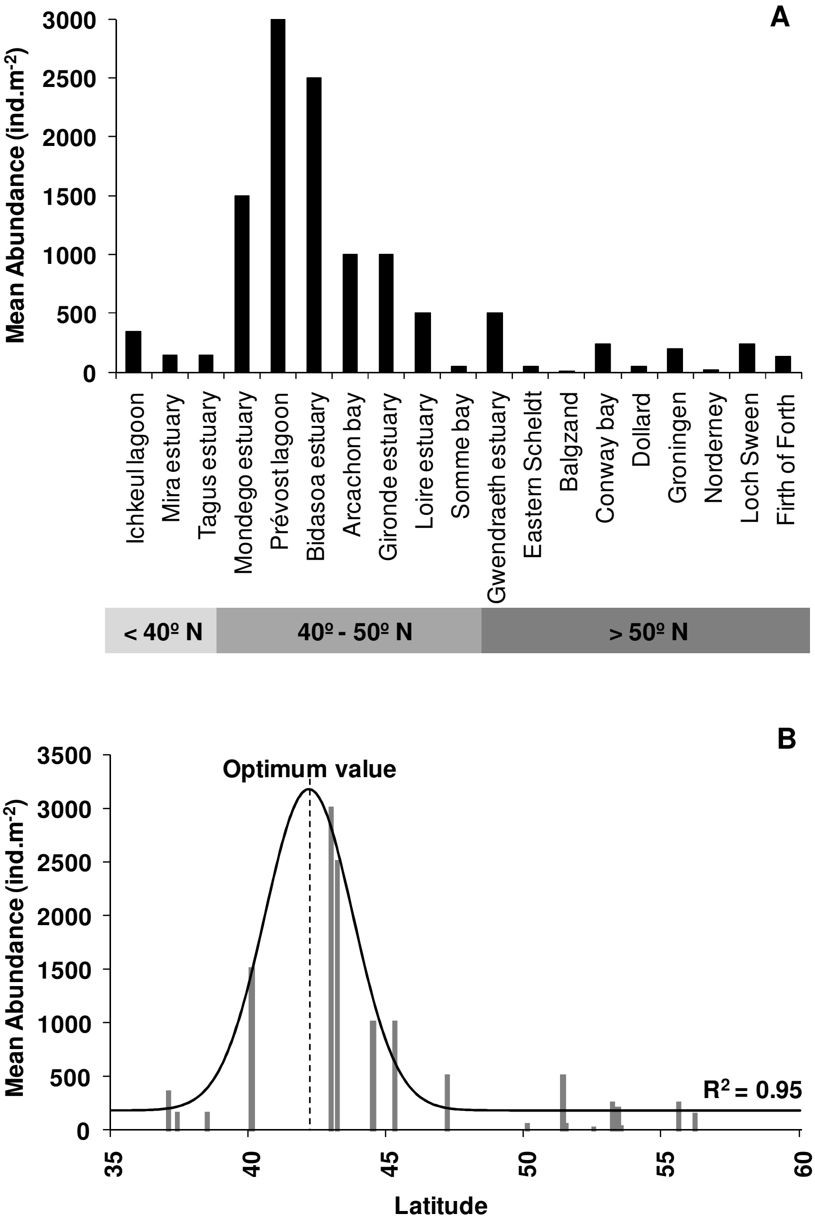

Fig. 6 - Mean abundance of *Scrobicularia plana* and the estimated Gaussian distribution along a latitudinal gradient.

Further South (< 40º N), the mean population abundance values decreased again. *Scrobicularia plana*'s population abundance seems to follow a Gaussian distribution along latitude (Fig. 6 B), with maximum values near 42º N and decreasing both towards North and South, which was established after the application of the Gauss equation to abundance results: $y = a + b^{(- 0.5 ((x - c) / d2)}$, in which: y – abundance; x – latitude.

From the analysis of the estimated von Bertalanffy growth model, from the resultant growth coefficient – *k* (Fig. 7), and from the resulting total length reached at year 1 and year 2 (Fig. 8 A), we can observe different growth patterns on populations along latitude. Individual growth in *S. plana* is usually higher during the first years of life, declining with age, although in Northern populations (> 50º N) that difference is not much clear. In this area, growth is slower and individuals show lower growth rates and extended life span (Fig. 7). The two UK populations (Conway bay and Gwendraeth estuary) showed similar growth patterns, with constant low growth: *k* = 0,194 and *k* = 0,108, respectively, and reaching ~10 mm on the 1st year and ~20 mm on the 2nd. Populations on the Gulf of Biscay show a different growth pattern, with higher *k* values (Gironde estuary: k = 0,625; Arcachon bay: k = 0,322; Bidasoa estuary: k = 0,815) and an accentuated growth especially during the 1st and 2nd years, reaching ~ 18 mm and ~ 28 mm, respectively. On the Mediterranean (Prévost lagoon) growth is particularly intense, reaching 22 mm during the 1st year and 33 mm on the 2nd (Fig. 8 A). On the Portuguese populations, growth is more intense during the 1st and 2nd years, reaching ~ 22 mm, and growing ~ 8 mm in the following years. Growth rates seem to be somewhat intermediate between the UK and the Gulf of Biscay and Mediterranean populations, with *k* values between 0.2 and 0.41. When analysing the resulted growth at year 2, along latitude, we can observe a Gaussian variation, with higher values near 43º N (Fig. 8 B), estimated from the application of the Gauss equation to length results: $y = a + b^{(- 0.5 ((x - c) / d2)}$, in which: y – length at year 2; x – latitude.

