Impacts of Climate Variability and Anthropogenic Stressors on Estuarine Macrobenthic Assemblages

Socio-economic Implications and Human Health Risk Assessment

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UNIVERSIDADE DE COIMBRA

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Impacts of Climate Variability and Anthropogenic Stressors on Estuarine Macrobenthic Assemblages

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CONTENTS

ABSTRACT 9

RESUMO 13

GENERAL INTRODUCTION 17

- Importance of estuarine ecosystems and major threats 17
- Climate forcing and system's changes 19
- Importance of long-term studies 21
- The Mondego estuary – a case study 22
- The Ria de Aveiro coastal lagoon 27
- Assessing contamination by polychlorinated biphenyls (PCBs) and hexachlorobenzene (HCB) 28
- General Objectives 33
- References 35

CHAPTER I 43

Effects of extreme climate events on the macrobenthic communities' structure and functioning of a temperate estuary

- Abstract 45
- Introduction 45
- Materials and Methods 47
- Results 51
- Discussion 64
- Acknowledgements 69
- References 69

CHAPTER II 73

Implications of *Zostera noltii* recolonization on *Hydrobia ulvae* population structure success

- Abstract 75

Introduction 76
Materials and Methods 77
Results 82
Discussion 88
Acknowledgements 92
References 92

CHAPTER III **97**

Organochlorine accumulation on a highly consumed bivalve (*Scrobicularia plana*) and its main implications for human health

Abstract 99
Introduction 100
Materials and Methods 102
Results 109
Discussion 117
Acknowledgements 126
References 126

CHAPTER IV **131**

Uptake and decontamination of PCB-153 in the edible shrimp *Palaemonetes varians*

Abstract 133
Introduction 133
Materials and Methods 136
Results 141
Discussion 145
Acknowledgements 150
References 150

GENERAL DISCUSSION **155**

Eutrophication and environmental quality degradation (1993-1998) 155

Estuary recovery and climate variability (1999-2008) 158

Zostera noltii recolonization and *Hydrobia ulvae* population recovery (2008-2010) 161

Organochlorine assessment in Ria de Aveiro 163

Investigating bioaccumulation and detoxification kinetics of PCB-153 165

FINAL REMARKS **168**

FUTURE PERSPECTIVES **169**

REFERENCES **170**

AGRADECIMENTOS | ACKNOWLEDGEMENTS **179**

ABSTRACT

Among the most productive, valuable and dynamic systems across the world are estuaries, supporting a wide variety of fauna and flora exposed to large natural fluctuations. The major aim of the present thesis was to understand the multiple stressors (e.g. eutrophication, climate variability and chemical pollution) impacting estuarine ecosystems, through direct or indirect effects on their macrobenthic assemblages and key species, and to integrate it in order to assess potential human health risks. The Mondego estuary and Ria de Aveiro served as a basis to perform this work, by constituting estuarine systems that have experienced distinct human disturbances over time, which made these areas of exceptional study interest.

The first chapter explores the structural and functional changes occurred in the macrobenthic communities of the Mondego's south arm in response to the eutrophication history, subsequent restoration measures implementation in 1998 and extreme climate episodes (floods, droughts and heat waves). Progressive environmental quality degradation characterized the pre-restoration. A replacement and rapid decline of *Zostera noltii* beds by opportunistic macroalgal blooms was observed, concomitant with significant reduction of macrobenthic abundance, biomass, production and species biodiversity. After restoration, macrobenthic communities showed some signs of recovery but a succession of extreme weather events appeared to delay it. The largest floods (2000/01) and the prolonged drought (2004/05) seemed to be the most negative events impacting macrobenthic assemblages of the *Z. noltii* beds, in opposition to the mud- and sandflat areas which were mostly affected by the heat wave (2003). All climate phenomena promoted a general abundance, production and biodiversity impoverishment and further distinct changes at trophic and taxonomic levels. On the other hand, total community biomass seemed to be less susceptible to climate stressors exhibiting a slight recovery tendency.

The second chapter focuses on the seagrass *Z. noltii* recolonization, in the former most eutrophic area, after its disappearance for more than 20 years, and its subsequent implications on a mud snail *Hydrobia ulvae* population. A decade after restoration measures implementation, *Z. noltii* began to gradually recolonize the sandflat area, coinciding with significant increases in *H. ulvae* abundance, biomass and production. The seagrass provided long-term protection and abundant food resources for *H. ulvae* reproductive adults, contrarily to the ephemeral macroalgae, very common during the pre-restoration phase. Through time, large size individuals increased considerably, becoming the population more stable and structured, comparatively to the pre-restoration existing population, which was very unstable and juveniles dominated.

In the third chapter, a different anthropogenic stress source (chemical pollution by polychlorinated biphenyls – PCBs – and hexachlorobenzene – HCB) was investigated in a nearby system, the Ria de Aveiro. Contamination by organochlorines varied along a spatial gradient, being mostly restricted to the entrance point of industrial effluents in the system. Water and SPM presented residual levels but deeper sediments exceeded quality guidelines, inducing toxic effects to biota. It is evident a PCBs bioaccumulation trend throughout *Scrobicularia plana* lifespan, being the bivalve able to incorporate annually almost half of a gram of PCBs from the sediments, which was consequently free for higher trophic levels. There is no danger by consuming *S. plana*, at least in a short-term, as they exhibited PCB concentrations complying the European regulation. However, consumption of contaminated bivalves over many years and after reaching a steady-state concentration in the body (bioaccumulation) could be dangerous to the human health.

The fourth chapter addresses a mesocosms laboratorial experiment concerning PCB-153 bioaccumulation and detoxification by the edible and economic important shrimp *Palaemonetes varians* over a 30-day period, using the water as the contamination pathway. Depending on the exposure concentrations, different bioaccumulation kinetics and uptake rates were observed. For low PCB-153 levels, accumulation followed a saturation model, reaching an apparent steady state after 15 days exposure, whilst for intermediate and high PCB-153 levels, accumulation was faster and best fitted using simple linear regressions. Even after decontamination, the shrimps were not able to recover completely background values, retaining into their tissues levels of contamination harmful to the environment and inclusively prohibited for human consumption.

Overall, this study provides a valuable contribute towards estuarine sustainable management and human and ecological health risk assessment.

RESUMO

Os estuários são dos ecossistemas mais produtivos, dinâmicos e de inestimável valor, suportando uma ampla variedade de fauna e flora, expostas a grandes oscilações, características destes ambientes aquáticos. A presente tese teve como objectivo principal a compreensão e integração do impacto de múltiplos factores de stress nos ecossistemas estuarinos, nomeadamente a eutrofização, a variabilidade climática e a poluição química, através de efeitos directos ou indirectos ao nível das comunidades macrobentónicas e em espécies chave, de forma a avaliar potenciais riscos para a saúde humana. O estuário do Mondego e a Ria de Aveiro serviram de base à realização deste trabalho, constituindo áreas de particular relevância de estudo, visto terem sofrido diferentes formas de perturbação antropogénica ao longo do tempo.

O primeiro capítulo aborda as alterações estruturais e funcionais observadas nas comunidades macrobentónicas do braço sul do estuário do Mondego, em resposta ao histórico problema de eutrofização e à posterior introdução de medidas de mitigação, em 1998, e ocorrência de fenómenos climáticos extremos (cheias, períodos de seca e ondas de calor). No período anterior à implementação de medidas de mitigação, assistiu-se a uma progressiva degradação da qualidade ambiental do estuário, visível através da substituição e rápido declínio dos bancos de *Zostera noltii* por blooms de macroalgas, o que resultou numa considerável diminuição da abundância, biomassa, produção secundária e diversidade de espécies. A introdução de medidas de mitigação traduziu-se numa ligeira recuperação das comunidades macrobentónicas, a qual parece ter sido retardada devido a uma série de eventos climáticos extremos. As intensas cheias de 2000/01 e a severa e prolongada seca de 2004/05 foram os fenómenos climáticos cujo impacto foi mais negativo nas comunidades de macrofauna dos bancos de *Z. noltii*, contrariamente às zonas vasosa e arenosa que foram mais afectadas pela onda de calor de 2003. Estes fenómenos contribuíram para um geral decréscimo da abundância, produção e biodiversidade, além de alterações significativas aos níveis trófico e taxonómico. Por outro lado, a biomassa total aparentou ser menos susceptível ao impacto dos factores climáticos, apresentando uma ligeira tendência de recuperação.

No segundo capítulo são focados os factores subjacentes à recolonização de *Z. noltii*, após mais de 20 anos de ausência da macrófita na zona arenosa do braço sul do estuário do Mondego e quais as suas principais implicações na população do gastrópode *Hydrobia ulvae*. Uma década após a implementação das medidas de mitigação assistiu-se à recolonização gradual da zona arenosa por *Z. noltii*, o que coincidiu com um aumento significativo da abundância, biomassa e produção de *H. ulvae*. Contrariamente às macroalgas oportunistas e de curta duração, cuja ocorrência era frequente durante a fase de pré-mitigação, os bancos de *Z. noltii* proporcionaram protecção a longo prazo bem como abundantes recursos alimentares essenciais ao estabelecimento de indivíduos adultos de *H. ulvae* com capacidade reprodutiva. Ao longo do tempo verificou-se um aumento considerável da biomassa, atingindo os indivíduos dimensões progressivamente superiores, tendo-se constituído uma população mais estável e estruturada comparativamente à população de *H. ulvae* existente durante a fase de pré-mitigação, muito instável e maioritariamente dominada por juvenis.

O terceiro capítulo explora uma forma de perturbação antropogénica distinta da observada no estuário do Mondego - a poluição química por bifenis policlorinados (PCBs) e hexaclorobenzeno (HCB) na Ria de Aveiro. A contaminação por compostos organoclorados variou ao longo de um gradiente espacial, estando fundamentalmente restrita ao ponto de entrada de efluentes industriais no sistema. Níveis residuais de contaminação foram encontrados na água e na matéria particulada em suspensão mas em sedimentos de camadas profundas os níveis observados excederam as directrizes de qualidade, induzindo efeitos tóxicos nas formas de vida aí presentes. Verificou-se uma clara tendência de bioacumulação de PCBs ao longo da vida do bivalve *Scrobicularia plana*, evidenciando esta espécie a capacidade de incorporação anual de aproximadamente metade de um grama de PCBs a partir dos sedimentos, a qual estaria disponível para transferência para níveis tróficos superiores. A curto prazo, não existem perigos associados à ingestão de bivalves desta espécie, visto que os níveis de PCBs analisados foram inferiores às normas europeias vigentes. Contudo o seu consumo ao longo de muitos anos poderá ser nocivo para a saúde humana, dada a sua capacidade de bioacumulação.

O quarto capítulo visa uma experiência laboratorial de 30 dias, usando a água como vector de contaminação, e cujo objectivo foi estudar os padrões de bioacumulação e destoxificação de um dos congéneres de PCBs mais abundantes no ambiente – PCB-153 – numa espécie comestível de camarão, de elevada importância económica, *Palaemonetes varians*. Diferentes cinéticas e taxas de acumulação foram observadas dependendo das concentrações de exposição ao contaminante. Para baixos níveis de PCB-153, a acumulação seguiu um modelo de saturação, atingindo um aparente "estado estacionário" ao fim de 15 dias de exposição, enquanto que para concentrações intermédias e elevadas, a acumulação comportou-se de forma linear e mais rápida. Após descontaminação, os camarões não recuperaram os valores de base de PCB-153, retendo nos seus tecidos concentrações nocivas para níveis tróficos superiores e inclusivamente legalmente proibidas para consumo humano.

Globalmente, este trabalho representa um valioso contributo para a gestão sustentável dos ecossistemas estuarinos e para a avaliação de potenciais riscos para a saúde pública.

GENERAL INTRODUCTION

Importance of estuarine ecosystems and major threats

Estuaries are transition zones formed by mixing of freshwater from land drainage with saltwater of the ocean, constituting unique and important coastal environments, both ecologically and with respect to human settlement and use. As transition areas, estuaries are strongly affected by tidal action, where land and sea merge into a dynamic natural complex, with highly interrelated processes between their physicochemical and biological components (Elliott, 2002). Along with tropical rainforests and coral reefs, estuaries rank as the most productive and valuable ecosystems on Earth (Costanza et al., 1997; Kennish, 2002; McLusky and Elliott, 2004), supporting a great variety of fauna and flora (Elliott and McLusky, 2002; Agardy et al., 2005; Wilson and Farber, 2009). Their high productivity is based on primary production from phytoplankton, macrophytes (seaweeds, seagrasses and marsh grasses), benthic microphytes, as well as inputs of organic matter from rivers (Savage et al., 2012). This broad primary productivity is the keystone of the estuarine food chain, providing abundant food resources for their inhabitants (Elliott et al., 2002; McLusky and Elliott, 2004).

Allied to plentiful food availability, estuaries provide an extraordinary diversity of habitats including sand and mudflats, oyster beds, saltmarshes and seagrass meadows (McLusky and Elliott 2004). These ensure optimal settlement conditions for a wide range of invertebrates (Cardoso et al., 2004, 2008a,b; Dolbeth et al., 2007; Bordalo et al., 2011; Grilo et al., 2012), larval, young and adult forms of some marine fish species (Martinho et al., 2007; Nyitrai et al., 2012; Primo et al., 2012), and top predators including shore birds, either for estuarine residency, as nursery areas or as migration routes (Beck et al., 2001; Vasconcelos et al., 2007). Moreover, several other goods and services vital to global society are provided by estuarine environment, such as decomposition and nutrient cycling, contaminants and water regulation, shoreline protection against natural catastrophes and also recreational and tourism activities (Costanza et al., 1997; McLusky and Elliott 2004; Wilson and Farber, 2009; Barbier et al., 2011).

Given these goods and service benefits, it is not surprising that coastal estuaries have long served as a focus of human concentration on the planet. Throughout history, humans have favoured estuarine and coastal zones as attractive locations to live and explore, which made these environments under increasing pressure and subsequent quality deterioration (Martínez et al., 2007; Wilson and Farber, 2009). Nowadays are few if any coastal systems that have not been affected in some way by human intervention (Agardy et al., 2005). Population growth associated with rapid urbanization and industrialization collides with the ecological function of estuaries (McLusky and Elliott, 2004; Vasconcelos et al., 2007). Approximately half of the salt marshes, 35% of mangroves and 30% of coral reefs and seagrasses are either lost or degraded worldwide (Barbier et al., 2011), raising significant challenges for planners and decision makers in the near future.

In fact, the most common threats to estuarine and coastal ecosystems are nutrient enrichment, and consequently eutrophication and habitat loss (Valiela et al., 1997; Kennish, 2002; Dolbeth et al., 2003; Cardoso et al., 2004; Philippart et al., 2007; Grilo et al., 2009), along with overexploitation of natural resources (Vasconcelos et al., 2007) and contamination by heavy metals (Caeiro et al., 2005; Dauvin, 2008; Zhou et al., 2008) and persistent organic pollutants (UNEP 2001; Otchere et al., 2005; Moon et al., 2009). More recently, the oceanic biosphere is facing climate variability (Harley et al., 2006; Badjeck et al., 2010) and progressive acidification, promoted by the increasing uptake of anthropogenic carbon dioxide (CO_2) from the atmosphere (Chen, 2008; Doney et al., 2012), both factors hypothesized as a potential direct consequence of global climate warming. Synergistic effects of climate variability and human induced stressors may determine profound changes in the structure and functioning of ecosystems (Emmerson et al., 2005; Houghton, 2005; Kotta et al., 2009) and contribute to the loss of species and hence of biodiversity (Cardoso et al., 2008a).

Climate forcing and systems' changes

Until the industrial revolution, atmospheric concentrations of naturally occurring greenhouse gases, namely carbon dioxide (CO_2), methane, nitrous oxide and ozone had been relatively stable. However, with the advent of fossil fuels to support the industry, coupled with expansion of other human activities, large amounts of additional greenhouse gases have been released to the atmosphere, increasing nearly 80% between 1970 and 2004 (IPCC, 2007). Actually, there is overwhelming evidence that anthropogenic activities are driving several rapid changes, which are already occurring within the world's oceans with serious impacts (Hoegh-Guldberg and Bruno, 2010). Rising atmospheric carbon dioxide (CO_2) is one of the most critical problems since its effects are globally pervasive and irreversible on ecological timescales (Doney et al., 2012). During the past century, increasing concentrations of CO_2 in the atmosphere have driven to a rise in the global oceans' average temperature by 0.74°C and sea level by 17 cm, and have depleted seawater carbonate concentrations by nearly 30 $\mu mol.kg^{-1}$ seawater and acidity by 0.1 pH unit (IPCC, 2007). Climbing temperatures generate a host of further changes, such as rising sea level, increased ocean stratification, decreased sea-ice extent and altered patterns of ocean circulation, precipitation, and freshwater input (Doney et al., 2012) (Fig. 1).

In fact, climate change projections estimate an increase in the frequency and severity of extreme weather events (e.g. floods, droughts and heat waves) in the future, inducing alterations in the Earth's global oceanic and atmospheric circulation patterns (Hoegh-Guldberg and Bruno, 2010; IPCC, 2012). For example, circulation models predict that oxygen concentrations in the upper layers of the ocean will probably decline as a result of increasing stratification (Diaz and Rosenberg, 2008), leading to massive extinction events, as occurred in the past (Kump et al., 2005). Additionally, changes in wind and ocean currents are accordingly to interact with overfishing and eutrophication, further increasing the incidence of hypoxic and anoxic events and consequently the risk of mass mortalities (Hoegh-Guldberg and Bruno, 2010; Doney et al., 2012). Understanding the interaction between overexploitation and changing climate is a key challenge for global ecology and a primary concern in sustainability science. Thus, face to global warming, both scientists and stakeholders have the responsibility of developing adaptation and management strategies to ensure conservation of terrestrial and marine ecosystems.

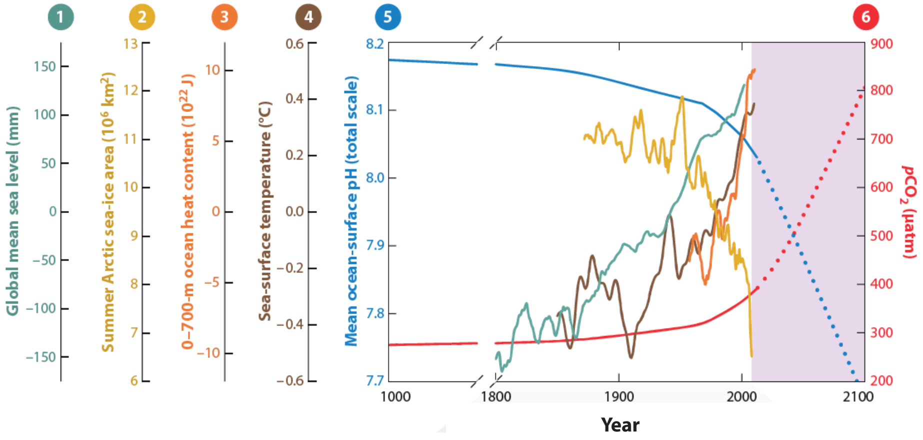

Fig. 1. Changes occurred over time in (1) global mean sea level (*teal line*); (2) summer Arctic sea-ice area (*yellow line)*; (3) 0–700 m ocean heat content (*orange line*); (4) sea-surface temperature (*brown line*); (5) mean ocean-surface pH (*blue line*) and (6) atmosphere *p*CO2 (*red line*). Light purple shaded region denotes projected changes in pH and *p*CO2 consistent with the IPCC twenty-first-century A2 emissions scenario with rapid population growth (figure from Doney et al., 2012).

Due to their sensitiveness in reflecting anthropogenic and natural stressors, macrobenthic communities are assumed to be good indicators of climate change in the marine environment (Borja et al., 2003; Dauvin et al., 2007; Begun et al., 2010; Joydas et al., 2011). Macrobenthic fauna possess life history traits and functions allowing relatively rapid responses to a wide range of stressors (Mackay et al., 2010). Major impacts of climate change in estuarine ecosystems are usually related with fluctuations in river flow and consequently salinity gradient changes, either by flood or drought events, inducing important shifts in distribution, abundance, species composition, functioning and productivity (Strydom et al., 2002; Ysebaert et al., 2003; IPCC, 2012). Several studies have demonstrated how variations in precipitation patterns and freshwater runoff may influence benthic communities. For instance, periods of prolonged drought promote reduced species richness, production and trophic diversity, as well as changes in the structure and composition of estuarine planktonic, macrobenthic and fish communities (Currie and Small, 2005; Marques et al., 2007; Martinho et al., 2007b; Cardoso et al., 2008a,b; Grilo et al., 2011; Cyrus et al., 2011; Dolbeth et al., 2011).

Despite the great efforts that have been taken worldwide, the knowledge concerning climate change impacts in estuarine systems is still insufficient. For instance, responses of benthic communities to climate variability are difficult to predict and fully understand in a short-term scale, requiring continuous monitoring to detect potential alterations.

Importance of long-term studies

Long-term ecological studies have been a critical element in oceanography for more than fifty years, providing valuable insights in ecology, environmental change, natural resource management and biodiversity conservation (Cardoso et al. 2005, Neto et al., 2010; Lindenmayer et al., 2012).

Over time, there has been great discussion about what constitutes "long-term" in ecological research. After all, what should be considered a long-term ecological study? According to Lindenmayer et al. (2012), long-term ecological studies are *those that systematically and regularly collect field data from a particular site or set of sites for more than 10 years*. This somewhat arbitrary time component is justifiable by Lindenmayer et al. (2012) based on the evidence that it is representative of *repeated sampling of annual growth cycles and it includes some longer-term bioclimatic cycles that operate over decadal periods*. In contrast to short-term observations, there is greater likelihood of observing or helping to explain slow processes, rare climate events and subtle or complex changes in natural environments through longer studies (Jackson and Füreder, 2006).

Five key values of long-term ecological studies were proposed by Lindenmayer et al. (2012) and can be summarized as: (1) quantifying ecological responses to environmental change; (2) understanding complex ecosystem phenomena that occur over a prolonged period; (3) providing core ecological data for developing theoretical models and to parameterize and validate simulation models; (4) acting as platforms for collaborative studies, thus promoting multidisciplinary research; (5) providing data and understanding to support evidence-based policy, decision making and management of ecosystems.

Despite undoubtedly value of long-term ecological perspectives, the collection of long-term data is still limited by funding constraints, personal or institutional changes in research directions and the absence or inaccessibility of comparable data from older research (Jackson and Füreder, 2006). Also, recent trends in ecological research, with much emphasis on approaches using meta-analysis, data mining and modeling, may difficult long-term field studies, since these tools are very powerful and generally cheaper and quicker to generate trends and publications than traditional field-based, empirical ecology (Lindenmayer et al., 2012).

The Mondego estuary – a case study

The study presented in this thesis was partly conducted in the Mondego estuary and also in a nearby system, Ria de Aveiro, afterwards described. The Mondego estuary, located in a warm temperate region on the Atlantic coast of Portugal (40º08'N, 8º50'S), is a small estuary of 8.6 km^2 (Fig. 2). The source of the Mondego river is situated at Serra da Estrela and extends along 227 Km, providing an average freshwater flow rate of 79 m^3.s^{-1}. The Mondego river forms a vast alluvian plain, the Lower Mondego region, consisting of 15.000 ha of agricultural land, where is essentially cultivated rice (Neto et al., 2010).

In its terminal part, the estuary comprises two arms of different hydrological characteristics, north and south, separated by an alluvium form island – the Murraceira Island. The north arm is deeper (4-8 m during high tide, tidal range of 1-3 m), highly hydrodynamic, constituting the main navigation channel and the location of the Figueira da Foz harbour, with regular dredging activities. The south arm is shallower (2-4 m deep at high tide, tidal range of 1-3 m), less hydrodynamic, hence with higher residence time. Until 2006, this channel was largely silted up, mainly in the upstream areas, so that most of the freshwater flow occurred essentially through the north arm. The water circulation was mostly dependent on the tides and on the freshwater input from a small tributary, the Pranto river, which was artificially controlled by a sluice according to the water needs of surrounding rice fields in the Mondego valley (Martins et al., 2001; Cardoso et al., 2004). This freshwater input represented a relevant source of nutrients into the south arm (Grilo et al., 2009), which was partly responsible for the eutrophication symptoms observed until the end of the 1990s.

The south arm is mostly constituted by large intertidal mudflats (75%), covered in downstream sections by the seagrass *Zostera noltii* and salt marshes dominated by *Spartina maritima*. In opposition, salt marshes of the upstream region are essentially composed by *Scirpus maritimus* (Lillebø et al., 2006). Regarding special protection legislation, the estuary has been classified as an Important Bird Area (IBA, PT039) as well as a RAMSAR site (site no. 1617).

The estuary supports a variety of industrial activities, saltworks and aquacultures (old salt-ponds currently converted to semi-intensive aquacultures), mercantile and fishing harbors, as well as great urban pressures from Figueira da Foz, a center of seasonal tourism activity (Neto et al., 2010). Approximately 900,000 people live in the Mondego basin and over 60,000 individuals are estimated to live nearby, in the Figueira da Foz city (Pinto et al., 2010).

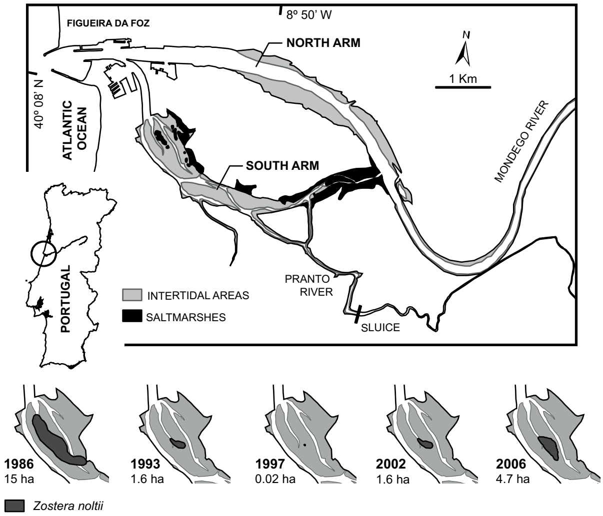

Fig. 2. The Mondego estuary, with indication of the two arms, intertidal areas, salt marshes and the seagrass bed (*Zostera noltii*) evolution in the south arm over the period 1986-2006.

Industrial and agricultural activities, from which resulted excessive nutrient loadings into the south arm, allied to favourable physical conditions, as the shallow depth of the system and high water residence time (5 to 7 days) (Cardoso et al., 2005), culminated in a long and severe eutrophication process in the early 1990's (Martins et al., 2001; Cardoso et al., 2004; Verdelhos et al., 2005; Grilo et al., 2009, Dolbeth et al., 2011).

Eutrophication has been greatly impacting ecological processes in marine coastal waters worldwide (Valiela et al., 1997; Cloern, 2001; Cardoso et al., 2004; Philippart et al., 2007), often coinciding with shifts in species composition within the primary producer community and further implications on the underlying macrobenthic assemblages (Philippart et al., 2007). The most noted effect of anthropogenic nutrient enrichment consists in the replacement of slow-growing rooted macrophytes by dense mats of opportunistic fast-growing macroalgae and phytoplankton, through competition for increased availability of nutrients and decreased light levels (Valiela et al., 1997; Cardoso et al., 2004; Lillebø et al., 2005; Burkholder et al., 2007; Schmidt et al., 2012). In a short-term scale, low controlled amounts of macroalgae may have beneficial effects, since they increase habitat heterogeneity, providing alternative habitats and thereby protection and food resources for establishment and recruitment of some macroinvertebrate species (Norkko et al., 2000; Cardoso et al., 2004; Grilo et al., 2009). However, when in excess, macroalgae can form compact mats, in which decay and drift result in anoxia and hypoxia events, with negative consequences to the related macrofauna (Norkko et al., 2000). Significant macroalgal blooms were reported in the south arm of the Mondego estuary (Martins et al. 2001; Cardoso et al., 2004), constituting the main factor motivating gradual decline in biomass and spatial extent of the *Zostera noltii* meadows. From an approximate coverage area of 15 ha in 1986 to 1.5 ha in 1993 and 0.02 ha in 1997 (Lillebø et al., 2005; Cardoso et al., 2008b; Dolbeth et al., 2011) (Fig. 2).

It is well known the important ecological role played by seagrasses in the marine environment. They represent highly productive and diverse areas that support complex food webs as well as critical habitat functions, such as spawning and nursery areas for commercially important invertebrate and fish species (Duarte, 2002; Baeta et al., 2011). A sudden decline in the area and biomass of the seagrass beds community of the Mondego estuary has naturally imposed negative impacts on the composition and structure of the associated macrobenthic assemblages (Cardoso et al., 2004, 2008a, 2010; Lillebø et al., 2005),

Face to alarming south arm's environmental quality degradation, a restoration program was implemented, in 1998, in order to minimize and potentially reverse the historical eutrophication issues on the system and thus to restore the original seagrass bed (Lillebø et al., 2005; Cardoso et al., 2004; Grilo et al., 2009; Dolbeth et al., 2011). Introduction of restoration measures resulted in a significant nutrient loading decline, mainly in nitrogen forms (Lillebø et al., 2005), by reduction of the Pranto sluice-opening regime, diverting most of the nutrient enriched freshwater by another sluice placed further upstream in the north arm. The water residence time was reduced to just 1 day, through enlarging the south arm riverhead connection. In addition, seagrass beds area was limited with wooden stakes to prevent human disturbance and several public education forums were undertaken, emphasizing the ecological importance of intertidal vegetation conservation for the health and related socio-economic activities of the estuary (Lillebø et al., 2005, Cardoso et al., 2004, 2008a,b; Grilo et al., 2009; Dolbeth et al., 2011).

At present, overall environmental quality of the south arm appears to be gradually improving. This is supported by the absence of macroalgal blooms since implementation of restoration measures and consequent recovery of the *Z. noltii* beds, both in biomass and areal extent, achieving almost 5 ha in 2006 (Dolbeth et al., 2011) (Fig. 2). Furthermore, restoration efforts enabled the recolonization of the seagrass *Z. noltii* in the inner most disturbed areas, where it was absent for more than 20 years, facilitating fixation and growth of a more stable and structured population of the gastropod *Hydrobia ulvae* (Grilo et al., 2012). Nevertheless, system recovery would have been faster if a succession of different climate extreme events had not affected the region so intensively (Cardoso et al., 2008a,b; Grilo et al, 2011).

In the last years, great instability in the climate of Portugal has been recorded, comparatively to the general climate for the last century (IPMA – Portuguese Institute for Ocean and Atmosphere, http://www.ipma.pt/ and INAG – Portuguese Water Institute, http://snirh.inag.pt/). There was a high variability in precipitation regime, with unusual heavy precipitation events and prolonged periods of drought. For instance, in the winter 2000/01, unprecedented high precipitation values were registered, causing one of the largest floods of the century. On the other hand, precipitation in 2004/05 was 45-50% below average (487.8 mm against the mean annual 1971-2000 of 905.1mm), resulting in the worst drought since 1931 (IPMA). In addition, and similarly to a global tendency, it was observed an increase of the mean air temperature and also several episodes of at least 6 consecutive days, in which the daily maximum temperature was higher in 5ºC than the mean daily value for the reference period (heat waves), mainly in 2003 (IPMA).

The Ria de Aveiro coastal lagoon

Ria de Aveiro is a coastal lagoon adjacent to the Atlantic Ocean (40º38′N, 08º45′W), in northwestern of Portugal (Fig. 3). It comprises a combined shallow estuary-coastal lagoon with a complex morphology and productive ecosystem. The topography of Ria de Aveiro consists of channels radiating from the mouth with several branches, islands and mudflats. The four main branching channels (Ovar, Murtosa, Ílhavo and Mira) are connected to the ocean by a single tidal outlet, via an intervening tidal lagoon (da Silva et al., 2004). Ria de Aveiro is 45 km long with a maximum width of 10 km, covering an area of 83 km^2 and 66 km^2, depending on the high and low tide (Abrantes et al., 2006; Pereira et al., 2009). The average depth is roughly 1 m, excluding navigation channels where dredging activities maintain a depth of about 7 m. The fresh water input is relatively small and ensured by Vouga and Antuã rivers. This mesotidal system, with predominantly semi-diurnal tides, presents a tidal range of around 2 m.

A strong reduction in seagrasses within Ria de Aveiro is occurring as a consequence of changes in tidal related physical forcing, salinity, turbidity, sedimentation and dredging operations (da Silva et al, 2004). Although the overall nutrient status of the system is classified as in “moderate low” eutrophic condition range, the trophic status of inner areas is evidently higher (Lopes et al, 2007), promoting macroalgal growth.

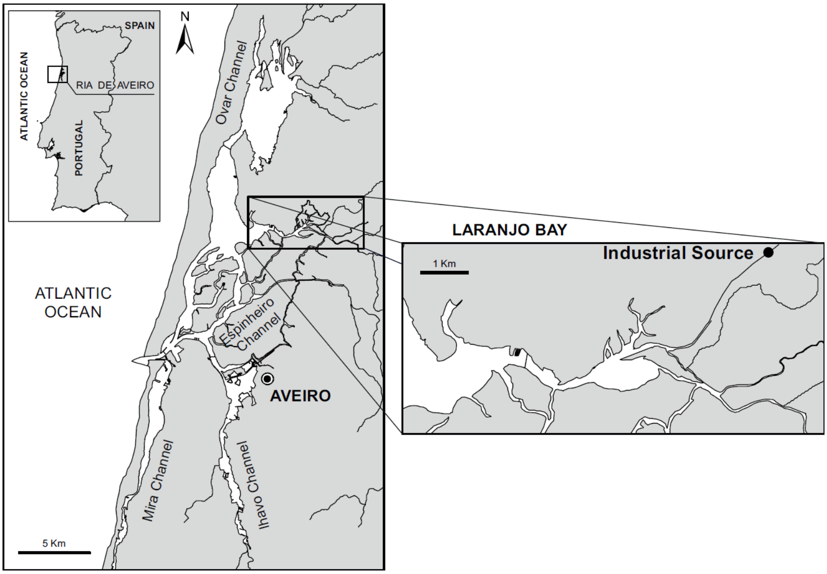

Fig. 3. The Ria de Aveiro coastal lagoon and location of the Laranjo bay.

Assessing contamination by polychlorinated biphenyls (PCBs) and hexachlorobenzene (HCB)

Ria de Aveiro supports a population of 250.000 inhabitants in the watershed area, and its main municipality (Aveiro town) is located 15 km south from an industrial cluster in Estarreja, a city in the vicinity of the Murtosa channel. The system was for decades the endpoint of effluents discharged from this industrial complex, in which chlorine and aromatic compounds containing benzene were produced. Continuous releasing of untreated effluents has induced an environmental contamination gradient inside the lagoon, most pronouncedly in the sediments of the Laranjo bay (Coelho et al., 2006; Pereira et al., 2009) (Fig. 3). Due to its semi-enclosed characteristic, the Laranjo bay constitutes itself a small inner lagoon of 2 km^2, showing a distinct west-east salinity gradient between saline water, from the interior of the lagoon, and freshwater from rivers and other water sources (industrial and waste waters) (Lopes et al, 2001).

Contamination by organochlorine compounds, particularly polychlorinated biphenyls (PCBs) and hexachlorobenzene (HCB) (Fig. 4), constitutes a relevant environmental issue worldwide. PCBs ($C_{12}H_{10-x}Cl_x$) were manufactured by chlorination of biphenyl, a molecule composed by two benzene rings, in the presence of a suitable catalyst. Synthesis of hexachlorobenzene (C_6Cl_6) involves the chlorination of benzene at 150–200°C with a ferric chloride catalyst or distillation of residues from the production of tetrachloroethylene (WHO, 1997). Both compounds are identified as priority hazardous substances, belonging to a broad family of human-made organic chemicals, defined by the Stockholm Convention for human health and environment protection (UNEP, 2001) as persistent organic pollutants (POPs). Both synthetic pollutants (PCBs and HCB) exhibit very low water solubility, but are extremely liposoluble, persisting for long periods and bioaccumulating in the environment.

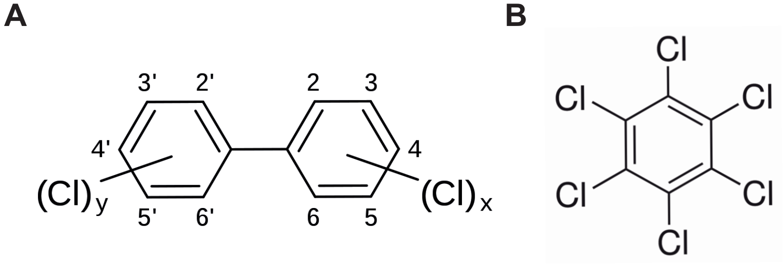

Fig. 4. Chemical structure of (A) polychlorinated biphenyls and (B) hexachlorobenzene.

Theoretically, 209 PCB congeners of different physicochemical and toxicological properties are possible, however statistically and mechanically, it is unlikely that all occur in nature (Hutzinger et. al, 1974) and only 130 congeners are probable to be present in commercial products.

PCBs were discovered before the turn of the 19th century, in 1865, and the first PCB was synthesized 16 years later. Their usefulness for industry was recognized early, mostly due to intrinsic properties, such as resilience to degradation, low flammability and vapour pressure, chemical and thermal stability, and high dielectric constants. Since the early 1930s, PCBs have been used commercially as lubricants in transformers and capacitors, stabilizers in paints, hydraulic and heat-exchange fluids, polymers and as adhesives (Bellas et al, 2011). Over than 2 million tons of PCBs were produced worldwide in the form of technical mixtures of 60–90 congeners, such as Aroclor (USA and UK), Ascarel (Brazil), Kaneclor (Japan), Clophen (Germany), Pyralene (France), and Sovol (former USSR) (Kim et al., 2004; Szlinder-Richert et al., 2009).

In contrast, HCB was only used since the 1940s and essentially as a fungicide on grain seeds, such as wheat. It also had industrial uses, particularly in manufacturing processes (aluminium and graphite rods) and in organic synthesis as a raw material for synthetic rubber, fireworks and munitions (Environmental Portuguese Agency, 2010).

By the mid-1960s, the general scientific community became aware of PCBs as a serious environmental problem. Jensen (1966) reported PCBs in wildlife samples, highlighting their highly prevalence in the environment and capacity to bioaccumulate along the food chain, staying strongly adsorbed into the fatty tissues of the organisms. Top food chain animals, like whales, polar bears, dolphins and humans can store PCBs at highly concentrated levels, constituting great matter of concern. Posteriorly, many other authors raised interest in studying PCBs toxicity in the environment and ultimately in human beings (Wang et al., 1998; Covaci et al., 2002; de Felip et al., 2004; Okumura et al. 2004; Croes et al., 2012).

Human exposure to organochlorines has resulted largely from the consumption of contaminated food (Moon et al. 2009; Nunes et al., 2011; Fillos et al. 2012), but also from inhalation and skin absorption in work environments. Depending on the concentration at which the organism was subjected, susceptibility of each individual and duration of contaminants exposure, several toxic effects are expected. The skin and liver are the major sites of pathology, but the gastrointestinal tract and the immune, reproductive and nervous systems are also preferential targets (Voorspoels et al., 2004; Jaikanlaya et al., 2009).

