

**Ecological status and functioning evaluation of sandy
beach ecosystems:
A macrobenthic community based approach**

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Contents

Abstract	1
Resumo	3
General introduction	5
General aims and thesis outline	11

Chapter I

Changes in macrofaunal assemblages as response indicator to potential long-term human pressures on exposed sandy beaches.....	13
---	----

Introduction	16
Methodology	18
Results	21
Discussion	29

Chapter II

Niche segregation amongst sympatric species at exposed sandy shores with contrasting wrack availabilities illustrated by stable isotopic analysis.....	33
--	----

Introduction	36
Methodology	38
Results	41
Discussion	49

Chapter III

Behavioural adaptations of two sympatric sandhoppers living on a mesotidal European Atlantic sandy beach.....	53
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Introduction	56
Methodology	58
Results	60
Discussion	66

Behaviour of <i>Talitrus saltator</i> (Crustacea: Amphipoda) in a rehabilitated sandy beach on the eastern Atlantic Coast (Portugal)	71
Introduction	72
Methodology	74
Results	77
Discussion	85
 Chapter IV	
Sandy beach macrofaunal assemblages as indicators of anthropogenic impacts on coastal dunes	89
Introduction	92
Methodology	94
Results	96
Discussion	102
General discussion	107
Suggestions for further research	112
References	113
Acknowledgments/ Agradecimentos	126

Abstract

The primary goal of the research presented in this thesis is to provide a deep knowledge of the ecological status and functioning of exposed sandy beaches on the Portuguese Atlantic coast. Because benthic invertebrates are structurally and functionally important in beach ecosystems (e.g., nutrient cycling, trophic links), this assessment was based on macrobenthic communities' structure, composition and adaptations to the beach environment. In the context of the increasingly human pressures on the sandy beach ecosystems, the use of key species as potential indicators of environmental changes was also evaluated. For this purpose, this work specifically focuses on the assessment of ecological status of sandy beaches subjected to long-term human pressures (Chapter I), on the analyses of niche segregation strategies of crustaceans (Chapter II) and on the behavioural adaptations of talitrid amphipods under different environmental scenarios (Chapter III). The last Chapter (IV) proposes a potential bioindicator of anthropogenic impacts on beach-dune ecosystems.

Chapter I evaluates whether changes in the macrobenthic communities were a response indicator of ten years of increased human pressures (tourism activities and engineering actions) at Cabedelo beach, by comparing it with an undisturbed reference beach in Quiaios. In this sense, the physical environment and the macrofaunal communities' structure at both beaches in 1999–2000 and 2010–2011 were compared. The detected faunal changes (decrease in the abundance of the talitrid *Talitrus saltator* and the tyloid *Tylos europaeus*) were consistent with an interpretation of increased human pressures at Cabedelo beach. In contrast, Quiaios beach presented a similar pattern in key ecological measures in the two periods considered. The observed trends in Cabedelo are suggested to be an effect of human pressures, however, our findings emphasises the need of further effective ecological impact assessments.

Chapter II describes the niche segregation strategies of three peracarid crustaceans from Cabedelo and Quiaios beaches, which are characterised by contrasting food availabilities, i.e. wrack (macroalgae, carrion and dune plants). To attain this goal, stable isotopic analyses of primary consumers and potential food sources were performed and the zonation patterns recorded for the winter and summer conditions. The results of the diets and distribution of these peracarid species revealed spatio-temporal differences in consumers' niches. In Quiaios, winter and wrack-limited conditions allowed for niche segregation of primary consumers, in contrast, at Cabedelo beach, higher beach-wrack deposits induced generalist diets of the three crustaceans studied. Therefore, partitioning of forage resources by these species may vary according to food availability and also environmental constraints.