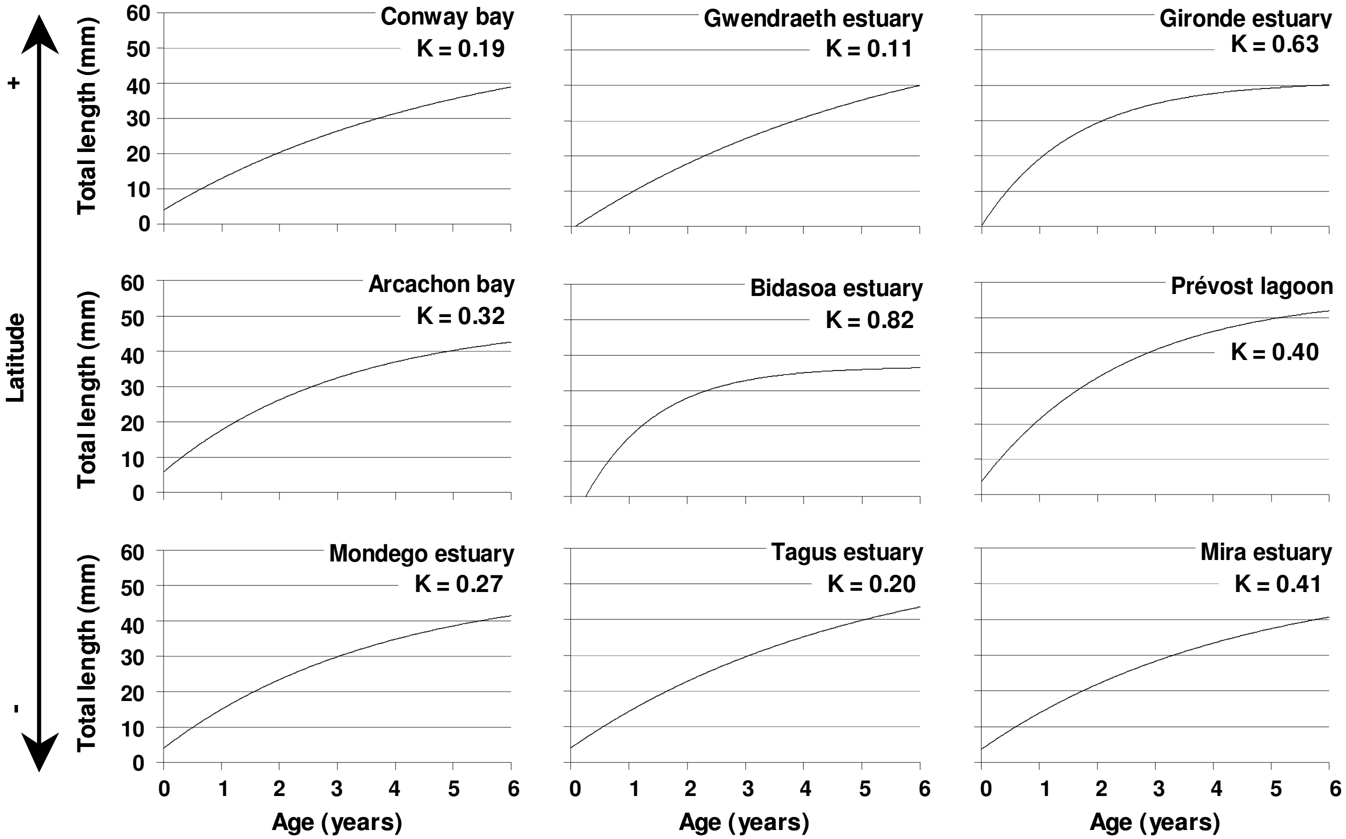

Fig. 7 – Growth estimation using the von Bertalanffy equation on different *Scrobicularia plana* populations, along a latitudinal gradient.

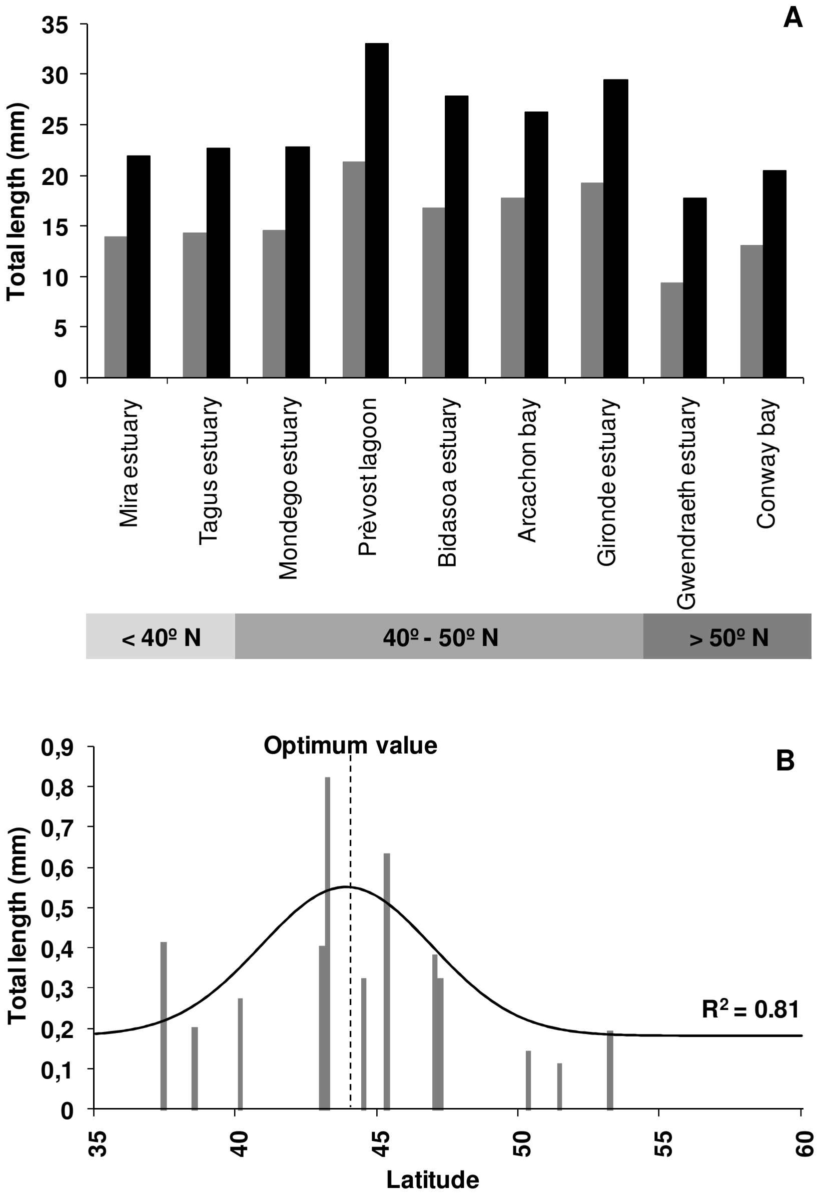

Fig. 8 – *Scrobicularia plana* total length at year 1 (▨) and year 2 (■), obtained from the von Bertalanffy growth estimation model applied on different *S. plana* populations and Gaussian distribution of the total length of *Scrobicularia plana*, at year 2, along a latitudinal gradient.