Face to this global threat, many industrial nations implemented restrictive measures to control the flow of PCBs and HCB into the environment. By the late 1970s, PCBs were banned for most uses. An exception is sometimes made for mono- and dichloro-PCBs, probably because are more easily degraded and are less toxic than highly chlorinated PCBs (Rodziewicz et al., 2004). Regarding HCB, its use as fungicide was virtually banned in the United States, Canada and some European countries almost simultaneously with PCBs restriction. Nevertheless, both compounds are still present in the environment. Hundreds of thousands tons of PCBs continue currently in use, but almost entirely restricted in closed systems, such as isolating oils in transformers, capacitors and other electrical systems (Zhou et al., 2001; Otchere, 2005). Also, HCB is yet present as an impurity in several pesticides, namely pentachlorophenol, dacthal, atrazine, picloram, pentachloronitrobenzene, chlorthalonil, and lindane (Draft PBT, 2000).

Given the status of both substances as highly priority pollutants, some guidelines have been issued in order to protect human health. For example, EPA set a maximum contaminant level (MCL) of PCBs and HCB in drinking water at 0.5 $\mu g.L^{-1}$ and 1 $\mu g.L^{-1}$, respectively. The Food and Drug Administration (FDA) has set safety limits for PCBs in various foods, requiring a threshold of 2 $\mu g.g^{-1}$ in fish and shellfish (edible portions). Moreover, the European Union (Commision Regulation No. 1259/2011) established a maximum limit of 75 $ng.g^{-1}$ (wet weight basis) for the six ecological PCB congeners (CBs 28, 52, 101, 138, 153, 180) in muscle meat of fish and fishery products.

GENERAL OBJECTIVES

In a scenario of global change, estuarine environments are facing multiple interacting natural and anthropogenic stressors, manifested at individual, population and community levels differently. Intrinsic natural environmental variability coupled with increasing human induced modifications (e.g. nutrient enrichment and chemical pollution) can potentially result in abrupt estuarine ecosystem shifts. Understanding how these impacts are affecting the structure and functioning of key components of estuarine ecosystems, particularly intertidal vegetation communities and related macrozoobenthic assemblages, and their main consequences to human health, constitute the central challenge of this thesis. Further, distinguishing and integrating the effects of those stressors is essential for understanding and managing coastal biotic resources, providing a holistic overview about complexity and value of estuarine habitats to society. Accordingly, several field studies (3) plus a laboratory experiment were conducted and are presented in the following chapters.

Chapter I

This chapter deals with the impact of multiple consecutive stochastic weather episodes on the Mondego's intertidal macrobenthic community. A centenary flooding event in the winter of 2000/01, a heat wave in the summer of 2003 and a prolonged and severe drought between 2004-2005 were analyzed. It was explored a 16-year time series to evaluate how different extreme climate events can affect distinct functional groups and the community structure, as well as to understand how these consecutive episodes may have single, cumulative or synergistic effects on the overall system stability.

Chapter II

An approach regarding the effects of seagrass *Z. noltii* recolonization on a gastropod *H. ulvae* population dynamics and secondary production is demonstrated in this chapter. The ecological response of a mud snail *H. ulvae* population to the restoration efforts conducted in the Mondego estuary that subsequently enabled seagrass recolonization was investigated. A comparison between different time periods was established, the eutrophication phase (1993-1998) and the recovery process (1999-2010). Some pertinent questions were addressed: would this system and associated flora and fauna recover after 20 years of profound modifications, in an expected trajectory? Would the mud snail population structure completely recover to the original condition, following the seagrass expansion?

Chapter III

This chapter provides a detailed overview about contamination by polychlorinated biphenyls (PCBs) and hexachlorobenzene (HCB) in both biotic and abiotic components of the Ria de Aveiro coastal lagoon and its main consequences to the human health. Organochlorine accumulation was studied in water, muddy sediments and in the highly consumed bivalve species *Scrobicularia plana*. Determination of the annual PCBs bioaccumulation pattern during *S. plana* lifespan and the potential PCBs transfer from contaminated sediments to economically important species was achieved. Human health risks intrinsic to the consumption of bivalves from Ria de Aveiro were also assessed.

Chapter IV

This chapter focuses on a mesocosms experiment concerning PCBs uptake and decontamination by edible and economic important shrimp *Palaemonetes varians*. Over a 30-day period, it was studied the bioaccumulation and decontamination processes of *P. varians*, when exposed to different CB-153 concentrations, using the water as the contamination pathway. In the case of eventual PCBs leakage to coastal waters will the shrimps be able of releasing the contaminant presumably accumulated and reach the legal limits allowed for food consumption?

At the end of this thesis, a summarizing discussion integrates the information present in the previous chapters, providing an overview of multiple stressors impacts and human health risks assessment.

References

Abrantes I, Dias JM, Rocha F (2006) Spatial and temporal variability of suspended sediments concentration in Ria de Aveiro and fluxes between the lagoon and the ocean. Journal of Coastal Research 39: 718-723.

Agardy T, Alder J, Dayton P, Curran S, Kitchingman A, Wilson M A, Catenazzi A, Restrepo J, Birkeland C, Blaber S, Saifullah S, Branch G, Boersma D, Nixon S, Dugan P, Davidson N, Vorosmarty C (2005) Coastal Systems: An assessment of ecosystem services. In Ecosystems and Human Well-Being: Current States and Trends, ed. (Millennium Ecosystem Assessment), 515-549 pp. Island Press. Washington, DC.

Badjeck MC, Allison EH, Halls AS, Dulvy NK (2010) Impacts of climate variability and change on fishery-based livelihoods. Marine Policy 34: 375-383.

Baeta A, Niquil N, Marques JC, Patrício J (2011) Modelling the effects of eutrophication, mitigation measures and an extreme flood event on estuarine food webs. Ecological Modelling 222: 1209-1221.

Barbier EB, Hacker SD, Kennedy C, Koch EW, Stier AC, Silliman BR (2011) The value of estuarine and coastal ecosystem services. Ecological Monographs 81: 169-193.

Beck MW, Heck Jr. KL, Able KW, Childers DL, Eggleston DB, Gillanders BM, Halpern B, Hays CG, Hoshino K, Minello TJ, Orth RJ, Sheridan PF, Weinsetin MP (2001). The identification, conservation, and management of estuarine and marine nurseries for fish and invertebrates. BioScience 51: 633-641.

Begun T, Teacă A, Gomoiu MT (2010) State of the macrobenthos within Modiolus phaseolinus biocoenosis from Romanian Black Sea continental shelf. Geo-Eco-Marina 16: 5-18.

Bellas J, Gonzalez-Quijano A, Vaamonde A, Fumega J, Soriano JA, Gonzalez JJ (2011) PCBs in wild mussels (*Mytilus galloprovincialis*) from the N–NW Spanish coast: current levels and long-term trends during the period 1991–2009. Chemosphere 85: 533-541.

Bordalo MD, Ferreira SMF, Cardoso PG, Leston S, Pardal MA (2011) Resilience of an isopod population (*Cyathura carinata*) to multiple stress factors in a temperate estuarine system. Hydrobiologia 671: 13-25.

Burkholder JM, Tomasko DA, Touchette BW (2007) Seagrasses and eutrophication. Journal of Experimental Marine Biology and Ecology 350: 46-72.

Borja A, Muxika I, Franco J (2003) The application of a Marine Biotic Index to different impact sources affecting soft-bottom benthic communities along European coasts. Marine Pollution Bulletin 46: 835-845.

Caeiro S, Costa MH, Ramos TB, Fernandes F, Silveira N, Coimbra A, Medeiros G, Painho M (2005) Assessing heavy metal contamination in Sado Estuary sediment: An index analysis approach. Ecological Indicators 5: 151-169.

Cardoso PG, Pardal MA, Lillebø AI, Ferreira SM, Raffaelli D, Marques JC (2004) Dynamic changes in seagrass assemblages under eutrophication and implications for recovery. Journal of Experimental Marine Biology and Ecology 302: 233-248.

Cardoso PG, Brandão A, Pardal MA, Raffaelli D, Marques JC (2005) Resilience of *Hydrobia ulvae* populations to anthropogenic and natural disturbances. Marine Ecology Progress Series 289: 191-199.

Cardoso PG, Raffaelli D, Lillebø AI, Verdelhos T, Pardal MA (2008a) The impact of extreme flooding events and anthropogenic stressors on the macrobenthic communities' dynamics. Estuarine, Coastal and Shelf Science 76: 553-565.

Cardoso PG, Raffaelli D, Pardal MA (2008b) The impact of extreme weather events on the seagrass *Zostera noltii* and related *Hydrobia ulvae* population. Marine Pollution Bulletin 56: 483-492.

Cardoso PG, Leston S, Grilo TF, Bordalo MD, Crespo D, Raffaelli D, Pardal MA (2010) Implications of nutrient decline in the seagrass ecosystem success. Marine Pollution Bulletin 60: 601-608.

Chen CTA (2008) Effects of climate change in marine ecosystems. Fisheries for Global Welfare and Environment, 5th World Fisheries Congress 307-316.

Cloern JE (2001) Our evolving conceptual model of coastal eutrophication problem. Marine Ecology Progress Series 210: 223-253.

Coelho JP, Rosa M, Pereira ME, Duarte AC, Pardal MA (2006) Pattern and annual rates of *Scrobicularia plana* mercury bioacumulation in a human induced mercury gradient (Ria de Aveiro, Portugal). Estuarine, Coastal and Shelf Science 69: 629-635.

Commission Regulation (EU) No 1259/2011 of 2 December 2011 amending Regulation (EC) No 1881/2006 as regards maximum levels for dioxins, dioxin-like PCBs. (http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2011:320:0018:0023:EN:PDF).

Costanza R, d'Arge R, de Groot R, Farber S, Grasso M, Hannon B, Naeem S, Limburg K, Paruelo J, O'Neill RV, Raskin R, Sutton P, ven den Belt M (1997) The value of the world's ecosystem services and natural capital. Nature 387: 253-260.

Covaci A, Ryan JJ, Schepens P (2002) Patterns of PCBs and PCDD/PCDFs in chicken and pork fat following a Belgian food contamination incident. Chemosphere 47: 207-217.

Croes K, Colles A, Koppen G, Govarts E, Bruckers L, Van de Mieroop E, Nelen V, Covaci A, Dirtu AC, Thomsen C, Haug LS, Becher G, Mampaey M, Schoeters G, Van Larebeke N, Baeyens W (2012) Persistent organic pollutants (POPs) in human milk: A biomonitoring study in rural areas of Flanders (Belgium). Chemosphere 89: 988-994.

Currie DR and Small KJ (2005) Macrobenthic community responses to long-term environmental change in an eastern Australian sub-tropical estuary. Estuarine, Coastal and Shelf Science 63: 315-331.

Cyrus, DP, Jerling H, MacKay F, Vivier l (2011) Lake St Lucia, Africa's largest estuarine lake in crisis: Combined effects of mouth closure, low levels and hypersalinity. South African Journal of Science 107: 59-71.

da Silva JF, Duck RW, Catarino JB (2004) Seagrasses and sediment response to changing physical forcing in a coastal lagoon. Hydrology and Earth System Sciences 8: 151-159.

Dauvin JC, Ruellet T, Desroy N, Janson AL (2007) The ecological quality status of the Bay of Seine and the Seine estuary: use of biotic indices. Marine Pollution Bulletin 55: 241-257.

Dauvin JC (2008) Effects of heavy metal contamination on the macrobenthic fauna in estuaries: The case of the Seine estuary. Marine Pollution Bulletin 57: 160-169.

de Felip E, di Domenico A, Miniero R, Silvestroni L (2004) Polychlorobiphenyls and other organochlorine compounds in human follicular fluid. Chemosphere 54:1445-1449.

Diaz RJ, Rosenberg R (2008) Spreading dead zones and consequences for marine ecosystems. Science 321: 926-929.

Dolbeth M, Pardal MA, Lillebø AI, Azeiteiro UM, Marques JC (2003) Short- and long-term effects of eutrophication on the secondary production of an intertidal macrobenthic community. Marine Biology 143:1229-1238.

Dolbeth M, Cardoso PG, Ferreira SM, Verdelhos T, Raffaelli D, Pardal MA (2007) Anthropogenic and natural disturbance effects on a macrobenthic estuarine community over a 10-year period. Marine Pollution Bulletin 54: 576-585.

Dolbeth M, Cardoso PG, Grilo TF, Bordalo MD, Raffaelli D, Pardal MA (2011) Long-term changes in the production by estuarine macrobenthos affected by multiple stressors. Estuarine, Coastal and Shelf Science 92: 0-18.

Doney SC, Ruckelshaus M, Duffy JE, Barry JP, Chan F, English CA, Galindo HM, Gremeier JM, Hollowed AB, Knowlton N, Polovina J, Rabalais, NN, Sydeman WJ, Talley LD (2012) Climate change impacts on marine ecosystems. Annual Review of Marine Science 4: 11-37.

Draft PBT National Action Plan For Hexachlorobenzene (HCB) for Public Review (2000) Prepared by The USEPA Persistent, Bioaccumulative and Toxic Pollutants (PBT) HCB Workgroup.

Duarte CM (2002) The future of seagrass meadows. Environment Conservation 29: 192-206.

Ducklow HW, Doney SC, Steinberg DK (2009) Contributions of long-term research and time-series observations to marine ecology and biogeochemistry. Annual Review of Marine Science 1: 279-302.

Elliott M, McLusky DS (2002) The need for definitions in understanding estuaries. Estuarine, Coastal and Shelf Science 55: 815-827.

Elliott M (2002) The role of the DPSIR approach and conceptual models in marine environmental management: an example for offshore wind power. Marine Pollution Bulletin 44, III-VII.

Emmerson M, Bezemer M, Hunter MD, Jones TH (2005) Global change alters the stability of food webs. Global Change Biology 11: 490–501.

Environmental Portuguese Agency (2010) National Implementation Plan for the Stockholm Convention. 72 pp. Available from: http://www.apambiente.pt/_zdata/LRA/UNEP-POPS-NIP-Portugal-1.English.pdf

Fillos D, Scott LL, De Sylor MA, Grespin M, Luksemburg WJ, Finley B (2012) PCB concentrations in shrimp from major import markets and the United States. Environmental Toxicology and Chemistry 31: 1063-71.

Grilo TF, Cardoso PG, Dolbeth M, Pardal MA (2009) Long-term changes in amphipod population dynamics in a temperate estuary following ecosystem restoration. Hydrobiologia 630: 91-104.

Grilo TF, Cardoso PG, Dolbeth M, Bordalo MD, Pardal MA (2011) Effects of extreme climate events on the macrobenthic comunities' structure and functioning of a temperate estuary. Marine Pollution Bulletin 62: 303-311.

Grilo TF, Cardoso PG, Pardal MA (2012) Implications of *Zostera noltii* recolonization on *Hydrobia ulvae* population structure success. Marine Environmental Research 73: 78-84.

Harley CDG, Hughes AR, Hultgren KM, Miner BG, Sorte CJB, Thornber CS, Rodriguez LF, Tomanek L, Williams SL (2006) The impacts of climate change in coastal marine system. Ecology Letters 9: 228-241.

Hoegh-Guldberg O and Bruno JF (2010) The impact of climate change on the world's marine ecosystems. Science 328:1523-28.

Houghton J (2005) Global warming. Reports on Progress in Physics 68: 1343-1403.

Hutzinger O, Safe S, Zitko V (1974) The chemistry of PCB's. CRC Press, Boca Raton, Florida.

Instituto da Água (Portuguese Water Institute) http://snirh.inag.pt/ (accessed January 2013).

Instituto Português do Mar e da Atmosfera (Portuguese Institute for Ocean and Atmosphere) http://www.ipma.pt/ (accessed January 2013).

IPCC (2007) Climate Change 2007: The Physical Science Basis. Contribution of Working Group I to the Fourth Assessment. Report of the Intergovernmental Panel on Climate Change [Solomon, S., D. Qin, M. Manning, Z. Chen, M. Marquis, K.B. Averyt, M. Tignor, H.L. Miller (Eds.)]. Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA, 996 pp.

IPCC (2012) Managing the Risks of Extreme Events and Disasters to Advance Climate Change Adaptation. A Special Report of Working Groups I and II of the Intergovernmental Panel on Climate Change [Field, C.B., V. Barros, T.F. Stocker, D. Qin, D.J. Dokken, K.L. Ebi, M.D. Mastrandrea, K.J. Mach, G.-K. Plattner, S.K. Allen, M. Tignor, and P.M. Midgley (eds.)]. Cambridge University Press, Cambridge, UK, and New York, NY, USA, 582 pp.

Jackson JK and Füreder L (2006) Long-term studies of freshwater macroinvertebrates: a review of the frequency, duration and ecological significance. Freshwater Biology 51: 591-603.

Jaikanlaya C, Settachan D, Denison MS, Ruchirawat M, van den Berg M (2009) PCBs contamination in seafood species at the Eastern Coast of Thailand. Chemosphere 76: 239-249.

Jensen S (1966) Report of a new chemical hazard. New scientist 32: 612.

Joydas TV, Krishnakumar PK, Qurban MA, Ali SM, Al-Suwailem A, Al-Abdulkader K (2011) Status of macrobenthic community of Manifa–Tanajib Bay System of Saudi Arabia based on a once-off sampling event. Marine Pollution Bulletin 62: 1249-1260.

Kennish MJ (2002) Environmental threats and environmental futures of estuaries. Environmental Conservation 29:78-107.

Kim M, Kim S, Yun S, Lee M, Cho B, Park J, Son S, Kim O (2004) Comparison of seven indicator PCBs and three coplanar PCBs in beef, pork, and chicken fat. Chemosphere 54: 1533-1538.

Kotta J, Kotta I, Sunm M, Pollupüü M (2009) Separate and interactive effects of eutrophication and climate variables on the ecosystem elements of the Gulf of Riga. Estuarine, Coast and Shelf Science 84: 509-518.

Kump L, Pavlov A, Arthur M (2005) Massive release of hydrogen sulphide to the surface ocean and atmosphere during intervals of oceanic anoxia. Geology 33: 397-400.

Lillebø AI, Neto JM, Martins I, Verdelhos T, Leston S, Cardoso PG, Ferreira SM, Marques JC, Pardal MA (2005) Management of a shallow temperate estuary to control eutrophication: the effect of hydrodynamics on the system's nutrient loading. Estuarine, Coastal and Shelf Science 65: 697-707.

Lillebø AI, Flindt MR, Pardal MA, Marques JC (2006) The effect of *Zostera noltii*, *Spartina maritima* and *Scirpus maritimus* on a sediment pore-water profiles in a temperate intertidal estuary. Hydrobiologia 555: 175-183.

Lindenmayer DB, Likens GE, Andersen A, Bowman D, Michael CB, Burns E, Dickman CR, Hoffmann AA, Keith DA, Liddell MJ, Lowe AJ, Metcalfe DJ, Phinn SR, Russell-Smith J, Thurgate N, Wardle GM (2012) Value of long-term ecological studies. Austral Ecology 37: 745-757.

Lopes CB, Pereira ME, Vale C, Lillebø AI, Pardal MA, Duarte AC (2007) Assessment of spatial environment quality status in Ria de Aveiro (Portugal). Scientia Marina 71: 293-304.

Lopes JF, Dias JM, Dekeyser I (2001) Influence of tides and river inputs on suspended sediment transport in the Ria de Aveiro lagoon, Portugal. Physics and Chemistry of the Earth, Part B: Hydrology, Oceans and Atmosphere 26: 729-734.

Mackay F, Cyrus D, Russell K-L (2010) Macrobenthic invertebrate responses to prolonged drought in South Africa's largest estuarine lake complex. Estuarine, Coastal and Shelf Science 86: 553-567.

Marques SC, Azeiteiro UM, Martinho F, Pardal MA (2007) Climate variability and planktonic communities: The effect of an extreme event (severe drought) in a southern European estuary. Estuarine, Coastal and Shelf Science 73: 725-734.

Martínez ML, Intralawan A, Vázquez G, Pérez-Maqueo O, Sutton P, Landgrave R (2007) The coasts of our world: ecological, economic and social importance. Ecological Economics 63: 254-272.

Martinho F, Leitão R, Neto JM, Cabral HN, Marques JC, Pardal MA (2007a) The use of nursery areas by juvenile fish in a temperate estuary, Portugal. Hydrobiologia 587: 281-290.

Martinho F, Leitão R, Viegas I, Dolbeth M, Neto JM, Cabral HN, Pardal MA (2007b) The influence of an extreme drought event in the fish community of a southern Europe temperate estuary. Estuarine, Coastal and Shelf Science 75: 537-546.

Martins I, Pardal MA, Lillebø AI, Flindt MR, Marques JC (2001) Hydrodynamics as a major factor controlling the occurrence of green macroalgal blooms in a eutrophic estuary: a case study on the influence of precipitation and river management. Estuarine, Coastal and Shelf Science 52: 165-177.

McLusky DS, Elliott M (2004) The estuarine ecosystem: Ecology, threats and management. 3rd Ed. Oxford University Press, Oxford, 214 pp.

Moon HB, Kim HS, Choi M, Yu J, Choi HG (2009) Human health risk of polychlorinated biphenyls and organochlorine pesticides resulting from seafood consumption in South Korea, 2005-2007. Food and Chemical Toxicology 47: 1819-1825.

Norkko J, Bonsdorff E, Norkko A (2000) Drifting algal mats as an alternative habitat for benthic invertebrates: species specific responses to a transient resource. Journal of Experimental Marine Biology and Ecology 248: 79-104.

Neto JM, Teixeira H, Patrício J, Baeta A, Veríssimo H, Pinto R, Marques JC (2010) The response of estuarine macrobenthic communities to natural-and human-induced changes: dynamics and ecological quality. Estuaries and Coasts 33: 1327-1339.

Nunes M, Marchand P, Vernisseau A, Le Bizec B, Ramos F, Pardal MA (2011) PCDD/Fs and dioxin-like PCBs in sediment and biota from the Mondego estuary (Portugal). Chemosphere 83: 1345-1352.

Nyitrai D, Martinho F, Dolbeth M, Baptista J, Pardal MA (2012) Trends in estuarine fish assemblages facing different environmental conditions: combining diversity with functional attributes. Aquatic Ecology 46: 201-214.

Okumura Y, Yamashita Y, Khono Y (2004) Bioaccumulation of pcdd/fs and co-pcbs in lower-trophic-level organisms in Sendai bay, Japan. Water, Soil and Air Pollution 159: 291-212

Otchere FA (2005) Organochlorines (PCBs and pesticides) in the bivalves *Anadara (Senilis) senilis*, *Crassostrea tulipa* and *Perna perna* from the lagoons of Ghana. Science of Total Environment 348: 102-114.

Pereira ME, Lillebø AI, Pato P, Válega M, Coelho JP, Lopes CB, Rodrigues S, Cachada A, Otero M, Pardal MA, Duarte AC (2009) Mercury pollution in Ria de Aveiro (Portugal): a review of the system assessment). Environmental Monitoring and Assessment 155: 39-49.

Philippart CJM, Beukema JJ, Cadée GC, Dekker R, Goedhart PW, van Iperen KM, Leopold MF, Herman PMJ (2007) Impacts of nutrient reduction on coastal communities. Ecosystems 10: 96-119.

Pinto R, Patrício J, Neto JM, Salas F, Marques JC (2010) Assessing estuarine quality under the ecosystem services scope: ecological and socio-economic aspects. Ecological Complexity 7: 389-402.

Primo AL, Azeiteiro UM, Marques SC, Martinho F, Baptista J, Pardal MA (2012) Colonization and nursery habitat use patterns of larval and juvenile flatfish species in a small temperate estuary. Journal of Sea Research 76: 126-134.

Rodziewicz M, Kaczmarczyk A, Niemirycz E (2004) Polychlorinated biphenyls in the sediments of the Odra River and its tributaries. Polish Journal of Environmental Studies 13: 203-208.

Savage C, Thrush SF, Lohrer AM, Hewitt JE (2012) Ecosystem Services Transcend Boundaries: Estuaries Provide Resource Subsidies and Influence Functional Diversity in Coastal Benthic Communities. PLoS ONE 7(8): e42708.

Schmidt AL, Wysmyk JKC, Craig SE, Lotze HK (2012) Regional-scale effects of eutrophication on ecosystem structure and services of seagrass beds. Limnology and Oceanography 57: 1389-1402.

Strydom NA, Whitfield AK, Paterson AW (2002) Influence of altered freshwater flow regimes on the abundance of larval and juvenile *Gilchristella aestuaria* (Pisces: Clupeidae) in the upper reaches of two South African estuaries. Marine and Freshwater Research 53: 431-438.

Szlinder-Richert J, Barska I, Mazerski J, Usydus Z (2009) PCBs in fish from the southern Baltic Sea: Levels, bioaccumulation features, and temporal trends during the period from 1997 to 2006. Marine Pollution Bullettin 58: 85-92.

UNEP. Stockholm Convention (2001) Final Act of the Conference of Plenipotentiaries on the Stockholm Convention on Persistent Organic Pollutants. Geneva, Switzerland. 44 pp.

Valiela I, Malelland J, Hauxwell J, Behr PJ, Hersh D, Foreman K (1997) Macroalgal blooms in shallow estuaries: controls and ecophysiological and ecosystem consequences. Limnology and Oceanography 42: 1105-1118.

Vasconcelos RP, Reis-Santos P, Fonseca V, Maia A, Ruano M, França S, Vinagre C, Costa MJ, Cabral HN (2007) Assessing anthropogenic pressures on estuarine fish nurseries along the Portuguese coast: A multi-metric index and conceptual approach. Science of the Total Environment 374: 199-215.

Verdelhos T, Neto JM, Marques JC, Pardal MA (2005) The effect of eutrophication abatement on the bivalve *Scrobicularia plana*. Estuarine, Coastal and Shelf Science 63: 261-268.

Voorspoels S, Covaci A, Maervoet J, De Meester I, Schepens P (2004) Levels and profiles of PCBs and OCPs in marine benthic species from the Belgian North Sea and the Western Scheldt Estuary. Marine Pollution Bulletin 49: 393-404.

Wang JS, Chou HN, Fan JJ, Chen CM (1998) Uptake and transfer of high PCB concentrations from phytoplankton to aquatic biota. Chemosphere 36: 1201-1210.

WHO (1997) Hexachlorobenzene (Environmental Health Criteria 195), Geneva, International Programme on Chemical Safety.

Wilson MA and Farber S (2009) Accounting for ecosystem goods and services in coastal estuaries. In: Pendleton, L.H. (ed.), The Economic and Market Value of Coasts and Estuaries: What's at Stake? Arlington, Virginia: Restore America's Estuaries 13-32.

Ysebaert T, Herman PMJ, Meire P, Craeymeersch J, Verbeek H, Heip CHR (2003) Large-scale spatial patterns in estuaries: estuarine macrobenthic communities in the Schelde estuary, NW-Europe. Estuarine, Coastal and Shelf Science 57: 335-355.

Zhou JL, Maskaoui K, Qiu YW, Hong HS, Wang ZD (2001) Polychlorinated biphenyl congeners and organochlorine insecticides in the water column and sediments of Daya Bay. China Environmental Pollution 113: 373-84.

Zhou Q, Zhang J, Fu J, Shi J, Jiang G (2008) Biomonitoring: An appealing tool for assessment of metal pollution in the aquatic ecosystem. Analytica Chimica Acta 606: 135-150.

CHAPTER I

Effects of extreme climate events on the macrobenthic communities′ structure and functioning of a temperate estuary

Keywords

Climate Change • Floods • Droughts • Heat Waves • Recovery • Macrobenthos

ABSTRACT

The Mondego estuary (Portugal) experienced profound structural and functional modifications due to eutrophication, which was exacerbated by consecutive weather extremes that compromised a previous restoration project. This work explores multiple climate impacts on macrobenthic communities' structure and functioning and its implications on ecosystem's recovery. Floods and heat waves had a stronger negative effect on macrobenthic assemblages than the droughts, imposing a total abundance decline. Contrarily, biomass was not so affected by climate events, being stable and even increased in a mudflat area, where seagrass is re-colonizing. Bivalves and oligochaetes decreased with the flooding episodes, likewise subsurface-deposit feeders and suspension feeders, while crustaceans were particularly sensitive to heat waves. Species richness declined with the floods and heat waves, whilst evenness increased in sandflat area, constituting a positive sign towards recovery. Succession of different climate extremes affected ecosystem structure and functioning, delaying its recovery with possible consequent effects at higher trophic levels.

INTRODUCTION

Human pressure associated to population growth and economic development are affecting the health and the ecological balance of marine environments, making them one of the most endangered ecosystems on Earth. In addition to anthropogenic pressure, the oceanic biosphere is now, also, experiencing rapid global climate change, which constitutes an unprecedented threat to human and natural systems.

Given their ecological and socio-economical importance, coastal marine ecosystems are a major focus of concern regarding the potential dramatic impacts of climate change on its structure and functioning (Houghton, 2005; Harley et al., 2006).

Climate model projections ensure that frequency and severity of extreme weather events such as floods, droughts and heat waves will increase in the near future which may amplify the risk of abrupt and non-linear changes in many ecosystems and therefore affect their composition, function, biodiversity and productivity (IPCC, 2007). For mainland Portugal these climate-related changes have been particularly evident during the last decades, essentially on annual precipitation and atmospheric temperature patterns. Precipitation presented a generalized decreasing trend compared to the annual mean values for the period 1971-2000 and significant rainfall differences were observed between seasons, with a systematic reduction of spring precipitation. Additionally, in the second half of the 20th century, it was observed an increase in the frequency/intensity of extreme weather events, namely flooding episodes (Santos et al., 2002). Climate data for mainland also revealed a progressive increase in mean air temperature since 1970 (0.33ºC/decade) and a higher frequency of heat waves in the last 10 years (2003, 2005 and 2006), coupled with an intensification in frequency of severe drought periods (Portuguese Institute for Ocean and Atmosphere; Santos et al., 2002).

Previous studies developed in the Mondego estuary, concerning global climate change, have focused only on the impact of isolated events (e.g. floods) on the entire macrobenthic community (Cardoso et al., 2008a) or in specific taxa, such as the gastropod *Hydrobia ulvae* (Cardoso et al., 2008b) or the amphipods' population (Grilo et al., 2009). However, this paper has a holistic overview, since it deals with the impact of multiple consecutive stochastic weather episodes on the whole intertidal macrobenthic community at structural and functional levels. Here, we explore a 16-year time series to evaluate how different extreme climate events can affect distinct functional groups and the community structure, as well as understand how these consecutive episodes may have single, cumulative or synergistic effects on the overall system stability.

MATERIALS AND METHODS

Study area

Located on the Atlantic coast of Portugal (40°08′N, 8°50′W), the Mondego estuary is a warm-temperate intertidal system of 8.6 km^2, which comprises in its terminal part two distinct arms separated by an alluvium-formed island (Murraceira Island) (Fig. 1). For further detailed information about the system see Cardoso et al. (2005) and Dolbeth et al. (2007).

Since the end of the 1980s, anthropogenic activities combined with specific physical characteristics (low hydrodynamics, excessive nutrient release and high salinity) have contributed to a gradual increase of eutrophication, which led to a dramatic decline in area and biomass of the *Z. noltii* bed. At present, the south arm is progressively recovering from the negative effects caused by organic pollution, thanks to a restoration project implemented in the estuary, which major aim was to restore a previously existing healthy state condition of the system. This program included measures to reduce nutrient loading, improve water dynamics by enlarging the upstream connection between the two arms and the physical protection of the seagrass patches from human disturbance (Cardoso et al., 2005, 2008a; Grilo et al., 2009).

Along the south arm, three sampling areas were selected (Fig. 1): 1) the *Z. noltii* bed, located towards the marine end of the estuary and characterized by muddy sediments and covered with the macrophyte *Z. noltii*; 2) a mudflat area placed just upstream of the previous sampling station, showing a recolonization of the seagrass; and 3) a sandflat area, in the inner part of the estuary, characterized by the absence of rooted macrophytes for more than 20 years and seasonally covered by some green opportunist macroalgae (Cardoso et al., 2010).

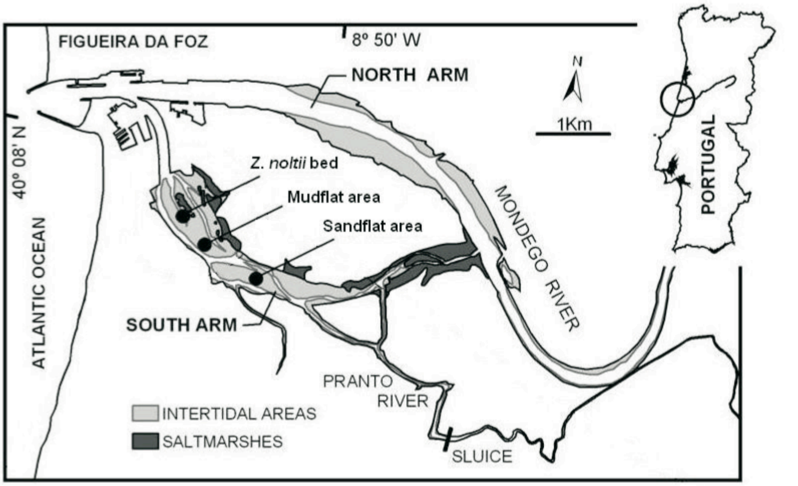

Fig. 1. Location of the Mondego estuary and sampling areas.

Sampling program and laboratory procedures

The macrobenthic communities were monitored during two different periods: from January 1993 to September 1995 and March 1999 to November 2008, with a sampling gap between these periods in order to facilitate recovery of the seagrass bed. In the first 18 months samples were collected fortnightly and monthly thereafter. At each sampling occasion, 6-10 sediment cores (141 cm^2 surface area) were randomly taken to a depth of 20 cm, by using a manual corer. Samples were washed in estuarine water through a 500 μm mesh sieve and the material retained (sediment, rooted macrophytes, algae and fauna) preserved in 4% buffered formalin. Temperature, oxygen, pH and salinity were measured *in situ* in the intertidal pools and sediment samples were collected to quantify the organic matter content. In the laboratory, plant material was sorted and separated into green macroalgae and *Zostera noltii*. Animals were separated and transferred to 70% ethanol, identified to the lowest possible taxon and counted. For fauna, plant material and sediment the ash-free dry weight (AFDW) was assessed, after combustion for 8 h at 450ºC.

Data analysis

Macrobenthic taxonomic group assignments

Each macrobenthic taxon was assigned to the respective taxonomic group, according to its relative position in the organisms' classification system. Five main taxonomic groups were studied for all areas (Bivalvia, Crustacea, Gastropoda, Oligochaeta and Polychaeta) since these groups are the most abundant and representative. The contribution of the remaining groups, identified as "others", for total macrobenthic community is almost insignificant, less than 2%. Anthozoa, Echinodermata, Insecta, Nemertinea, Polyplacophora, Sipunculida and Zoantharia species composed "Others". As in previous works (Cardoso et al. 2008a) we decided to present the results of the analysis excluding the snail *Hydrobia ulvae* since it usually occurs in very high numbers and its inclusion masks changes in other species.

Macrobenthic trophic group assignments

Based on feeding behaviour and food type, each macrobenthic taxon was assigned to a trophic group. Trophic groups used in this study were detritivores (D), carnivores (C), herbivores (H) and omnivores (O). Since detritivores are the main trophic group, we subdivided it into surface deposit feeders (SDF), subsurface-deposit feeders (SsDF) and suspension feeders (SuF) in order to better identify which specific detritivores groups were affected by the extreme climate events studied. Once again, the trophic composition was analysed without the gastropod *Hydrobia ulvae*, since the dominance of the species masked changes in other species.

Macrofaunal diversity

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 Pielou's evenness measures (Krebs, 1999), excluding the most abundant species (*Hydrobia ulvae*).

Multivariate approach: Redundancy Analysis (RDA)

A detrended correspondence analysis (DCA) was performed with the biological data (species density) to evaluate which ordination method, linear or unimodal, was suitable to apply. Since a linear response was detected, a redundancy analysis (RDA) was carried out to examine the relationships between biological and environmental parameters to each study area. Originally, nine environmental parameters were tested (annual values): green macroalgal biomass, mean estuarine dissolved oxygen, mean water temperature, mean salinity, mean pH, maximum absolute air temperature registered in the whole year, runoff, organic matter content and *Z. noltii* mean biomass (only in the seagrass bed and mudflat area). Prior to the analysis, the species density data was square root transformed, in order to scale down the scores of the highly abundant species, and whenever necessary environmental variables were also transformed to reduce possible outliers. Co-linearity between environmental parameters was checked (Draftsman plot and variation inflation factors) and the model forward selection with Monte Carlo permutation tests was used to identify the minimal significant subset of environmental variables ($p < 0.05$) needed to explain the observed patterns of community change. The permutation test is a special case of randomization tests that randomly reassigns environmental variables to species composition and tests for significance. The software used for the statistical analysis was PRIMER v.5 and CANOCO 4.5 (Ter Braak and Similaeur 1998).

RESULTS

Climate variability

The analysis of atmospheric temperature and precipitation patterns throughout the 16-year period revealed the existence of several atypical climate episodes, comparing to the mean values registered for central Portugal for the period 1971-2000 (Fig. 2). During the last decade it was observed an increase in the frequency of extreme weather events in the Mondego region. Two unusual rainfall events occurred during winters of 1995/96 and 2000/01, being the latter the most atypical, since it was characterized by long periods of intense flooding. It is considered the 3rd rainiest winter of the last 30 years (Portuguese Institute for Ocean and Atmosphere). In contrast, several periods with extremely low precipitation values compared to the climate normal of 1971-2000 were also observed. Annual precipitation reached minimum values in 2005 (487.8 mm against 905.1 mm for 1971-2000 mean), but 2004, 2007 and 2008 also presented below-mean precipitation values, being considered as dry, extremely dry and very dry years, respectively. According to the Portuguese Institute for Ocean and Atmosphere, 2005 was the driest year since 1931, followed by 2007 that registered the second lowest annual precipitation value in the last 60 years.

Mean air temperature showed a clear seasonal variation pattern, with higher values during summer and lower temperatures in the winter months, which is typical of a Mediterranean temperate climate (Fig. 2B). Throughout the study period, mean temperature varied more or less concordantly to values observed for the period between 1971 and 2000, except during the summers of 1995 and 2003 and the spring/summer of 2005, which presented higher mean monthly temperatures compared to the climate normal of 1971-2000. Besides, for some days in these years the maximum absolute air temperature reached more than 40ºC. Occurrence of several periods of at least 6 days in which maximum atmospheric temperature exceeded 5ºC relatively to the mean daily value of the reference period 1971-2000 are defined as heat waves (Portuguese Institute for Ocean and Atmosphere).