In Chapter III, the behavioural adaptations of talitrid amphipods were assessed in two different ecological scenarios. In the first case, the behaviour (sun orientation) and distribution of two sympatric talitrids were analysed under the undisturbed environment described at Quiaios beach. Despite the stable conditions offered by this mesotidal beach, different behavioural strategies were described for each species. *Talorchestia brito* showed a precise

sun orientation and occurred mainly on the intertidal area, while *T. saltator* revealed a more flexible distribution and orientation. The study suggested that individual habitat requirements and tolerances of these species might be reflected in their behavioural flexibility in a specific environment. In the second study comprehended in Chapter III, the behaviour (orientation and locomotor endogenous rhythms) of *T. saltator* were investigated at a recently restored beach (Leirosa) to test their behavioural adaptations to the new landscape - the artificial foredune. The results revealed a precise orientation of these talitrids, which use the sun compass and visual cues (landscape) to find the optimal zone on the beach, and a well defined free running rhythm (with a tidal component), suggesting a stable behaviour of this population at Leirosa beach.

Finally, in Chapter IV, the composition and structure of the macrofaunal community in the artificial dune zone were compared with that in a natural dune in the same beach (Leirosa). The total density, taxa richness and diversity of macrofauna were similar between sites, but the artificial zone harboured a significantly lower density of *T. saltator*, coinciding with the zone where the geotextiles were placed in the beach. Therefore, this species was suggested to be a suitable bioindicator of disturbances on coastal dunes.

A general discussion section synthesised and discussed the thesis contribution to understanding the use of macrobenthic communities to assess ecological status and functioning of exposed sandy beaches, suggesting future research challenges.

Keywords: exposed sandy beaches, Atlantic coast, macrobenthic communities, human pressures, niche segregation, behavioural adaptations, Peracarid crustaceans, bioindicators.

Resumo

O principal objetivo do trabalho apresentado nesta tese é aumentar o conhecimento do estado ecológico e funcionamento de praias arenosas expostas na costa Atlântica Portuguesa. Os invertebrados bentónicos são estrutural e funcionalmente importantes nestes ecossistemas (e.g. reciclagem de nutrientes, relações tróficas), deste modo esta avaliação foi baseada na estrutura, composição e adaptações das comunidades macrobentónicas ao ambiente da praia. No contexto do crescente aumento de pressões humanas a atuar nestes ecossistemas, foi avaliado o uso de espécies chave como potenciais indicadores de distúrbios ambientais. Deste modo, este trabalho centra-se particularmente na avaliação do estado ecológico de praias arenosas sujeitas a pressões humanas (Capítulo I), na análise das estratégias de segregação de nichos em crustáceos (Capítulo II) e nas adaptações comportamentais de anfípodes Talitridae sujeitos a diferentes cenários ambientais (Capítulo III). No último capítulo (IV) é proposto um potencial bioindicador de impactos antropogénicos nos ecossistemas duna-praia.

O Capítulo I avalia se alterações nas comunidades macrobentónicas foram indicador de pressões humanas a longo prazo (atividades de turismo e obras de engenharia) na praia do Cabedelo, em comparação com uma praia de referência em Quiaios. Neste sentido, o ambiente físico e a estrutura das comunidades macrofaunais foram comparadas entre praias em 1999–2000 e 2010–2011. As alterações detetadas na macrofauna (diminuição da abundância do anfípode *Talitrus saltator* e do isópode *Tylos europaeus*) foram consistentes com a interpretação do aumento das pressões humanas na praia do Cabedelo. Em contraste, na praia em Quiaios foi descrito um padrão semelhante na estrutura ecológica nos períodos considerados. No entanto, apesar do padrão encontrado na praia do Cabedelo ser sugerido como resultado de pressões humanas, este resultado salienta a necessidade de posteriores avaliações ecológicas efetivas.