Results from different studies on *S. plana* production are highly variable, either between populations of different geographic regions and even in the same area (Table 3). Production (P) and productivity ($P/\overline{B}$) estimates are dependent on the sampling procedure and calculation methodology, local conditions and on the populations' intrinsic characteristics (e.g. dynamics, structure, biomass, growth) and therefore are difficult to analyse and compare. From the given results, we can observe the highest production (P) and productivity ($P/\overline{B}$) values in areas between 40º N and 50º N, particularly in the Bidasoa estuary (P = 83.62 g.m^{-2}.year^{-1} ; $P/\overline{B}$ = 1.21) and in the Prévost lagoon (P = 81.05 g.m^{-2}.year^{-1} ; $P/\overline{B}$ = 3.65).

Table 3 – Mean Population Biomass ($\overline{B}$, g AFDW m-2), Production (P, g AFDW m-2 y-1) and Productivity ($P/\overline{B}$, y-1) on different *Scrobicularia plana* populations, along a latitudinal gradient.

	Latitude	Habitat	B	P	$P/\overline{B}$
Conway bay (Wales – UK)	**53º 15' N**	Seaward	46.24	13.41	0.29
		Marshward	4.37	2.97	0.68
Tamar estuaries (Cornwall – UK)	**50º 25' N**		2.15	0.48	0.22
Arcachon bay (Gironde – France)	**44º 50' N**	Interior Area (Fine Sand)	9.65	8.34	0.86
Bidasoa estuary (San Sebastian – Spain)	**43º 22' N**	Estuary mouth (Mud)	69.20	83.62	1.21
Prévost lagoon (Marseille – France)	**43º 20' N**	Seaward (Fine Sand)	22.02	81.05	3.68
		Interior Area (Muddy Sand)	1.31	6.54	4.99
		Interior Area (Muddy Sand)	2.04	4.64	2.28
Mondego estuary (Coimbra – Portugal)	**40º 07' N**		16.17	9.41	0.58
Tagus estuary (Lisboa – Portugal)	**38º 50' N**	Interior area (Marsh)	26.10	6.78	0.26
Mira estuary (Alentejo – Portugal)	**37º 43' N**	Estuary mouth (Sand)	4.75	1.76	0.37
		Interior area (Sandy mud)	3.74	0.14	0.04
		Interior area (Mud)	7.67	2.40	0.31
Ichkelul lagoon (Tunisia)	**37º 10' N**	Interior area (Bare bottom)	34.27	12.17	0.36
		Channel mouth (Marsh)	22.54	17.41	0.77

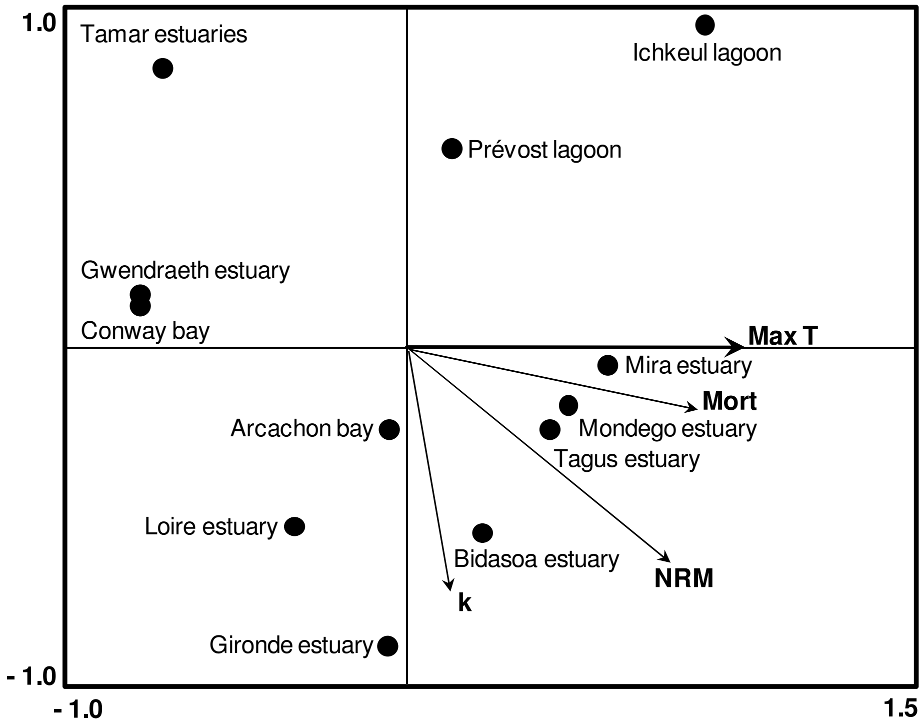

Fig. 9 – Ordination plot of Principal Components Analysis (PCA) for maximum temperature (MaxT), population dynamics (Mortality; NRM – number of recruitment months) and growth (k) patterns in the 12 *Scrobicularia plana* populations.

Data on reproduction patterns (number of reproduction months – NRM), mortality (Mort) and growth (*k*) were comparatively analysed using a Redundancy Analysis (RDA), after running a Detrended Components Analysis (DCA), in order to compare twelve *S. plana* populations relate them with temperature conditions, in order to outline the similarities of different geographic areas. A significant relation between maximum temperature (Max T) and the biological variables was obtained ($p<0.05$). By analysing the resulted ordination diagram (Fig. 9), similarities among populations within the same geographic area are highlighted and populations are grouped according to their characteristics. The UK populations (Conway bay, Gwendraeth estuary and Tamar estuaries) form a group characterised by short reproduction periods during summer, winter mortality and low *k*, reflecting low growth rates. The Gulf of Biscay (Gironde estuary, Arcachon bay, Bidasoa estuary) populations are characterised by longer reproduction periods, summer mortality and high growth rates, forming another group. The Loire population is plotted near this group, resulting from similarities in the reproduction pattern. However, the existing differences in growth and mortality period differentiate them in the plot. The Portuguese populations (Mondego estuary, Tagus estuary and Mira estuary) are grouped together, showing longer reproduction periods and summer mortality, while *k* is smaller than in the previous group. However, *k* values state the differences within this group. Finally, the Mediterranean populations (Prévost lagoon and Ichkeul lagoon) are plotted together and isolated from other groups.