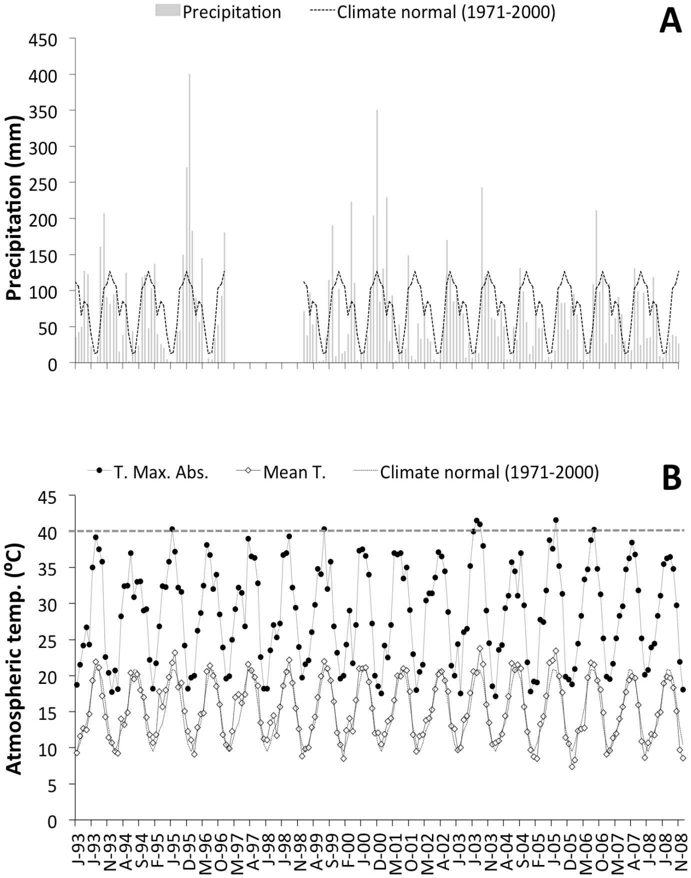

Fig. 2. Long-term monthly variation in (A) precipitation and (B) atmospheric temperature (monthly means and absolute maximum value registered) compared to the climate normal of 1971-2000 for the centre of Portugal. Climate normal of a climatic element is the mean value correspondent to a sufficient number of years to admit that it represents the predominant value of that element in a certain place. The World Meteorological Organization fixed that number in 30 years, starting in the first year of each decade (e.g. 1971–2000). The climate normal of 1971–2000 is considered a reference (IPMA – Portuguese Institute for Ocean and Atmosphere, http://www.ipma.pt/).

Frequency of these events increased in mainland Portugal especially during the last 5 years of the study period: a heat wave was registered in July-August of 2003 (lasted 16-17 days and was the longest heat wave ever recorded in Portugal), two heat waves in June of 2005 and three in June, July and August 2006 (for the Mondego region only the heat waves in July and August 2006 had significance, lasting 6-7 days and 8-9 days, respectively – Portuguese Institute for Ocean and Atmosphere). In this paper higher relevance is given to the heat wave occurred in 2003 since it was an exceptional episode never observed in Portugal not only for its duration but also due to the extremely high temperatures recorded.

Macrobenthic assemblages' dynamics

Changes in density and biomass

Macrobenthic communities responded differently to the impact of extreme weather events at the three sampling areas. Despite some annual cyclic oscillations, both density and biomass decreased over time in the *Z. noltii* bed (Fig. 3A). In fact, the mean density and biomass in the pre-management period was substantially higher than in the post-management period. Total density started to increase immediately after restoration measures. From 1999 onwards, both density and biomass had a slight increasing tendency, which seemed to be always interrupted by the occurrence of the extreme weather events (Fig. 3A). This trend became clearer for the analysis of density without the gastropod *H. ulvae* (Fig. 3D). A closer inspection of density and biomass trends during occurrence of extreme climate events, allowed us to conclude about their negative impacts on community structure, more specifically on abundance. The intense floods in the winter of 2000/01 implied a decrease in density, which declined even more due the heat wave in the summer of 2003 (Fig. 3A, D). During the severe drought of 2005, density kept low and seemed to increase only after the influence of this stochastic episode, reaching the highest values of all post-management period since 2006 (Fig. 3A, D). On the contrary, total biomass without *H. ulvae* slightly increased from the pre-management to the post-management period, maintaining relatively constant until the end of the study period (Fig. 3D).

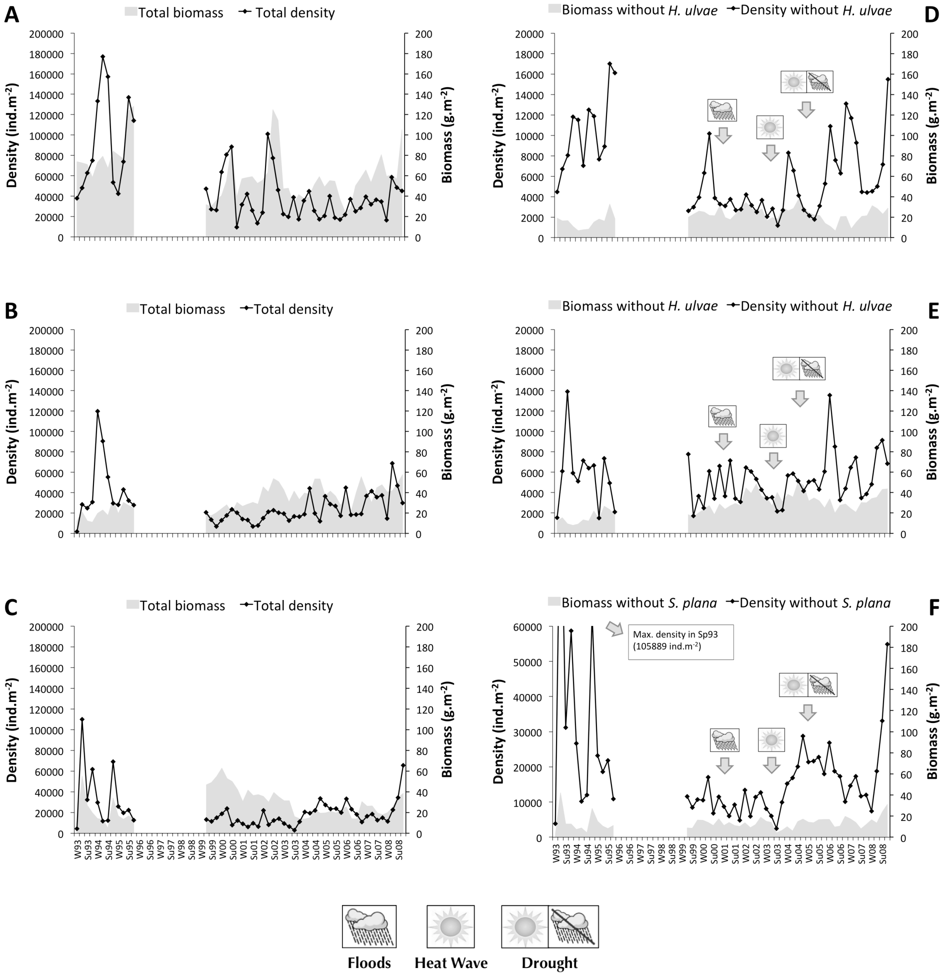

Fig. 3. Variation of total density, biomass and density/biomass without *H. ulvae* and *S. plana* of the macrobenthic community from 1993 to 2008. (A, D) *Z. noltii* bed; (B, E) Mudflat area; and (C, F) Sandflat area.

In the mudflat area, there was a decrease in density from the pre- to the post-management period. After the management measures, density seemed to gradually increase, especially in the latest years of the study (Fig. 3C). With regard to biomass, there was a sustained increase throughout the whole 16-year period (Fig. 3B). These trends were also observed when the dominating *H. ulvae* was removed from the analysis (Fig. 3E). Among the extreme climate events, the heat wave of 2003 seemed to be the event with the most pronounced and negative effects on macrobenthic assemblages in the mudflat area, resulting in decreases in both density and biomass (Fig. 3B,E).

For the sandflat area, density changes were broadly similar to those in the mudflat area. However biomass increased considerably in the beginning of the post-management period and showed a decreasing trend afterwards (Fig. 3C). Re-analysing data excluding the large abundant bivalve *Scrobicularia plana*, the biomass pattern of the remaining species was relatively stable throughout the post-management period, whilst density appeared to be more sensitive than biomass to the stochastic events, mainly to the heat wave in the summer of 2003 when have reached the lowest value ever (2481 ind.m^{-2}) (Fig. 3F). Contrarily to the remaining areas, during the drought occurrence there was no evident decrease in density and biomass (Fig. 3C, F).

Taxonomic groups

Taxonomic structure differed for the three areas, despite a common dominance of polychaetes for all zones. In the seagrass bed the dominant groups were polychaetes (46%), oligochaetes (26%) and bivalves (16%) (Fig. 4A). These groups responded differently to climate extremes, with oligochaetes relative abundance being severely affected by the floods and the heat wave, contrarily to polychaetes that reinforced their dominance during these periods. Since the summer of 2005, the percentage of oligochaetes in the community considerably increased until the end of the study period. Bivalves' importance in the community became slightly lower during the floods and seemed to be not affected by the heat wave and the drought, although the lowest relative values observed during autumn and winter of 2005/06. On the other hand, crustaceans decreased considerably after the impact of the heat wave and were relatively stable during the remaining stochastic episodes. Gastropods were almost inexistent when *H. ulvae* was removed from the analysis and contribution of other classes was equally negligible, less than 2% (Fig. 4A).

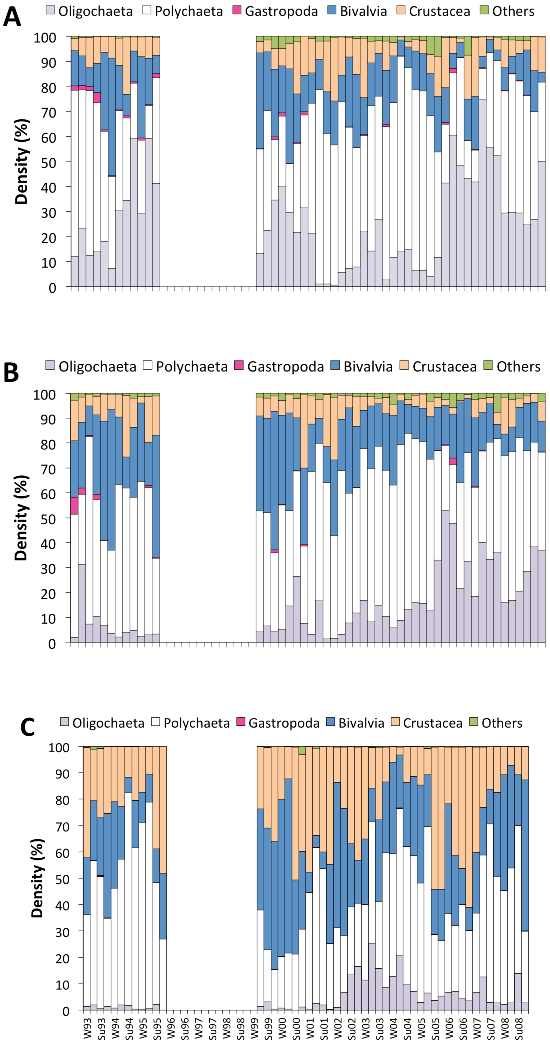

Fig. 4. Taxonomic structure of the macrobenthic assemblages of the Mondego estuary. Values are percentages of total individuals. (A) Z. *noltii* bed; (B) Mudflat area; and (C) Sandflat area.

In the mudflat area, the dominance of polychaetes was even more evident than in the *Z. noltii* bed (51%), followed by bivalves (23%), oligochaetes (16%), crustaceans (8%) and other classes (2%). There was a general increase in oligochaetes over time and the opposite pattern was observed for bivalves and crustaceans (Fig. 4B). Oligochaetes and bivalves relative abundance decreased during the occurrence of the large floods, while polychaetes reached the lowest values during drought periods (summer and autumn of 2005). As verified for the *Z. noltii* bed, oligochaetes appreciably increased since 2005 (Fig. 4B).

In the sandflat area, polychaetes were again the dominating group, but had lower percentages (41%) than in the other areas, especially in the post-management period (Fig. 4C). Both bivalves and crustaceans followed as dominant groups, with 27% and 26%, respectively (Fig. 4C). Over time only polychaetes decreased, whilst oligochaetes and bivalves' proportion increased. Extreme climate events induced to different community compositions: polychaetes dominance became lower during the drought of 2005; bivalves percentages decreased during floods and the drought; and the heat wave was the most dramatic event for crustaceans, causing a significant decline in their relative abundance. Gastropods and other classes represented less than 1% of total community (Fig. 4C).

Trophic groups

Similarly to the previous sections, preliminary analysis which included *Hydrobia ulvae* were difficult to interpret, due to the masking effect of this dominant species, so it was decided only to present results for trophic groups excluding that gastropod.

For both *Z. noltii* bed and the mudflat area, detritivores and omnivores were the dominant groups and evidenced an opposite response to the extreme weather events (Fig. 5A,B). Short after the floods of 2000/01 and the 2003 heat wave occurrence and also during summer of 2007, detritivores dominance in the community decreased significantly in both areas, contrarily to omnivores that increased their proportion during these periods, reaching maximum values. While the floods and the heat wave seemed to cause a similar decline in detritivores for the *Z. noltii* bed, the same did not occur for the mudflat area, where the heat wave had a stronger effect, causing the minimum value ever. Interestingly to note the considerably increase in detritivores abundance in the last 3 years for both sampling areas, but especially in the seagrass bed.

In the sandflat area, detritivores represented even a higher fraction of total community, followed by carnivores with almost 15% (Fig. 5C). Contribution of both herbivores and omnivores for total abundance was small, less than 5%. Evaluation of the impact of extreme climate events in this area was not so evident as in the previous zones.

Analysing in detail the detritivores' assemblage, surface-deposit feeders (SDF) declined during the first period (from 1993 to 1995) (Fig. 5D), concomitantly to the reduction in area and biomass of *Z. noltii* (Cardoso et al., 2008a). On the other hand, subsurface-deposit feeders (SsDF) (mainly small polychaetes like *Heteromastus filiformis*, *Capitella capitata*, *Tubificoides* sp.) increased during the same period, until the large floods of 2000/01 when sharply declined (from nearly 60% to the minimum value ever – 8%). Contrarily, surface-deposit feeders dominance increased, constituting about 81% of the community after the intense floods. Since then, surface-deposit feeders gradually decreased, consistently to periods of higher temperatures, namely during the heat wave of 2003 and the severe drought of 2005 when presented low abundance values. Subsurface-deposit feeders proportion increased between mid 2001 and the summer of 2003, when it decreased again probably due to the heat wave's influence. Afterwards, subsurface-deposit feeders started to become dominant, reaching the highest relative values of all study period since 2006. Contribution of suspension feeders (SuF) to the community trophic composition was low during the floods and increased during the heat wave, when achieved the highest relative value (35%)

of the entire 16 year-period. Since then, their dominance progressively declined as observed with surface-deposit feeders (Fig. 5D).

In the mudflat area, despite some differences during the pre-management period, the relative composition of main detritivores groups was generally similar to that one described for the *Z. noltii* bed (Fig. 5E).

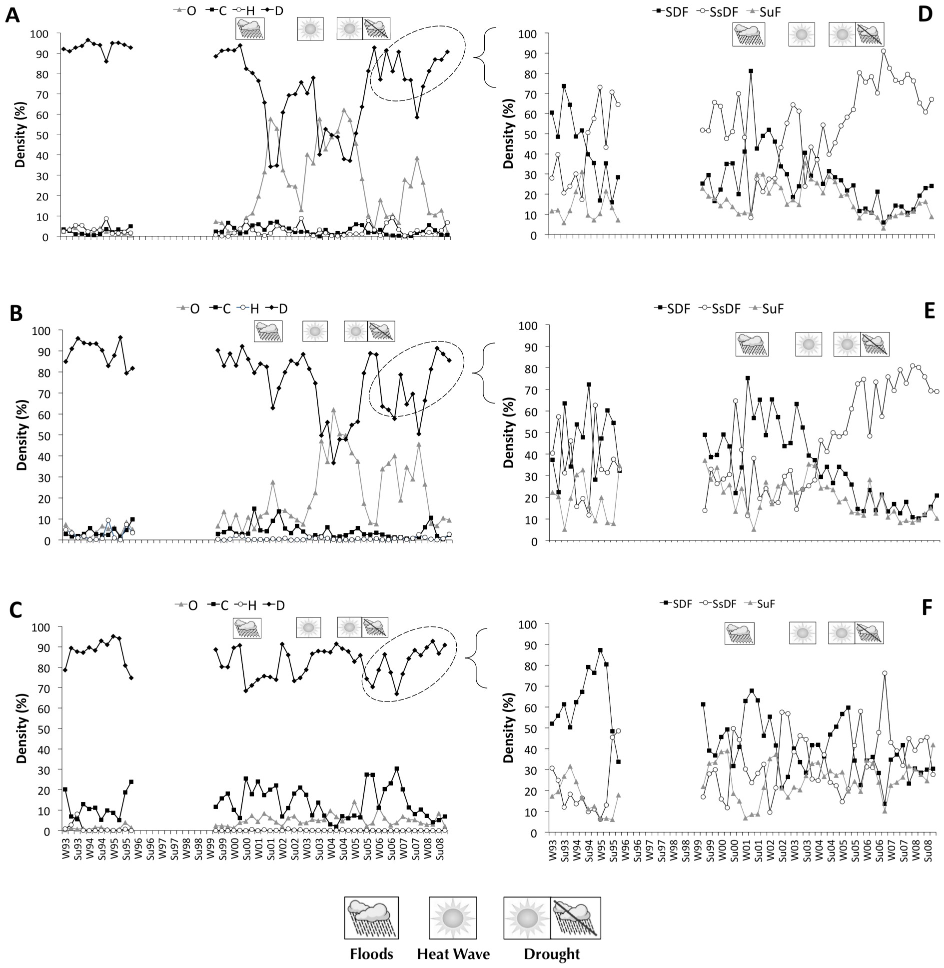

Fig. 5. Trophic structure of the macrobenthic assemblages of the Mondego estuary: omnivores (O), carnivores (C), herbivores (H), detritivores (D), surface-deposit feeders (SDF), subsurface-deposit feeders (SsDF) and suspension feeders (SuF). Values are percentages of total individuals. (A,D) Z. *noltii* bed; (B,E) Mudflat area; and (C, F) Sandflat area.

Trophic structure in the sandflat area was quite different than in the seagrass bed and the mudflat area, being characterized by a better balance within the three detritivores groups (Fig. 5F). There was a gradual increase in subsurface-deposit feeders and suspension feeders over the 16-year period and a general decrease in surface-deposit feeders. The occurrence of the floods in 2000/01 resulted in strong declines in subsurface-deposit feeders and suspension feeders in opposition to surface-deposit feeders that became dominant in the community. The influence of the heat wave of 2003 was not so evident in this area for the three detritivores groups. During the drought of 2005, surface-deposit feeders relative importance had a significant increase while subsurface-deposit feeders declined. After the drought, subsurface-deposit feeders started to recover in the community, displaying a slight increase tendency until 2008, but never recording values similar to those verified for the previous study areas (Fig. 5F).

Changes in diversity

Several differences were observed regarding diversity of macrobenthic assemblages over time. Generally, the *Z. noltii* bed supported more species than the remaining areas, however during the post-management period all the areas became more similar (Fig. 6). Species richness declined from 1993 to 1995 for all sampling sites but its subsequent recovery was faster in the mud- and sandflat areas than in the seagrass bed (Fig. 6). With the unprecedented high floods of 2000/01, species richness decreased in the three areas, increasing again until the summer of 2003 when the heat wave occurred. At this time, species richness declined and reached among the lowest values of the entire study period. Interestingly, the floods and the heat wave seemed to have a more negative impact on species richness than the drought periods (Fig. 6). Previous studies indicated that *H. ulvae* is highly dominant taxon, especially in the *Zostera* area (Cardoso et al. 2008a), masking the results of the whole community. So, evenness was studied without *H. ulvae* and resulted higher in the seagrass bed and mudflat area (Fig. 6 A,B), but evenness recovery became more noticeable and rapid during the last years in the sandflat area (Fig. 6C).

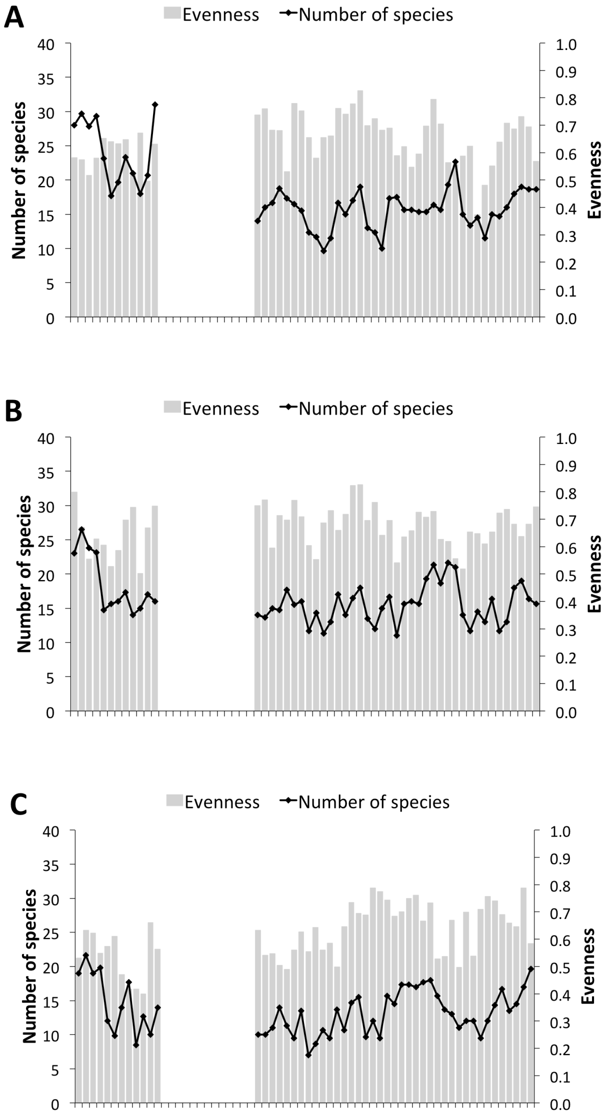

Fig. 6. Variation of the biological indices (evenness and number of species) in the three sampling stations from 1993 to 2008. (A) Z. *nolti* bed; (B) Mudflat area; and (C) Sandflat area.

Redundancy analysis (RDA)

Species-environment relationships were evaluated to each study area with Redundancy Analysis (RDA). In the seagrass bed, four of the nine environmental parameters tested were statistically significant (*Zostera noltii* biomass, salinity, maximum absolute air temperature and runoff), with the resulting constraint ordination explaining 74.9% of the variability found in the data. Samples from the pre-mitigation period (1993-1995) were quite separated from those of the post-management period (1999-2008), evidencing the low similarities between samples from different periods (Fig. 7A). Macrobenthic samples from 1993, 1994 and 1995 were similar being associated to higher *Z. noltii* biomass. The runoff was the main environmental variable conditioning spatial distribution of samples from 2000/2001, in agreement with the occurrence of the largest floods. Consistently to the severe drought, samples from 2005 were closely tied to maximum absolute air temperatures and higher salinities. Samples from 2003, 2006 and 2007 presented high similarities between them as well as samples from 1999 and 2008 (Fig. 7A).

In the mudflat area, four environmental parameters were statistically significant (green macroalgal biomass, *Zostera noltii* biomass, maximum absolute air temperature and mean water temperature) with the resulting constraint ordination explaining 78.9% of the variability found in the data. Samples from the mudflat area were more scattered than those of the previous area, probably indicating more heterogeneity in time and space, perhaps reflecting less stability in this area. Samples from the pre-management period (1993-1995) were isolated from the remaining years, being associated to higher green macroalgal biomasses (Fig. 7B). Biological samples from 1999 to 2003 presented high similarities between them and were correlated to maximum absolute air temperatures. Samples from 2005 were naturally associated to higher maximum absolute temperature (Fig. 7B), which also occurred during the drought. Consistently to the seagrass cover increase, samples from 2006, 2007 and especially 2008 were related to higher *Z. noltii* mean biomass (Fig. 7B).

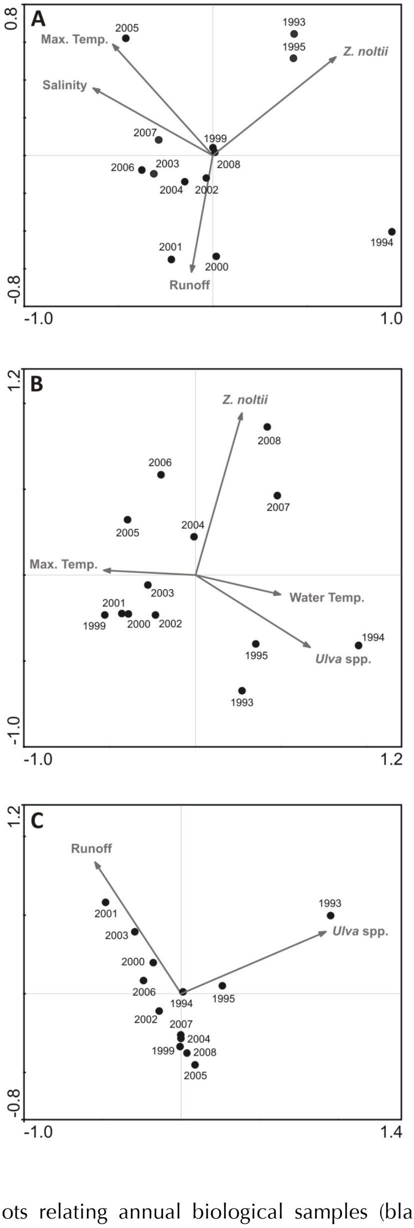

Fig. 7. RDA ordination biplots relating annual biological samples (black symbols) and significant environmental variables (after Monte Carlo permutation tests, grey vector lines) along the study period. (A) Z. *noltii* bed; (B) Mudflat area; and (C) Sandflat area.

For the sandflat area, only the green macroalgal biomass and the runoff were statistically significant with the resulting constraint ordination explaining 58.2% of the variability found in the data. The spring macroalgal bloom occurred in 1993 (for more detailed information see Grilo et al. 2009) was the principal factor influencing macrobenthic community, thus biological samples from 1993 were associated to higher green macroalgal biomasses (Fig. 7C). Remaining samples were quite differentiated from those of 1993, evidencing changes in macrobenthic community after the macroalgal bloom influence. Biological samples from 2001 were closely related to the runoff in agreement with the flooding episodes. The ordination also revealed that recent samples (2007 and 2008) were closer to samples collected immediately after restoration measures (1999), indicating a higher faunal similarity between them (Fig. 7C).

DISCUSSION

Over the last decade, the Mondego estuary has been recovering from an intense eutrophication process, however, the occurrence of consecutive extreme weather events (floods, droughts and heat waves), represented an additional stressor to full recovery of macrobenthic communities. The available climate data reveal an increase both in the frequency of heavy precipitation events and in the frequency of severe droughts, as well as heat waves, especially over the last 10 years for mainland Portugal (Santos et al., 2002; Cardoso et al., 2008a).

Following the introduction of management measures, different scenarios were observed among the three study areas with regard to macrobenthic abundance and biomass. Compared to the pre-management period a general decline in total density was observed for all areas while total biomass varied differently along the three sampling sites, being lower than in the previous period (1993-1995) for the seagrass bed and higher for the mudflat and sandflat areas. However, removing the dominant species in each area (the gastropod *Hydrobia ulvae* for the seagrass bed and mudflat area and the bivalve *Scrobicularia plana* for the sandflat area), distinct considerations could be made regarding abundance and biomass patterns and extreme climate events' impacts on the macrobenthic assemblages. Closer inspection revealed a density's increasing tendency after the management for the three sampling areas, despite the great instability caused by the succession of extreme climate events that retarded the system's return to its previous state. In both seagrass bed and sandflat area total macrobenthic biomass displayed a stable pattern, contrarily to the mudflat area where biomass showed an appreciably increase, probably boosted by the gradual cover increase of the macrophyte *Z. noltii* in that area, which constitutes a positive sign towards recovery.

Among the different stochastic events occurred in the Mondego region, the intense floods (2000/2001) and the heat wave (2003) seemed to be the most negative climate episodes directly impacting macrobenthic assemblages without dominating species, leading to minimum density values and a pronounced biodiversity impoverishment in all sampling areas. Interestingly, biomass did not present great oscillations for all areas over time, appearing to be not affected by any specific extreme weather event.

Extreme climate events seemed to affect distinct areas of the estuarine system, differently. Whilst the floods imposed a huge decline in total density in the *Z. noltii* bed, the mud- and sandflat areas were affected in a more slightly way displaying only few changes on density level. Disturbances, particularly flooding, have an important role as regulators of macroinvertebrates distribution, abundance and coexistence (Miserendino, 2009). Floods' specific effects are difficult to predict since distinct communities and habitats may respond differently to heavy rainfall events, depending on the ecology and feeding habits of the species (Cardoso et al., 2008a). For instance, severe rain episodes and flooding are generally associated to high water flow rates, which could cause some macrobenthic fauna to be flushed away. Additionally, it could result in catastrophic deposition of fine terrigenous sediments and subsequent anoxic conditions, which may have profound negative implications at structural and functional levels of some macrobenthic communities (Norkko et al., 2002). On the contrary, other communities dominated by opportunistic species may thrive under flood conditions (Salen-Picard and Arlhac, 2002; Salen-Picard et al., 2003).

From a global perspective, literature regarding the effects of droughts on estuaries is scarce, and the mechanisms by which they conditioned these ecosystems are poorly understood (Pillay and Perissinotto, 2008). Nevertheless, the effects of drought conditions usually include intra and inter site variability in macrobenthic species abundance and composition (Jones, 1990), the rapid colonization of opportunistic marine associated species with increasing salinity (Hastie and Smith, 2006) and changes in species dominance due to altered salinity and substrate type (MacKay et al., 2010). The severe drought of 2005, as a result of long periods of below-average rainfall, coupled with high temperatures have weakly affected macrobenthic communities from the sandflat area, causing a slight density's increase, while biological assemblages from the seagrass bed and the mudflat area maintained low density values until the end of the drought influence.

Macroinvertebrate assemblages at the three sampling areas were dominated by deposit-feeding species, indicative that detritus are a basal resource in the estuary, as also suggested by Baeta et al. (2009). Following the floods and the heat wave, there seemed to be a replacement of detritivores by omnivores in both *Z. noltii* bed and mudflat area, evidencing the opposite responses of these distinct functional groups facing climate extremes. Within detritivores, the subsurface-deposit feeders and suspension feeders were the most impacted by the largest floods, suffering a strong decline, which in the case of suspension feeders it was probably related to the clogging of their feeding structures resulting from high turbidity caused by the floods (Norkko et al., 2002).

Feeding types were closely associated to environmental variables. Physical parameters such as hydrodynamics, sediment grain size or quantity/quality of the available food have been indicated as factors determining the dominance of feeding types (García-Arberas and Rallo, 2002). For example, subsurface deposit-feeders are clearly dominant in sediments with high percentages of coarse elements and higher levels of organic matter (Rosenberg, 1995; García-Arberas and Rallo, 2002). This fact is consistent with our results, since the areas where subsurface-deposit feeders predominate (seagrass bed and mudflat area) presented higher organic matter content, more than double than in the sandflat area (unpublished data). Thus, the success of subsurface-deposit feeders in recent years can be related to the highest levels of organic matter retained on sediments of the *Z. noltii* bed and the mudflat area. Besides, the last years were characterized by mild, smooth, stable climate conditions, instead severe climate extreme episodes, which might be favored growth and reproduction of opportunistic species (*Capitella* sp., *Heteromastus* sp., *Tubificoides* sp.). Evidences from other European estuaries revealed that opportunistic polychaetes, namely some capitellids with a subsurface-deposit feeding strategy are very sensitive to cold winter temperatures, disappearing or reducing their number during these conditions (Pearson and Rosenberg, 1978). This suggests that lower temperatures may limit the survivor of these organisms and probably their growth and reproductive efficiency were higher in mild/warmer environmental conditions. As a result of subsurface-deposit feeders' success, there was a decline in surface-deposit feeders, which may be explained based on the fact that surface feeders commonly rely on newly deposited organic matter, which was not available at superficial depths, or that predation at the surface favoured animals living and feeding deeper in the sediment (Rosenberg, 1995).

With respect to taxonomic composition, polychaetes were the dominant group and apparently were not markedly affected by any extreme event studied, contrarily to other taxonomical groups. Bivalves and oligochaetes generally declined after the heavy floods and the heat wave, reducing their percentage in the macrobenthic assemblages. A possible reason for bivalves decline may be the high salinity variations in the system, both temporally and spatially, which might have negatively affected bivalves, as verified in a previous work of Pillay and Perisinotto (2008) where high densities of bivalves were recorded in stable marine salinities (25-50) while low abundances were observed during low salinity conditions. It is therefore possible that the decline of *Scrobicularia plana*, one of the dominant species of the bivalve community in the Mondego estuary (Dolbeth et al., 2007), may have been attributed to unpredictable and rapid changes in salinity recorded in the system. Furthermore, the high temperatures registered in intertidal water pools were probably an additional cause for the observed decline of the bivalve *S. plana* at the sandflat area. Lethal temperatures were determined for this species at 27.5ºC in summer conditions (Wilson, 1981), which adds to explain the species' decline during the heat wave and the drought. Crustaceans also showed a considerable reduction concomitantly to the occurrence of the heat wave, being the isopod *Cyathura carinata* the species mostly impacted (Bordalo et al., 2011).

Despite natural and anthropogenic disturbances, the sandflat area presented positive signs of recovery by displaying an appreciable and faster increase in species richness and diversity in recent years, mainly visible through changes in evenness.

Based on a longer data series, our findings reinforce the results from previous studies and allow to conclude that different groups of organisms and habitats may respond differently to flooding and/or drought conditions, depending on the ecology and feeding habits of the species, which makes difficult to accurately predict the biological responses of the macrobenthic communities to future catastrophic events. Moreover, the succession of different extreme weather events (floods, droughts and heat waves) seems to have a synergistic effect on the global stability of the ecosystem, delaying the recovery of the estuarine system by severely impacting the macrobenthic community. These extreme climate episodes not only have implications for biodiversity conservation of the estuarine system, but also for the livelihoods of the people who directly depend on the goods provided by the system, since the food provision has decreased, mainly commercially important species such as the bivalve *S. plana*. The predicted rapid changes in climate over the next century may be such that the subsequent changes in habitat, species composition and diversity will alter ecosystems' structure and functioning as well as important ecosystem goods and services such as food production and recreational opportunities. Therefore, it is important to continuing monitoring these habitats to better understand their functioning and alert the authorities about the rapid and profound modifications that ecosystems are suffering which can compromise the biodiversity conservation.

ACKNOWLEDGEMENTS

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REFERENCES

Baeta A, Valiela I, Rossi F, Pinto R, Richard P, Niquil N, Marques JC (2009) Eutrophication and trophic structure in response to the presence of the eelgrass *Zostera noltii*. Marine Biology 156: 2107-2120.

Bordalo MD, Ferreira SMF, Cardoso PG, Leston S, Pardal MA (2011) Resilience of an isopod population (*Cyathura carinata*) to multiple stress factors in a temperate estuarine system. Hydrobiologia 671: 13-25.

Cardoso PG, Brandão A, Pardal MA, Raffaelli D, Marques JC (2005) The resilience of *Hydrobia ulvae* populations to anthropogenic and natural disturbances. Marine Ecology Progress Series 289: 191-199.

Cardoso PG, Raffaelli D, Pardal MA (2008a) The impact of extreme flooding events and anthropogenic stressors on the macrobenthic communities' dynamics. Estuarine, Coastal and Shelf Science 76: 553-565.

Cardoso PG, Raffaelli D, Pardal MA (2008b) The impact of extreme weather events on the seagrass *Zostera noltii* and related *Hydrobia ulvae* population. Marine Pollution Bulletin 56: 483-492.

Cardoso PG, Leston S, Grilo TF, Bordalo MD, Crespo D, Raffaelli D, Pardal MA (2010) Implications of nutrient decline in the seagrass ecosystem success. Marine Pollution Bulletin 60: 601-608.

Dolbeth M, Cardoso PG, Ferreira SM, Verdelhos T, Raffaelli D, Pardal MA (2007) Anthropogenic and natural disturbance effects on a macrobenthic estuarine community over a 10-year period. Marine Pollution Bulletin 54: 576-585.

García-Arberas L, Rallo A (2002) The intertidal soft-bottom infaunal macrobenthos in three Basque estuaries (Gulf of Biscay): a feeding guild approach. Hydrobiologia 475/476: 457-468.

Grilo TF, Cardoso PG, Dolbeth M, Pardal MA (2009) Long-term changes in amphipod population dynamics in a temperate estuary following ecosystem restoration. Hydrobiologia 630: 91-104.

Harley CDG, Hughes AR, Hultgren KM, Miner BG, Sorte CJB, Thornber CS, Rodriguez LF, Tomanek L, Williams SL (2006) The impacts of climate change in coastal marine system. Ecology Letters 9: 228-241.

Hastie BF, Smith SDA (2006) Benthic macrofaunal communities in intermittent estuaries during a drought: Comparisons with permanently open estuaries. Journal of Experimental Marine Biology and Ecology 330: 356-367.

Houghton J (2005) Global warming. Reports on Progress in Physics 68: 1343-1403.

Instituto Português do Mar e da Atmosfera (Portuguese Institute for Ocean and Atmosphere) http://www.ipma.pt/ (accessed January 2013).

IPCC (2007) Climate Change 2007: The Physical Science Basis. Contribution of Working Group I to the Fourth Assessment. Report of the Intergovernmental Panel on Climate Change [Solomon, S., D. Qin, M. Manning, Z. Chen, M. Marquis, K.B. Averyt, M. Tignor, H.L. Miller (Eds.)]. Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA, 996 pp.

Jones AR (1990) Zoobenthic variability associated with a flood and drought in the Hawkesbury Estuary, New South Wales: some consequences for environmental monitoring. Environmental Monitoring and Assessment 14: 185-195.

Krebs CJ (1999) Ecological Methodology, second ed. Addison-Wesley Educational Publishers, Inc., Menlo Park, CA.

MacKay F, Cyrus D, Russell K-L (2010) Macrobenthic invertebrate responses to prolonged drought in South Africa's largest estuarine lake complex. Estuarine, Coastal and Shelf Science 86: 553-567.

Miserendino ML (2009) Effects of flow regulation, basin characteristics and land-use on macroinvertebrate communities in a large arid Patagonian river. Biodiversity Conservation 18: 1921-1943.

Norkko A, Thrush SF, Hewitt JE, Cummings VJ, Norkko J, Ellis JI, Funnell GA, Schultz D, MacDonald I (2002) Smothering of estuarine sandflats by terrigenous clay: the role of wind-wave disturbance and bioturbation in site-dependent macrofaunal recovery. Marine Ecology Progress Series 234: 23-41.

Pearson TH, Rosenberg R (1978) Macrobenthic Succession in relation to Organic Enrichment and Pollution of the Marine Environment. Oceanography and Marine Biology – An Annual Review 16: 229-311.

Pillay D, Perissinotto R (2008) The benthic macrofauna of the St. Lucia Estuary during the 2005 drought year. Estuarine, Coastal and Shelf Science 77: 35-46.

Salen-Picard C, Arlhac D (2002) Long-term changes in a Mediterranean benthic community relationships between the polychaete assemblages and hydrological variations of the Rhône River. Estuaries 25: 1121-1130.

Salen-Picard C, Arlhac D, Alliot E (2003) Responses of a Mediterranean soft bottom community to short-term (1993-1996) hydrological changes in the Rhone river. Marine Environmental Research 55: 409-427.

Santos FD, Forbes K, Moita R (2002) Climate Change in Portugal. Scenarios, Impacts and Adaptation Measures e SIAM Project. Gradiva Publishers, Lisbon, Portugal, 456 pp.

Rosenberg R (1995) Benthic marine fauna structured by hydrodynamic processes and food availability. Netherlands Journal of Sea Research 34: 303-317.

Ter Braak CJF, Similaeur P (1998) Canoco Reference Manual and User's Guide to Canoco for Windows. Software for Canonical Community Ordination (version 4). Centre for Biometry Wageningen, Netherlands.

Wilson JG (1981) Temperature tolerance of circatidal bivalves in relations to their distribution. Journal of Thermal Biology 6: 279-286.

CHAPTER II

Implications of *Zostera noltii* recolonization on *Hydrobia ulvae* population structure success

Keywords

Eutrophication • Ecological Restoration • *Zostera noltii* Recolonization • *Hydrobia ulvae* • Habitat Recovery

ABSTRACT

Over 1990-1998 the Mondego estuary, Portugal, experienced profound modifications due to eutrophication, culminating in the disappearance and replacement of *Zostera noltii* by opportunistic macroalgae in the inner most disturbed areas. A decade after restoration measures implementation, *Z. noltii* started to gradually recolonize the inner parts, following 20 years of absence. This work explores the factors underlying successful *Z. noltii* recolonization and its subsequent implications on a mud snail *Hydrobia ulvae* population. During the macroalgal bloom, highest values in *H. ulvae* abundance, biomass and production were recorded, strongly declining afterwards. Three recovery attempts characterized the post-restoration phase, with considerably increases in *H. ulvae* abundance, biomass and production since *Z. noltii* reappearance. The seagrass provided long-term protection and abundant food resources for reproductive adults, contrarily to the ephemeral macroalgae. Through time, large size individuals increased, becoming the population more stable, structured and similar to the one inhabiting the "original" *Z. noltii* meadows.

INTRODUCTION

Seagrasses are unique marine flowering plants commonly found on tidal mudflats in estuaries and other nearshore coastal areas, playing a recognizable key ecological role in the marine environment. Seagrass meadows represent highly productive and diverse areas that support complex food webs as well as critical habitat functions, such as spawning and nursery areas for commercially important invertebrate and fish species (Duarte, 2002; Ganassin and Gibbs, 2008; Baeta et al., 2011).

Influenced by a wide range of physical and environmental factors, seagrasses are particularly susceptible to various natural and human-induced disturbances

(Schanz and Asmus, 2003; Ganassin and Gibbs, 2008; Dolch and Reise, 2010). Large-scale rapid declines of seagrass habitats have been broadly documented worldwide (Duarte, 2002; Borum et al., 2004; Orth et al., 2006a; Burkholder et al., 2007), being often attributed to a combination of serious threats. During the 1930s, for example, 'wasting disease' decimated the eelgrass *Z. marina* meadows along the Atlantic Coast of North America and Europe with over 90% loss. This epidemic was caused by infection with a marine slime mould-like protist, *Labyrinthula zosterae* (Muehlstein, 1989). Nowadays, other factors act as potential threats to conservation of seagrass meadows, such as overexploitation, physical modifications, nutrient and sediment pollution, introduction of nonnative species and global climate change (Waycott et al., 2009). In fact, both natural and anthropogenic disturbances imply not only loss of seagrass habitats, but have also impacts on abundance and diversity of associated macrofaunal communities (Whanpetch et al., 2010; Grilo et al., 2011). Usually, this leads to changes in estuarine food webs, shifts in species composition, reductions in density and diversity of estuarine fish and crustaceans as well as increases in turbidity and erosion (Orth et al., 2006a).

Several modifications in the local physical environment occurred when seagrass plants are lost, such as further loss of sediments, especially fine particles, and sediment resuspension (Baeta et al., 2011). This means that natural recovery of a seagrass bed from disturbance is often a slow process, which can take decades to centuries (Bryars and Neverauskas, 2004; Cardoso et al. 2005; Ganassin and Gibbs, 2007). Therefore efficient monitoring and management strategies are highly necessary to reverse loss of seagrass beds.

The restoration efforts developed to improve the overall environmental quality of the Mondego estuary (Portugal), after a long and severe period of eutrophication (1990-1998), enabled the recolonization of the seagrass *Z. noltii* in the inner most disturbed areas, where it was absent for almost 20 years (M.A. Pardal, personal observation). Facing the natural seagrass recovery it is crucial to understand the response of the associated macrobenthic communities to that habitat modification. The mud snail *Hydrobia ulvae* is the most abundant epibenthic faunal species of the Mondego estuary, being frequently found in the downstream *Z. noltii* meadows. At this habitat, it presents a stable and well-structured population, represented by individuals of all age classes (Cardoso et al., 2005). Hydrobiids feed on benthic material and on periphyton attached on seagrass leaves, constituting an important link in the estuarine food web (Cardoso et al., 2005; Casagranda et al., 2005). Therefore, the present study focus on the effects of *Z. noltii* recolonization on *H. ulvae* population dynamics and secondary production, by comparing two different time periods, the eutrophication period (1993-1998) and the recovery phase (1999-2010). The novelty of this work dwells on the response of the ecosystem, specifically the mud snail *H. ulvae*, which is an excellent ecological indicator, to the restoration process: would this system and associated flora and fauna recover, after 20 years of profound modifications, in an expected trajectory or not? Would the mud snail totally recover to the original condition, following the seagrass expansion?

MATERIALS AND METHODS

Study area

The Mondego estuary is a relatively small intertidal system of 8.6 km^2 located in a warm temperate region on the Atlantic coast of Portugal (40°08′N, 8°50′W). It is constituted by two arms (north and south) with distinct hydrological characteristics and separated by the Murraceira Island (Fig. 1). The northern arm, where the Figueira da Foz harbour is located, is deeper (4-8m during high tide, tidal range 1-3 m) and highly hydrodynamic. The southern arm is shallower (2-4m during high tide, tidal range 1-3 m) being characterized by large areas of exposed intertidal flats during low tide (Cardoso et al., 2005; Dolbeth et al., 2011).

Over the period 1990-1998, south arm’s ecological quality declined due to several human activities (harbour facilities, aquaculture farms, intensive agriculture with abusive fertilizers use in nearby rice fields, salt-work) that contributed to an ongoing eutrophication process, culminating in the disappearance and progressive replacement of the seagrass *Z. noltii* by opportunistic green macroalgae (*Ulva* sp.) in the inner areas. At present, the south arm is gradually recovering from the negative effects imposed by organic pollution, due to the implementation of a restoration program in 1998, which major aim was to reverse the historical eutrophication issues and to restore the original condition of the system. Application of physical restoration measures, coupled with public education, highlighting the ecological importance of intertidal vegetation conservation for the health and related socioeconomic activities of the estuary, resulted in: 1) significant nutrient loading reduction; 2) improvement of water dynamics and transparency, by re-establishment of the south arm riverhead connection; and 3) seagrass bed protection from human disturbance (Cardoso et al., 2005, 2008; Grilo et al., 2009; Dolbeth et al., 2011).

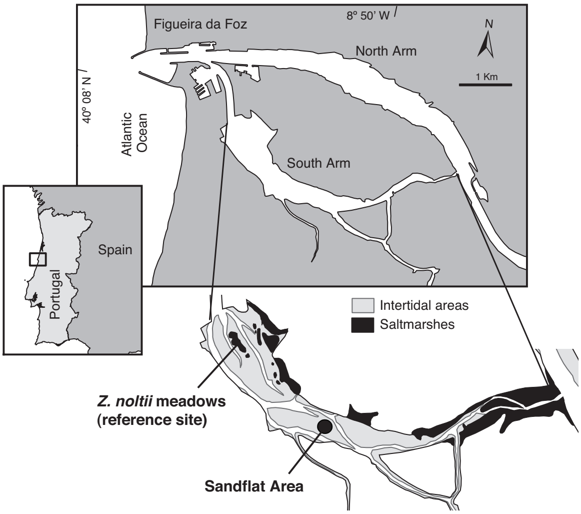

Fig. 1. Location of the Mondego estuary showing sampling station and the *Z. noltii* meadows (reference site).

A sandflat area (which represents 1/3 of the original condition of *Z. noltii* bed), in the inner part of the estuary, was selected as our study site (Fig. 1). In the mid 1980s, this area was covered by the seagrass *Z. noltii*, however following the eutrophication process, the seagrass disappeared and was restricted to a small patch located downstream (Fig. 1). Even the reference area (*Z. noltii* bed located approximately 1.5 km downstream of the sandflat area) (Fig. 1) was affected by the eutrophication though in a smaller scale, since the area occupied by the seagrass was also reduced and the associated fauna negatively affected (Cardoso et al. 2005, Grilo et al. 2011). The sandflat area is characterized by sandy sediments with lower organic matter content (mean 3.0% ± 1.14), lower water flows (0.8 – 1.2 $m.s^{-1}$), being often seasonally covered by green macroalgae *Ulva* sp. (Dolbeth et al., 2011). In the last years, as a result of the restoration measures, the macrophyte Z. *noltii* started to recolonize this area, after an absence of almost 20 years.

Sampling program and biological material processing

The *H. ulvae* population was monitored in a sandflat area during low tide from 1993 to 2010, with a 2-year sampling gap (between 1997 and 1998) for the implementation of restoration measures. In the first 18 months, samples were collected fortnightly and monthly thereafter. On each sampling episode, 6-10 sediment cores (141 cm^2 surface area) were taken in a haphazard manner to a depth of 20 cm, by using a PVC manual corer (Bordalo et al., 2011). Samples were washed in estuarine water through a 500 µm mesh sieve and the material retained (sediment, rooted macrophytes, algae and fauna) was preserved in 4% buffered formalin. Plant material was sorted and separated into green macroalgae (*Ulva* sp.) and Z. *noltii*. *H. ulvae* individuals were separated and transferred to 70% ethanol, being later counted and measured. Two different shell measurements were obtained: total shell length (TSL) and maximum width (MW), being the last one the most appropriate measure, since a great number of snails presented damaged shells. Based on previous works, a conversion equation was used ($MW = 0.4369\ TSL + 0.2091$, $n = 339$, $r = 0.97$; Lillebø et al., 1999). Length–weight relationships were determined for production estimates. Preliminary ANOVA of length x AFDW relationships indicated no significant seasonal differences, and an overall regression equation was used ($AFDW = 0.0564TSL^{2.2381}$, $n = 191$, $r = 0.98$; Lillebø et al., 1999). For both fauna and plant material the ash-free dry weight (AFDW) was assessed, after combustion for 8 h at 450ºC.

The macrobenthic communities were monitored during two different periods: from January 1993 to September 1995 and March 1999 to November 2008, with a sampling gap between these periods in order to facilitate recovery of the seagrass bed. In the first 18 months samples were collected fortnightly and monthly thereafter. At each sampling occasion, 6-10 sediment cores (141 cm^2 surface area) were randomly taken to a depth of 20 cm, by using a manual corer. Samples were washed in estuarine water through a 500 µm mesh sieve and the material retained (sediment, rooted macrophytes, algae and fauna) preserved in 4% buffered formalin. Temperature, oxygen, pH and salinity were measured *in situ* in the intertidal pools and sediment samples were collected to quantify the organic matter content. In the laboratory, plant material was sorted and separated into green macroalgae and *Zostera noltii*. Animals were separated and transferred to 70% ethanol, identified to the lowest possible taxon and counted. For fauna, plant material and sediment the ash-free dry weight (AFDW) was assessed, after combustion for 8 h at 450ºC.

Statistical analysis

Relationships between *H. ulvae* density/biomass and plants biomass were evaluated through linear regression analysis for the period 1993-1995 for green macroalgal biomass and from 2008 to 2010 for *Z. noltii* biomass. The software used for the statistical analysis was SigmaPlot 11.0. Growth rates of the mud snail

H. ulvae were estimated by tracking recognizable cohorts in size–frequency distributions over successive sample dates using FAO-ICLARM Stock Assessment Tool package (FISAT II software) (Gayanilo and Pauly, 1997).

Secondary production

Secondary production represents a direct measure of food provision delivered by an ecosystem, with a clear socio-economic value. Furthermore, production is also a measure of ecosystem function and may provide a better perception of ecosystem shift in a restoration scenario than static parameters, such as diversity, density or biomass (Dolbeth et al., 2011). Secondary production (P) was estimated based on cohort recognition, as described in Dauvin (1986). Total values of P for *H. ulvae* population were expressed as:

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

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

$$\bar{B} = \left(\frac{1}{T}\right) \sum_{n=1}^{N} (\bar{B}_{n^{t}})$$

where T is the period of study (annual cycles); N is the number of successive cohorts in the period T (year); $\bar{B}_n$ is the mean biomass of cohort n; and t is the duration of the cohort n (in our case, 16 to 20 months, according to Cardoso et al., 2005).

RESULTS

Seagrass and macroalgal biomass

The occurrence of a remarkable spring macroalgal bloom in 1993 resulted in a maximum green macroalgal biomass peak (342 g.AFDW.m^{-2}), which rapidly declined afterwards (Fig. 2). A new increase in green macroalgal biomass was detected in 1995, but never comparable to the values previously registered. After implementation of restoration measures, green macroalgal biomass was substantially reduced and no more macroalgal bloom events were recorded. Surprisingly, after almost 20-year absence period, the seagrass *Z. noltii* started to recolonize the bare sediments of the sandflat area in 2008, forming small and few patches irregularly distributed. Since then, the biomass of the seagrass *Z. noltii* never stopped increasing (Fig. 2), as well as the area covered by the macrophyte that became larger and more compact at the end of 2010, covering actually about 50-60% of the total sandflat area (personal observation). Despite this tendency, the total biomass of *Z. noltii* still only represents a small percentage (≈ 25%) of the mean biomass for the reference area (see Cardoso et al., 2010).

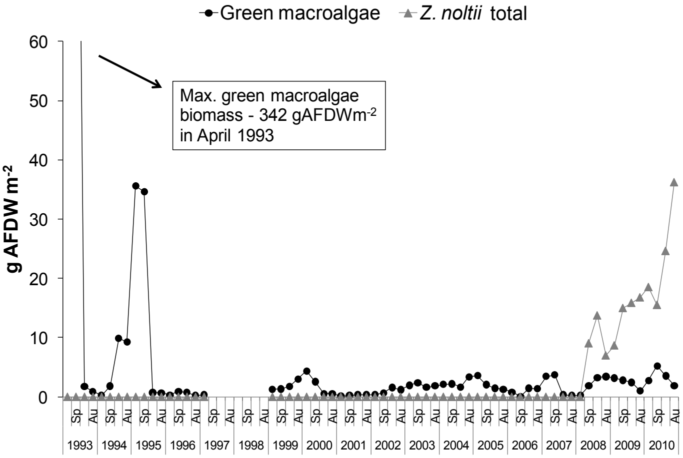

Fig. 2. Seasonal variation of seagrass *Z. noltii* and macroalgal biomass from 1993 to 2010.

H. ulvae population density and biomass

In general, both *H. ulvae* density and biomass decreased until 1997. Afterwards several recovery attempts should be highlighted looking to the results (Fig. 3). Analysing both periods separately, it is evident a sharply decline in density and biomass during the pre-restoration period, probably as a result of the macroalgal dynamics observed at this area. Highest values were achieved during the spring macroalgal bloom of 1993, being promptly reduced in the next years. In fact, a significant relation was obtained between *H. ulvae* and green macroalgal biomass ($R^2 = 0.266$, p-value = 0.003) at the sandflat area, being expressed as:

ln (H. ulvae biomass + 1) = 0.868 + 0.246 ln (green macroalgal biomass + 1)

After restoration measures, both density and biomass started to increase, which may represent the first recovery attempt of *H. ulvae* population. However, a sequence of several atypical extreme climate events, namely the centenary floods in 2000/01 and the longest heat wave ever recorded since 1941, in 2003, caused a clear depletion in both density and biomass (Cardoso et al., 2008). After the harmful impact of such extreme weather episodes, *H. ulvae* biomass and especially density evidenced a second recovery attempt between 2004 and 2005, which was suddenly interrupted by a physical anthropogenic intervention initiated in 2006, which aimed to amplify the artificial re-establishment of the upstream connection between the two arms of the Mondego estuary (Neto et al., 2010). Immediately after human intervention's end, *H. ulvae* population displayed a noteworthy evolution, which may constitute the third recovery attempt, showing a huge increase in density during 2008/09, reaching at this time the highest value (31,527 ind.m^{-2}) of the entire post-restoration period. Until the autumn of 2009, biomass varied similarly to the pattern described for density, but since then, density declined to values slightly lower than those observed in

2004/05 – perhaps motivated by the rainy winter (IPMA – Portuguese Institute for Ocean and Atmosphere, http://www.ipma.pt/) that have contributed to the elimination of great part of *H. ulvae* young individuals, being dragged out of the estuary. On the other hand, biomass continued sustainably increasing, culminating in a maximum record in November 2010 (11.09 g.AFDW.m^{-2}), just comparable to values observed in the beginning of the pre-restoration phase (Fig. 3).

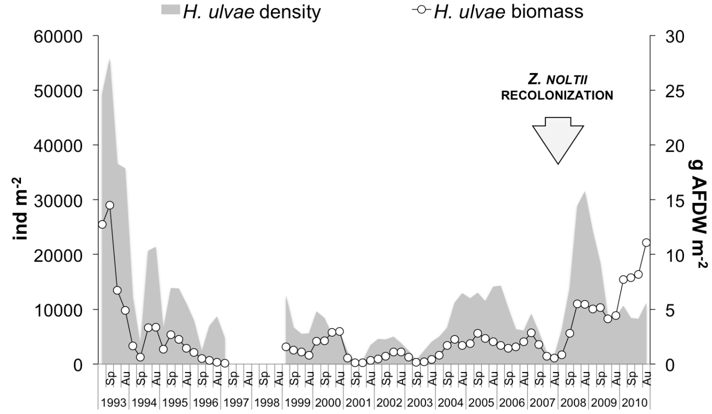

Fig. 3. Seasonal variation of total density and biomass of a *H. ulvae* population from 1993 to 2010.

Despite the great increment of larger individuals, the actual *H. ulvae* biomass is still far from the one observed at the reference site (*Z. noltii* meadows), corresponding to nearly 35% (see Cardoso et al., 2008). Considering the period in which *Z. noltii* started to recolonize the sandflat area, a positive linear relation was established between *H. ulvae* and *Z. noltii* biomasses ($R^2 = 0.368$, p-value < 0.001), being expressed as:

$$\ln (H.\ ulvae\ biomass + 1) = 0.968 + 0.308 \ln (Z.\ noltii\ biomass + 1)$$

Population Structure

Taking into account the long-term data series and considering that the mud snail *H. ulvae* presents four recruitments per year at the Mondego estuary (Cardoso et al., 2002, 2005), it was difficult to graphically represent the 68 recognizable cohorts, thus it was decided to represent only the maximum width (MW) achieved by *H. ulvae* individuals of each cohort, which aimed to reflect differences in *H. ulvae* population structure over the 18-year period (Fig. 4A).

Distinct scenarios in *H. ulvae* population structure were observed at the sandflat area (Fig. 4A,B). Until 1999, the population exhibited great instability, being difficult to follow any cohort from birth to extinction. During the short period regarding the massive macroalgal bloom of 1993, juveniles (< 1.0 mm MW) and young individuals (1.0-1.5 mm MW) were clearly dominant, with adults (> 1.5 mm MW) representing a small fraction (~5%) (Fig. 4B). This trend was also observed during the remaining years of the pre-restoration period, becoming juveniles even more dominant, constituting approximately 2/3 of total population (Fig. 4B). The maximum size reached by *H. ulvae* individuals progressively declined from 1993 to 1996 and no individuals larger than 2 mm (MW) were found during the pre-restoration period (Fig. 4A). Generally, dominance of juveniles persisted in the post-restoration period, although it was less evident comparatively to the previous period (Fig. 4B). From 1999 to 2001, *H. ulvae* individuals showed a great width increment, exceeding 2 mm (MW), and adults' proportion gradually increased, which is coincident to the first recovery attempt described for total density and biomass (Fig 4A,B). Interestingly, the population structure observed in 1999 was significantly different (χ^2 test, $p < 0.05$) from the one in 1993, presenting a higher juveniles' percentage. During the period between 2003 and 2006, the maximum width achieved by *H. ulvae* individuals stabilized around 1.5 mm (Fig. 4A) and percentage of juveniles was nearly 60% (Fig. 4B). It was only by the end of 2006 that larger snails reappeared in the size–frequency distributions, being observed significant statistically differences in the population structure between 1999 and 2006 (χ^2 test, $p < 0.05$). In 2007, young individuals and adults' percentages increased considerably, totalizing almost 60% of total population, contrarily to juveniles' proportion that gradually declined since 2005, losing more than 25% during this 2-year period (Fig. 4B). Three years following *Z. noltii* recolonization, *H. ulvae* population became more stable and structured, being majorly formed by larger individuals. Juveniles represented only 1/3 of total population, which constituted the lowest percentage of the entire study period (Fig. 4B). At this time, population significantly differed from that one in 2006 (χ^2 test, $p < 0.05$) and individuals reached a maximum width of approximately 2.5 mm, which is closer to the values reported for the reference *Z. noltii* bed (see Cardoso et al., 2005) (Fig. 4A).

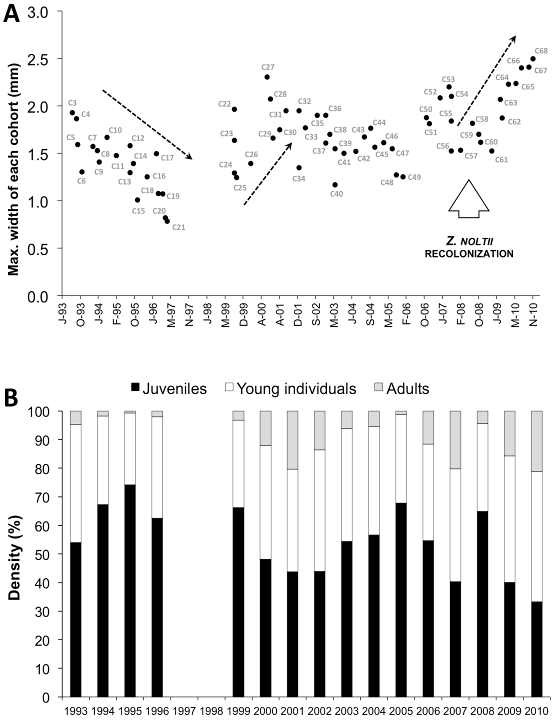

Fig. 4. (A) Estimations of the maximum width reached by each cohort of *H. ulvae* over the period 1993-2010. Each black circle represents a new different cohort (Cx). (B) *H. ulvae* population structure from 1993 to 2010. Values are percentages of total individuals.

Secondary production

Numerous changes were observed regarding *H. ulvae* production and annual mean biomass, comparing the two distinct time periods (Table 1). In general, both parameters strongly declined in the first period, reversing this trend with implementation of restoration measures. They present a slow but continued increasing pattern, despite some oscillations attributed to consecutive climate extremes and some human disturbances. Consistently to the spring macroalgal bloom, when food resources were highly available, both production and mean population biomass reached a maximum in 1993 (45.73 / 9.37 g.AFDW.m^{-2}.year^{-1} respectively), which markedly collapsed in the following years. As described for *H. ulvae* abundance and biomass, three distinct recovery attempts were recognizable in the second studied period. Between 1999 and 2000, production and mean biomass almost triplicated, decreasing afterwards to minimum values in 2001 and 2003, probably as a result of the floods and the heat wave's impact, respectively. Since then, both parameters started a new increasing cycle, ending in 2007, presumably due to the anthropogenic intervention made in the estuary. Simultaneously to the *Z. noltii* recolonization, secondary production reached values never seen since the last decade, achieving in 2009 the highest record (16.94 g.AFDW.m^{-2}.year^{-1}) of the entire post-restoration period. Similarly to production, the mean population biomass also registered a maximum peak in 2009, corresponding to half of the mean biomass observed in 1993, when the massive algal bloom occurred. $P/\bar{B}$ ratios were highest in the first two years of the study period, due to the dominance of young individuals with higher growth rates, while during the remaining years the turnover ranged between 2 and 4.

Table 1. Secondary production of *H. ulvae* population at the sandflat area between 1993- 2010.

Year	P (g AFDW m^{-2} y^{-1})	$\bar{B}$ (g AFDW m^{-2})	$P/\bar{B}$	Events	
1993	45.73	9.37	4.87	**EUTROPHICATION**	Macroalgal bloom
1994	10.86	2.38	4.56		
1995	8.88	2.76	3.22		
1996	2.81	0.78	3.59		
1997	-	-	-	**RECOVERY**	Restoration measures
1998	-	-	-		
1999	3.21	1.09	2.94	1st Recovery attempt	
2000	8.19	2.51	3.27		
2001	1.30	0.36	3.66		Centenary floods
2002	4.46	1.32	3.39		
2003	1.43	0.44	3.26		Longest heat wave
2004	6.12	1.54	3.97	2nd Recovery attempt	
2005	8.45	3.49	2.42		Drought + Heat wave
2006	6.17	2.21	2.79		
2007	3.79	1.85	2.04		
2008	9.89	2.73	3.62	3rd Recovery attempt	*Z. noltii* recolonization
2009	16.94	4.54	3.73		
2010	9.90	3.00	3.30		

DISCUSSION

In the Mondego estuary, the introduction of restoration measures contributed to the improvement of water quality, determining a considerable decline in the opportunistic macroalgal biomass. Moreover no massive algal bloom events occurred since then, which may have facilitated recolonization by seagrass years later. Besides nutrient loading reduction and macroalgal biomass depletion, other conditions had to be suitable for seagrass return, including a nearby source of seeds (Erftemeijer et al., 2008; Vaudrey et al., 2010). As documented by Bryars and Neverauskas (2004) the reappearance of seagrasses must be a result of recolonization via seeds and not by regeneration from existing original rhizomes, which maybe were no longer viable. In the case of the Mondego estuary, it was apparently the downstream seagrass beds located 1.5 km far from the sandflat area that provided seeds for the reestablishment and proliferation of *Z. noltii*. As documented in other coastal systems (Philippart et al., 2007; Vaudrey et al., 2010), it seems that such overall environmental quality improvement, effectively allowed the seagrass *Z. noltii* to restore its former distribution, after almost 20 years of absence.

Zostera species are able to increase water transparency in their local environment by ensuring particle settlement, reducing re-suspension and taking up nutrients (Moore and Short, 2006; Baeta et al., 2011). Once plants are lost from a system, water quality requirements for recovery can actually be greater than those needed for maintenance of existing populations (Kemp et al., 2004; Moore and Short, 2006). Likewise, propagule supply of both vegetative material and seeds in established beds may provide a higher recovery capacity from episodic pressures than areas with no established plants, which have to rely on seeds or propagules from other regions (Orth et al., 2006b). These differences could explain why the macrophyte *Z. noltii* took so much time to recolonize the inner areas of the Mondego estuary, comparing to the downstream locals where the seagrass recovery after nutrient enrichment reversal was faster (Cardoso et al., 2008, 2010; Grilo et al., 2009; Dolbeth et al., 2011).

Over time, there were clear differences in the dynamics and structure of the *H. ulvae* population before and after the introduction of restoration measures. In the spring of 1993, during the macroalgal bloom, the population density, biomass and production reached the highest values of the entire study period, based on the great food availability and shelter provided by the green macroalgae. In fact, hydrobiid snails are known to be highly mobile, actively migrating in the water column to exploit alternative habitats and new fresh patches of food (Norkko et al., 2000; Bolam et al., 2004; Van Colen et al., 2008). This suggests that abundance of *H. ulvae* at the sandflat area was associated to the presence of green macroalgae, clearly demonstrating the opportunistic feeding behaviour of the species (Cardoso et al., 2002; Bolam et al., 2004). At this time, population was majorly formed by juveniles (< 1 mm MW) and young individuals (1.0–1.5 mm MW) due to the combined effect of stronger benthic recruitments occurred at the sandflat area and dispersion of juveniles proceeding from the *Z. noltii* meadows placed downstream (Cardoso et al., 2005). This is in agreement with Van Colen et al. (2008), confirming that colonization of large-scale disturbed habitats is initiated and dominated by post-larval and juvenile recruitment. The absence of larger individuals (> 1.5 mm MW) during most of the study period could be related to predation pressure by shorebirds and/or fishes (Cardoso et al., 2002, 2005 and references therein), since *Hydrobia* individuals were particularly exposed on bare unvegetated sediments. According to Sfriso et al. (2001), the presence of high *Ulva* biomass creates very unstable and unsuitable conditions leading to subsequent anoxic crises that strongly reduce biodiversity and biomass of macrofauna communities. Similar results were observed at the sandflat area after the macroalgal bloom influence, when *H. ulvae* abundance, biomass and production abruptly declined, keeping low until the end of the pre-restoration period.

The benefits of seagrass habitats for ecosystems' health and functioning were broadly documented. For instance, Sheridan (2004) reported that, once seagrasses start to cover dredged sediments, increases in densities of the associated mobile macrofauna would be expected. Moreover, accordingly to Asmus and Asmus (2000) the presence of intertidal seagrasses potentially increases food availability for endo and epibenthic organisms by acting as a sink for organic matter. Additionally, Casagranda et al. (2005) reinforced that macrophyte cover represents the most important factor governing the temporal and spatial patterns of *Hydrobia* density, being observed much higher abundances on vegetated tidal flats than on bare substrata. These evidences support the results obtained for the Mondego estuary, in which the recolonization of seagrass vegetation initiated in

2008 had positive implications on the dynamics, structure and production of the *H. ulvae* population. Abundance, biomass and production started to increase with *Z. noltii* reestablishment, but since 2009, while biomass continued increasing, both density and production decreased, perhaps motivated by the unusual rainy conditions that characterized the winter of 2009/10, classified as the 11º most rainy since 1931/32. (IPMA – Portuguese Institute for Ocean and Atmosphere, http://www.ipma.pt/). Furthermore, distinct changes in population structure were also found simultaneously to seagrass recolonization, becoming young individuals and adults' dominant in detriment of juveniles. This means that the population is getting more structured, thus more similar to the one existing at the reference *Z. noltii* meadows (Cardoso et al., 2005, 2008), being clear the increment of large size individuals. Results obtained by Sheridan (2004) reinforced that increasing seagrass cover in unvegetated substrata began to provide structure and shelter for surrounding macrofaunal communities. This confirms observations in the Mondego estuary in which *H. ulvae* success seems to depend on seagrass vegetation area to form a stable and well-structured population, since reproductive adults were almost exclusively found when *Z. noltii* started to recolonize the sandflat area. Therefore, seagrass recolonization helped *H. ulvae* population recovery by providing suitable protection against predators, abundant food resources, through algal epiphytes attached on *Zostera* leaves (Philippart, 1995; Schanz et al., 2002), as well as acting as a buffer, reducing water current velocity and sediment erosion. Despite the remarkable *H. ulvae* population recovery in the last three years of study, this is a very slow process that can take some years to decades to reach the original condition of the system. These results are in agreement with Sheridan (2004) for the Upper Laguna Madre (Texas, USA), where mollusc densities in seagrasses that had colonized dredging sediments required at least 10 years to become similar to communities in adjacent natural seagrass beds. In the future, face to the present results and depending on the scale of possible natural and anthropogenic disturbances in such intertidal Mondego's habitat, it is expected that the gradual increase in both *Z. noltii* biomass and areal extent may accelerate *H. ulvae* population recovery.

In conclusion, this work highlights the relevance of seagrass vegetation to the recovery success of *H. ulvae* population and also as an indicator of estuarine health, such as in preserving biodiversity and ecosystem functions.

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REFERENCES

Asmus H, Asmus R (2000) Material exchange and food web of seagrass beds in the Sylt-Rømø Bight: how significant are community changes at the ecosystem level? Helgoland Marine Research 54: 137-150.

Baeta A, Niquil N, Marques JC, Patrício J (2011) Modelling the effects of eutrophication, mitigation measures and an extreme flood event on estuarine food webs. Ecological Modelling 222: 1209-1221.

Bolam SG, Whomersley P, Schratzberger M (2004) Macrofaunal recolonization on intertidal mudflats: effect of sediment organic and sand content. Journal of Experimental Marine Biology and Ecology 306: 157-180.

Bordalo MD, Ferreira SMF, Cardoso PG, Leston S, Pardal MA (2011) Resilience of an isopod population (*Cyathura carinata*) to multiple stress factors in a temperate estuarine system. Hydrobiologia 671: 13-25.

Borum J, Duarte CM, Krause-Jensen D, Greve TM (2004) European Seagrasses: An Introduction to Monitoring and Management. Copenhagen: The M&MS Project, 95 pp.

Bryars S, Neverauskas V (2004) Natural recolonisation of seagrasses at a disused sewage sludge outfall. Aquatic Botany 80: 283-289.

Burkholder JM, Tomasko DA, Touchette BW (2007) Seagrasses and eutrophication. Journal of Experimental Marine Biology and Ecology 350: 46-72.

Cardoso PG, Lillebø AI, Pardal MA, Ferreira SM, Marques JC (2002) The effect of different primary producers on *Hydrobia ulvae* population dynamics: a case study in a temperate intertidal estuary. Journal of Experimental Marine Biology and Ecology 277: 173-195.

Cardoso PG, Brandão A, Pardal MA, Raffaelli D, Marques JC (2005) Resilience of *Hydrobia ulvae* populations to anthropogenic and natural disturbances. Marine Ecology Progress Series 289: 191-199.

Cardoso PG, Raffaelli D, Pardal MA (2008) The impact of extreme weather events on the seagrass *Zostera noltii* and related *Hydrobia ulvae* population. Marine Pollution Bulletin 56: 483-492.

Cardoso PG, Leston S, Grilo TF, Bordalo MD, Crespo D, Raffaelli D, Pardal MA (2010) Implications of nutrient decline in the seagrass ecosystem success. Marine Pollution Bulletin 60: 601-608.

Casagranda C, Boudouresque CF, Francour P (2005) Abundance, Population Structure and Production of *Hydrobia ventrosa* (Gastropoda: Prosobranchia) in a Mediterranean brackish lagoon, Lake Ichkeul, Tunisia. Archiv für Hydrobiologie 164: 411-428.

Dauvin JC (1986) Dynamique de la population d'*Abra prismatica* (Mollusque, Bivalve) de la baie de Morlaix (Manche Occidentale). Annales de l'Institut Oceanographique 62: 1-12.

Dolbeth M, Cardoso PG, Grilo TF, Bordalo MD, Raffaelli D, Pardal MA (2011) Long-term changes in the production by estuarine macrobenthos affected by multiple stressors. Estuarine, Coastal and Shelf Science 92: 10-18.

Dolch T., Reise K. (2010) Long-term displacement of intertidal seagrass and mussel beds by expanding large sandy bedforms in the northern Wadden Sea. Journal of Sea Research 63: 93-101.

Duarte CM (2002) The future of seagrass meadows. Environment Conservation 29: 192-206.

Erftemeijer PLA, Van Beek JKL, Ochieng CA, Jager Z, Los HJ (2008) Eelgrass seed dispersal via floating generative shoots in the Dutch Wadden Sea: a model approach. Marine Ecology Progress Series 358: 115-124.

Ganassin C, Gibbs PJ (2008) A review of seagrass planting as a means of habitat compensation following loss of seagrass meadow. NSW Department of Primary Industries – Fisheries Final Report Series No. 96. 41pp. ISSN 1449-9967.

Gayanilo FCJr., Pauly D (1997) The FAO-ICLARM Stock Assessment Tools (FISAT) Reference Manual. FAO Computerized Information Series (Fisheries). No. 8. Rome, FAO, 262 pp.

Grilo TF, Cardoso PG, Dolbeth M, Pardal MA (2009) Long-term changes in amphipod population dynamics in a temperate estuary following ecosystem restoration. Hydrobiologia 630: 91-104.

Grilo TF, Cardoso PG, Dolbeth M, Bordalo MD, Pardal MA (2011) Effects of extreme climate events on the macrobenthic communities' structure and functioning of a temperate estuary. Marine Pollution Bulletin 62: 303-311.

Kemp WM, Batiuk R, Bartleson R, Moore K, Bergstrom P, Carter V, Gallegos C, Humnley W, Karrh L, Koch EW, Landwehr J, Moore K, Murray L, Naylor M, Rybicki N, Stevenson JC, Wilcox D (2004) Habitat requirements for submerged aquatic vegetation in Chesapeake Bay: Water quality, light regime, and physical-chemical factors. Estuaries 27: 363-377.

Lillebø AI, Pardal MA, Marques JC (1999) Population structure, dynamics and production of *Hydrobia ulvae* (Pennant) (Mollusca: Prosobranchia) along an eutrophication gradient in the Mondego estuary (Portugal). Acta Oecologica 20: 289-304.

Moore KA, Short FT (2006) *Zostera*: biology, ecology and management. In: T. Larkum, R. Orth and C. Duarte (eds.), Seagrasses: Biology, Ecology and Conservation. Springer 361-386.

Muehlstein LK (1989) Perspectives on the wasting disease of eelgrass *Zostera marina*. Diseases Of Aquatic Organisms 7: 211-221.

Neto JM, Teixeira H, Patrício J, Baeta A, Veríssimo H, Pinto R, Marques JC (2010) The response of estuarine macrobenthic communities to natural-and human-induced changes: dynamics and ecological quality. Estuaries and Coasts 33: 1327-1339.

Norkko J, Bonsdorff E, Norkko A (2000) Drifting algal mats as an alternative habitat for benthic invertebrates: species specific responses to a transient resource. Journal of Experimental Marine Biology and Ecology 248: 79- 104.

Orth RJ, Carruthers TJB, Dennison WC, Duarte CM, Fourqurean JW, Heck Jr. KL, Hughes AR, Kendrick GA, Kenworthy WJ, Olyarnik S, Short FT, Waycott M, Williams SL (2006a) A global crisis for seagrass ecosystems. BioScience 56: 987-996.

Orth RJ, Harwell MC, Inglis JG (2006b) Ecology of Seagrass Seeds and Seagrass Dispersal Processes. In: T. Larkum, R. Orth and C. Duarte (eds.), Seagrasses: Biology, Ecology and Conservation. Springer 111-133.

Philippart CJM (1995) Effect of periphyton grazing by *Hydrobia ulvae* on the growth of *Zostera noltii* on a tidal flat in a Dutch Wadden Sea. Marine Biology 122: 431-437.

Philippart CMJ, Beukema JJ, Cadée GC, Dekker R, Goedhart PW, van Iperen, JM, Leopold MF, Herman PMJ (2007) Impacts of nutrient reduction on coastal communities. Ecosystems 10: 95-118.

Schanz A, Polte P, Asmus H (2002) Cascading effects of hydrodynamics on an epiphyte- grazer system in intertidal seagrass beds of the Wadden Sea. Marine Biology 141: 287-297.