DISCUSSION

Latitude is a surrogate for several environmental variables, such as temperature, precipitation, seasonality and ecosystem energy fluxes (Willig et al., 2003; Giangrande and Licciano, 2004), defining a general North - South gradient. Ecological patterns generally follow this latitudinal gradient that influence species diversity, individual growth, body size and productivity (Crame, 2000, 2002; Rex et al., 2000; Roy et al., 2000a, b; MacPherson, 2002). Throughout its distribution range, diverse populations have to face different habitats, resulting from the interaction of environmental variables, both dependent on latitudinal gradient and on local environmental conditions, that define its main characteristics (e.g. temperature and precipitation regimes, food availability, sediment) (Sola, 1997). As a result, variable patterns of population dynamics, reproduction, growth rates and productivity are observed.

Analysing the studies on *Scrobicularia plana* along the Western European and Mediterranean coast, diverse results were obtained and different patterns observed. As in other bivalve species, reproduction patterns in *S. plana* are latitude related and dependent on temperature, influencing the reproductive cycle. The temperature increment after the cold winter months triggers *S. plana*'s gonad development (Paes da França, 1956; Hughes, 1971; Sola, 1997; Guerreiro, 1998; Rodriguez-Rúa, 2003; Raleigh and Keegan, 2006; Mouneyrac et al., 2008), from a threshold value of ~ 8º C (Hughes, 1971; Sola, 1997; Raleigh and Keegan, 2006). Additionally, the time extent of this development seems to be dependent on temperature, which varies with latitude, lasting longer on higher latitudes and shortening towards South.

On one hand, the time available for gonadal development decreases with increased latitude, until there is insufficient time to complete maturation (Hughes, 1971; Rodriguez-Jaramillo et al., 2001), near the geographical limits of the species. On the other hand, spawning depends on temperature (Paes da França, 1956; Hughes, 1971; Sola, 1997; Guerreiro, 1998; Rodriguez-Rúa, 2003; Raleigh and Keegan, 2006; Mouneyrac et al., 2008), occurring when favourable temperature values are observed. As a consequence, spawning shows a clear latitudinal trend as a response to temperature, and usually occurs later on Northern populations, on short periods during Summer, while further South it may start earlier, prolonging for longer time periods. Here, spawning may show one or two main peaks, which is reflected on benthic recruitment, originating one or two cohorts per year (Paes da França, 1956; Hughes, 1971; Sola, 1997; Guerreiro, 1998; Rodriguez-Rúa, 2003; Raleigh and Keegan, 2006; Mouneyrac et al., 2008).

High post-recruitment mortality is quite common in bivalve populations, once spat living on the sediment surface is highly vulnerable to extreme values of temperature, salinity and are easily removed by strong water flows. Benthic recruitment depends on the planktonic larvae survival, finding appropriate location to settle and surviving from settlement to recruitment (Ripley and Caswell, 2006). Its success is thus dependent on habitat conditions, such as temperature, salinity, sediment type and adult density (Hughes, 1970a). On the Mediterranean populations, for instance, post-summer recruitment was registered, instead of occurring in warmer months, probably due to extremely high summer temperature causing massive mortality and reducing recruitment success (Guelorget and Mazoyer-Mayère, 1983; Casagranda and Boudouresque, 2005).

Usually, on this species, whenever a highly successful recruitment occurs, the following years may exhibit a decline in recruitment success, reduced or even suppressed by the resulting dominant cohort, essentially as a result of intraspecific competition for food and space (Hughes, 1970a; Casagranda and Boudouresque, 2005). Reproduction patterns, along with habitat and environmental conditions can, therefore, influence the whole population dynamics.

Northern *S. plana* populations (> 50º N) often registered low abundance values, depending on short lasting successful recruitments, occurring in favourable years, whose originated cohorts usually dominate the population structure for several years (Green, 1957; Hughes, 1970a; Essink et al., 1991). During severe winters, periods of massive mortality were registered, resulting from harsh conditions such as extreme cold, high freshwater flows and low salinity (Hughes, 1970a; Essink et al., 1991). More abundant and structured populations were observed in warmer areas, resulting from more frequent successful recruitments and lower mortality (Bachelet, 1981; Guelorget and Mazoyer-Mayère, 1983; Essink et al., 1991; Sola, 1997; Verdelhos et al., 2005). Further South, smaller recruitment peaks are described in the Tagus and Mira estuaries (Guerreiro, 1998), leading to lower density populations, but stable and well structured, probably related to lower mortality when compared to Northern populations. In the Mediterranean (Prévost and Ichkeul lagoons), the population structure is usually dominated by juveniles, depending on successful recruitments, which are much higher in the Prévost lagoon leading to high density population (Guelorget and Mazoyer-Mayère, 1983; Casagranda and Boudouresque, 2005). Massive mortality occurs during summer, certainly caused by extreme high temperature and salinity values, and low oxygen conditions (Casagranda and Boudouresque, 2005). As a general trend, the most abundant populations were found between 40º N and 50º N, near the middle of the geographic distribution of the species, decreasing towards the edges, following a Gaussian distribution.

Several methods were used on *S. plana* growth estimation: simple equation models (Green, 1957; Guelorget and Mazoyer-Mayère, 1983); Ford-Walford plots applied to annual length increments (Hughes, 1970a) and von Bertalanffy equation models (Bachelet, 1981; Sola, 1997; Guerreiro, 1998). In this study, we fitted the available data on a von Bertalanffy growth model, using the same method as Bachelet (1981), in order to compare growth rates on diverse populations. The resulted k value (Bachelet, 1981) is a merely growth coefficient and should not be regarded as a growth rate *per se* (MacDonald and Thompson, 1988) and so, care must be taken when analysing and comparing different populations. In fact, the highest growth rate was observed on the Prévost lagoon (France), with individuals growing up to 33 mm in the second year, although the resulted $k = 0.404$ was lower than in other populations.