Schanz A, Asmus H (2003) Impact of hydrodynamics on development and morphology of intertidal seagrasses in the Wadden Sea. Marine Ecology Progress Series 261: 123-134.

Sfriso A, Birkemeyer T, Ghetti PF (2001) Benthic macrofauna changes in areas of Venice lagoon populated by seagrasses or seaweeds. Marine Environmental Research 52: 323-349.

Sheridan P (2004) Recovery of floral and faunal communities after placement of dredged material on seagrasses in Laguna Madre, Texas. Estuarine Coastal and Shelf Science 59: 441-458.

Van Colen C, Montserrat F, Vincx M, Herman PMJ, Ysebaert T, Degraer S (2008) Macrobenthic recovery from hypoxia in an estuarine tidal mudflat. Marine Ecology Progress Series 372: 31-42.

Vaudrey JMP, Kremer JN, Branco BF, Short FT (2010) Eelgrass recovery after nutrient enrichment reversal. Aquatic Botany 93: 237-243.

Waycott M, Duarte CM, Carruthers TJB, Orth RJ, Dennison WC, Olyarnik S, Calladine A, Fourqurean JW, Heck Jr. KL, Hughes AR, Kendrick GA, Kenworthy WJ, Short FT, Williams SL (2009) Accelerating loss of seagrasses across the globe threatens coastal ecosystems. Proceedings of the National Academy of Sciences of the United States of America 106, 12377–12381.

Whanpetch N, Nakaoka M, Mukai H, Suzuki T, Nojima S, Kawai T, Aryuthaka C (2010) Temporal changes in benthic communities of seagrass beds impacted by a tsunami in the Andaman Sea, Thailand. Estuarine, Coastal and Shelf Science 87: 246-252.

CHAPTER III

Organochlorine accumulation on a highly consumed bivalve (*Scrobicularia plana*) and its main implications for human health

Keywords

PCBs • HCB • *Scrobicularia plana* • Bioaccumulation
• Trophic Transfer • Human Health

ABSTRACT

Contamination by polychlorinated biphenyls (PCBs) and hexachlorobenzene (HCB) was investigated along a spatial gradient in water, sediments and in commercially important bivalve species *Scrobicularia plana*, from Ria de Aveiro (Portugal). Organochlorines dissolved in water were below detection limit and concerning suspended particulate matter, only PCBs were quantified, ranging from 3.8 to 5.8 ng.g^{-1} dw (Σ_{13}PCB). There was a distinct spatial gradient regarding PCBs accumulation in sediments. The highest concentrations were found in deeper layers and closest to the pollution source, decreasing gradually along a 3 km area. Contamination in sediments exceeded the Canadian and Norwegian sediment quality guidelines, inducing potential toxic effects in related biota. PCBs tended to bioaccumulate throughout *S. plana* lifespan but with different annual rates along the spatial gradient. The maximum values were found in older individuals up to 3+ years old, reaching 19.4 ng.g^{-1} dw. HCB concentrations were residual and no bioaccumulation pattern was evident. Congeners 138, 153 and 180 were the most accumulated due to their abundance and long-term persistence in the environment. In the inner area of the Laranjo bay (0.6 km^2), the species was able to remove up to 0.4 g of PCBs annually from sediments into their own tissues, which is consequently free for trophic transfer (biomagnification). Concerning human health, and despite the high concentrations found in sediments, PCB levels in bivalves do not exceed the limit established by the European Union for fishery products and are largely below tolerable daily intake. Although PCBs in *Scrobicularia plana* are present at low levels, their impact to human health after consumption over many years might be harmful and should be monitored in future studies.

INTRODUCTION

Environmental contamination by persistent organic pollutants (POPs) has received increasing concern worldwide, reflecting the impact of anthropogenic activities over time. Organochlorine compounds such as polychlorinated biphenyls (PCBs) and hexachlorobenzene (HCB) are defined as POPs by the Stockholm Convention, which acts as a global treaty to protect human health and the environment from these chemicals (UNEP, 2001).

Ranking among the top 5 priority hazardous substances along with arsenic, lead, mercury and vinyl chloride (ATSDR, 2007), PCBs were manufactured commercially in Europe from 1929 until the mid-1980s (OSPAR, 2010) and were primarily used in industry due to their insulating and flame retardant properties. According to the Water Framework Directive (2000/60/EC), HCB is also identified as a priority hazardous substance and has been synthesized and used from the 1940s to the late 1970s as a fungicide for a variety of crops, being also applied in several manufacturing processes such as aluminum and graphite rods. Although PCBs are no longer produced, hundreds of thousands of tons are still in use in electrical transformers and other equipment (Zhou et al., 2001; Otchere, 2005), giving the governments until 2025 to phase out these uses and arrange alternative PCB-free replacements (UNEP, 2001). Likewise, HCB production has now virtually ceased in Europe but it may still be released into the environment, since it is currently formed as an inadvertent by-product at trace levels in the production of chemical solvents, several pesticides and by combustion of chlorinated waste material.

Due to their toxicity, potential to bioaccumulate on fatty tissues and biomagnification through the food chain, exposure to these pollutants is a topic of huge concern (Otchere, 2005; Tomza et al., 2006). In fact, POPs have been directly related with deleterious health problems, including endocrine disruption, reproductive disorders, cardiovascular diseases, carcinogenicity and neurotoxicity (Amodio et al., 2012 and references therein).

Ria de Aveiro, a lagoon adjacent to the Atlantic Portuguese coast, comprises in its vicinity a large industrial complex besides extensive agriculture fields, in which great amounts of fertilizers and pesticides are applied. Considering the use of PCBs and HCB in industrial electrical equipment, pesticide extenders and fungicides respectively, it is crucial to determine if there exists a contamination by organochlorines in the inner part of the lagoon, the Laranjo bay. Thus, a survey was conducted in order to investigate their occurrence along a spatial contamination gradient in water, muddy sediments and biota, specifically in the bivalve *Scrobicularia plana* from three selected locations of Ria de Aveiro.

As sedentary filter-feeders, bivalves are known to be effective bioaccumulators of chemical substances (Coelho et al., 2006; Lobo et al., 2010; Cardoso et al., 2012, 2013), providing a time integrated indication of environmental contamination as well as reliable information on the potential impact of seafood consumption on public health (Fang, 2004; Otchere, 2005).

The worldwide-distributed benthic bivalve *S. plana* is a deposit feeding species of high commercial interest and key importance in the structure and functioning of estuarine ecosystems. It is a crucial prey item to higher trophic levels (crabs, fishes and birds), playing an important role in biogeochemical cycles of both nutrients and contaminants, as a consequence of its bioturbation activity. This species presents a lifespan of 5 years, growth rates of 1 cm.yr^{-1} (Verdelhos et al., 2005) and has been extensively used as a relevant model organism for biomonitoring programs and toxicity tests (Coelho et al., 2006; Bonnard et al., 2009; Cardoso et al., 2012). Despite being a well-studied edible species, there is no information regarding PCB and HCB accumulation throughout its life cycle. Therefore, the major aims of this paper were: (1) to study PCB and HCB accumulation in water, muddy sediments and in the bivalve *S. plana*; (2) to assess the annual PCBs bioaccumulation pattern during *S. plana* lifespan; (3) to evaluate the potential PCB transfer from contaminated sediments to economically important species; and (4) to infer about the risks for human health intrinsic to the consumption of bivalves from locations with different concentrations of PCBs and HCB.

MATERIALS AND METHODS

Study area and sample collection

The sampling campaigns were conducted in Ria de Aveiro (40º38′N, 8º45′W). This coastal lagoon is located in the northwestern part of Portugal (Fig. 1) and is permanently connected to the sea, receiving inputs from agriculture, urban and industrial activities. Ria de Aveiro supports a population of 250,000 inhabitants in the watershed area, and its main municipality (Aveiro town) is located 15 km south from an industrial complex, in which chlorine and aromatic compounds containing benzene are produced. From the 1950s until the present, Ria de Aveiro continuously receives effluents coming from this industrial complex, inducing an environmental contamination gradient inside the lagoon and pronouncedly in the sediments of the Laranjo bay (Coelho et al., 2006; Pereira et al., 2009). Three sampling locations were selected along a spatial contamination gradient in the Laranjo bay, regarding the distance to the point where industrial effluents were discharged – Station 1, Station 2 and Station 3 (Fig. 1).

Water, sediments and biological samples were collected in the spring of 2011, on the mudflats during low tide conditions. On each study area, water samples were collected from intertidal pools by using pre-washed glass bottles, free of organochlorine residues, and maintained on ice during transportation to the laboratory. A composite sediment sample was obtained from the upper 5 cm, consisting of three randomly replicate cores (141 cm^2 surface area) pooled together with the purpose to account for within-site variability. An additional composite sediment sample was obtained from a deeper layer (15 – 20 cm), with equal number of replicates as described above, in order to examine if there were differences in organochlorine contamination according to depth. Supplementary sediment samples were taken for determination of the total organic matter (TOM) content. Sediments were previously dried at 60°C and combusted at 500°C for 4 h (Lobo et al., 2010).

S. plana individuals were collected by hand, washed *in situ* and transported in a thermic box. At the laboratory, individuals were left in clean seawater at 20°C for 24h to depurate, then measured in terms of total length and dissected. Afterwards, bivalve samples were freeze-dried at -60ºC and 1.0 bar, and homogenised for later PCB extraction. Prior to the analysis, the samples were grouped into three size classes (1-2, 2-3 and 3-4 cm, corresponding to 1+, 2+, and 3+ year old individuals, respectively) (Verdelhos et al., 2005). Few individuals below 1 cm and above 4 cm were found for all locations, making impossible to obtain sufficient body mass to perform organochlorines analysis and quantification in these age classes.

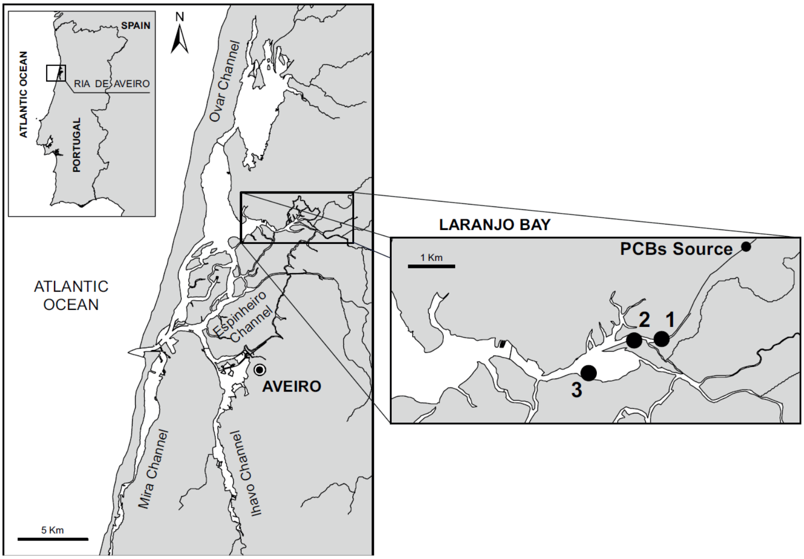

Fig. 1. The Ria de Aveiro coastal lagoon and sampling areas location.

Sample preparation and PCBs and HCB extraction

Water and suspended particulate matter (SPM)

Water samples from intertidal pools were filtered through pre-washed (hexane) glass fiber filters (Whatman GF/F, Ø 47 mm, Schleicher & Schuell), before heating to 350 ºC for 10h (adapted from Antunes et al., 2007). This procedure was used in order to eliminate possible traces of organic compounds and also to separate dissolved and particulate fractions. While the dissolved fraction was collected in glass flasks and analysed within two days, the glass fiber filters were packed into aluminum foil and stored frozen for latter suspended particulate matter (SPM) analysis. SPM samples were transferred to cellulose thimbles and Soxhlet extracted for 16 h with a mixture of *n*-hexane:acetone (1:1) (adapted from Antunes et al., 2007). Afterwards, resulting extracts were cleaned up following the methodology described for the sediments. Dissolved fraction samples (aliquots of 1000 mL) were spiked with CB209 and extracted following the USEPA Method 3510C (using hexane as extraction solvent). The extraction was repeated three times, the combined hexane extract was concentrated in a rotary evaporator and submitted to SPE cleanup in a column with Florisil (using hexane for conditioning and elution) and anhydrous sodium sulphate. After evaporation in a rotary evaporator and under a gentle nitrogen flow, the extract was stored at -20ºC until analysis.

Sediments

Sediment samples were freeze-dried, sieved to < 1 mm, homogenized and wrapped in aluminium foil. Representative aliquots of 10 g of sediment were Soxhlet-extracted with 180 ml of hexane/acetone (1:1) for 8 h, at a rate of 10 cycles/h, in a prewashed glass fiber thimble (adapted from USEPA, 1996). For both sediment and SPM resulting extracts, activated copper granules were added to remove elemental sulphur. The extracts were concentrated using a rotary evaporator (at 30 °C) and submitted to a clean-up using solid-phase extraction cartridges filled with 1 g of silica (Supelclean® LC-Si), 2 g of neutral alumina (Supelclean® LC-AL-N), both 3% deactivated, and 0.5 g of anhydrous sodium sulphate at the top, using a solid phase extraction (SPE) system. The elution was made with 20 ml of hexane/dichloromethane (9:1) and 10 ml of hexane/dichloromethane (2:1). The eluate was then concentrated down to 1 mL using a rotary evaporator and dried under a gentle stream of nitrogen. The extracts were re-dissolved in hexane and further experienced a clean-up with 2 g of acidic silica (30% concentrated H_2SO_4, w/w), eluted with 15 ml of hexane and the solvent changed to iso-octane before analysis in a gas chromatographer with mass spectrometry detector (GC-MS).

Bivalves

Regarding bivalves, individuals from the same size class were homogenized and combined into a single composite, and then sub-sampled for analysis. This procedure was done in order to obtain enough body mass to accurately determine PCB concentration in bivalves. According to the mass available for each age class from distinct locations, sub-samples of 3 g were weighted, followed by the addition of polychlorinated CB 209 to monitor extraction efficiency. Subsequently, samples were extracted by sonication (Selecta®) with a *n*-hexane:acetone (1:1) mixture (30 mL), for 20 minutes. Extract was decanted and the process was repeated thrice. Solvent volume was reduced by using a rotary evaporator. Lipid content was gravimetrically determined using 10% of the extract. A soft stream of nitrogen was used to reduce solvent volume by evaporation. Lipids were removed with sulphuric acid (97%). Afterwards, the extract passed through a multilayered column packed with florisil (1g of Supelclean® Florisil) and anhydrous sodium sulphate, and organochlorines eluted with 15 mL of hexane. All samples were dried, under a gentle stream of nitrogen, and solvent exchanged to iso-octane before analysis.

PCB and HCB analysis and quantification

Before the gas chromatography coupled to mass spectrometry (GC-MS) analysis, internal standards were added (HCB-^{13}C, CB 34, 62, 119, 131 and 173) and the extract was reconstituted to 200 µL of iso-octane for analysis. All resultant extracts (water, SPM, sediments and bivalves) were analysed by using a gas chromatographic system (Shimadzu Corporation GC/MS-QP5050A) equipped with a ZB5-MS column (30 m × 0.25 mm i.d., 0.25 µm film thickness), helium as carrier gas (0.9 ml/min) and a MS detector using electron impact ionization. Samples were injected (1 µL) in splitless mode and the analytes were separated with the following oven temperature program: 2 min at 40 °C, first ramp at 10 °C min^{-1} to 180 °C, second ramp at 6 °C min^{-1} to 310 °C (held for 10 min). The injector was operated at 280 °C and the interface at 310°C.

HCB and thirteen PCB congeners (IUPAC nos. 18, 28 and 31- tri; 44 and 52 –tetra; 101 and 118-penta; 138, 149 and 153-hexa; 170 and 180-hepta and 194-octachlorobiphenyl) according to EN 12766/CEN and EN 61619 were analysed. Within these, seven congeners were considered as priority marine pollutants by ICES (1987) and have ecological relevance (IUPAC nos. 28, 52, 101, 118, 138, 153 and 180). However, according to recent recommendations from the European Commission (Commission Regulation (EU) No., 1259/2011), only six congeners should be considered as ecological indicators ($\sum_6$ PCBs), being the CB118 exclusively classified as a dioxin-like PCB (dl-PCB). Based on the latest regulations, the inclusion of CB118 was not considered in the results' section, specifically in PCBs indicators analysis.

The detector was operated in selected ion monitoring (SIM) acquisition and the ions monitored were: 284 for HCB and 290 for HCB-^{13}C; **256**, 258 for tri-CBs; **292**, 290 for tetra-CBs; **326**, 328 for penta- CBs; **360**, 362 for hexa-CBs; **394**, 396 for hepta-CBs; **430**; 428 for octa-CBs. The ions highlighted in bold were used for quantification and the others for confirmation. Besides the confirmation ions, the identifications of PCB congeners were also made on the basis of their retention times relative to the internal standards.

Quality assurance and quality control (QA/QC) procedures

The method performance was assessed by daily check of calibration curves, method blanks, analysis of certified reference materials (CRMs), fortification of samples and recoveries of surrogate (CB 209). Method blanks were done in every forth sample in order to detect possible interferences from the reagents, glassware and other processing hardware. In fortified water samples recoveries varied between 78 and 104 % and in sample recoveries of CB209 were 82 ± 5 % (95% confidence interval). The PCB recoveries for the sediment CRM CNS-300 (freshwater sediment) ranged between 83% and 99% and for the CRM-141 (freshwater sediment) recoveries ranged between 81% and 110% for PCBs and HCB. For spiked sediment samples, analytes recoveries were between 74-105%. The results of PCBs in the NIST certified material SRM 2977 (mussel tissue) were within 74-108% of the certified values and in spiked bivalve samples the recoveries of analytes ranged from 78-112%. Both for sediments and biota, surrogate recoveries ranged from 68% to 115%. Limits of detection for individual congeners and HCB ranged between 0.6 and 0.8 $ng.L^{-1}$ in water, from 0.3 to 1.4 $ng.g^{-1}$ in SPM samples, from 0.04 to 0.10 $ng.g^{-1}$ in sediments and from 0.15 to 0.40 $ng.g^{-1}$ in bivalves.

Secondary production

Secondary production is a useful tool of ecosystem function, representing a direct measure of food provision delivered by an ecosystem, with a clear socioeconomic value. Calculation of secondary production was performed by using data obtained by monthly sampling campaigns undertaken from September 2010 to September 2011 in the same locations. Secondary production was estimated using the Brey (2001) method version 4-04 (worksheet in Brey, 2001; www.awi-bremerhaven.de/Benthic/Ecosystem/FoodWeb/Handbook/main.htm), considered the best alternative empirical method to estimate secondary production (Dolbeth et al., 2005; 2011). No value was given to the depth, since the species is intertidal and conversions from ash-free dry weight (AFDW) to dry weight (DW) were done based on Dolbeth et al. (2005). Determination of the annual production of *S. plana*, will allow to estimate the quantity of PCBs removed from the sediments (otherwise not bioavailable), and consequently free for trophic transfer to predators. This quantification is essential to evaluate the amount of PCBs that could reach higher trophic levels endangering the aquatic environment.

Bioaccumulation and biota-sediment accumulation factors

The partitioning of compounds between the organisms and abiotic environmental compartments, in which they inhabit may be described by the bioaccumulation factor (BAF). Since this study aimed to measure PCBs in different layers of the sediment profile, it was assumed that the use of the mean value of PCB concentration between superficial and deeper layers for calculation of BAF was more appropriate. In addition, because the HCB concentration in bivalves was mostly below the detection limit, it was only possible to calculate BAF for PCBs, according to the formula (Lee, 1992; Lobo et al., 2010):

$$BAF = \frac{C_o}{C_s}$$

where C_o represents the PCBs concentration in the organism expressed in $ng.g^{-1}$ dry weight of tissue and C_s is the PCB concentration in the sediment expressed in $ng.g^{-1}$ dry weight of sediment. Biota-sediment accumulation factors (BSAF) was also calculated and is essentially the BAF normalized to the total organic matter content of the sediment (TOM, given in % relative to sediment dw) (adapted from USEPA, 1995; Lobo et al., 2010):

$$BSAF = \frac{C_o}{(C_s/TOM)}$$

Statistical analysis

All data were initially checked for normality and for homogeneity of variances using Shapiro-Wilk and Bartlett's tests respectively (Zar, 1996). Data not meeting these criteria were transformed appropriately and checked again for normality and homoscedasticity (Zar, 1996). Differences in PCBs and HCB concentrations between stations and sediment depths were analysed with a multivariate two-way ANOVA (stations x depths). A similar statistical methodology was used to determine differences in PCBs concentrations between study areas and bivalves' age classes. Pairwise multiple comparisons (stations, sediment depths and *S. plana* age classes) were made using the Tukey's honestly significant difference (HSD) test, except whenever the number of replicates differed among bivalves' age classes of different stations. In this case, the Unequal N HSD was applied. This test is a modification of the Tukey HSD test and provides an acceptable test of differences in group means if group *n's* are not too discrepant (Winer et al., 1991). All statistical analyses were performed with Statistica 7 software (StatSoft).

RESULTS

Organochlorines in water, SPM and sediments

Levels of PCBs and HCB in water of the three sampling locations were vestigial and below the limit of detection (LOD), never exceeding 0.80 ng.L^{-1} for all analysed congeners. All suspended particulate matter (SPM) samples presented also HCB levels below detection limit. Concentrations of PCBs in SPM samples were low ranging between 3.77 ± 0.43 ng.g^{-1} dw in station 3 (sum of CBs 138 and 153) and 5.84 ± 0.52 ng.g^{-1} dw in station 1 (sum of CBs 101, 138, 149 and 153).

PCB accumulation in sediments showed a distinct spatial gradient, with station 1 presenting significantly much higher contamination than the remaining areas (two-way ANOVA: $F_{2,11} = 183.1$, $p < 0.001$) (Fig. 2). In addition, there were statistical differences in PCB concentrations according to depth (two-way ANOVA: $F_{1,11} = 391.3$, $p < 0.001$) and a significant interaction was also found between factors (stations x depths) (two-way ANOVA: $F_{2,11} = 136.5$, $p < 0.001$). Sediment superficial layers (0-5 cm) of stations 1 and 2 presented similar PCB content, below 5 ng.g^{-1} dw (Fig. 2A,B), while superficial deposits of station 3 had approximately half of that concentration (Fig. 2C). However, no relevant statistical differences were found (Tukey test, $p > 0.05$). Concerning PCB concentrations in the deepest strata (15-20 cm), it was observed that sediments from station 1 were 4 and 17 times more contaminated than those from stations 2 and 3, respectively (Tukey test, $p < 0.05$). Congeners composition analysis showed that CBs 153, 138 and 180 were the most abundant, independent from the sampling station and/or the depth, accounting for 50% of the total studied congeners. On the other hand, trichlorobiphenyl congeners (18, 28 and 31) were very rare, except in deeper sediments from station 2 in which the CB18 was the third most represented (Fig. 2B). The octachlorobiphenyl CB194 was not detected in superficial sediments from the three selected locations and in the less contaminated site, it was even undetectable in 15-20 cm depth (Fig 2C).

Distribution of HCB in sediments followed a similar tendency described for PCBs (Table 1), despite values being substantially smaller. Significant statistical differences were observed between stations (two-way ANOVA: $F_{2,11} = 27.0$, $p < 0.001$) and sediment depths (two-way ANOVA: $F_{1,11} = 33.5$, $p < 0.001$). The highest concentrations were reported nearby the industrial complex, decreasing steadily along a 3 km gradient. Deeper sediment layers contained higher HCB levels, except in station 3 where contamination was vestigial and no differences in HCB concentration were recognised along sediment depth profile.

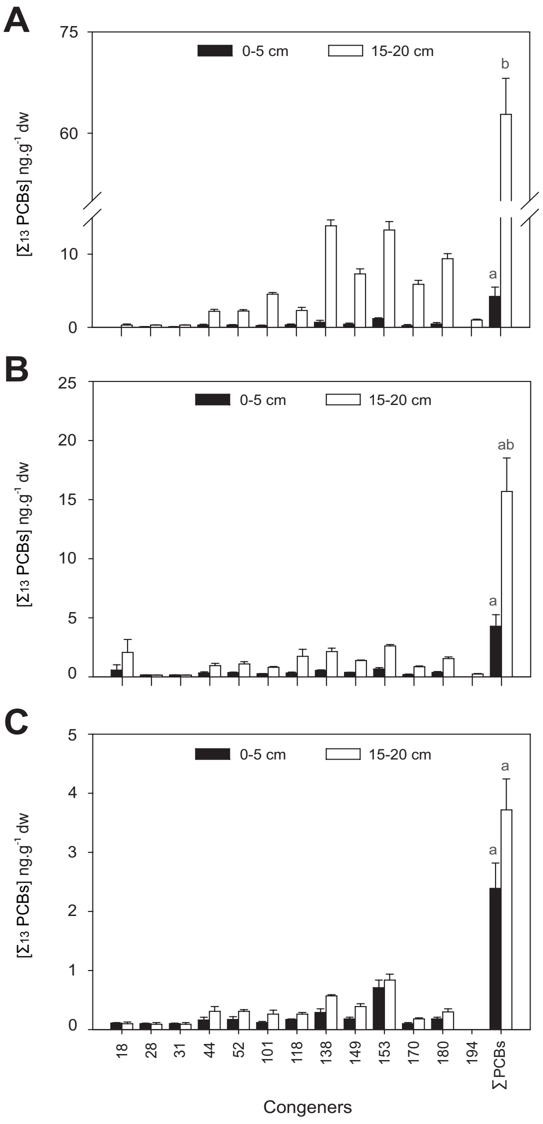

Fig. 2. Mean Σ_{13} PCB concentrations ($ng.g^{-1}$ dry weight) at distinct sediments depths (in centimeters) from different sampling locations: (A) Station 1; (B) Station 2; and (C) Station 3. Error bars represent standard error. Tukey's test results are presented: different letters indicate significant differences among treatments ($p < 0.05$).

Organochlorines in *S. plana*

In accordance with the spatial gradient described for the sediments, the bivalves from the station closer to the point of discharge of industrial effluents evidenced higher contamination, decreasing significantly with the distance to the PCB source (two-way ANOVA: $F_{2,10} = 6.3$, $p < 0.05$) (Fig. 3A). Likewise, when the considered PCB concentration was expressed in a lipid weight basis, an identical spatial gradient emerged (Fig. 3B).

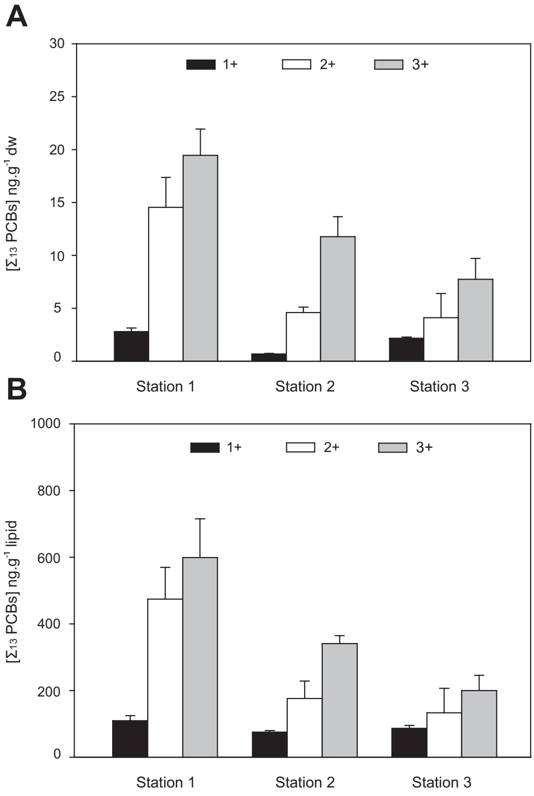

Fig. 3. Mean Σ_{13} PCB concentrations in different age classes of *S. plana* from distinct sampling locations: (A) expressed in ng.g^{-1} dry weight; and (B) expressed in a lipid weight basis (ng.g^{-1} lipid). Error bars represent standard error.

In average, individuals from station 1 presented twice the lipid normalized PCB concentration than the ones inhabiting the moderately contaminated area, and 2.5 times more PCBs than bivalves from the less contaminated area. For all locations, smaller individuals presented lower contamination values, while larger bivalves tended to progressively accumulate higher levels, suggesting a bioaccumulation trend throughout *S. plana* lifespan (Fig. 3A,B). Moreover, PCB concentration varied significantly between age classes (two-way ANOVA: $F_{2,10} = 10.4$, $p < 0.05$) and no interaction between study areas and age classes was detected (two-way ANOVA: $F_{4,10} = 1.0$, $p > 0.05$).

Analysis of the six congeners with ecological relevance (Σ_6 PCB) showed a well-defined bioaccumulation pattern during *S. plana* lifespan, common to all sampling locations (Fig. 4). The single ecological congener detected in bivalves grouped into the class 1+ was CB153, however with age, five remaining indicator congeners tended to be gradually accumulated. Data revealed that the most represented and incorporated congeners by the bivalves were CBs 153, 138 and 52, which is consistent with results obtained for the sediments, except for the latest congener. Within this group of six congeners, CB28 was not detected in bivalves from station 3 (Fig. 4C) and accounted for less than 1% in the two most contaminated areas, being only detected in individuals larger than 2 cm (Fig 4A,B).

For all sampling areas, no HCB residues were detected (< LOD) in the smallest bivalves. In the less contaminated area was only possible to quantify HCB in individuals larger than 3 cm. Contamination levels were quite lower and almost negligible compared to PCBs. Also, a bioaccumulation trend during *S. plana* lifespan was not evident (Table 1).

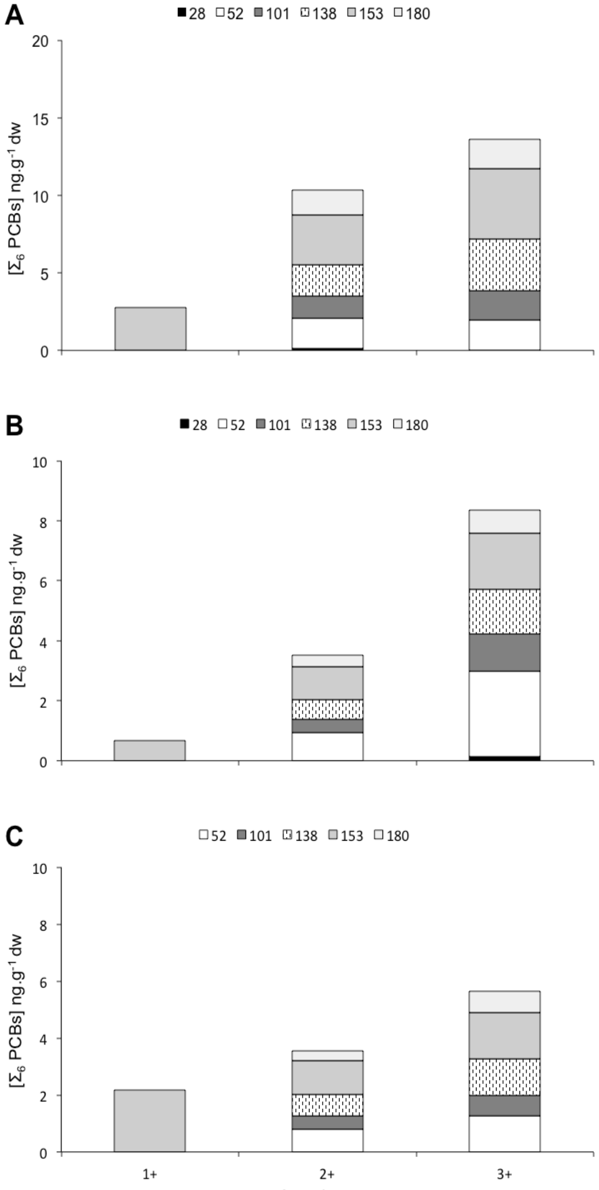

Fig. 4. Mean Σ_6 PCB concentrations (ng.g^{-1} dry weight) in different age classes of *S. plana* along a spatial contamination gradient: (A) Station 1; (B) Station 2; and (C) Station 3.

Table 1. HCB concentration (ng.g^{-1} dry weight) at distinct sediment depths (in centimeters) and different age classes of the bivalve *S. plana* (mean ± SE). Tukey's test results are presented: different letters indicate significant differences among treatments ($p < 0.05$).

Location	Sediment (ng.g^{-1} dw)		*Scrobicularia plana* (ng.g^{-1} dw)		
	0-5	**15-20**	**1+**	**2+**	**3+**
St. 1	3.79 ± 2.03^{a}	9.49 ± 0.31^{b}	bdl	0.312 ± 0.028	0.316 ± 0.026
St. 2	2.00 ± 0.16^{a}	5.89 ± 0.58^{a}	bdl	0.234 ± 0.002	0.178 ± 0.011
St. 3	0.25 ± 0.05^{a}	0.23 ± 0.02^{a}	bdl	bdl	0.151 ± 0.046

bdl – below detection limit (< LOD)

Annual rates of PCB bioaccumulation, secondary production, transfer to the trophic chain and biota-sediment accumulation factors

Annual PCB bioaccumulation rates through *S. plana* lifespan varied differently in result to the environmental organochlorine levels. Bioaccumulation followed a linear trend in all locations, with annual rates increasing with the proximity to the pollution source (Fig. 5). In the most contaminated station (8.3 ng.g^{-1}.yr^{-1}), the annual bioaccumulation rate was 1.5 times greater than the estimated rate for the intermediate station (5.5 ng.g^{-1}.yr^{-1}) and approximately 3 times higher than the estimated rate for the station farther away from the contamination source (2.8 ng.g^{-1}.yr^{-1}).

In order to assess the role of *S. plana* on the PCB transfer to the estuarine food webs, the annual production of this benthic sediment dwelling invertebrate was calculated in the three selected locations. Production was considerably higher in the moderately and less contaminated stations (155 g.DW.m^{-2}.yr^{-1} and 147 g.DW.m^{-2}.yr^{-1}, respectively), while in the most contaminated area the annual production was roughly half of those values (Table 2). Considering the annual bioaccumulation rates, this bivalve was responsible for the bulk PCB removal from the sediment, incorporating almost 1 μg.m^{-2}.yr^{-1} in the moderately contaminated station (Table 2). Three kilometers away from the effluent discharge point, such PCB removal declined 50%. In fact, extrapolating the results from the mean associated PCBs in the three sampling locations to the approximate 0.6 km^2 of intertidal flats existing in the inner Laranjo Bay, this species is responsible for the annual incorporation of almost 0.4 g of PCBs from the sediments.

Regarding BAF and BSAF, a common increasing tendency farther from the contamination source and through *S. plana* lifespan was discernible (Table 2), meaning that higher BAF and BSAF were registered in lower PCB contaminated environmental sites and in larger bivalves.

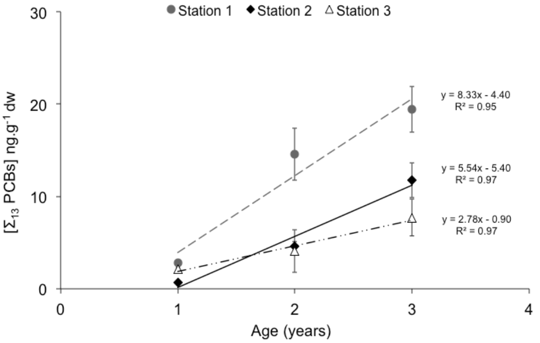

Fig. 5. PCB accumulation during *S. plana* lifespan. The trendlines calculated from average levels correspond to the annual net accumulation of PCBs by bivalves for each sampling location. Error bars represent standard error.

Table 2. Annual production, quantity of PCBs available for trophic transfer and bioaccumulation and biota-sediment accumulation factors in *S. plana*.

Location	Annual production (g.DW.m^{-2}.yr^{-1})	Associated PCBs (ng.m^{-2}.yr^{-1})	BAF			BSAF		
			1+	**2+**	**3+**	**1+**	**2+**	**3+**
St. 1	78	650	0.1	0.4	0.6	0.7	3.5	4.7
St. 2	155	851	0.1	0.5	1.2	0.5	3.4	8.6
St. 3	147	411	0.7	1.4	2.7	5.6	10.6	20.0

DISCUSSION

The assessment of organochlorine contamination in abiotic and biotic environmental compartments of the Laranjo bay, in Ria de Aveiro, revealed that the highest concentrations were found closer to the site where industrial effluents were discharged, decreasing sharply along a 3 km extent area. Concentrations found in intertidal water pools were negligible and below the detection limit for both PCBs and HCB. For numerous aquatic systems across the world, PCB levels in the dissolved phase were vestigial, except in the Seine estuary (France) and particularly in Daya bay (China), where high concentrations were reported (Table 3). Residual levels of HCB in water were also documented in the Black Sea (Maldonado and Bayona, 2002), never exceeding 0.022 $ng.L^{-1}$. The results obtained in the Laranjo bay concerning levels of organochlorines dissolved in intertidal water were in a certain way expected, based on the hydrophobic nature of these pollutants. In fact, PCBs easily volatilize from water surfaces in spite of their low vapour pressure, and partly as a result of their hydrophobicity (Ritter et al., 1995).

Due to that intrinsic property, these contaminants have great affinity with particulate materials, remaining strongly adsorbed to suspended particulate matter (SPM). PCB concentrations found in particulate fractions of the Laranjo bay intertidal water were similar to those found in East Java, Indonesia (Hillebrand et al., 1989) and within the range determined by Chen et al. (2011) in Pearl River estuary, in China (Table 3). Several European and Asian aquatic systems presented substantially lower PCBs in SPM than the Laranjo bay and in some locations, like Daya bay (China), no congeners were even quantified. On the other hand, values documented in the Seine estuary were remarkably higher compared to the range estimated in the present study (Table 3). PCB levels found in SPM were slightly higher than the concentrations measured in superficial sediments, which may be related with transport of particles from the upstream more contaminated areas to the Laranjo bay and also with plankton's ability to bioaccumulate PCBs (Quental et al., 2003). Furthermore, preferential accumulation of PCBs with finest sediment fractions (< 63 μm) (Piérard et al., 1996), which remain suspended in the water column for a longer time compared to coarser particles, could also explain these results.

Sediment analysis constitutes a tool of extreme importance in aquatic ecosystems quality assessment, since sediments can reflect long-term contamination levels, acting as reservoirs and thus a source of contamination to benthic organisms (Fang, 2004; He et al., 2006). Chemistry of bottom sediments is influenced by numerous natural and anthropogenic variables and their heterogeneity and spatial variation are undoubtedly linked to erosion phenomena, transport and deposition, granulometry and organic matter content. The latter is particularly relevant in the context of accumulation of hydrophobic organic contaminants since it represents a factor directly influencing the concentration of PCBs in bottom sediments. For instance, Tomza et al. (2006) stated that sediments rich in organic matter (muddy deposits) accumulated more PCBs than sandy ones.