Within species, latitudinal clines are determined genetically and serve to offset the effects of temperature on growth rates, which show a positive correlation (Clarke, 2003). As in other bivalve species, growth in *S. plana* appears to follow not a linear latitudinal gradient, but a tendency to decrease towards both its Northern and Southern distribution limits (Hummel et al., 1998), showing a Gaussian distribution, with higher values between 40º N and 50º N. In general, growth rate is lowest on Northern populations (> 50º N), with individuals growing until ~10 mm on the 1st year and ~20 mm on the 2nd, and k values resulting between 0.1 and 0.2. Near the Gulf of Biscay and Mediterranean, growth rates reach the highest values, growing up to ~ 18 mm on the 1st year and ~ 28 mm on the 2nd, with k between 0.3 and 0.8. *Scrobicularia plana´s* growth rate decreases again on the Portuguese Atlantic coast, reaching ~ 12 mm on the 1st year and ~ 22 mm on the 2nd, with k between 0.3 and 0.6.

Production and productivity estimates of a population are good evaluation methods of the functional importance of a species on the ecosystem (Bachelet, 1982; Casagranda and Boudouresque, 2005). Production is dependent on habitat conditions, population structure, stability and growth rates, being also highly dependent on the sampling and estimation methods (Bachelet, 1982; Sola, 1997; Guerreiro, 1998; Casagranda and Boudouresque, 2005; Verdelhos et al., 2005). Productivity is a measurement of the biomass renewal rate of the population (Bachelet, 1982) and shows high intraspecific variability, related to the species' biological cycle on different locations. Moreover, variable results were observed on different sampling stations of the same ecosystem (Verdelhos et al. 2005). In Conway bay and Ichkeul lagoon, higher $P/\overline{B}$ was estimated in stations with plant coverage, indicating more dynamic populations in marsh areas (Hughes, 1971; Casagranda and

Boudouresque, 2005). High production values were obtained in the Bidasoa estuary and the Prévost lagoon, which may be related with both high recruitment success and growth of the species in those locations. On the other hand, exceptionally high $P/\overline{B}$ values observed in the Prévost lagoon, in accordance to the high growth rates and shorter life span found in the lagoon (Guelorget and Mazoyer-Mayère, 1983). Usually, $P/\overline{B}$ values tend to decrease both towards Northern or Southern populations.

By analysing ecological parameters on a RDA, a general North - South gradient was observed, defined by temperature. However, similarities between populations of close geographic areas are highlighted, grouping populations according to reproduction patterns, mortality and growth: UK (Conway bay, Gwendraeth and Tamar estuaries); Loire, near the Gulf of Biscay group (Gironde estuary, Arcachon bay and Bidasoa estuary); Portugal (Mondego, Tagus and Mira estuaries); and finally, the Mediterranean populations (Prévost lagoon and Ichkeul lagoon) form a group quite separated from the others, despite the similar high growth rate and registered mortality period, enhancing the importance of temperature and reproduction patterns on the analysis.

Apparently, *S. plana* shows different life strategies along its distributional range, reflected on the reproduction patterns, population abundance and dynamics, growth and production. Populations in latitudes between 40º N and 50º N seem to have the highest ecological performance for this species, showing extremely successful recruitments and the highest abundance values, growth rates, production and productivity, particularly near 42º/43º N. The ecological conditions on these areas appear to be optimal for this species, with less climatic extremes of temperature and precipitation, favourable temperature regime for gonadal development and growth, favouring highly energetic life patterns (Clarke, 2003).

In contrast, populations further North and South show “slower” life strategies, with less successful recruitments, lower abundance, significant mortality episodes and lower growth and productivity. This is certainly a result of poorer ecological conditions in areas closer to the edges of the species geographic distribution. In fact, animals living at the biogeographic limits of the species distribution are assumed to live on the limits of their adaptation capacities, showing poorer ecological performance and higher sensitivity to stress, when compared to animals living at the centre of its distribution (Hummel et al., 1998).

With increasing climate change scenarios, variations in temperature patterns are expected, altering both the latitudinal gradient and seasonality, which may lead to significant changes in the population dynamics throughout its geographic range, as already observed for *Mytilus* sp. (Jansen et al., 2007). Moreover, by understanding the influence of temperature and seasonality, it will be reasonable to predict possible responses to ongoing climate change.

Most latitudinal gradient studies provide a powerful tool that can be used to understand temperature-dependent ecological patterns, as well as to predict adaptive tolerance and responses to climate change (Jansen et al., 2007). Major impacts are expected on populations living on the edges of their geographic distribution, affecting abundance, population dynamics and even causing shifts on distribution limits of a species (Dekker and Beukema, 1999; Jansen et al., 2007). Moreover, increased knowledge on the ecological patterns and life strategies is achieved with this kind of studies, which may be useful to future approaches on *S. plana* ecology assessment.

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GENERAL DISCUSSION AND CONCLUSIONS

WHY FOCUS ON ESTUARIES?

Estuaries are widely recognised as highly important ecosystems, either ecologically to several invertebrate, fish and bird species (Elliott, 2002; Kennish, 2002; McLusky and Elliott, 2004; Cardoso et al., 2004; Lopes et al., 2006; Dolbeth et al., 2007; Martinho et al., 2007), as well as socio-economically to mankind, being essential focal points where human populations settle and develop (Kennish et al., 2002; McLusky and Elliott, 2004; Martínez et al., 2007; Svensson et al., 2007; Vasconcelos et al., 2007).

The species inhabiting these ecosystems are naturally subjected to a unique and biologically challenging environment. With the increasing anthropogenic and natural pressures (e.g. pollution, eutrophication, habitat loss, resources exploitation, extreme climate events), severe impacts are expected on estuarine populations and communities, colliding with the ecological functions of these ecosystems and threatening their long-term integrity (Kennish, 2002; McLusky and Elliott, 2004; Martínez et al., 2007; Vasconcelos et al., 2007).

It is then a key issue to ecologists to understand the ecosystem dynamics and the ecological responses facing multiple stressors, which can impact resources through single, cumulative and synergistic processes (Adams, 2005). All these stressors may lead to changes on population dynamics, growth, production, and even on the geographical range of distribution of a species (Vinebrooke et al., 2004; Adams, 2005; Cardoso et al., 2005; Dolbeth et al., 2007). The responses of ecosystems will depend on their sensitivity, adaptive capacity and vulnerability (Houghton, 2005).