An increase in PCB and HCB concentrations was observed in the sediment depth profile. By analysing organochlorines in deeper strata, a large discrepancy was found in relation to the superficial sediments, particularly in the two most contaminated sites. Deeper layers presented much higher concentrations than the uppermost sediments, reflecting PCB and HCB past emissions. Several activities related with agro-industrial development undertaken in the past may be responsible for the observed increase in the sediment core. Nevertheless, the lower levels obtained in the superficial sediments may indicate that the influence of organochlorine compounds in the study area is now decreasing. Compared to other systems worldwide, the results obtained in Ria de Aveiro regarding PCBs in sediments were identical to those found in the coast of Barcelona (Castells et al., 2008), but quite superior to those in Singapore's coast (Wurl and Obbard, 2006) or in Manila bay, in Philippines (Villeneuve et al., 2010) (Table 3). Besides, HCB levels were similar to those reported by Kim et al. (2009) in surface sediments from the Han River, in Korea, and rather higher than levels found in the Gulf of Bothnia (Strandberg et al., 2000). Although, when compared to the Ya-Er Lake, China, where PCB concentrations in sediments reached almost 6,000 ng.g^{-1} dw and HCB levels up to 58,000 ng.g^{-1} dw, the results in the Laranjo bay seemed almost irrelevant (Table 3). The Tagus estuary, in Portugal, displayed PCB values (17.7 ng.g^{-1} dw) (IAEA/MEL/ 67, 1999) analogous to those found in sediments from the intermediate sampling station, while contamination in the Mondego estuary was residual, varying in the range of 0.81-2.36 ng.g^{-1} dw (Baptista et al., 2013).

Canadian sediment quality guidelines (CCME, 2002) can be used to assess the degree to which adverse biological effects are likely to occur as a consequence of exposure to PCBs in sediments. The present study revealed that concentrations detected in most contaminated sediments exceeded the PCB threshold effect level (TEL), 21.5 ng.g^{-1}, but were below PCB probable effect level (PEL), 189 ng.g^{-1}. Therefore, this location may be considered a highly stressful environment, since PCB toxic effects may occur on benthic biota at this contamination level, which is reflected by its lowest annual production. In fact, the bivalve *Cerastoderma edule*, was found to regulate and eliminate organic contaminants under adverse conditions by synthesis of metallothioneins (Lobo et al., 2010). A similar physiological response might be expected regarding the species *S. plana*. According to the Norwegian sediment quality guideline (NSQG; Bakke et al., 2010) for PCBs, sediments closest to the contamination source are included in class III, causing toxic effects to biota following chronic exposure. Based on NSQG, HCB levels in sediments from the Laranjo bay were not problematic, since no toxic effects were suggested. Furthermore, according to the Portuguese legislation for dredged material (Decreto-Lei no. 226-A/2007, 31 May; Portaria no. 1450/2007, 12 November), sediments collected nearby the point of discharge of industrial effluents are classified as slightly contaminated for both PCBs and HCB (25 – 100 ng.g^{-1} and 2.5 – 10 ng.g^{-1} respectively) and in the case of immersion of the dredged sediments it requires a thorough study about the deposition local and subsequent monitoring.

Regarding the contribution of individual congeners to Σ_{13}PCB in sediments and bivalves, the most abundant were generally the hexa- (CBs 138 and 153) and heptachlorinated (CB180) followed by the pentachlorinated ones (CBs 101 and 118), which is in accordance with observations reported by Strandberg et al. (2000), Wurl and Obbard (2006) and Villeneuve et al. (2010). That contribution was primarily related to their abundant release into the environment associated to its lower volatility (Ritter et al., 1995) and higher long-term environmental persistence compared to less chlorinated congeners.

The bivalve *S. plana* was found to respond to the human induced PCB gradient, by incorporating organic compounds parallel to environmental contamination levels. Through its filter and deposit feeding strategies, *S. plana* accumulated contaminants adsorbed in particulate matter (Coelho et al., 2006) and the bottom sediment. The species revealed to be an important vehicle of PCB transference from sediments to the trophic web, incorporating into their tissues approximately 0.4 g of PCBs, annually, in the inner area of the Laranjo bay (0.6 Km^2). This amount may seem residual and irrelevant, but considering that it can be transferred to higher trophic levels by predation and exported to other areas of the system, it may be significant, since this species represents an important prey item to estuarine predators such as crabs, fishes, and birds. Therefore, the importance of these findings to the understanding of biomagnification processes is evident.

Bioaccumulation and biota-sediment accumulation factors increased farther from the pollution source and in lower environmental contaminated areas. In average, levels of PCBs in sediments from station 1 are 3 and 10 times higher than values observed in stations 2 and 3, respectively. However, in average, bivalves collected closer to the PCB source only reached accumulations twice the levels found in the less contaminated sites. This means that accumulation is not proportional and bivalves under higher environmental contamination demonstrated lower ability to accumulate PCBs, suggesting the eventual existence of detoxification strategies in the most contaminated area. Similar observations were reported by Lobo et al. (2010) investigating trace metals and organic compounds bioaccumulation potential in the edible cockle *C. edule* and also by Cardoso et al. (2013) evaluating mercury bioaccumulation kinetics in the same species.

Comparisons of PCB concentrations in biota among different aquatic systems are typically difficult and require particular attention, because there is a wide variation in the number of quantified congeners and the basis in which the concentration is expressed may also vary (wet weigh, dry weight and lipid weight) (Table 3). For instance, in a lipid basis, results from the Laranjo bay were comparable to PCB concentrations found in mussels *Perna viridis*, in South China (Fang, 2004). Moreover, *S. plana* accumulated higher PCB concentration than the blue mussel *Mytilus edulis* and the oyster *Crassostrea talienwhanensis* from the Chinese Bohai Sea (Wang et al., 2008), even though the number of quantified congeners between locations was different (13 CBs in this study against 25 CBs in Chinese Bohai Sea). Using different Arctic bivalve species and analyzing 16 CBs, Vieweg et al. (2012) reported lower lipid normalized concentrations than levels obtained in the Laranjo bay, as well as Tomza et al. (2006) investigating the uptake of 7 CBs in freshwater mussels *Anodonta complanata*, in Poland. On the other hand, there are numerous aquatic systems around the world in which PCB concentrations in bivalves broadly exceed levels observed in the Laranjo bay. For example, in *Mytilus galloprovincialis* from the Galician coast, Carro et al. (2010) estimated PCB concentrations (wet weight) up to 50 times the maximum concentration found in *S. plana*. Similar observations were documented in mussels from the Egyptian Red Sea Coast (Khaled et al., 2004) and from Algiers bay (Fouial-Djebbar et al., 2011). The latter stated an evident tendency of PCB bioaccumulation along different size classes of *M. galloprovincialis*, with larger mussels (> 8 cm) presenting higher concentrations than the small ones. Similar observations were found in *S. plana* throughout its lifespan, which is confirmed by Σ_{13}PCB and also when it was only considered the contribution of the six ecological indicator congeners (Σ_6PCB).

At present the non-dioxin-like PCBs (ndl-PCBs) lack international evaluation, and hence a health-based maximum intake level has not been established. The sum of the six indicator congeners (CBs 28, 52, 101, 138, 153, and 180) comprises about 50% of the amount of total ndl-PCBs present in feed and food, being considered by the European Food Safety Authority (EFSA, 2010) a suitable indicator for occurrence and human exposure to ndl-PCBs. Therefore, it is appropriate to set maximum levels as a sum of these 6 PCBs. According to Commision Regulation (EU) No 1259/2011, there is no danger for human health by consuming this bivalve species, since contamination values among locations and age classes varied in the range of 0.1-2.3 ng.g^{-1} wet weight, which is widely below the limit established for muscle meat of fish and fishery products (75 ng.g^{-1} wet weight).

Although PCBs are present at low background levels, their harmful impact to human health after consumption over many years and after reaching a steady-state concentration in the body (bioaccumulation) is recognized. Thus, it is extremely important to determine the tolerable daily intake (TDI) for such contaminants. The French Food Safety Agency (AFSSA) and the Norwegian Scientific Committee for Food Safety (VKM) estimated a Maximum Permissible Risk level, TDI for the sum of the six ecological indicator PCBs of 10 ng.kg^{-1} body weight per day (AFSSA, 2007; VKM, 2008). Since there are no values in literature regarding gross consumption (GC) of shellfish in Portugal, it was reasonable to assume the GC calculated for bivalves in Italy (2.3 g.day^{-1}) (Pompa et al., 2003), due to similarities in the Mediterranean diet common to both countries. Considering this average daily bivalve consumption, using a mean human body weight of 65 kg and the mean Σ_6 PCB concentrations in *S. plana* from the Laranjo bay (0.93 ng.g^{-1} wet weight), the estimated Σ_6 PCB daily intake for the Portuguese population would be 0.033 ng.kg^{-1} body weight. Thus, the estimated daily intake does not exceed the established TDI and is inclusively about 300 times lower than that limit.

This research may have important implications for management policies and biomonitoring programs, by providing a better insight into the organochlorine bioaccumulation process in the bivalve *S. plana*, itself an important food resource for human population and a crucial contaminant transfer vehicle (biomagnification) to other economically important and exploited natural resources.

Table 3. Comparison of PCB concentrations in water (ng.L^{-1}), suspended particulate matter (SPM, ng.g^{-1} dry weight), sediments (ng.g^{-1} dry weight) and bivalves (ng.g^{-1} dry weight) from various aquatic systems worldwide.

Location	**No of CBs**	**Σ PCBs**				**Reference**
		Water	**SPM**	**Sediment**	**Bivalves**	
Africa						
Egyptian Red Sea Coast	7				6.7-66.4 ^{a}	Khaled et al. (2004)
Algiers Bay, Algeria	17 / 7	4.0-18.8			64-222	Fouial-Djebbar et al. (2011)
Asia						
Manila Bay, Philippines	13			0.1-1.5	7-73	Villeneuve et al. (2010)
Daya Bay, China	12	91-1355	bdl	0.85-27.4		Zhou et al. (2001)
Chinese Bohai Sea	25				1.84-3.75	Wang et al. (2008)
Pearl River Estuary, China	12	0.02-7.18	0.02-15^{c}			Chen et al. (2011)
Pearl River Delta, China	-			16.4-198.6	41-729 ^{b}	Fang (2004)
Ya-Er Lake, China	9			10.7-5970		Wu et al. (1997)
East Java, Indonesia	-	0.2-3.5	4			Hillebrand et al. (1989)
Singapore's Coast	38	0.06-6.9	0.04-3.8^{c}	0.6-1.3		Wurl and Obbard (2006)
Europe						
Danube Estuary, Romania	10	0.02-0.1	0.002-0.06^{c}			Maldonado and Bayona (2002)
Odra River, Poland	7	1.4-3.1		2.6-17.9	33-66^{b}	Tomza et al. (2006)

^{a} ng.g^{-1} wet weight ^{b} ng.g^{-1} lipid weight ^{c} ng.L^{-1} bdl – below detection limit (< LOD)

Table 3. Comparison of PCB concentrations in water ($ng.L^{-1}$), suspended particulate matter (SPM, $ng.g^{-1}$ dry weight), sediments ($ng.g^{-1}$ dry weight) and bivalves ($ng.g^{-1}$ dry weight) from various aquatic systems worldwide (Cont.)

Location	No of CBs	Σ PCBs				Reference
		Water	SPM	Sediment	Bivalves	
Svalbard, Norway	16				55.4-78.1^{b}	Vieweg et al. (2012)
Gulf of Bothnia, Sweden	12			9.0-9.3		Strandberg et al. (2000)
Seine Estuary, France	8	2.0-21.2	58-463			Cailleaud et al. (2007)
Arrone River, Rome	58	6-13		11-196		Bazzanti et al., 1997
Coast of Barcelona, Spain	12			2.3-44.0		Castells et al. (2008)
Galician Coast, Spain	10				0.62-107.5^{a}	Carro et al. (2010)
Sado Estuary, Portugal	18			1.2-17.6		Lobo et al. (2010)
Ria de Aveiro, Portugal	13	bdl	3.8-5.8	2.4-62.8	1.0-19.4 0.1-2.3^{a} 75-599^{b}	Present study

^{a} $ng.g^{-1}$ wet weight ^{b} $ng.g^{-1}$ lipid weight ^{c} $ng.L^{-1}$ bdl – below detection limit (< LOD)

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REFERENCES

AFSSA (2007) Opinion of the French Food Safety Agency (AFSSA) on the establishment of relevant maximum levels for non dioxin-like polychlorobiphenyls (NDL-PCB) in some foodstuffs, Afssa – Request No. 2006-SA-0305. Maisons-Alfort.

Amodio E, Turci R, Massenti MF, Di Gaudio F, Minoia C, Vitale F, Firenze A, Calamusa G (2012) Serum concentrations of persistent organic pollutants (POPs) in the inhabitants of a Sicilian city. Chemosphere 89: 970-974.

Antunes P, Gil O, Reis-Henriques MA (2007) Evidence for higher biomagnification factors of lower chlorinated PCBs in cultivated seabass. Science of the Total Environment 377: 36-44.

ATSDR (2007) CERCLA Priority list of hazardous substances. Agency for toxic substances and disease registry, Atlanta, GA. Available from: http://www.atsdr.cdc.gov/SPL/index.html

Bakke T, Källqvist T, Ruus A, Breedveld GD, Hylland K (2010) Development of sediment quality criteria in Norway. Journal of Soil and Sediments 10: 172-178.

Baptista J, Pato P, Pereira E, Duarte AC, Pardal MA (2013) PCBs in the fish assemblage of a southern European estuary. Journal of Sea Research 76: 22-30.

Bazzanti M, Chiavarini S, Cremisini C, Soldati P (1997) Distribution of PCB congeners in aquatic ecosystems: a case study. Environment International 23: 799-813.

Bonnard M, Roméo M, Amiard-Triquet C (2009) Effects of copper on the burrowing behavior of estuarine and coastal invertebrates, the polychaete *Nereis diversicolor* and the bivalve *Scrobicularia plana*. Human and Ecological Risk Assessment 15: 11-26.

Brey T (2001) Population dynamics in benthic invertebrates. A virtual handbook. Version 01.2. Alfred Wegener Institute for Polar and Marine Research, Germany. Available from: http://www.awibremerhaven.de/Benthic/Ecosystem/FoodWeb/Handbook/main.html.

Cailleaud K, Forget-Leray J, Souissi S, Hilde D, LeMenach K, Budzinski H (2007) Seasonal variation of hydrophobic organic contaminants concentrations in the watercolumn of the Seine estuary and their transfer to a planktonic species *Eurytemora affinis* (Calaïnoda, copepoda). Part 1: PCBs and PAHs. Chemosphere 70: 270-280.

[CCME] Canadian Council of Ministers of the Environment (2002) Canadian sediment quality guidelines for the protection of aquatic life: Summary tables. Updated. In: Canadian environmental quality guidelines (1999). Canadian Council of Ministers of the Environment, Winnipeg. Available from: http://www.ecy.wa.gov/programs/eap/psamp/BoundaryBay/PSAMP-BBAMP%20documents/Canadian%20guidelines%20for%20water%20quality/SedimentProtAquaticLifeSummaryTables(en).pdf.

Cardoso PG, Pereira E, Grilo TF, Duarte AC, Pardal MA (2012) Kinetics of mercury bioaccumulation in the polychaete *Hediste diversicolor* and in the bivalve *Scrobicularia plana*, through a dietary exposure pathway. Water, Air and Soil Pollution 223: 421-428.

Cardoso PG, Grilo TF, Pereira E, Duarte AC, Pardal MA (2013) Mercury bioaccumulation and decontamination kinetics in the edible cockle *Cerastoderma edule*. Chemosphere 90:1854-1859.

Carro N, García I, Ignacio M, Mouteira A (2010) Spatial and temporal trends of PCBs (polychlorinated biphenyls) in mussel from Galician coast (1998–2008). Environment International 36: 873-879.

Castells P, Parera J, Santos FJ, Galceran MT (2008) Occurrence of polychlorinated naphthalenes, polychlorinated biphenyls and short chain chlorinated paraffins in marine sediments from Barcelona (Spain). Chemosphere 70: 1552-1562.

Chen M-Y, Yu M, Luo X-J, Chen S-J, Mai B-X (2011) The factors controlling the partitioning of polybrominated diphenyl ethers and polychlorinated biphenyls in the water-column of the Pearl River Estuary in South China. Marine Pollution Bulletin 62: 29-35.

Coelho JP, Rosa M, Pereira E, Duarte A, Pardal MA (2006) Pattern and annual rates of *Scrobicularia plana* mercury bioaccumulation in a human induced mercury gradient (Ria de Aveiro, Portugal). Estuarine Coastal and Shelf Science 69: 629-635.

Commission Regulation (EU) No 1259/2011 of 2 December 2011 amending Regulation (EC) No 1881/2006 as regards maximum levels for dioxins, dioxin-like PCBs. (http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2011:320:0018:0023:EN:PDF).

Decreto-Lei no. 226-A/2007, 31 May; Portaria no. 1450/2007, 12 November. Available from: http://dre.pt/pdf1sdip/2007/11/21700/0837208382.pdf.

Dolbeth M, Lillebø AI, Cardoso PG, Ferreira SM, Pardal MA (2005) Annual production of estuarine fauna in different environmental conditions: An evaluation of the estimation methods. Journal of Experimental Marine Biology and Ecology 326: 115-127.

Dolbeth M, Cardoso PG, Grilo TF, Bordalo MD, Raffaelli D, Pardal MA (2011) Long-term changes in the production by estuarine macrobenthos affected by multiple stressors. Estuarine, Coastal and Shelf Science 92: 10-18.

European Food Safety Authority (EFSA) Journal 2010; 8 (7): 1701. Available from: http://www.efsa.europa.eu/en/efsajournal/doc/1701.pdf.

Fang ZQ (2004) Organochlorines in sediments and mussels collected from coastal sites along the Pearl River Delta, South China. Journal of Environmental Sciences 16: 321-327.

Fouial-Djebbar D, Djebbar R, Yacine A, Badjah-Hadj-Ahmed AY, Budzinski H (2011) Level of polychlorinated biphenyls in marine environment of Algiers Bay, Algeria. Analytical Letters 44: 2438–2456.

He M-c, Sun Y, Li X-r, Yang Z-f (2006) Distribution patterns of nitrobenzenes and polychlorinated biphenyls in water, suspended particulate matter and sediment from mid- and down-stream of the Yellow River (China). Chemosphere 65: 365-374.

Hillebrand MTHJ, Everaarts JM, Razak H, Moelyadi Moelyo D, Stolwijk L Boon JP (1989) Input of selected chlorinated hydrocarbons into the coastal area of east Java and adjacent waters: Distribution patterns in the dissolved and suspended phase. Netherlands Journal of Sea Research 23: 369-377.

ICES (1987) Guidelines for Temporal Trend Analysis of Data on Contaminants in Shellfish. Report of the ICES Advisory Committee on Marine Pollution. Cooperative Research Reports. 142 pp.

International Atomic Energy Agency, Marine Environment Laboratory – MESL, Report IAEA/AL/ 121 (IAEA/MEL/ 67) (1999) Worldwide and regional intercomparison for the determination of organochlorine compounds, petroleum hydrocarbons and sterols in the sediment sample IAEA-408, Monaco

Khaled A, El Nemr A, Said TO, El-Sikaily A, Abd-Allah AMA (2004) Polychlorinated biphenyls and chlorinated pesticides in mussels from the Egyptian Red Sea coast. Chemosphere 54: 1407-1412.

Kim KS, Lee SC, Kim KH, Shim WJ, Hong SH, Choi KH, Yoon JH, Kim JG (2009) Survey on organochlorine pesticides, PCDD/Fs, dioxin-like PCBs and HCB in sediments from the Han River, Korea. Chemosphere 75: 580-587.

Lee H (1992) Models, muddles, and mud: predicting bioaccumulation of sediment associated pollutants. In: Burton AG (ed) Sediment toxicity assessment. CRC, Boca Raton, FL, 267-293.

Lobo J, Costa PM, Caeiro S, Martins M, Ferreira AM, Caetano M, Cesário R, Vale C, Costa MH (2010) Evaluation of the potential of the common cockle (*Cerastoderma edule* L.) for the ecological risk assessment of estuarine sediments: bioaccumulation and biomarkers. Ecotoxicology 19: 1496-512.

Maldonado C, Bayona JM (2002) Organochlorine compounds in the North-western Black sea water: distribution and water column processes. Estuarine, Coastal and Shelf Science 54: 527-540.

[NOAA] National Oceanic and Atmospheric Administration (1999) Screening quick reference tables (SquiRTs). Available from: http://response.restoration.noaa.gov/cpr/sediment/squirt/squirt.html.

OSPAR (2010) Quality status report 2010. OSPAR commission. London. 176 pp.

Otchere FA (2005) Organochlorines (PCBs and pesticides) in the bivalves *Anadara (Senilis) senilis*, *Crassostrea tulipa* and *Perna perna* from the lagoons of Ghana. Science of the Total Environment 348: 102-114.

Pereira ME, Lillebø AI, Pato P, Válega M, Coelho JP, Lopes CB, Rodrigues S, Cachada A, Otero M, Pardal MA, Duarte AC (2009) Mercury pollution in Ria de Aveiro (Portugal): a review of the system assessment. Environmental Monitoring and Assessment 155: 39-49.

Piérard C, Budzinski H, Garrigues P (1996) Grain-Size distribution of polychlorobiphenyls in coastal sediments. Environmental Science and Technology 30: 2776-2783.

Pompa G, Caloni F, Fracchiolla ML (2003) Dioxin and PCB contamination of fish and shellfish: assessment of human exposure. Review of the international situation. Veterinary Research Communications 27: 159-167.

Quental T, Ferreira AM, Vale C (2003) The distribution of PCBs and DDTs in seston and plankton along the Portuguese coast. Acta Oecologica 24: S333-S339.

Ritter L, Solomon K, Forget J (1995) A Review of Selected Persistent Organic Pollutants: DDT - Aldrin - Dieldrin - Endrin - Chlordane - Heptachlor- Hexachlorobenzene - Mirex - Toxaphene - Polychlorinated Biphenyls - Dioxins - Furans. The International Programme on Chemical Safety (IPSC) within the framework of the Inter-Organization Programme for the Sound Management of Chemicals (IOMC).

Strandberg B, Bandh C, van Bavel B, Bergqvist PA, Broman D, Ishag R, Näf C, Rappe Ch (2000) Organochlorine compounds in the Gulf of Bothnia: sediment and benthic species, Chemosphere 40: 1205-1211.

Tomza A, Witczak A, Ciereszko W (2006) Uptake of polychlorinated biphenyls in freshwater mussels *Anodonta complanata* from the lower Odra river. Polish Journal of Environmental Studies 15: 603-608.

UNEP. Stockholm Convention (2001) Final Act of the Conference of Plenipotentiaries on the Stockholm Convention on Persistent Organic Pollutants. Geneva, Switzerland. 44 pp.

United States Environmental Protection Agency (USEPA) (1995) CRITFC exposure study. Water quality criteria and standards Newsletter. USEPA, Washington, DC.

United States Environmental Protection Agency (USEPA). EPA Method 3510C: Separatory Funnel Liquid-Liquid Extraction. Report Number: SW-846 Ch 4.2.1. Available from: http://www.caslab.com/EPA-Methods/PDF/EPA-Method-3510C.pdf.

United States Environmental Protection Agency (USEPA) (1996) Method 3540C: Soxhlet Extraction. US Environmental Protection Agency, Washington, DC.

VKM (2008) Opinion of the Panel on Contaminants of the Norwegian Scientific Committee for Food Safety. Risk assessment of non dioxin-like PCBs in Norwegian food. Available from: http://www.vkm.no/dav/c29e178d9c.pdf.

Verdelhos T, Neto JM, Marques JC, Pardal MA (2005) The effect of eutrophication abatement on the bivalve *Scrobicularia plana*. Estuarine, Coastal and Shelf Science 63: 261-268.

Vieweg I, Hop H, Brey T, Huber S, Ambrose Jr WG, Locke V WL, Gabrielsen GW (2012) Persistent organic pollutants in four bivalve species from Svalbard waters. Environmental Pollution 161: 134-142.

Villeneuve J-P, Cattini C, Bajet CM, Navarro-Calingacion MF, Carvalho FP (2010) PCBs in sediments and oysters of Manila Bay, the Philippines. International Journal of Environmental Health Research 20: 259-269.

Wang Y, Wang T, Li A, Fu J, Wang P, Zhang Q, Jiang G (2008) Selection of Bioindicators of Polybrominated Diphenyl Ethers, Polychlorinated Biphenyls, and Organochlorine Pesticides in Mollusks in the Chinese Bohai Sea. Environmental Science & Technology 42: 7159-7165.

Winer BJ, Brown DR, Michels, KM (1991) Statistical principles in experimental design. (3rd ed.). New York: McGraw-Hill

Wurl O, Obbard JP (2006) Distribution of organochlorine compounds in the sea-surface microlayer, water column and sediment of Singapore's coastal environment. Chemosphere 62: 1105-1115.

Wu WZ, Schramm KW, Henkelmann B, Xu Y, Yediler A, Kettrup A (1997) PCBs, HCHs and HCB in sediments and soils of Ya-er Lake area in China: results on residual levels and correlation to the organic carbon and the particle size. Chemosphere 24: 191-202.

Zar J (1996) Biostatistical analysis. 3rd edition. Upper Saddle River, NJ: Prentice-Hall.

Zhou JL, Maskaoui K, Qiu YW, Hong HS, Wang ZD (2001) Polychlorinated biphenyl congeners and organochlorine insecticides in the water column and sediments of Daya Bay, China. Environmental Pollution 113: 373-84.

CHAPTER IV

Uptake and decontamination of PCB-153 in the edible shrimp *Palaemonetes varians*

Keywords

Palaemonetes varians • PCB-153 • Bioaccumulation • Decontamination • Food Safety • Human Health

ABSTRACT

A medium-term exposure mesocosms experiment was conducted in order to study the bioaccumulation and detoxification of a hepatoxin and carcinogenesis promoter – PCB-153 – in the edible shrimp *Palaemonetes varians*. Over the 15-day exposure period, shrimps under different contamination treatments exhibited a significant increase in CB-153 concentration comparatively to control organisms. Distinct bioaccumulation patterns and uptake rates were observed depending on the contamination treatments. For low CB-153 levels (0.25 $\mu g.L^{-1}$), accumulation followed a saturation model, reaching an apparent steady state after 15 days exposure. For intermediate (2.5 $\mu g.L^{-1}$) and high CB-153 levels (25 $\mu g.L^{-1}$), accumulation was faster and linear. In addition, the bioaccumulation rate was not proportional to the CB-153 concentration, being observed higher bioconcentration factors for intermediate contamination levels. Regarding the decontamination phase, *P. varians* lost up to 30% of the CB-153 after 72h, continuing slowly to decrease the contaminant's concentration until the end of the experiment. However, shrimps did not reach the original condition, and those exposed to moderate and high CB-153 concentrations presented inclusively contamination levels much higher than the legal limit regulating human food consumption (75 $ng.g^{-1}$ ww for Σ_6 PCB). These observations are of critical significance, representing a matter of concern to both humans and wildlife.

INTRODUCTION

Among persistent organic pollutants (POPs), polychlorinated biphenyls (PCBs) are recognized as one of the largest groups of widespread anthropogenic contaminants, comprising a total of 209 congeners with different physicochemical and toxicological properties.

By the 1970s owing to serious concerns pertaining to their human toxicity, suspected carcinogenicity and environmental persistence, several countries limited the use and production of PCBs, but it was only in 1985 that their use and marketing in the European Community were heavily restricted. Since PCBs are highly lipophilic, they can bioaccumulate in the adipose tissues of exposed organisms, being present in various compartments, both biotic (from plankton to humans) (Wang et al., 1998; Voorspoels et al., 2004; Helm et al., 2008; Croes et al., 2012) and abiotic (air, water, sediments, soil) (Howell et al., 2008; Han et al., 2010; Cachada et al., 2012). Furthermore, biomagnification through the food chain has been well documented, posing serious risks to both wildlife and humans (Borga et al., 2001; Skarphedinsdottir et al., 2010; Nunes et al., 2011).

Since the main pathway of human exposure to PCBs is through dietary intake, namely by ingestion of edible marine species (Nunes et al., 2011; Fillos et al., 2012), the consumers and the food agencies are particularly concerned about the potential health effects associated with their consumption (Bodin et al., 2007). Crustaceans are a diverse group of marine organisms with enormous importance in the food webs, tending to accumulate large amounts of lipophilic contaminants in their tissues (Voorspoels et al., 2004; Bodin et al., 2007). Within crustaceans, shrimps have singular interest since they are an important prey for both ichthyo and avifauna and represent a highly appreciated and consumed food item worldwide. The Food and Agriculture Organization estimated a global annual production of 3.4 million tons of cultivated marine shrimps commonly fed on fresh food items, farm-made and/or commercially compounded aquafeeds, valued at more than US$ 14 million (FAO, 2011). Ditch shrimp, *Palaemonetes varians* (Leach, 1814), is a small decapod caridean species commonly found in salt pounds and estuaries worldwide, demonstrating high tolerance to a broad range of temperatures and salinities (Oliphant et al., 2010). It is a species of great economic importance, used essentially as bait for fishery and for human consumption. In Portugal, its production in extensive systems reaches 200-300 $kg.ha^{-1}.yr^{-1}$ with a nearly average price of 10 $eur.kg^{-1}$.

Considering that coastal areas are subjected to great human pressure and are frequently under episodic contaminant events, it is crucial to understand the bioaccumulation trends in marine species, such as *P. varians*, and their ability to detoxify after a hypothetical industrial discharge. For this purpose, it was used the most abundant PCB congener in marine biota, shown to be a good indicator in monitoring programs – PCB-153 (2, 2′, 4, 4′, 5, 5′ - hexachlorobiphenyl) (Danis et al., 2005). It has been suggested as a congener responsible to induce neurobehavioral deficits via gestational and lactational transfer, being also known as a hepatotoxin and carcinogenesis promoter (Lee et al., 2002; Chubb et al., 2004). The selection of this specific congener lies on its highly abundance and prevalence in significant quantities in the environment and human serum and tissues (Chubb et al., 2004; Johansen et al., 2011), besides it is considered as a congener representative of all the others, constituting nearly 10% of the total amount of PCBs and about 33% of the summed six indicator non-dioxin-like PCBs (Ilyina, 2007; VKM, 2008).

Studies on PCBs uptake and depuration by edible decapod crustaceans are rather scarce or even inexistent, being mostly restricted to experiments using laboratory-contaminated sediments as a source of contamination (Rubinstein et al., 1983; Tatem, 1986; Danis et al., 2005 and references therein). In addition, few studies have taken into account essential species, upon which depends the ecosystem structure and functioning. Further investigation is required in order to estimate biomagnification through the food chain and consequently to assess the risks to human health. Therefore, the major aim of this work is to study the bioaccumulation and decontamination processes of the ditch shrimp *P. varians*, when subjected to different CB-153 concentrations through a mesocosms experiment. It is also our goal to determine if the organisms will be able to detoxify and reach the legal limits allowed for food consumption after a 15-day decontamination period.

MATERIALS AND METHODS

Biological samples collection

The organisms (*Palaemonetes varians*) and the water used in the experiment were collected in the Gala, Mondego estuary (40º08′ N, 8º50′ W), located on the Atlantic coast of Portugal (Fig. 1). Since no local source of PCB contamination is known (Nunes et al., 2011), this location was considered as a reference condition. PCB-153 baseline concentrations were vestigial, both in the water (< 5 ng.L^{-1}) as in the shrimps *P. varians* (2 ng.g^{-1} wet weight).

In the laboratory, the shrimps were measured in terms of total length and all the organisms used in the experiment ranged between 2 and 3 cm. These organisms were left in clean seawater at 20ºC during four days to acclimate and depurate. Oxic conditions were ensured by air-bubbling the water. Shrimps were fed daily with *Artemia* sp. during both acclimation, exposure and decontamination periods. Before being administrated as a food source *Artemia* sp. was previously tested for possible residues of CB-153, which were vestigial and below 0.5 ng.g^{-1} dry weight.

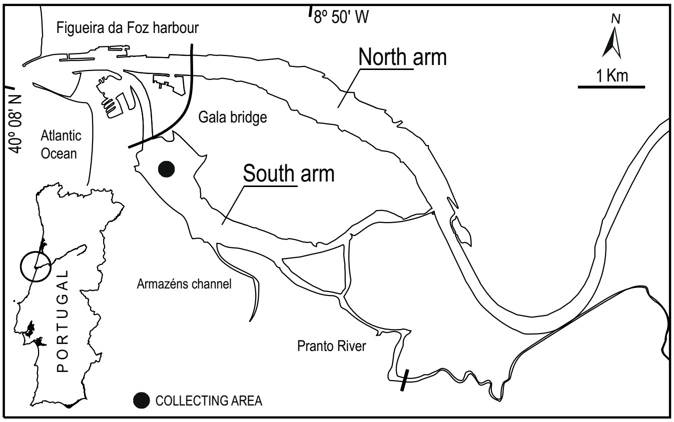

Fig. 1. The Mondego estuary and collecting location of shrimps and water.

Experimental set-up

The experiment was performed in pre-washed glass containers of 3 litres (14 cm Ø), free of any PCB contamination residues. The water collected in the Mondego Estuary (reference condition) was filtered through 0.45 μm pore size Millipore filters, in order to remove particles. Four different treatments were applied: control condition without CB-153; treatment I - low CB-153 contamination, corresponding to 0.25 $\mu g.L^{-1}$ (half the maximum limit stated by the Environmental Protection Agency for PCBs in drinking waters), treatment II - intermediate CB-153 contamination (2.5 $\mu g.L^{-1}$) and treatment III - high CB153 contamination (25 $\mu g.L^{-1}$), corresponding to a concentration 50 times higher than the legal limit allowed for drinking waters. All the solutions were prepared using a PCB-153 (2, 2′, 4, 4′, 5, 5′ – hexachlorobiphenyl) OEKANAL® standard solution (Sigma-Aldrich) diluted in acetone. Since PCBs are hydrophobic, acetone was used as a carrier solvent to facilitate their addition to seawater (Roesijadi et al., 1976).

Mesocosms were established by transferring 1 L of solution, according to the different treatments to each glass container, always maintained under oxic conditions. The glass containers were randomly distributed in an acclimated room, with temperature maintained at 20ºC and water salinity in the range of 30-32. The containers were maintained in these conditions during 24 hours to stabilize before introducing the organisms. In each of the glass containers 15 individuals of *P. varians* were introduced, according to the mean densities observed in the field (Gregg and Fleeger, 1998).

The experiment was divided in two phases: contamination and decontamination. For each of the four studied conditions (reference – non-contamination, low, intermediate and high CB-153 contamination) two different sampling times were chosen during contamination phase (t_1 – 3 days; t_2 – 15 days). At each contamination condition and sampling time, 6 replicates were carried out, which were posteriorly combined in pairs, in order to obtain 3 composite replicates (consisting of a maximum of 30 individuals each composite replicate) and consequently sufficient body mass to perform subsequent PCB quantification. In parallel, other 48 flasks, each one with 15 individuals, were maintained under the same conditions of CB-153 accumulation and used afterwards for the decontamination study. Therefore, the experimental setup (contamination and depuration) included 96 (6x4x4) containers (sub-experiments). After 15 days, the organisms were transferred from the contaminated containers to others filled with clean seawater, free of contamination residues. During this phase two sampling times were chosen (t_1 – 3 days; t_2 – 15 days). Over the entire experimental period (15 days of contamination + 15 days of decontamination), the medium of each flask was totally renewed twice a week, to avoid re-contamination by the faeces.

At the pre-defined sampling times, the organisms were removed from the glass containers, rinsed with estuarine water and placed in constantly aerated clean estuarine water at 20°C for 24 hours to allow depuration (to remove pseudo-faecal and faecal material from the digestive tract) (Blust et al., 1992; Cardoso et al., 2013 and references therein). Afterwards, the organisms were freeze-dried for later CB-153 analysis and quantification.

CB-153 quantification in the flasks water was performed regularly to ensure that concentration remained constant throughout the experiment. Any decrease of CB-153 concentration due to adsorption and absorption mechanisms was compensated by addition of CB-153 solution, in accordance with other studies (Cardoso et al., 2013 and references therein).

CB-153 extraction and quantification

Water samples (aliquots of 100 mL) spiked with CB-209 were extracted by liquid-liquid extraction with hexane following the USEPA Method 3510C. The three organic extracts of each sample were combined, dried with anhydrous sodium sulfate, followed by evaporation to dryness using rotary evaporator and a stream of nitrogen. The extract was stored at -20ºC and at the moment of instrumental analysis, internal standard (CB131) was added to the extract and reconstituted to 200 µL of iso-octane. Shrimp samples were extracted by sonication (Selecta®) with a *n*-hexane:acetone (1:1) mixture (30 mL), for 20 minutes. Extract was decanted and the process was repeated three times. Solvent volume was reduced by using a rotary evaporator. Lipid content was gravimetrically determined using 10% of the extract. A soft stream of nitrogen was used to reduce solvent volume by evaporation. Lipids were removed with sulphuric acid (97%). Afterwards, the extract passed through a multilayered column packed with florisil (1g of Supelclean® Florisil) and anhydrous sodium sulphate and eluted with 15 mL of hexane. All samples were dried, under a gentle stream of nitrogen, before instrumental analysis internal standard (CB131) was added and solvent exchanged to iso-octane before analysis.

The quantification was performed using a gas chromatographic system (Shimadzu Corporation GC/MS-QP5050A) equipped with a ZB5-MS column (30 m × 0.25 mm i.d., 0.25 µm film thickness), a mass spectrometry detector using electron impact ionization and selected ion monitoring (SIM) acquisition. Helium was used as carrier gas (constant flow-rate of 0.9 ml/min). The PCBs were separated with the following GC oven temperature program: 2 min at 40ºC, first ramp at 10°C $\min^{-1}$ to 180°C, second ramp at 6 °C $\min^{-1}$ to 310°C (held for 10 min). The injector was held at 280°C and the interface at 310°C. Detector acquisition was programmed to monitor m/z 360 for quantification of CB153 and the internal standard and m/z 362 for confirmation.

Quality assurance and quality control (QA/QC) procedures

The method performance was assessed by daily check of calibration curves, method blanks, fortification of samples and recoveries of surrogate. Method blanks were done in every forth sample in order to detect possible interferences from the reagents, glassware and other processing hardware. Recoveries of CB-209 in water samples were 78 ± 6 % (95% confidence interval) and the method performance was also assessed by the recoveries of CB-153 in fortification experiments, which were 85 ± 5 % (95% confidence interval). For tissue samples, recoveries obtained for the NIST certified material SRM 2977 (mussel tissue) were 81 ± 7 % (95% confidence interval).

Data analysis

Uptake kinetics were expressed in terms of change of CB-153 concentration over time. Variations were described using either a simple linear regression model [Eq. (1)] or by a simple first order exponential kinetic model [Eq. (2)] when observed a pattern tended to reach a steady-state:

$$C_t = k_u t \quad (1)$$

$$C_t = C_{ss} - (C_{ss} - C_0)*\exp(-k_u t) \quad (2)$$

where C_t and C_{ss} are the concentrations at time t (d) and at steady-state, respectively; k_u is the uptake rate constant (d^{-1}); C_0 is the concentration at time 0 (Cardoso et al., 2013 and references therein).

For treatment I, a biological half-life (the time it takes to reach half of the equilibrium value) was calculated ($T_{b1/2}$) from the corresponding constant uptake rate constant (k_u), according to the relation $T_{b1/2} = \ln 2/k_u$.

To infer differences between treatments, all data were initially checked for normality using the Kolmogorov–Smirnov test and for homogeneity of variances using the Levene's test (Zar, 1996). However, whenever data did not meet these criteria, even after transformation, non-parametric tests were applied. It was used the Scheirer Ray-Hare test (equivalent to two-factor ANOVA (time x treatments)) in order to determine differences in CB-153 concentrations between treatments and sampling times (Dytham, 2003). Differences in shrimps' survival among treatments were investigated through a parametric one-way ANOVA test. Statistical analyses were performed using the SigmaPlot 11.0 software package and the IBM SPSS Statistics 20.0.

Additionally, bioconcentration factors (BCF_{15d}) were determined dividing the total accumulated concentration of CB-153 in the shrimps by the concentration of that contaminant in the water.

RESULTS

CB-153 bioaccumulation and decontamination rates

Over the entire exposure period, shrimps under different contamination treatments exhibited an increased CB-153 concentration comparatively to control organisms, which maintained vestigial concentrations all over the time (Fig. 2). However, distinct accumulation patterns were observed regarding contamination treatments. Organisms subjected to low CB-153 contamination were best fitted using a first order kinetic model (Fig. 2A), while shrimps experiencing intermediate and high CB-153 levels were best fitted using simple linear regressions (Fig. 2B, C). Uptake rate constants (k_u) increased according with the CB-153 contamination, being around 100 times faster in the organisms exposed to treatment II than in the shrimps under treatment I (Table 1). In addition, shortly before 2 days exposure, shrimps subjected to treatment I reached half of the equilibrium concentration (Table 1). Significant differences in CB-153 concentrations were found between contamination treatments and the controls, as well as between sampling times (Table 2).

For all the treatments, a strong CB-153 accumulation was observed in the first 3 days exposure (treatment I: 800%; treatment II: 6,000%; treatment III: 45,500%). During the following 12 days exposure, the accumulation corresponded to values around 30% to the treatment I and 500% for both treatments II and III. After 15 days of CB-153 exposure, concentration factors (CFs) were highest for intermediate concentrations (2.5 μg.L^{-1}) and minimum for lower contamination levels (0.25 μg.L^{-1}) (Table 3).

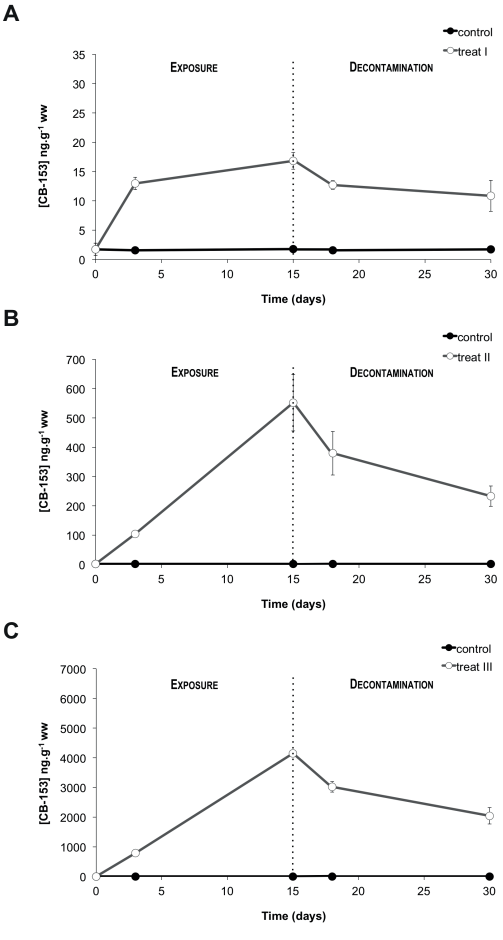

Fig. 2. Uptake and decontamination kinetics of the shrimp *Palaemonetes varians* under CB-153 exposure (mean ± SE): (A) Treatment I; (B) Treatment II; and (C) Treatment III.

During the decontamination phase, evident declines in CB-153 concentrations were observed after 72 h, for all the contamination treatments (Fig. 2). This loss was identical in the shrimps exposed to the treatments I and III (25%)

and slightly higher in the organisms subjected to intermediate CB-153 levels (30%). Until the end of the experiment, independently of the contamination treatments at which the shrimps were subjected, they still continued to lower the CB-153 previously incorporated. Nevertheless, the initial concentration values were never reached. Thus, even after 15 days in clean seawater, the organisms exposed to different contamination treatments still retained substantial CB-153 levels compared with controls and inclusively higher than those allowed by the European legislation (Regulation No 1259/2011) for muscle meat of fish and fishery products (75 ng.g^{-1} wet weight).

Table 1. Estimated uptake kinetic parameters for the shrimp *P. varians*, exposed for 15 days to CB-153 in seawater. Css: concentration at steady state; k_u: uptake rate constant (d^{-1}); R^2: determination coefficient; and $T_{b1/2}$: biological half-life (d).

	C_{ss}	K_u	R^2	$T_{b1/2}$
Treat. I	16.90	0.36	0.99	1.94
Treat. II	-	36.84	0.99	-
Treat. III	-	277.01	0.99	-

Table 2. Statistical results of the Scheirer Ray–Hare test.

	SS	SS/MS_{total}	df	*P*-value
A	1919.7	12.7	4	*
B	2942.4	19.5	3	***
A x B	775.3	5.1	12	ns

Factor A – time, factor B – treatments, AxB – interaction
ns – not significant
* $p < 0.05$
*** $p < 0.001$

Table 3. Bioconcentration factors (BCF_{15d}) of *P. varians* in the three contaminated treatments considering 15 days exposure. BCF – ratio between CB-153 concentration in the organism/water. Values are the mean of three replicates with standard error in brackets.

Treat. I (0.25 μg.L^{-1})	Treat. II (2.5 μg.L^{-1})	Treat. III (25 μg.L^{-1})
67.3 (± 4.9)	220.6 (± 38.4)	165.7 (± 1.6)

Shrimp survival rates

Survival decreased during CB-153 exposure in all the treatments (Fig. 3). After being transferred to clean seawater, survival at day 18 was identical to the observed at day 15 and slightly higher than the registered at the end of the experiment. Overall survival was around 80% in both control and CB-153 treatments and no significant differences were observed between them (one-way ANOVA, $p > 0.05$). Hence, shrimps mortality seemed to be not connected with CB-153 toxicity.

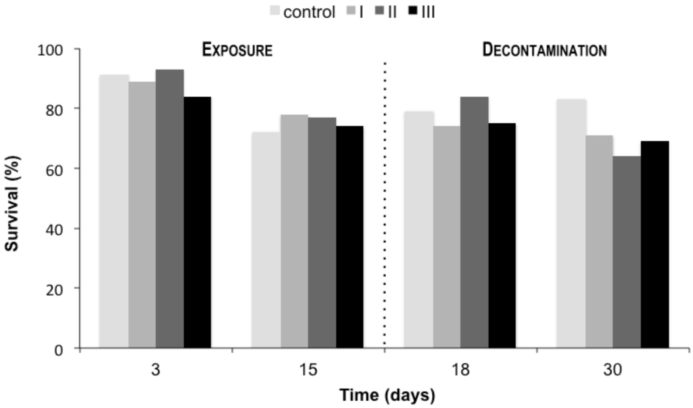

Fig. 3. Survival rates of *P. varians* under different CB-153 treatments (control, treatment I, treatment II, treatment III).

DISCUSSION

Toxic effects inherent to direct exposure to PCBs or indirectly by ingestion of contaminated food constitute an important concerning issue for both humans and wildlife. In fact, several studies demonstrated that exposure to PCBs may interfere with normal physiology and biochemistry of the organisms resulting in numerous adverse health alterations, namely skin lesions, neuro and immunotoxicity, endocrine disruption, chronic reproductive effects, with an increasing risk of infertility, and carcinogenicity (Çok and Şartiroğlu, 2004; Jaikanlaya et al., 2009; Meeker and Hauser, 2010). Thus, it is crucial to understand the mechanisms underlying bioaccumulation and detoxification of the marine fauna, particularly of species playing key role in the ecosystems' structure and functioning and simultaneously with great socio-economic interest, like the edible shrimp *P. varians*. This species lives in close association with aquatic plants and occasionally browses on the substrate surface. Although, it is more representative of the water column habitat, being primarily susceptible to water-mediated uptake (Rubinstein et al., 1983).

Grass shrimp *Palaemonetes* spp. is considered a good estuarine bioindicator of anthropogenic impacts (Key et al., 2006), being more sensitive to heavy metals compared to estuarine fish (Downs et al., 2001). Heavy metals are usually stored in detoxified form, being therefore unavailable to interact with sites in metabolism where they might exert a toxic effect (Rainbow and Luoma, 2011). In contrast to heavy metals, the toxicity of organic contaminants to the accumulating organisms is typically directly related to the total accumulated concentration (Rainbow and Luoma, 2011). This in turn closely associated with the local bioavailability of that contaminant (McLeese et al., 1980).

The present study revealed that ditch shrimp *P. varians* exhibited different kinetics of accumulation depending on the concentration of CB-153. For intermediate and higher CB-153 levels, shrimps presented a linear accumulation kinetics, while for low CB-153 contamination the organisms followed a saturation model. However, independently of the contamination treatments at which the shrimps were subjected, significant accumulations occurred during the first 72h of exposure. Shrimps experiencing low CB-153 levels demonstrated a slower constant uptake rate, reaching an apparent steady state after 15 days exposure, contrarily to organisms exposed to intermediate and high CB-153 concentrations.

Comparisons with other studies focusing on accumulation of PCBs on marine and freshwater fauna are normally difficult, since they are relatively scarce plus the contaminants (Aroclors mixtures and/or different individual congeners), their intrinsic toxicity and the sources of contamination are in most cases very distinct. Nevertheless, a similar behavior to the observed in the present study was already demonstrated in the grass shrimp *Palaemonetes pugio* when exposed to different water contaminated treatments (Nimmo et al., 1974). For lower concentrations of Aroclor 1254 (0.09 $\mu g.L^{-1}$), accumulation became more evident on the third day, appearing to stabilize after 21 days of exposure. On the other hand, for a higher concentration of Aroclor 1254 (0.62 $\mu g.L^{-1}$), accumulation began earlier, only after 8h exposure, and tended to follow a linear model. This indicates that somewhere in the range of 0.09-0.62 $\mu g.L^{-1}$, accumulation of Aroclor 1254 follow a more linear pattern instead of a saturation model. The same analogy may be transposed to our results.

In addition, observations reported by Tatem (1986), investigating bioaccumulation of PCBs from contaminated sediments by freshwater Palaemonidae prawn *Macrobrachium rosenbergii*, also corroborate our results. When exposed to sediments containing low concentrations of Aroclor 1254, *M. rosenbergii* presented an accumulation described by a first order kinetic model, reaching a steady state after 16 days of exposure. Moreover, the uptake rate (k_u) from a mixture of 10% contaminated sediment and 90% of clean sand was identical to the obtained in the present study, for the lowest contamination treatment.

On the contrary, Rubinstein et al. (1983) reported discrepant results from those described above. By using four different harbor PCB contaminated sediments, in which *P. pugio* was exposed for 58 days, Rubinstein et al. (1983) observed that accumulation in shrimps did not show a well-defined pattern, varying erratically, independently of the sediment at which they were subjected. Also, organisms under a treatment containing 10-fold higher PCBs than other, reached similar maximum concentrations on their tissues after approximately 26-days exposure. From that time onwards, PCBs concentration decreased for all treatments, possibly indicating a detoxification mechanism (Rubinstein et al., 1983).

Bioconcentration factors suggest that CB-153 accumulation after a 15-day period is not proportional for all levels of contamination, since shrimps exposed to treatment II, which corresponds to a concentration 10 times higher than those of treatment I, reached accumulations 30 times higher than those of treatment I. Likewise, organisms exposed to treatment III, corresponding to a concentration 10-fold higher than the treatment II and 100 times higher than the treatment I, achieved accumulations of 7.5 and 250 times higher than those of treatments II and I, respectively.

Apparently, none of the CB-153 concentrations was lethal for the shrimps. However, increases in respiration of shrimps exposed to the CB-153 might have occurred, since toxic stress is almost instantaneous, even though the PCBs concentration was very low (Reyes et al., 2003). Several authors have suggested that shrimps showed great susceptibility to PCBs, especially during moult (Duke et al., 1970; Nimmo et al., 1971). The concentration of a chemical in air or water, which is expected to cause death in 50% of the test animals in a given time period is known as LC_{50}. This measure has been estimated for several shrimp species to assess their response to a wide range of contaminants. For example, LC_{50} of Aroclor 1254 for *Palaemon adspersus* is 1 $mg.L^{-1}$ (Reyes et al, 2003) while for the shrimp *P. kadiakensis* is considerably smaller, only 3 $\mu g.L^{-1}$. Furthermore, a mortality of 51% was observed in pink shrimps (*Penaeus duorarum*) within 15 days of continuously exposure to 0.94 µg.L^{-1} of Aroclor 1254 (Stalling and Mayer, 1972). This means that response to Aroclor 1254 may be species specific and reliant on the physiological susceptibility of each species. With this respect, the behavior demonstrated by *P. varians*, when subjected to different CB-153 levels, appears to be more similar to the one observed in *P. adpsersus*, since they can tolerate higher concentration ranges.

Regarding the decontamination period, shrimps transferred to clean seawater began immediately to lose CB-153, as observed by Tatem (1986). Similarly to the verified in the bivalve *Cerastoderma edule* concerning mercury decontamination (Cardoso et al., 2013), this decline was faster in the first 3 days, especially for treatment II. Overall, after 15 days of decontamination, shrimps lost around 35%, 60% and 50% of the CB-153 previously accumulated, corresponding to treatments I, II and III respectively. Thus, higher losses were recorded in shrimps under intermediate contamination levels, which is consistent with results of Tatem (1986) for a depuration period slightly longer (20 days) and for both Aroclors 1242 and 1254. In opposition, Nimmo et al. (1974) reported higher losses of Aroclor 1254 in *P. pugio* after 14 days of decontamination (50-80%).

Despite great declines in CB-153, it is important to highlight that even after 15 days in clean seawater, the organisms were not able to recover completely, presenting at the end of the experiment levels comparable to those after 3 days exposure to the contaminant. According to Bodin et al. (2007) the high levels and prevalence of PCBs in crustacean tissues reflect their low rates of biotransformation, and particularly the inability of these organisms to metabolize congeners with chlorine atoms in positions 2, 4, and 5 in one (CB-118 and 138) or both rings (CB-153 and 180). Even after decontamination, shrimps that were exposed to a minimum CB-153 concentration of 0.25 µg.L^{-1}, which represent half the maximum limit allowed for PCB congeners in drinking waters, still maintained 11 $ng.g^{-1}$ ww in their tissues.

The Norwegian Scientific Committee for Food Safety stated that CB-153 corresponds roughly to 1/3 of the summed six indicator congeners (Σ_6 PCB) (VKM, 2008). On the other hand, Σ_6 PCB comprises about 50% of the amount of total non-dioxin-like PCBs (ndl-PCBs) (Σ_{197} PCB) present in food (EFSA, 2010). Taking this into account the organisms accumulated about 33 $ng.g^{-1}$ ww and 66 $ng.g^{-1}$ ww, extrapolating directly as Σ_6 PCB and Σ_{197} PCB, respectively. This evidence is of critical significance, since exposure of shrimps to half the PCBs concentration allowed for drinking waters, determined an accumulation of approximately 50% the maximum limit of Σ_6 PCB established by the European Union (Regulation No 1259/2011) for muscle meat of fish and fishery products (75 $ng.g^{-1}$ ww). Similarly, for intermediate and high levels of CB-153, organisms largely exceeded that limit, incorporating around 700 / 1,400 $ng.g^{-1}$ ww and 6,135 / 12,270 $ng.g^{-1}$ ww, by direct conversion as Σ_6 PCB and Σ_{197} PCB, respectively. Based on these formulations and knowing that accumulation of CB-153 was not proportional, it is reasonable to infer that for a CB-153 concentration equal to the threshold for drinking waters, shrimps will likely exceed the maximum limit of Σ_6 PCB imposed for human consumption. Nevertheless, there is no consensus among European and United States agencies relatively to regulations of PCBs in food. For instance, according to the United States Food and Drug Administration (US-FDA) only concentrations achieved by shrimps under high CB-153 levels may be harmful to human health, by far exceeding tolerance level of 2000 $ng.g^{-1}$ for PCBs in shellfish.

These findings constitute a great matter of concern, since most organisms did not have the ability to return to safety values (for food consumption). Facing a hypothetic PCBs leakage, here simulated by the intermediate and high CB-153 treatments, the consequences for the ecosystem and ultimately to human health might be dangerous and the recovery of the habitat would be compromised.

Further research on the transfer of PCBs from abiotic compartments (e.g. water and sediments) to marine edible species is needed, in order to estimate levels of contaminants that will accumulate in their predators and consequently to assess their impact on the biomagnification process through the food webs.

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REFERENCES

Blust R, Kockelbergh E, Baillieul M (1992) Effect of salinity on the uptake of cadmium by the brine shrimp *Artemia franciscana*. Marime Ecology Progress Series 84:245-254.

Bodin N, Abarnou A, Le Guellec AM, Loizeau V, Philippon X (2007) Organochlorinated contaminants in decapod crustaceans from the coasts of Brittany and Normandy (France). Chemosphere 67: S36-S47.

Borga K, Gabrielsen GW, Skaare JU (2001) Biomagnification of organochlorines along a Barents Sea food chain. Environmental Pollution 113: 187-198.

Brown-Peterson N, Manning C, Patel V, Denslow N, Brouwer M (2008) Effects of cyclic hypoxia on gene expression and reproduction in a grass shrimp, *Palaemonetes pugio*. The Biological Bulletin 214: 6-16.

Cachada A., Pato P., Rocha-Santos T., Ferreira da Silva E., Duarte A.C., 2012. Levels, sources and potential human health risks of organic pollutants in urban soils. Science of the Total Environment 430: 184-192.

Cardoso PG, Grilo TF, Pereira E, Duarte AC, Pardal MA (2013) Mercury bioaccumulation and decontamination kinetics in the edible cockle *Cerastoderma edule*. Chemosphere 90:1854-1859.

Chubb LS, Andersen ME, Broccardo CJ, Billings RE, Dean CE, Hanneman WH (2004) Regional induction of CYP1A1 in rat liver following treatment with mixtures of PCB 126 and PCB 153. Toxicologic Pathology 32: 467-73.

Çok I, Şartiroğlu MH (2004) Polychlorinated biphenyl levels in adipose tissue of primiparous women in Turkey. Environment International 30: 7-10.

Commission Regulation (EU) No 1259/2011 of 2 December 2011 amending Regulation (EC) No 1881/2006 as regards maximum levels for dioxins, dioxin-like PCBs. (http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2011:320:0018:0023:EN:PDF).

Croes K, Colles A, Koppen G, Govarts E, Bruckers L, Van de Mieroop E, Nelen V, Covaci A, Dirtu AC, Thomsen C, Haug LS, Becher G, Mampaey M, Schoeters G, Van Larebeke N, Baeyens W (2012) Persistent organic pollutants (POPs) in human milk: A biomonitoring study in rural areas of Flanders (Belgium). Chemosphere 89: 988-994.

Danis B, Cotret O, Teyssié JL, Bustamante P, Fowler SW, Warnau M (2005) Bioaccumulation of PCBs in the sea urchin *Paracentrotus lividus*: seawater and food exposures to a ^{14}C-radiolabelled congener (PCB 153). Environmental Pollution 135: 11-16.

Downs C, Fauth JE, Woodley CM (2001) Assessing the health of grass shrimp (*Palaeomonetes pugio*) exposed to natural and anthropogenic stressors: a molecular biomarker system. Marine Biotechnology 3: 380-397.

Duke TW, Lowe JI, Wilson AJ (1970) A polychlorinated biphenyl (Aroclor 1254) in the water, sediment, and biota of Escambia Bay, Florida. Bulletin of Environmental Contamination and Toxicology 5: 171-180.

Dytham C (2003) Choosing and Using Statistics. A Biologist's Guide. Blackwell Publishing.

European Food Safety Authority (EFSA) Journal 2010; 8 (7): 1701, http://www.efsa.europa.eu/en/efsajournal/doc/1701.pdf.

Fillos D, Scott LL, De Sylor MA, Grespin M, Luksemburg WJ, Finley B (2012) PCB concentrations in shrimp from major import markets and the United States. Environmental Toxicology and Chemistry 31: 1063-71.

Food And Agriculture Organization Of The United Nations (FAO) (2011) Tacon A. G.J., Hasan, M.R., Metian, M. Demand and supply of feed ingredients for farmed fish and crustaceans. Trends and prospects. FAO Fisheries And Aquaculture Technical Paper, 102 pp.

Gregg CS, Fleeger JW (1998) Grass shrimp *Palaemonetes pugio* predation on sediment- and stem-dwelling meiofauna: field and laboratory experiments. Marine Ecology Progress Series 175: 77-86.

Han W, Feng J, Gu Z, Wu M, Sheng G, Fu J (2010) Polychlorinated biphenyls in the atmosphere of Taizhou, a major e-waste dismantling area in China. Journal of Environmental Sciences 22: 589-597.

Helm PA, Gewurtz SB, Whittle DM, Marvin CH, Fisk AT, Tomy GT (2008) Occurrence and biomagnification of polychlorinated naphthalenes and nonand mono-ortho PCBs in Lake Ontario sediment and biota. Environmental Science & Technology 42: 1024-1031.

Howell NL, Suarez MP, Rifai HS, Koenig L (2008) Concentrations of polychlorinated biphenyls (PCBs) in water, sediment, and aquatic biota in the Houston Ship Channel, Texas. Chemosphere 70: 593-606.

Ilyina T (2007) Modelling the fate of persistent toxic substances in the North Sea: γ -HCH and PCB 153 multiyear simulations. Pollution Of The Sea - Prevention And Compensation Hamburg Studies on Maritime Affairs 10: 15-22.

Jaikanlaya C, Settachan D, Denison MS, Ruchirawat M, van den Berg M (2009) PCBs contamination in seafood species at the Eastern Coast of Thailand. Chemosphere 76: 239-249.

Johansen EB, Knoff M, Fonnum F, Lausund P, Walaas SI, Wøien G, Sagvolden T (2011) Postnatal exposure to PCB 153 and PCB 180, but not to PCB 52, produces changes in measures of attention, activity level and impulsivity in outbred male Wistar Kyoto rats. Behavioral and Brain Functions 7:18.

Key PB, Wirth EF, Fulton MH, (2006) A review of grass shrimp, *Palaemonetes* spp., as a bioindicator of anthropogenic impacts. Environmental Bioindicators 1: 115-128.

Lee SK, Ou YC, Yang RSH (2002) Comparison of pharmacokinetic interactions and physiologically based pharmacokinetic modeling of PCB 153 and PCB 126 in non pregnant mice, lactating mice, and suckling pups. Toxicological Sciences 65: 26-34.

McLeese DW, Metcalfe CD, Pezzack DS (1980) Uptake of PCBs from sediment by *Nereis virens* and *Crangon septemspinosa*. Archives of Environmental Contamination and Toxicology 9: 507-518.

Meeker JD, Hauser R (2010) Exposure to polychlorinated biphenyls (PCBs) and male reproduction. Systems Biology in Reproductive Medicine 56: 122-131.

Nimmo DR, Blackman RR, Wilson AJ, Forester J (1971) Toxicity and distribution of Aroclor 1254 in the pink shrim *Penaeus duorarum.* Marine Biology 11: 191-197.

Nimmo DR, Forester J, Heitmuller PT, Cook GH (1974) Accumulation of Aroclor 1254 in grass shrimp *(Palaemonetes pugio)* in laboratory and field exposures. Bulletin of Environmental Contamination and Toxicology 11: 303-308.

Nunes M, Marchand P, Vernisseau A, Le Bizec B, Ramos F, Pardal MA (2011) PCDD/Fs and dioxin-like PCBs in sediment and biota from the Mondego estuary (Portugal). Chemosphere 83: 1345-1352.

Oliphant A, Thatje S, Brown A, Morini M, Ravaux J, Shillito B (2011) Pressure tolerance of the shallow-water caridean shrimp Palaemonetes varians across its thermal tolerance window. The Journal of Experimental Biology 214: 1109-1117.

Rainbow PS, Luoma SN (2011) Metal toxicity, uptake and bioaccumulation in aquatic invertebrates – Modelling zinc in crustaceans. Aquatic Toxicology 105: 455-465.

Reyes GG, Verdugo JM, Cassin D, Carvajal R (2003) Pollution by polychlorinated biphenyls in an estuaryof the Gulf of California. Their toxicity and bioaccumulation in shrimp *Litopenaeus vannamei*. Marine Pollution Bulletin 46: 959-963.

Roesijadi G, Anderson JW, Glam CS (1976) Osmoregulation of the grass shrimp *Palaemonetes pugio* exposed to polychlorinated biphenyls (PCBs). II. Effect on free amino acids of muscle tissue. Marine Biology 38: 357-363.

Rubinstein NI, Lores E, Gregory NR (1983) Accumulation of *PCBs,* mercury, and cadmium by *Nereis virens, Mercenaria mercenaria,* and *Palaemonetes pugio* from contaminated harbor sediments. Aquatic Toxicology 3: 249-260.

Skarphedinsdottir H, Gunnarsson K, Gudmundsson GA, Nfon E (2010) Bioaccumulation and biomagnification of organochlorines in a marine food web at a pristine site in Iceland. Archives of Environmental Contamination and Toxicology 58: 800-809.

Stalling DL, Mayer FL Jr. (1972) Toxicities of PCBs to fish and environmental residues. Environmental Health Perspectives, April, 159-164.

Tatem HE (1986) Bioaccumulation of polychlorinated biphenyls and metals from contaminated sediment by fresh-water prawns, *Macrobrachium rosenbergii* and clams, *Corbicula fluminea*. Archives of Environmental Contamination and Toxicology 15: 171-183.

United States Environmental Protection Agency (USEPA). EPA Method 3510C: Separatory Funnel Liquid-Liquid Extraction. Report Number: SW-846 Ch 4.2.1. Available from: http://www.caslab.com/EPA-Methods/PDF/EPA-Method-3510C.pdf.

VKM (2008) Opinion of the Panel on Contaminants of the Norwegian Scientific Committee for Food Safety. Risk assessment of non dioxin-like PCBs in Norwegian food. Available from: http://www.vkm.no/dav/c29e178d9c.pdf.

Voorspoels S, Covaci A, Maervoet J, De Meester I, Schepens P (2004) Levels and profiles of PCBs and OCPs in marine benthic species from the Belgian North Sea and the Western Scheldt Estuary. Marine Pollution Bulletin 49: 393-404.

Wang JS, Chou HN, Fan JJ, Chen CM (1998) Uptake and transfer of high PCB concentrations from phytoplankton to aquatic biota. Chemosphere 36: 1201-1210.

Zar J (1996) Biostatistical analysis. 3rd edition. Upper Saddle River, NJ: Prentice-Hall. 662 pp.

Eutrophication and environmental quality degradation (1993-1996)

As ecotone areas between marine and freshwater habitats, estuaries are amongst the highest productive and dynamic environments on Earth, being characterized by salinity and nutrient gradients, which are important in regulating most ecological and biological processes occurring in these unique habitats (McLusky and Elliott 2004; Telesh al., 2010). They are supported by the flow of energy from primary producers at the base of the trophic webs through to intermediate consumers, top predators (including humans), and microorganisms, and then return again through decomposition and detrital pathways (Doney et al., 2012). The constantly changing mixture of salt and freshwater presents challenges to the physiology of the species that rely on this environment, which have to develop adaptation strategies to better resist to natural fluctuations typical of these systems (Mclusky and Elliott, 2004). Thus, estuarine communities represent biological networks in which the success of species depends direct or indirectly on various biological interactions (e.g. predator-prey relationships, competition, facilitation, mutualism). The combined effect of these interactions constitutes ecosystem function, through which coastal environments provide free natural benefits that society depends upon (Mclusky and Elliott, 2004; Wilson and Farber, 2009; Doney et al., 2012). Nevertheless, growing human pressures and natural variables are having profound consequences on estuarine ecosystems, including nutrient enrichment (eutrophication), habitat loss, overexploitation and chemical pollution. All these major threats can impact natural resources through single, cumulative or synergistic processes (Kennish, 2002; Smith, 2003; Mclusky and Elliott, 2004; Adams 2005; Dauvin, 2008; Moon et al., 2009).

Face to ongoing environmental quality deterioration of coastal systems worldwide, an increasing need of restoration programs and conservation directives began to be urgent (Teal and Peterson, 2005; Ducrotoy and Dauvin, 2008; Marquiegui and Aguirrezabalaga, 2009). The main purpose of these programs is to remedy long-standing adverse effects of human activities on estuarine ecosystems and thus to return these habitats to a previously existing natural condition. However, a return to the exact pre-existing condition is rarely achieved, particularly as the original state may be unknown (Elliott et al., 2007). In this respect, Doody (2003) advocate that habitat restoration may simply be a form of attenuating losses caused by human activity, suggesting that is not necessarily required to know the original condition of the natural habitat but only to distinguish which type of habitat was there (e.g. seagrass beds, saltmarshes), and to return it to the same general habitat type. Depending on the complexity and degree of modification, a habitat may be restored in a short or long-term scale (Elliott et al., 2007). Typically, the real effects of restoration projects can only be noticed and accurately assessed through time. Long-term monitoring plays an important role in this scenario, since it is determinant for a better understanding about ecological phenomena.

The Mondego estuary has a considerable long-term database on its macrobenthic community dynamics, which was explored in this thesis over a 17-year period. This enabled the study of at least two distinct major impacts of different origins occurring in this system: eutrophication, with subsequent seagrass *Z. noltii* decline, plus atypical climate-related episodes, which will be debated in the next topic of discussion.

Since the mid 1980s until the late 1990s, eutrophication led to strong environmental quality degradation of the Mondego's south arm (Cardoso et al., 2002, 2004; Dolbeth et al., 2003; Verdelhos et al., 2005). The occurrence of opportunistic green macroalgal blooms contributed to the total disappearance of rooted macrophytes in the inner areas and to a rapid decline of downstream seagrass beds. In a short-term, macroalgae may have a positive effect on macrofauna, by increasing habitat heterogeneity and thus providing temporary habitats (Norkko et al., 2000; Salovius et al., 2005; Dolbeth et al., 2007; Grilo et al., 2012). Nevertheless, these benefits are quite ephemeral, as the long-term effects necessarily imply negative consequences. According to Sfriso et al. (2001), the presence of high *Ulva* spp. biomass creates very unstable and unsuitable conditions leading to subsequent anoxic crises that strongly reduce biodiversity and biomass of macrofaunal communities. In the Mondego estuary, macroalgae stimulated temporarily macrobenthic community abundance, biomass and production. Attracted by plentiful food resources and suitable protection provided by the green macroalgae amphipod populations (e.g. *Ampithoe valida*, *Melita palmata*) exhibited highest abundance, biomass and production records during the influence of the macroalgal bloom (Grilo et al., 2009), likewise the population of the gastropod *H. ulvae* in the sandflat area (Grilo et al., 2012). This is indicative about the opportunistic feeding behavior of these species. Their spatial distribution may vary as a function of environmental changes, being able to actively disperse in the water column to exploit alternative habitats and new fresh patches of food (Norkko et al., 2000; Pardal et al., 2000; Grilo et al., 2009; 2012).

Following algal decomposition and hypoxia, clear changes were observed in the overall macrobenthic community (Cardoso et al., 2004, 2008b; 2010; Dolbeth et al., 2007, 2011; Grilo et al., 2011), consistently with other documented eutrophication scenarios (Bachelet et al., 2000; Karlson et al., 2002; Kemp et al., 2005). Total abundance, secondary production and species richness decreased remarkably from downstream to upstream areas, promoting a progressive impoverishment of the inner region of the Mondego estuary (Cardoso et al. 2008b; Dolbeth et. 2011; Grilo et al., 2011). Moreover, concerning important key species, such as the gastropod *H. ulvae*, a dramatic reduction in terms of abundance, biomass and production was observed (Cardoso et al., 2005, 2008b; Grilo et al., 2012).

Considering the trophic structure, a replacement of surface-deposit feeders by sub-surface deposit feeders was evident in the *Z. noltii* bed. The main changes in taxonomic composition occurred within oligochaetes, which became dominant over polychaetes in the seagrass beds, concurrently to increasing crustaceans and polychaetes dominance in detriment of bivalves in the sandflat area (Grilo et al., 2011). These were the main alterations occurred in the system during the pre-restoration period (1993-1996).

Estuary recovery and climate variability impacts (1999-2008)

On small systems, like the Mondego estuary, keystone species and ecological engineers (structural species) play a central role in effecting restoration (Fonseca et al., 2002; Hawkins, 2004). Implementation of restoration measures, in 1998, seemed to be partially effective (Lillebø et al. 2005; Cardoso et al., 2007; Dolbeth et al, 2007), resulting in the improvement of water quality (e.g. lower turbidity, nutrient dilution and reduced residence time), absence of macroalgal blooms and gradual recovery of *Z. noltii* in downstream areas, both in biomass and areal extent. Such overall environmental quality improvement had also positive repercussions in the macrobenthic communities, which demonstrated a recovery tendency in terms of abundance, biomass, production and species richness immediately after restoration. Moreover, a faster recovery of the total biomass compared to community abundance was observed. Species like the bivalve *S. plana* (Verdelhos et al., 2005) and the polychaete *Hediste diversicolor* (Cardoso et al., 2007, 2010) reached the highest biomasses in the post-restoration period, which means that invested more energy in their growth. This suggests that as the community recovered, opportunistic species with an *r*-strategy (e.g. *Capitella capitata*, *Alkmaria romijni*) have been replaced by larger slow growing species with a *K*-growth strategy (Cardoso et al., 2007, 2010). Despite this, macroinvertebrate assemblages were still dominated by deposit-feeding species (Grilo et al., 2011), indicative that detritus are a basal resource in the estuary, as also suggested by Baeta et al. (2009).

Such recovery seemed to be consecutively interrupted by a series of atypical climate events, retarding the estuarine system's return to its original condition.

The available climate data revealed an increasing frequency and severity of stochastic weather episodes such as flooding events, heat waves and prolonged droughts, particularly over the last 13 years for mainland Portugal (Santos et al., 2002; Cardoso et al., 2008a,b). Among different climate episodes affecting the region, the largest floods (2000/01) and the prolonged drought (2004/05) were apparently the most negative ones impacting macrobenthic assemblages in the *Z. noltii* beds. Contrarily, macrofaunal communities of the mud- and sandflat areas were mostly affected by the heat wave (2003).

The resilience of macrobenthic organisms to environmental change is manifested in their ability to withstand or adapt to changes in abiotic components (Cyrus et al., 2011). Disturbances, particularly flooding, have an important role as regulators of macroinvertebrates distribution, abundance and coexistence (Miserendino, 2009). Moreover, distinct groups of organisms and habitats may respond differently to flooding events, depending on the ecology and feeding habits of the species (Cardoso et al., 2008a). For instance, floods may favour communities dominated by opportunistic or tolerant species (Salen-Picard et al., 2003). On the contrary, high water flow rates resulting from heavily rain episodes and flooding may contribute to some macrobenthic fauna to be flushed away (Norkko et al., 2002). The observed pattern of *H. ulvae* population during flood events is a good example of that situation (Cardoso et al., 2005, 2008a). Moreover, flooding could result in deposition of fine terrigenous sediments and subsequent anoxic conditions, imposing negative implications at structural and functional levels of the macrobenthic communities (Norkko et al., 2002), as observed in the Mondego estuary. With this respect, bivalves were the main structural group reducing substantially their percentage in the macrobenthic assemblages after heavy flooding episodes. Clogging of bivalves' feeding structures resulting from high turbidity caused by the floods (Norkko et al., 2002) may explain their decline. Moreover, they have demonstrated great susceptibility to high salinity variations (Pillay et al., 2008), leading to hypothesize that decline of *S. plana*, one of the dominant species of the bivalve community in the Mondego estuary (Verdelhos et al., 2005; Dolbeth et al., 2007), may be related with unpredictable and rapid changes in salinity recorded for long periods in the system. Similarly, high temperatures measured in intertidal water pools, mostly above lethal temperature determined for the species (27.5ºC) (Wilson, 1981), might have been a further cause of its decline during the heat wave and the drought. Crustaceans also showed a considerable reduction during the 2003 summer heat wave, being the isopod *Cyathura carinata* the species mostly impacted, both in abundance and in secondary production (Bordalo et al., 2011).

In a common drought scenario various changes would be expected, including intra and inter site variability in macrobenthic species abundance and composition (Jones, 1990). For instance, rapid colonization of opportunistic marine associated species with increasing salinity (Hastie and Smith, 2006) and changes in species dominance due to altered salinity and substrate type (MacKay et al., 2010) may occur. This was particularly evident in the Mondego estuary concerning not only the macrobenthic fauna but also the intertidal vegetation. For example, biomass of *Z. noltii* was negatively affected by drought events, through direct alterations on its optimal salinity range, which in turn conditioned recovery of the related *H. ulvae* population (Cardoso et al., 2008a). Looking at functional level, detritivores increased their dominance in the macrobenthic community in detriment of omnivores. This increment was mostly reliant on success of opportunistic sub-surface deposit feeding species (e.g. *C. capitata*, *Heteromastus filiformis*, *Tubificoides* sp.), as a result of their growth and reproduction stimulation in the last years. On the contrary, under flooding episodes, omnivores became dominant over detritivores. Within these organisms, sub-surface deposit feeders and suspension feeders decreased in parallel with increasing prevalence of surface deposit feeding species. Interestingly, despite successive climate variability impacts, total biomass has been slowly increasing, showing to be a more resilient ecosystem measure than abundance.

Overall, different extreme climate events delayed ecosystem recovery and have contributed to a significant abundance decline and a pronounced biodiversity impoverishment, constituting a further stressor to a system already vulnerable due to an historic eutrophication problem. In fact, the loss of biodiversity following one stressor may enforce a decline in the resilience of a system to additional impacts, emphasizing the importance of ecological history in predicting responses to environmental change (Scheffer et al., 2001; Vinebrooke et al., 2004).

Z. noltii recolonization and *H. ulvae* population recovery (2008-2010)

More recently, as a consequence of the restoration efforts conducted in the estuary, the seagrass *Z. noltii* started to recolonize bare sediments of the inner most disturbed region, more than two decades after its disappearance (Grilo et al., 2012). Among others, nutrient loading decline and macroalgal biomass depletion were probably the main factors influencing seagrass expansion in downstream areas and its recolonization in the sandflat area. According to Fonseca et al. (2002), since the water column and the sediment conditions are appropriate, then seagrass recovery will follow. For instance, a suitable nutrient regime, water transparency, inundation period and substratum type for attachment will allow recolonization of seagrasses as long as their propagules are available, otherwise seagrass return may occur via a nearby source of seeds (Erftemeijer et al., 2008; Vaudrey et al., 2010). Considering that seagrass was absent from the sandflat area of the Mondego estuary for more than 20 years, it is unlikely that recolonization occurred by regeneration from existing original rhizomes (Bryars and Neverauskas, 2004), since they were perhaps no longer viable. Thus, the reestablishment and proliferation of the *Z. noltii* in the inner areas may be reliant on seeds transported from downstream seagrass beds, located roughly 1.5 km far (*Z. noltii* bed and mudflat area). It seems that such overall environmental quality improvement effectively allowed the seagrass *Z. noltii* to restore a small part of its former distribution, as documented in other coastal systems (Philippart et al., 2007; Vaudrey et al., 2010).