The evaluation of the ecological status on aquatic ecosystems has become a worldwide priority, due to the increasing pressure on natural aquatic ecosystems. The development of criteria and tools will allow implementing future restoration measures and improving the ecosystem ecological conditions (Lillebø et al., 2007; Teixeira et al., 2007). The general assessment methodology consists essentially in defining a threshold regarding a reference ecological condition (considered as a high quality status) and to analyse the ecosystem deviation from that threshold (Lillebø et al., 2007; Teixeira et al., 2007). The main purpose is to assess if an “estuary is still functioning as an estuary after a disturbance” and therefore several methods to study structural and functional symptoms are being recommended (Elliott et al., 2007).

In order to achieve a holistic view of the ecosystem ecological status, functioning and to understand how that ecosystem will respond to different stressors, multidimensional ecological approaches, through an integrative analysis of its different components, are required. Therefore, different approaches on population and community level processes, such as dynamic studies, production estimation or ecological modelation are essential, enlarging our scope on the assessment of the influence of different stressors on an ecosystem.

THE MONDEGO ESTUARY

The present work focuses on the impacts of anthropogenic and natural stressors – eutrophication and extreme climate events, on the Mondego estuary. A long-term monitoring program, from 1993 to 2005, provides a large data base, which allows the assessment of the ecosystem under different environmental scenarios:

- Eutrophication;
- Restoration;
- Extreme events of temperature and precipitation.

On this thesis we focused on the macrobenthic assemblages of the Mondego estuary and particularly on the bivalves *Scrobicularia plana* and *Cerastoderma edule*, assessing their long-term variability on dynamics and production, as a function of environmental changes. Furthermore, *S. plana* was studied under different ecological approaches, through the development of a population dynamics model and through the study of the different ecological patterns along its geographical distribution.

MACROBENTHIC ASSEMBLAGES

Macrobenthic communities are an essential component of estuarine ecosystems, in terms of its ecological dynamics and production (Dolbeth et al., 2003, 2007; Ysebaert et al., 2003). The macrobenthic community of the Mondego estuary was evaluated, regarding biodiversity, trophic groups, and dynamics, and through multivariate approaches – Principal Response Curves and Multi Dimensional Scaling, in order to analyse differences between areas. This community is clearly dominated by deposit feeding species, showing a characteristic trophic structure of unstable detritus/mineralization environments (Flindt et al., 1999; Dolbeth et al., 2003). This suggests that a great part of the energy/biomass enters the system via the detritus food chain (Dolbeth et al., 2003).

The seagrass bed has proven to be more functionally rich, when compared to the other areas, supporting higher percentages of other trophic groups, such as herbivores, carnivores and omnivores. Moreover, this area is characterised by higher species diversity, abundance, biomass and productivity (Pardal et al., 2000, 2004; Marques et al., 2003; Cardoso et al., 2005; Dolbeth et al., 2007). A clear spatial gradient was observed on the intertidal flats of the estuary, with the seagrass bed samples separated from those in the eutrophic area, by samples from the intermediate area (Chapter 1).

THE BIVALVES *SCROBICULARIA PLANA* AND *CERASTODERMA EDULE*

The bivalves *Scrobicularia plana* and *Cerastoderma edule* are two of the most important species of estuarine benthic communities worldwide, considering abundance, biomass and infaunal production (Mistri et al., 2000; Cusson and Bourget, 2005; Dolbeth et al., 2007). Benthic bivalves filter organic matter from the system, being an essential link between primary producers and consumers, affecting the food availability for the entire community and playing a central role in the energy flow of the ecosystem (de Montaudouin et al., 1999; Troost et al., 2008, 2009; Strayer et al., 1999; Vaughn and Hekenkamp, 2001). Here we analysed and compared the dynamics and production of these species on two distinct areas of the Mondego estuary, in order to understand its role on the ecosystem (Chapter 1).

When analysing the spatial distribution of *Scrobicularia plana* and *Cerastoderma edule* on the sampled areas, we observed clear differences between the seagrass bed and the eutrophic bare bottom. These areas can be considered as two completely distinct habitats arising from the long eutrophication problem that affected the Mondego estuary since the 80’s. In fact, differences in physical-chemical characteristics, plant coverage, biological composition and productivity between these two areas are quite clear and reflect a spatial eutrophication gradient (Lillebø et al., 2005; Cardoso et al., 2008; Dolbeth et al., 2007; Leston et al., 2008). In fact, abundant populations of both species were found on the seagrass bed, yet on the bare muddy sand-flat *S. plana* was clearly a dominant species, while *C. edule* showed a scarce population, probably related to settlement patterns and mortality (Hughes, 1970; Lee, 1996; de Montaudouin and Bachelet, 1996; de Montaudouin, 1997; de Montaudouin et al., 2003) (Chapter 1).

The evaluation of a species production is a good approach to assess its importance on the ecosystem and to obtain a quantitative measure of its functioning (Bachelet, 1982; Casagranda and Boudouresque, 2005; Dolbeth et al., 2003, 2005; Cusson and Bourget, 2005; Cusson et al., 2006; Brey, 2001). Since the species in study are economically important, it also allows to speculate on the carrying capacity of the estuary for these resources and it may change with anthropogenic and climate impacts. A correct evaluation is therefore important to avoid misinterpretations of the ecosystems. Usually, on studies focusing on the main species of the Mondego estuary, production was calculated using cohort increment summation method (Verdelhos et al., 2005; Cardoso et al., 2005; Dolbeth et al., 2005; Ferreira et al., 2007). Although, when cohort-based methods are not applicable, as in the case of *Cerastoderma edule*, other methods must be used. As such, in order to accurately compare the two bivalve populations, production was estimated using an empirical method, based on the sum of biomass increases from consecutive sampling dates (Dolbeth et al., 2005). These methods are a quicker and easier way to obtain production estimates, and allow comparisons between populations and communities (Brey et al., 2001; Cusson and Bourget, 2005; Dolbeth et al., 2005).