The benefits of seagrass meadows for ecosystems' health and functioning are well known worldwide. They provide suitable habitat and protection for many invertebrate and fish species as well as great food availability for benthic organisms by acting as a sink of organic matter (Asmus and Asmus, 2000; Duarte, 2002; Dolbeth et al., 2011). In a scenario of recolonization, once seagrasses start to cover bare substrata, increases in densities of the associated mobile macrofauna would be expected (Sheridan, 2004). In the Mondego estuary, the return of *Z. noltii* stimulated abundance, production and particularly the biomass of *H. ulvae*, contributing to the establishment of a more stable and structured population. The population started to be composed by individuals of different age classes, in opposition to population existing during pre-restoration, which was very unstable and juveniles dominated. In fact, as stated by Casagranda et al. (2005), macrophyte cover is the most important factor governing temporal and spatial patterns of *Hydrobia* spp. density, with vegetated tidal flats presenting much higher abundances than bare sediments.

Despite positive signs that are taking place, full recovery of the system was not yet entirely achieved in line with several other systems. For example, macrozoobenthic communities of Victoria Harbor (China) still showed signs of disturbance 3 years later eutrophication phenomena and subsequent environmental restoration (Shin et al., 2008), whilst in Orbetello lagoon (Italy) that period increased to 6 years (Lardicci et al., 2001). In addition, seagrasses from Tampa Bay (USA) did not yet reach original areal coverage existing in the 1950s (Greening and Janicki, 2006) and total recovery of seagrasses, benthos, and nekton after dredging in Laguna Madre (USA) was estimated to last between 4 to 8 years (Sheridan, 2004). According to Emu Ltd. (2004) recovery is truly successful when the ecosystem goods and services have been regained to the pre-impact state and the new community is now similar in species composition, population density and biomass structure to that previously existing before the impact. However, recovery may also be evaluated using single or sets of structural, functional or socio-economic indicators as suggested by Elliott et al., (2007). In this case the findings presented in this thesis clearly supported that the estuarine system is moving towards recovery, being highly necessary to continue monitoring it to better understand its complexity and ensure biodiversity conservation.

Organochlorine assessment in Ria de Aveiro

At present, there are no longer reservations about toxic and bioaccumulation potential of organochlorines, constituting a global concerning issue that even at extremely low levels represents a serious threat to the environment and ultimately to the human health. Ria de Aveiro received for decades large amounts of effluents from a closer industrial complex (Pereira et al., 2009), in which chlorine and aromatic compounds containing benzene were produced. Previous studies have reported the presence of organochlorines in Ria de Aveiro (Antunes et al., 2004, 2007). The Laranjo bay was the endpoint of effluents discharges, justifying its interest as an optimal location to study organochlorines, such as polychlorinated biphenyls (PCBs) and hexachlorobenzene (HCB). Highest concentrations were found closest to the location where industrial effluents were released, decreasing sharply along a 3 km extent area. Contamination by PCBs and HCB in intertidal water pools was negligible, always below detection limit, confirming low solubility of both compounds in water. In spite of their hydrophobic nature, these pollutants tend to remain strongly adsorbed to suspended particulate matter (SPM). Values of PCBs registered in the Laranjo bay are quite low in line with East Java, Indonesia (Hillebrand et al., 1989) and the Pearl River estuary, China (Chen et al., 2011).

By reflecting long-term contamination levels and acting as reservoirs and source of contamination to benthic organisms (Fang, 2004; He et al., 2006), sediments constitute a relevant tool to assess aquatic ecosystems quality. Increases in organochlorines were observed along sediment depth profile, reflecting agro-industrial activities undertaken in the past. Comparared with other Portuguese systems, levels of PCBs in most contaminated sediments of the Laranjo bay clearly exceed those reported for the Tagus estuary (IAEA/MEL/ 67, 1999) and were quite distant from residual concentrations found in the Mondego estuary (Baptista et al., 2013). This supports the evidence that no PCB contamination source was known, making the Mondego estuary a reference condition for several studies (Nunes et al., 2011). HCB levels from Ria de Aveiro were similar to those reported by Kim et al. (2009) in surface sediments from the Han River, in Korea, and rather higher than values found in the Gulf of Bothnia (Strandberg et al., 2000) and in the Scheldt estuary (Van Ael et al., 2012). Maximum HCB concentrations were around 7-fold lower than upper limit determined for PCBs.

According to both Canadian (CCME, 2002) and Norwegian (Bakke et al., 2010) sediment quality guidelines, concentrations detected in the most contaminated sediments of Ria de Aveiro exceed the PCB threshold effect level (TEL), 21.5 ng.g^{-1}, inducing toxic effects on benthic biota. In opposition, HCB levels may not be problematic, since no toxic effects were suggested accordingly to the Norwegian guidelines. Regarding the Portuguese legislation for dredged material (Decreto-Lei no. 226-A/2007, 31 May; Portaria no. 1450/2007, 12 November), sediments collected nearby the point of discharge of industrial effluents are classified as slightly contaminated for both PCBs and HCB, requiring further monitoring.

Concerning biota, the bivalve *S. plana* tended to bioaccumulate PCBs throughout its lifespan, but with different annual rates along the spatial contamination gradient. The maximum values were found in older individuals up to 3+ years old, reaching roughly 20 ng.g^{-1} dw. This bioaccumulation trend was also observed in mussels *Mytilus galloprovincialis* collected in the Algiers Bay (Fouial-Djebbar et al., 2011). However, the same bioaccumulation tendency was not perceived for HCB. Generally, the most abundant congeners both in sediments and bivalves were the hexa- (CBs 138, 153) and heptachlorinated (CB 180) followed by the pentachlorinated ones (CBs 101, 118). This is consistent to their profuse release into the environment allied with its lower volatility (Ritter et al., 1995) and higher long-term environmental persistence comparatively to less chlorinated congeners.

Interesting evidence resulting from this thesis is the great ability of *S. plana* to remove pollutants from the sediments. The species revealed to be an important PCB transfer vehicle from the sediments to the trophic web, incorporating annually about 0.4 g of PCBs, just in the inner area of the Laranjo bay (0.6 km^2). This amount may seem relevant, considering that it can be transferred to higher trophic levels and exported to other areas, since this species is an important prey item to various estuarine predators (Baeta et al., 2006; Dolbeth et al., 2008a).

Legislation regarding the 197 non-dioxin-like PCBs (ndl-PCBs) is presently lacking and a health-based maximum intake level has not yet been established. The European Food Safety Authority (EFSA, 2010) considers the sum of the six indicator congeners (CBs 28, 52, 101, 138, 153, 180) as a suitable indicator for occurrence and human exposure to ndl-PCBs and hence maximum levels were fixed for Σ_6 PCB. Based on the Regulation (EU) No 1259/2011, the analysed bivalves did not present PCB levels exceeding the limit established for muscle meat of fish and fishery products (75 ng.g^{-1} wet weight). Similarly, the estimated tolerable daily intake (TDI) for Σ_6 PCB was 300 times lower than the established TDI limit (10 ng.kg^{-1} body weight). According to these estimations, the consumption of *S. plana* from the Laranjo bay does not represent cause of concern on a short-term. Nevertheless, it is widely recognized the harmful human health impacts of PCBs after consumption over many years and after reaching a steady-state concentration in the body (European Commission, Health and Consumer Protection, 2000; Pompa et al., 2003).

Investigating bioaccumulation and detoxification kinetics of PCB-153

Coastal environments are exposed to great human pressure, being frequently under episodic contamination events (e.g. industrial discharges, PCBs leakage and oil spills), which have direct impacts on benthic fauna. As reinforced by the previous topic of discussion, PCB-153 is the most abundant congener in marine biota, demonstrating to be a good indicator in monitoring programs (Danis et al., 2005). It is vital to understand the bioaccumulation and detoxification processes of species with a key role in the ecosystems' structure and functioning and simultaneously with great socio-economic importance, such as the edible shrimp *P. varians*.

Depending on the CB-153 concentration at which the shrimps were exposed, they exhibited different kinetics of accumulation and uptake rates. For intermediate and higher CB-153 levels, shrimps presented a rapid linear accumulation pattern, while for low CB-153 contamination the organisms displayed a slower uptake rate, reaching an apparent steady state after 15 days exposure. However, independently of the contamination treatments, significant accumulations occurred during the first 72h of exposure. Other works confirmed these differences in bioaccumulation kinetics (Nimmo et al., 1974; Tatem, 1986).

None of the CB-153 concentrations was apparently lethal for the shrimps, although alterations in their respiration might have occurred, since toxic stress is almost instantaneous, even in low PCB concentrations (Reyes et al., 2003). In fact, the toxicity of organic contaminants to the accumulating organisms is commonly related to the total accumulated concentration (Rainbow and Luoma, 2011), which in turn is intimately associated with the local bioavailability of that contaminant (McLeese et al., 1980). Nevertheless, the order of magnitude between contaminated treatments was not completely reflected in the *P. varians* internal accumulation, as confirmed by the bioconcentration factors (BCFs). In fact, accumulation of CB-153 after a 15-day exposure period was not proportional for all levels of contamination, since the shrimps exposed to intermediate concentrations attained CB-153 levels 30 times higher than those under low concentrations, despite the order of magnitude among real concentrations was indeed 10-fold higher. Identical pattern was observed in the shrimps subjected to high concentrations by accumulating 7.5 times more CB-153 than the ones under moderate contamination levels.

After transfer to clean seawater, shrimps initiated immediately decontamination, losing great part (25-30%) of the accumulated CB-153 in the first 3 days, concordantly to Cardoso et al. (2013). Overall, the organisms lost around 35-60% of the contaminant adsorbed into their tissues, presenting at the end of the experiment levels analogous to those after 3 days exposure to the contaminant. So, even after 15 days in clean seawater, the shrimps were not able to return to safety values (for food consumption), which is probably related with low rates of biotransformation, and particularly the inability of these organisms to metabolize congeners with chlorine atoms in positions 2, 4, and 5 in both rings (CB-153) (Bodin et al., 2007).

Exposure of shrimps to half the PCB concentration allowed for drinking waters (0.25 $\mu g.L^{-1}$) determined an accumulation of nearly 50% the maximum limit of Σ_6 PCB established for muscle meat of fish and fishery products (75 $ng.g^{-1}$ ww) (EU Regulation No 1259/2011). Since CB-153 accumulation was not proportional, for a CB-153 concentration equal to the threshold for drinking waters, shrimps were likely exceed the maximum limit of Σ_6 PCB imposed for human consumption. In fact, our findings highlighted that exposure to moderate (2.5 $\mu g.L^{-1}$) and high CB-153 concentrations (25 $\mu g.L^{-1}$) determined an accumulation quite higher than the legal consumption limit (700 and 6,135 $ng.g^{-1}$ ww, respectively).

After transfer to clean seawater, shrimps initiated immediately decontamination, losing great part (25-30%) of the accumulated CB-153 in the first 3 days, in agreement to results reported by Cardoso et al. (2013) concerning mercury detoxification in the edible cockle *Cerastoderma edule*. Overall, the organisms lost around 35-60% of the contaminant, presenting at the end of the experiment levels analogous to those after 3 days exposure to the contaminant. So, even after 15 days in clean seawater, the shrimps were not able to return to safety values (for food consumption – 75 ng.g^{-1} ww), which is probably related with low rates of biotransformation, and particularly the inability of these organisms to metabolize congeners with chlorine atoms in positions 2, 4, and 5 in both rings (CB-153) (Bodin et al., 2007). This evidence is highly relevant in a scenario of hypothetic PCBs leakage, since deleterious consequences to the estuarine ecosystem and ultimately to the human health might be expected.

FINAL REMARKS

This work provides a holistic overview on dynamic changes of the macrobenthic communities of the Mondego estuary over a 17-year period, characterized by multiple stressors, both natural and anthropogenic, which may have synergistically compromised full ecosystem recovery. Restoration measures implemented to reverse eutrophication seemed to be partially effective, allowing seagrass recolonization in the inner region and its expansion in downstream areas, in parallel with slow macrobenthic assemblages' recovery and biomass increment. Nevertheless, extreme climate phenomena endangered estuarine biodiversity conservation and may have contributed to increasing social vulnerability, by affecting people's livelihoods and food provision services and goods. For instance, production of commercially important species (e.g. the shellfish *S. plana* and *C. edule*; and fishes *Dicentrarchus labrax*, *Solea solea*, *Platichthys flesus*) decreased in the estuary due to those impacts (Dolbeth et al., 2008b, 2011). In addition to food provisioning services decline, changes in community composition, structure and functioning were also observed, which may have influenced other ecosystem services and functions, such as nutrient cycling or carbon fluxes (Beaumont et al., 2007.

Assessment of human-induced organochlorines pollution (PCBs and HCB) in Ria de Aveiro highlighted that contamination was mainly restricted to the entrance point of chlorine and benzene industrial effluents in the system, containing the sediments levels exceeding quality guidelines and thus capable to induce toxic effects on biota. Despite the values of PCBs found in the sediment-related bivalves complied the European regulation, it is highly important to continue monitoring these areas in order to better understand their functioning, prevent further environmental damage and to control potential human health risks. This can be also achieved through laboratorial experiments, assumed to be valuable instruments to predict bioaccumulation and detoxification responses of estuarine species face to hypothetic industrial discharges in the environment.

Overall, the findings provided by this thesis add relevant information towards estuarine sustainable management and human susceptibility intrinsic with contaminated food consumption.

FUTURE PERSPECTIVES

As reinforced by this thesis, contribution of long-term studies for understanding of complex and slow ecological phenomena is unquestionable. In a constantly changing world, a continuing monitoring of the Mondego's macrobenthic community is the logical path to follow, in order to conclude about complete success of the restoration program and eventual upcoming impacts. Pertinent questions still remain to be answered in the future: would the sandflat area ever reach its original state after recent seagrass *Z. noltii* return? And the mudflat area would become more similar to the *Z. noltii* beds as expected? How predicted increasing frequency and intensity of climate extremes will impact sustainability of the estuarine system?

In addition, there is a relevant issue involving global warming and persistent organic pollutants (POPs) that should not be ignored. Environmental variables such as temperature, wind speed, precipitation and solar radiation influence directly or indirectly POPs environmental fate and transport (Teran et al., 2012). Temperature is one of the main climate drivers in determining POPs global distribution, through direct effects on their volatility, more rapid degradation and altered partitioning between phases (Lamon et al., 2009). A recent report advertise that releases of POPs trapped in soil, water and ice will increase due to rising global temperatures (UNEP/AMAP, 2011). This constitutes a concerning threat for global society, but particularly for people living in Northern latitudes, such as Arctic Indigenous people. The dispersal of POPs into freshwater and marine environments directly affects fish and marine mammals that represent the primary traditional foods for these Indigenous people, resulting in harmful health effects (UNEP/AMAP, 2011). This report also suggests that the expected increase in the incidence of vector-borne diseases (e.g. malaria) associated with climate change may increase demand for and release of the controversial POPs pesticide DDT in some regions. Concluding, climate variability may undermine global efforts aiming to reduce environmental and human exposure to POPs (Klanova et al., 2011; Ma et al., 2011).

An integration of climate change effects on POPs environmental behavior in chemicals' regulation is still missing (Teran et al., 2012). A comprehensive integration between the different international initiatives on the management of POPs would help to define a reference framework for POPs and other chemicals' assessment taking into consideration climate variability (Teran et al., 2012). Moreover, continuous monitoring activities are important as they could drive to the identification of new and unsuspecting POPs primary or secondary sources.

REFERENCES

Adams SM (2005) Assessing cause and effect of multiple stressors on marine systems. Marine Pollution Bulletin 51: 649-657.

Antunes P, Gil O (2004) PCB and DDT contamination in cultivated and wild sea bass from Ria de Aveiro, Portugal. Chemosphere 54: 1503-1507.

Antunes P, Gil O, Reis-Henriques MA (2007) Evidence for higher biomagnification factors of lower chlorinated PCBs in cultivated seabass. Science of the Total Environment 377: 36-44.

Asmus H, Asmus R (2000) Material exchange and food web of seagrass beds in the Sylt-Rømø Bight: how significant are community changes at the ecosystem level? Helgoland Marine Research 54: 137-150.

Bachelet G, de Montaudouin X, Auby I, Labourg PJ (2000) Seasonal changes in macrophyte and macrozoobenthos assemblages in three coastal lagoons under varying degrees of eutrophication. Ices Journal of Marine Science 57: 1495-1506.

Baeta A, Cabral HN, Neto JM, Marques JC, Pardal MA (2006) Biology, population dynamics and secondary production of the green crab *Carcinus maenas* (L.) in a temperate estuary. Estuarine, Coastal and Shelf Science 65: 43-52.

Baeta A, Valiela I, Rossi F, Pinto R, Richard P, Niquil N, Marques JC (2009) Eutrophication and trophic structure in response to the presence of the eelgrass *Zostera noltii*. Marine Biology 156: 2107-2120.

Bakke T, Källqvist T, Ruus A, Breedveld GD, Hylland K (2010) Development of sediment quality criteria in Norway, Journal of Soils and Sediments 10: 172-178.

Baptista J, Pato P, Pereira E, Duarte AC, Pardal MA (2013) PCBs in the fish assemblage of a southern European estuary. Journal of Sea Research 76: 22-30.

Beaumont N, Austen M, Atkins J, Burdon D (2007) Identification, definition and quantification of goods and services provided by marine biodiversity: implications for the ecosystem approach. Marine Pollution Bulletin 54: 253-265.

Bodin N, Abarnou A, Le Guellec AM, Loizeau V, Philippon X (2007) Organochlorinated contaminants in decapod crustaceans from the coasts of Brittany and Normandy (France). Chemosphere 67: S36-S47.

Bordalo MD, Ferreira SMF, Cardoso PG, Leston S, Pardal MA (2011) Resilience of an isopod population (*Cyathura carinata*) to multiple stress factos in a temperate estuarine system. Hydrobiologia 671: 13-25.

Bryars S, Neverauskas V (2004) Natural recolonization of seagrasses at a disused sewage sludge outfall. Aquatic Botany 80: 283-289.

Cardoso PG, Lillebø AI, Pardal MA, Ferreira SM, Marques JC (2002) The effects of different primary producers on *Hydrobia ulvae* population dynamics: a case study in a temperate intertidal estuary. Journal of Experimental Marine Biology and Ecology 277: 173-195.

Cardoso PG, Pardal MA, Lillebø AI, Ferreira SM, Raffaelli D, Marques JC (2004) Dynamic changes in seagrass assemblages under eutrophication and implications for recovery. Journal of Experimental Marine Biology and Ecology: 233–248.

Cardoso PG, Brandão A, Pardal MA, Raffaelli D, Marques JC (2005) Resilience of *Hydrobia ulvae* populations to anthropogenic and natural disturbances. Marine Ecology Progress Series 289: 191-199.

Cardoso PG, Bankovic M, Raffaelli D, Pardal MA (2007) Polychaete assemblages as indicators of habitat recovery in a temperate estuary under eutrophication. Estuarine, Coastal and Shelf Science 71: 301-308.

Cardoso PG, Raffaelli D, Lillebø AI, Verdelhos T, Pardal MA (2008a) The impact of extreme flooding events and anthropogenic stressors on the macrobenthic communities' dynamics. Estuarine, Coastal and Shelf Science 76: 553-565.

Cardoso PG, Raffaelli D, Pardal MA (2008b) The impact of extreme weather events on the seagrass *Zostera noltii* and related *Hydrobia ulvae* population. Marine Pollution Bulletin 56: 483-492.

Cardoso PG, Leston S, Grilo TF, Bordalo MD, Crespo D, Raffaelli D, Pardal MA (2010) Implications of nutrient decline in the seagrass ecosystem success. Marine Pollution Bulletin 60: 601-608.

Cardoso PG, Grilo TF, Pereira E, Duarte AC, Pardal MA (2013) Mercury bioaccumulation and decontamination kinetics in the edible cockle *Cerastoderma edule*. Chemosphere 90:1854-1859.

Casagranda C, Boudouresque CF, Francour P (2005) Abundance, Population Structure and Production of *Hydrobia ventrosa* (Gastropoda: Prosobranchia) in a Mediterranean brackish lagoon, Lake Ichkeul, Tunisia. Archiv für Hydrobiologie 164: 411-428.

[CCME] Canadian Council of Ministers of the Environment (2002) Canadian sediment quality guidelines for the protection of aquatic life: Summary tables. Updated. In: Canadian environmental quality guidelines, 1999, Canadian Council of Ministers of the Environment, Winnipeg. Available from: http://www.ecy.wa.gov/programs/eap/psamp/BoundaryBay/PSAMP-BBAMP%20documents/Canadian%20guidelines%20for%20water%20quality/SedimentProtAquaticLifeSummaryTables(en).pdf

Chen M-Y, Yu M, Luo X-J, Chen S-J, Mai B-X (2011) The factors controlling the partitioning of polybrominated diphenyl ethers and polychlorinated biphenyls in the water-column of the Pearl River Estuary in South China. Marine Pollution Bulletin 62: 29-35.

Commission Regulation (EU) No 1259/2011 of 2 December 2011 amending Regulation (EC) No 1881/2006 as regards maximum levels for dioxins, dioxin-like PCBs. Availbale from: (http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2011:320:0018:0023:EN:PDF.

Cyrus DP, Jerling H, MacKay F, Vivier l (2011) Lake St Lucia, Africa's largest estuarine lake in crisis: Combined effects of mouth closure, low levels and hypersalinity. South African Journal of Science 107: 59-71.

Danis B, Cotret O, Teyssié JL, Bustamante P, Fowler SW, Warnau M (2005) Bioaccumulation of PCBs in the sea urchin *Paracentrotus lividus*: seawater and food exposures to a ^{14}C-radiolabelled congener (PCB 153). Environmental Pollution 135: 11-16.

Dauvin JC (2008) Effects of heavy metal contamination on the macrobenthic fauna in estuaries: The case of the Seine estuary. Marine Pollution Bulletin 57: 160-169.

Decreto-Lei no. 226-A/2007, 31 May; Portaria no. 1450/2007, 12 November. Available from: http://dre.pt/pdf1sdip/2007/11/21700/0837208382.pdf.

Dolbeth M, Pardal MA, Lillebø AI, Azeiteiro UM, Marques JC (2003) Short- and long-term effects of eutrophication on the secondary production of an intertidal macrobenthic community. Marine Biology 143:1229-1238.

Dolbeth M, Cardoso PG, Ferreira SM, Verdelhos T, Raffaelli D, Pardal MA (2007) Anthropogenic and natural disturbance effects on a macrobenthic estuarine community over a 10-year period. Marine Pollution Bulletin 54: 576-585.

Dolbeth M, Martinho F, Leitão R, Cabral H, Pardal MA (2008a) Feeding patterns of the dominant benthic and demersal fish community in a temperate estuary. Journal of Fish Biology 72: 2500-2517.

Dolbeth M, Martinho F, Viegas I, Cabral H, Pardal MA (2008b) Estuarine production of resident and nursery fish species: conditioning by drought events? Estuarine, Coastal and Shelf Science 78: 51-60.

Dolbeth M, Cardoso PG, Grilo TF, Bordalo MD, Raffaelli D, Pardal MA (2011) Long-term changes in the production by estuarine macrobenthos affected by multiple stressors. Estuarine, Coastal and Shelf Science 92: 0-18.

Doney SC, Ruckelshaus M, Duffy JE, Barry JP, Chan F, English CA, Galindo HM, Gremeier JM, Hollowed AB, Knowlton N, Polovina J, Rabalais, NN, Sydeman WJ, Talley LD (2012) Climate change impacts on marine ecosystems. Annual Review of Marine Science 4: 11-37.

Doody P (2003) Coastal Habitat Restoration: Towards Good Practice. Introductory Unpublished Report Produced as Part of the LIFE Funded ‘Living with the sea project’ (Contract No. FST20-48-006 01/0384).

Duarte CM (2002) The future of seagrass meadows. Environment Conservation 29: 192-206.

Ducrotoy JP, Dauvin JC (2008) Estuarine conservation and restoration: The Somme and the Seine case studies (English Channel, France). Marine Pollution Bulletin 57: 208-218.

European Commission, Health and Consumer Protection Scientific Committee on Food (2000) Opinion of SCF on the Risk Assessment of Dioxins and Dioxin-like PCBs in Food. 141 pp.

European Food Safety Authority (EFSA) Journal 2010; 8 (7): 1701. Available from: http://www.efsa.europa.eu/en/efsajournal/doc/1701.pdf.

Elliott M, Burdon D, Hemingway KL, Apitz, SE (2007) Estuarine, coastal and marine ecosystem restoration: Confusing management and science – A revision of concepts. Estuarine, Coastal and Shelf Science 74: 349-366.

Emu Ltd. (2004) Marine Aggregate Restoration and Enhancement: Strategic Policy Overview. Report No. 04/J/01/06/0548/0437. Emu Ltd. on behalf of The British Marine Aggregate Producers Association, the Crown Estate and English Nature.

Erfteeijer PLA, Van Beek JKL, Ochieng CA, Jager Z, Los HJ (2008) Eelgrass seed dispersal via floating generative shoots in the Dutch Wadden Sea: a model approach. Marine Ecology Progress Series 358: 115-124.

Fang ZQ (2004) Organochlorines in sediments and mussels collected from coastal sites along the Pearl River Delta, South China. Journal of Environmental Sciences 16: 321-327.

Fonseca MS, Judson Kenworthy W, Julius BE, Shutler S, Fluke S (2002) Seagrasses. In: Perrow, M.R., Davy, A.J. (Eds.), Handbook of Ecological Restoration. Restoration in Practice, vol. 2. Cambridge University Press, Cambridge, pp. 149-170.

Fouial-Djebbar D, Djebbar R, Yacine A, Badjah-Hadj-Ahmed AY, Budzinski H (2011) Level of polychlorinated biphenyls in marine environment of Algiers Bay, Algeria. Analytical Letters 44: 2438-2456.

Greening H, Janicki A (2006) Toward reversal of eutrophic conditions in a subtropical estuary: Water quality and seagrass response to nitrogen loading reductions in Tampa Bay, Florida, USA. Environmental Management 38: 163-178.

Grilo TF, Cardoso PG, Dolbeth M, Pardal MA (2009) Long-term changes in amphipod population dynamics in a temperate estuary following ecosystem restoration. Hydrobiologia 630: 91-104.

Grilo TF, Cardoso PG, Dolbeth M, Bordalo MD, Pardal MA (2011) Effects of extreme climate events on the macrobenthic comunities' structure and functioning of a temperate estuary. Marine Pollution Bulletin 62: 303-311.

Grilo TF, Cardoso PG, Pardal MA (2012) Implications of *Zostera noltii* recolonization on *Hydrobia ulvae* population structure success. Marine Environmental Research 73: 78-84.

Hastie BF, Smith SDA (2006) Benthic macrofaunal communities in intermittent estuaries during a drought: Comparisons with permanently open estuaries. Journal of Experimental Marine Biology and Ecology 330: 356-367.

Hawkins SJ (2004) Scaling up: the role of species and habitat patches in functioning of coastal ecosystems. Aquatic Conservation: Marine Freshwater Ecosystems 14: 217-219.

Hillebrand MTHJ, Everaarts JM, Razak H, Moelyadi Moelyo D, Stolwijk L, Boon JP (1989) Input of selected chlorinated hydrocarbons into the coastal area of east Java and adjacent waters: Distribution patterns in the dissolved and suspended phase. Netherlands Journal of Sea Research 23: 369-377.

He M-c, Sun Y, Li X-r, Yang Z-f (2006) Distribution patterns of nitrobenzenes and polychlorinated biphenyls in water, suspended particulate matter and sediment from mid- and down-stream of the Yellow River (China). Chemosphere 65: 365-374.

International Atomic Energy Agency, Marine Environment Laboratory - MESL, Report IAEA/AL/ 121 (IAEA/MEL/ 67), 1999. Worldwide and regional intercomparison for the determination of organochlorine compounds, petroleum hydrocarbons and sterols in the sediment sample IAEA-408, Monaco.

Karlson K, Rosenberg R, Bonsdorff E (2002) Temporal and spatial large-scale effects of eutrophication and oxygen deficiency on benthic fauna in Scandinavian and Baltic waters – a review. Oceanography and Marine Biology: an Annual Review 40: 427-489.

Kemp WM, Boynton WR, Adolf JE, Boesch DF, Boicourt WC, Brush G, Cornwell JC, Fisher TR, Glibert PM, Hagy JD, Harding LW, Houde ED, Kimmel DG, Miller WD, Newell RIE, Roman MR, Smith EM, Stevenson JC (2005) Eutrophication of Chesapeake Bay: historical trends and ecological interactions. Marine Ecology Progress Series 303: 1-29.

Kennish MJ (2002) Environmental threats and environmental futures of estuaries. Environmental Conservation 29:78-107.

Klanova J, Diamond M, Jones K, Lammel G, Lohmann R, Pirrone N, Scheringer M, Balducci C, Bidleman T, Blaha K, Blaha L, Booij K, Bouwman H, Breivik K, Eckhardt S, Fiedler H, Garrigues P, Harner T, Holoubek I, Hung H, MacLeod M, Magulova K, Mosca S, Pistocchi A, Simonich S, Smedes F, Stephanou E, Sweetman A, Sebkova K, Venier M, Vighi M, Vrana B, Wania F, Weber R, Weiss P (2011) Identifying the research and infrastructure needs for the global assessment of hazardous chemicals ten years after establishing the Stockholm Convention. Environmental Science and Technology 45, 7617-7619.

Jones AR (1990) Zoobenthic variability associated with a flood and drought in the Hawkesbury Estuary, New South Wales: some consequences for environmental monitoring. Environmental Monitoring and Assessment 14: 185-195.

Lamon L, von Waldow H, MacLeod M, Scheringer M, Marcomini A, Hungerbuhler K, (2009) Modeling the global levels and distribution of polychlorinated biphenyls in air under a climate change scenario. Environmental Science and Technology 43: 5818-5824.

Lardicci C, Como S, Corti S, Rossi F (2001) Changes and recovery of macrozoobenthic communities after restoration measures of the Orbetello lagoon (Tyrrhenian coast, Italy). Aquatic Conservation: Marine Freshwater Ecosystems 11: 281-287.

Lillebø AI, Neto JM, Martins I, Verdelhos T, Leston S, Cardoso PG, Ferreira SM, Marques JC, Pardal MA (2005) Management of a shallow temperate estuary to control eutrophication: the effect of hydrodynamics on the system's nutrient loading. Estuarine, Coastal and Shelf Science 65: 697-707.

MacKay F, Cyrus D, Russell K-L (2010) Macrobenthic invertebrate responses to prolonged drought in South Africa's largest estuarine lake complex. Estuarine, Coastal and Shelf Science 86: 553-567.

Ma JM, Hung HL, Tian C, Kallenborn R (201). Revolatilization of persistent organic pollutants in the Arctic induced by climate change. Nature Climate Change 1: 255-260.

Marquiegui MA, Aguirrezabalaga F (2009) Colonization process by macrobenthic infauna after a managed coastal realignment in the Bidasoa estuary (Bay of Biscay, NE Atlantic). Estuarine, Coastal and Shelf Science 84: 598-604.

McLeese DW, Metcalfe CD, Pezzack DS (1980) Uptake of PCBs from sediment by *Nereis virens* and *Crangon septemspinosa*. Archives of Environmental Contamination and Toxicology 9: 507-518.

McLusky DS, Elliott M (2004) The estuarine ecosystem: Ecology, threats and management. 3rd Ed. Oxford University Press, Oxford, 214 pp.

Moon HB, Kim HS, Choi M, Yu J, Choi HG (2009) Human health risk of polychlorinated biphenyls and organochlorine pesticides resulting from seafood consumption in South Korea, 2005-2007. Food and Chemical Toxicology 47: 1819-1825.

Nimmo DR, Forester J, Heitmuller PT, Cook GH (1974) Accumulation of Aroclor 1254 in grass shrimp *(Palaemonetes pugio)* in laboratory and field exposures. Bulletin of Environmental Contamination and Toxicology 11: 303-308.

Norkko, A., Thrush, S.F., Hewitt, J.E., Cummings, V.J., Norkko, J., Ellis, J.I., Funnell, G.A., Schultz, D., MacDonald, I., 2002. Smothering of estuarine sandflats by terrigenous clay: the role of wind-wave disturbance and bioturbation in site-dependent macrofaunal recovery. Marine Ecology Progress Series 234: 23-41.

Norkko J, Bonsdorff E, Norkko A (2000) Drifting algal mats as an alternative habitat for benthic invertebrates: species specific responses to a transient resource. Journal of Experimental Marine Biology and Ecology 248: 79-104.

Nunes M, Marchand P, Vernisseau A, Le Bizec B, Ramos F, Pardal MA (2011) PCDD/Fs and dioxin-like PCBs in sediment and biota from the Mondego estuary (Portugal). Chemosphere 83: 1345-1352.

Pardal MA, Marques JC, Metelo I, Lillebø A, Flindt MR (2000) Impact of eutrophication on the life cycle, population dynamics and production of *Amphitoe valida* (Amphipoda) along an estuarine spatial gradient (Mondego Estuary, Portugal). Marine Ecology Progress Series 196: 207-219.

Pereira ME, Lillebø AI, Pato P, Válega M, Coelho JP, Lopes CB, Rodrigues S, Cachada A, Otero M, Pardal MA, Duarte AC (2009) Mercury pollution in Ria de Aveiro (Portugal): a review of the system assessment. Environmental Monitoring and Assessment 155: 39-49.

Philippart CMJ, Beukema JJ, Cadée GC, Dekker R, Goedhart PW, van Iperen JM, Leopold MF, Herman PMJ (2007) Impacts of nutrient reduction on coastal communities. Ecosystems 10: 95-118.

Pillay D, Perissinotto R (2008) The benthic macrofauna of the St. Lucia Estuary during the 2005 drought year. Estuarine, Coastal and Shelf Science 77: 35-46.

Pompa G, Caloni F, Fracchiolla ML (2003) Dioxin and PCB contamination of fish and shellfish: assessment of human exposure. Review of the international situation. Veterinary Research Communications 27: 159-167.

Rainbow PS, Luoma SN (2011) Metal toxicity, uptake and bioaccumulation in aquatic invertebrates – Modelling zinc in crustaceans. Aquatic Toxicology 105: 455-465.

Reyes GG, Verdugo JM, Cassin D, Carvajal R (2003) Pollution by polychlorinated biphenyls in an estuaryof the Gulf of California. Their toxicity and bioaccumulation in shrimp *Litopenaeus vannamei*. Marine Pollution Bulletin 46: 959-963.

Ritter L, Solomon K, Forget J (1995) A Review of Selected Persistent Organic Pollutants: DDT - Aldrin - Dieldrin - Endrin - Chlordane - Heptachlor- Hexachlorobenzene - Mirex - Toxaphene - Polychlorinated Biphenyls - Dioxins - Furans. The International Programme on Chemical Safety (IPSC) within the framework of the Inter-Organization Programme for the Sound Management of Chemicals (IOMC).

Salen-Picard C, Arlhac D, Alliot E (2003) Responses of a Mediterranean soft bottom community to short-term (1993-1996) hydrological changes in the Rhone river. Marine Environmental Research 55: 409-427.

Salovius S, Nyqvist M, Bonsdorff E (2005) Life in the fast lane: macrobenthos use temporary drifting algal habitats. Journal of Sea Research 53: 169-180.

Santos FD, Forbes K, Moita R (2002) Climate Change in Portugal. Scenarios, Impacts and Adaptation Measures e SIAM Project. Gradiva Publishers, Lisbon, Portugal, 456 pp.

Scheffer M, Carpenter SR, Foley JA, Folke C, Walter B (2001) Catastrophic shifts in ecosystems. Nature 413: 591-596.

Sfriso A, Birkemeyer T, Ghetti PF (2001) Benthic macrofauna changes in areas of Venice lagoon populated by seagrasses or seaweeds. Marine Environmental Research 52: 323-349.

Sheridan P (2004) Recovery of floral and faunal communities after placement of dredged material on seagrasses in Laguna Madre, Texas. Estuarine, Coastal and Shelf Science 59: 441-458.

Shin PKS, Lam NWY, Wu RSS, Qian PY, Cheung SG (2008) Spatio-temporal changes of marine macrobenthic community in sub-tropical waters upon recovery from eutrophication. I. Sediment quality and community structure. Marine Pollution Bulletin 56: 282-296.

Smith VH (2003) Eutrophication of freshwater and coastal marine ecosystems: A global problem. Environmental Science Pollution Research International 10: 126-139.

Strandberg B, Bandh C, van Bavel B, Bergqvist PA, Broman D, Ishag R, Näf C, Rappe C (2000). Organochlorine compounds in the Gulf of Bothnia: sediment and benthic species. Chemosphere 40: 1205-1211.

Tatem HE (1986) Bioaccumulation of polychlorinated biphenyls and metals from contaminated sediment by fresh-water prawns, *Macrobrachium rosenbergii* and clams, *Corbicula fluminea*. Archives of Environmental Contamination and Toxicology 15: 171-183.

Teal JM, Peterson SB (2005) Introduction to the Delaware Bay salt marsh restoration. Ecological Engineering 25:199-203.

Teran T, Lamon L, Marcomini M (2012) Climate change effects on POPs environmental behaviour: a scientific perspective for future regulatory actions. Atmospheric Pollution Research 3: 466-476.

UNEP/AMAP, 2011. Climate Change and POPs: Predicting the Impacts. Report of the United Nations Environmental Programme (UNEP) / Arctic Monitoring and Assessment Program (AMAP) Expert Group. Available from: http://library.arcticportal.org/1214/.

Van Ael E, Covaci, Blust R, Bervoets L (2012) Persistent organic pollutants in the Scheldt estuary: Environmental distribution and bioaccumulation. Envrionment International 48: 17-27.

Vaudrey JMP, Kremer JN, Branco BF, Short FT (2010) Eelgrass recovery after nutrient enrichment reversal. Aquatic Botany 93: 237-243.

Verdelhos T, Neto JM, Marques JC, Pardal MA (2005) The effect of eutrophication abatement on the bivalve *Scrobicularia plana*. Estuarine, Coastal and Shelf Science 63: 261-268.

Vinebrooke RD, Cottingham KL, Norberg J, Scheffer M, Dodson SI, Maberly SC, Sommer U (2004) Impacts of multiple stressors on biodiversity and ecosystem functioning: the role of species co-tolerance. Oikos 104: 451-457.

Wilson JG (1981) Temperature tolerance of circatidal bivalves in relations to their distribution. Journal of Thermal Biology 6: 279-286.

Wilson MA, Farber S (2009) Accounting for ecosystem goods and services in coastal estuaries. In: Pendleton, L.H. (ed.), The Economic and Market Value of Coasts and Estuaries: What's at Stake? Arlington, Virginia: Restore America's Estuaries 13-32.

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