ECOLOGICAL SCENARIOS

EUTROPHICATION

The Mondego estuary has experienced marked eutrophication over the last 20 year, which led to the replacement of the primary producers from slow growing macrophytes to fast and opportunistic macroalgae, with a consequent decline of the seagrasses (Dolbeth et al., 2003; Marques et al., 2003; Pardal et al., 2004; Lillebø et al., 2005; Leston et al., 2008). Seagrass beds usually support the richest macrofaunal compositions in terms of biodiversity and productivity (Marques et al., 2003; Dolbeth et al., 2007), by providing essential processes and services, such as nutrient cycling, detritus production and export, sediment stabilization and a wider variety of microhabitats, protection from predators and higher diversity of food resources, representing an optimal habitat for growth, survival and reproduction for many invertebrates, fish and bird species (Heck et al., 2003; Cunha et al., 2005; Polte et al., 2005; Marques et al., 2003; Pardal et al., 2000; Cardoso et al., 2002; Dolbeth et al., 2007).

The significant reduction of seagrass beds induced an overall decrease in the ecological integrity of the Mondego estuary, reflected on biodiversity decline and decreases in abundance, biomass and production of the macrobenthic community (Dolbeth et al., 2007; Cardoso et al., 2008b). Moreover, a significant structural change with declines in key species, such as the gastropod *Hydrobia ulvae* directly resulting from *Z. noltii* decline (Cardoso et al., 2008a) or the bivalve *S. plana* (Verdelhos et al., 2005), led to a global impoverishment of the macrobenthic assemblages (Pardal et al., 2000, 2004; Cardoso et al., 2004: Dolbeth et al., 2007). In fact, for *S. plana* the significant biomass and production reduction observed from 1993 to 1995 certainly relates to the instability caused by the eutrophication process. *C. edule*, instead, appears to be favoured on the seagrass bed, increasing its biomass and production during this period. The habitat changes resulted from eutrophication, particularly concerning to sediment characteristics and water turbidity, may have led to higher successful settlement and food availability to this suspension filter feeder (de Montaudouin, 1997; de Montaudouin et al., 2003), and the consequent impacts on other species may have given an ecological opportunity to this species (Chapter 1).

This process is unfortunately a common phenomenon worldwide, which brings the need to implement restoration programs to stop and reverse environmental quality decline and restore the ecosystem ecological integrity. This necessity is well expressed through several legislation that has been put out to to improve the water physicochemical and the ecological status of estuarine areas (e.g. European Union Water Framework Directive, European Marine Strategy, among others) (Lillebø et al. 2007, Teixeira et al. 2007). The Mondego estuary had been dwelling with this ecological problem when a management plan was implemented in 1998, with effective results in the environmental quality of the system.

RESTORATION

The restoration program was initiated in 1998, as a response to the ecological quality decline of the ecosystem, led to nutrient loading reduction reduced (Lillebø et al., 2005) and hydrodynamism improvement, reducing the water residence time. Consequently, macroalgal biomass was significantly reduced with an absence of blooms (Lillebø et al., 2005; Cardoso et al., 2008a; Leston et al., 2008).

Simultaneously, seagrass beds were protected and begun to recover, with higher biomass and coverage area (Cardoso et al., 2008a; Lillebø et al., 2005).

Within this brighter scenario macrobenthic assemblages were favoured, with increases in species richness, mean biomass and production (Dolbeth et al., 2007) Moreover, changes in the community were also reported with longer-living, large bodied species increasing (*Hediste diversicolor* and *S. plana*) and opportunistic species being reduced, suggesting a succession from r-strategists to K-strategists species (Dolbeth et al., 2007). This shift induced to the hypothesis that the system had higher stability and so an increase of the estuarine mean biomass was observed.

Looking at the bivalve populations more closely, we can see that *S. plana* population showed significant biomass and production increments, and presented a more stable and structured population, which indicates that the restoration program might have been beneficial. *C. edule* population, however, showed a different response, with considerable abundance and production decline, particularly on the seagrass bed, suggesting that the overall increase on the number and abundance of other benthic species might have been prejudicial as a result of higher competition pressure (Chapter 1).

EXTREME CLIMATE EVENTS

Regarding the climate in Portugal, since it has undergone major changes, the occurrence of episodes of flooding, drought and heat waves have increased. During the study period, extreme temperature and precipitation events have occurred, altering the system's hydrodynamics, salinity and water temperature (Cardoso et al., 2005). These events have clearly impacted the macrobenthic community, causing biodiversity loss and declines in abundance, biomass and production (Dolbeth et al., 2007), interrupting the recovery trend and slowing down the system's return to the previous state (Chapter 1).

The studied bivalve populations also seemed to be affected, showing abundance, biomass and production decrements when extreme events of of temperature or precipitation occurred. Intense floods may drastically affect juvenile recruitment and the caused high turbidity can affecting bivalves performance and survival (Norkko et al., 2002). Moreover, high salinity values during drought episodes and extreme temperatures during hot periods are expected to affect the health and survival of these species (Guelorget and Mazoyer-Mayère, 1983; Casagranda and Boudouresque, 2005).

The compounding effects of anthropogenic and climate stressors seem to lead to a significant negative impact on the resilience of macrofauna. The loss of biodiversity and reduced performance of individuals following the first stressor is probably the cause of resilience decline to a following stressor. In fact, macrofauna in the eutrophic area appears to be less resilient than the one present in the seagrass bed. Several authors believe that system resilience may be provided by biodiversity (Loreau et al., 2002; Marques et al., 2003; Raffaelli et al., 2003) and if it is severely reduced by one stressor (e.g. eutrophication), the effects of a subsequent stressor (e.g. extreme climate events) will be much greater than expected. The combined effects of the multiple stressors affecting the Mondego estuary seem to severely affect the recovery process, resulted from restoration, slowing the overall return to the undisturbed state.

POPULATION DYNAMICS MODEL

The Restoration process, which decreased the system's eutrophic state, provided an excellent opportunity to test an ecological model under different scenarios (eutrophication vs. restoration). A *S. plana* population dynamics model simulated the number of individuals on different environmental conditions as function of temperature, salinity and population density (Chapter 2).

The forcing functions used in the model were water temperature, salinity and population density, regulating mortality and recruitment. These seem the fundamental factors controlling the population in the area, which responded significantly to changes in the environmental conditions, in particular to high temperature and salinity values. Although *S. plana* supports a wide range of environmental conditions, temperature and salinity extremes cause mortality increase and may affect recruitment, influencing the dynamics of the population (Hughes, 1970; Guelorget and Mazoyer-Mayère, 1983; Casagranda and Boudouresque, 2005; Verdelhos et al., 2005).

This approach provides an important tool which allows us to understand *S. plana* responses to environmental changes. The model confirms the effective results of restoration. In fact, model simulations obtained for data during that period were more reliable, contrasting to simulations for data from the pre management period, which required more parameter calibration, concerning reproduction timing, minimum tolerated salinity and maximum tolerated temperature. This model was built for a specific region but with further recalibration, accounting for differences in local environmental characteristics and population parameters, it can be used for the same species or similar organisms in other ecosystems. Furthermore, the model seemed to be more sensitive to the occurrence of extreme environmental conditions and so it can be used for simulations predicting future environmental changes.

LATITUDINAL GRADIENTS ON *SCROBICULARIA PLANA*

Previous studies suggested the existence of latitudinal variation on the ecological patterns of *Scrobicularia plana*. latitudinal variations are usually related to temperature, that co-vary with latitude, influencing the recruitment success, survival and growth rates of a species and controlling primary producers and consequently food availability on the ecosystem (Macpherson, 2002; Willig et al., 2003; Angilletta and Sears, 2004; Giangrande and Licciano, 2004). This may result in different recruitment patterns, dynamics, growth and production patterns along the geographic range of distribution of a species, reflecting in different life strategies on different populations of the same species (Hughes, 1971; Bachelet, 1981, 1982; Essink et al., 1991; Sola, 1997; Clarke, 2003; Rodriguez-Rúa et al., 2003). Such variation was clearly observed for *Scrobicularia plana* populations, which showed higher ecological performances in areas near 40ºN, with more successful recruitments, higher density populations, higher growth rates and productivity, denoting favourable environmental conditions (Chapter 3). These areas, at the middle of the distribution range, may be considered as optimal habitats for this species.

With increasing climate change a variation of temperature pattern is expected, altering both the latitudinal gradient and seasonality, with significant impacts on population dynamics throughout its geographic range. Major impacts are expected on populations living closer to the edge of distribution, with a poorer ecological performance and thus more susceptible to environmental changes. Severe effects on the population dynamics, lowered tolerance to stressors and increased mortality are predictable, which may lead to shifts on the distribution of this species (Dekker and Beukema, 1999; Jensen et al., 2007). Such changes on the macrobenthic communities and in particular on commercially important populations such as *S. plana*, will have not only ecological impacts, but also significant economical effects to the local population, affecting valuable resources.

CONCLUSIONS

The present study assessed the impacts of anthropogenic and natural stressors on the Mondego estuary, evaluating long-term responses of the macrobenthic community and of the bivalves *Scrobicularia plana* and *Cerastoderma edule*, two of the most important species of the intertidal flats of the estuary, and focused on *S. plana* through different ecological approaches.

The Mondego is a highly productive ecosystem, of huge ecological and socioeconomic importance to local human populations. However, several human activities on the area affected the estuary, inflicting severe changes at the ecosystem level. **Eutrophication** is probably the most important anthropogenic stressor on the estuary over the last decades and it has unchained a series of impacts, compromising the overall integrity of the entire ecosystem, and **negative ecological responses** of the entire **macrobenthic community** and of ***S. plana*** seem quite clear.

Nowadays, ecological management is essential to the conservation of natural ecosystems worldwide. The **restoration** program implemented on the Mondego estuary brought effective results to the ecosystem, through eutrophication mitigation. *S. plana* and the macrobenthic assemblages were clearly favoured by the undertaken measures, initiating a recovery process, and the entire ecosystem showed an **overall ecological quality increase**.

Several extreme climate events occurred during the study period, impairing the health and fitness of resident biota. **Multiple stressors** (anthropogenic and natural) seem to interact through cumulative and synergistic processes, reducing the resilience of macrofauna, with **severe impacts on *S. plana* and on the macrobenthic assemblages**, effectively re-setting the recovery clock and slowing the overall return to the undisturbed state. Consequently, the ecosystem biodiversity conservation and production may be compromised, which can have profound implications for the livelihood of people who depend on the estuary.

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FUTURE PERSPECTIVES

During the development of the studies that resulted in the present thesis, diverse approaches have proven to be important in ecological studies, foreseeing a wide array of future research lines and scientific perspectives.

Ecological inquiries usually require extensive data sets over long-term periods in order to achieve knowledge on complex and slow processes. The long-term monitoring program of the Mondego estuary presents a good example, reflecting the importance of these studies to comprehend the responses of an ecosystem to environmental changes through time.

To continue the survey of the macrobenthic community seems an important path to follow as to monitor the success of the restoration measures and also to study more closely the effects of climatic variation and the occurrence of rare events. Also, the scope of the monitoring program may be widened to include other components of the ecosystem. Studies on valuables species to human populations, focusing on production, anthropogenic impacts and on the quantification of economic value seems to be an interesting approach to an integrated and sustainable use of the estuary.

The model developed on the population dynamics of *S. plana* revealed that some aspects were left aside or couldn’t be explained with the present knowledge. Therefore, further research on ecological and physiological parameters of the species is required, in order to improve the model. This knowledge can be achieved through experimental laboratory and field work, focusing on reproduction patterns, mortality rates, interactions between species (e.g. predation, competition), among other.

Moreover, the construction of dynamic models for other important species, such as the bivalve *Cerastoderma edule*, the gastropod *Hydrobia ulvae* or the isopod *Cyathura carinata* is potentially important to gain a better evaluation and understanding of the estuary. Furthermore, coupling several dynamic models of key species of the ecosystem will help us to better comprehend structural and functional features of the different components of the estuary, which with further research may enable the development of an integrated model of the ecosystem.

As discussed on the present thesis, the increasing rate of climate extremes may cause severe changes on the environmental conditions (e.g. temperature, precipitation) and is expected to impose additional stress on the ecosystem. So, the need to predict the impacts of change is crucial and modelling is an essential and reliable tool to achieve this goal. Therefore, the next step is the inclusion of environmental variables as forcing functions to simulate different climate pressure scenarios, especially under the present global climate change.

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