O Capítulo II descreve as estratégias de segregação de nichos em três crustáceos Peracarida nas praias de Cabedelo e Quiaios, que possuem diferentes disponibilidades de alimento (macroalgas, restos animais, plantas dunares). Neste sentido, foram efetuadas análises de isótopos estáveis de consumidores primários e potenciais fontes de alimento e o padrão de distribuição dos consumidores foi analisado no verão e inverno. Os resultados das dietas e da distribuição das espécies revelaram diferenças na segregação espacial e temporal dos nichos dos consumidores. Em Quiaios, as condições de inverno e a pouca disponibilidade de alimento, induziu à segregação dos nichos dos consumidores, pelo contrário, a maior disponibilidade de alimento na praia de Cabedelo permitiu uma dieta generalista destes crustáceos. Deste modo, a partição de recursos alimentares efetuada por estas espécies pode variar com a disponibilidade de alimento mas também com as condições ambientais.

No Capítulo III, foram avaliadas as adaptações comportamentais de anfípodes Talitridae em cenários ecológicos distintos. No primeiro caso, o comportamento (orientação

solar) e distribuição de duas espécies simpáticas foram analisados na praia de Quiaios. Apesar das condições estáveis oferecidas por esta praia mesotidal, cada espécie apresentou uma estratégia comportamental diferente. *Talorchestia brito* apresentou uma orientação solar definida e ocorreu principalmente na zona intertidal, enquanto que *T. saltator* apresentou uma distribuição e orientação mais flexíveis. Este estudo sugere que as restrições no habitat e as tolerâncias individuais de cada espécie podem determinar diferente flexibilidade comportamental no mesmo local. No segundo estudo do capítulo III, o comportamento (orientação e ritmos endógenos de locomoção) de *T. saltator* foi analisado numa praia recentemente restaurada (Leirosa) para testar as suas adaptações comportamentais à nova paisagem - duna frontal artificial. Os resultados revelaram uma orientação precisa para esta espécie que usa a bússola solar e indicações visuais (paisagem) para alcançar a zona ótima na praia, e um ritmo endógeno bem definido (com a componente de maré), o que sugere um comportamento estável desta população na praia da Leirosa.

Por fim, no Capítulo IV, a estrutura e composição das comunidades macrofaunais na zona de duna artificial foi comparada com a de uma zona de duna natural na mesma praia (Leirosa). A densidade total, a riqueza específica e diversidade da macrofauna foram semelhantes entre as duas zonas, no entanto, na zona artificial foram registadas as mais baixas densidades de *T. saltator*, que coincide com a zona artificial dos geotêxtis na praia. Deste modo, esta espécie foi sugerida como bioindicador de perturbações no sistema dunar.

A seção final de discussão geral sintetiza e discute o uso de comunidades macrobentónicas para avaliação do estado ecológico e funcionamento de praias arenosas expostas, com sugestões para futuros desafios científicos.

Palavras-chave: praias arenosas expostas, costa Atlântica, comunidades macrobentónicas, pressões humanas, segregação de nichos, adaptações comportamentais, crustáceos Peracarida, bioindicadores.

General introduction

“Fortunately the beaches of the world are cleaned every night by the tide. A fresh look always awaits the student, and every wave is a masterpiece of originality. It will ever be so. Go and see.”

Willard Bascom (1964)

The global need to protect sandy beaches

Ocean sandy beaches are among the most popular and attractive recreational venues for humans (Davenport and Davenport, 2006). However, they have been primarily valued for their amenity rather than for their ecological relevance. As a consequence, despite their importance as coastal ecosystems, studies on sandy beaches - the first significant studies were only published in the 1950's and 1960's (e.g., Parpi and Papi, 1953; Bascom, 1964; Clark, 1966) - did not contribute to the mainstream of ecological science in the same extent than studies on other types of systems.

At the time of the first International Sandy beach Symposium in 1983 held in South Africa, McLachlan and Erasmus (1983) posed the following questions: *“What environment could be more exciting to study than this sandy interface sea and land? And yet, how much do we know about sandy beaches?”* Sandy beach ecology research in that period was mainly based on quantitative studies of macrofauna ecology and ecophysiology (review in McLachlan and Erasmus, 1983). Since then, this science has advanced considerably, with a broad range of studies focusing on patterns, processes and regulatory mechanisms of sandy beach macrofauna, and has covered a large range of geographic areas, illustrating important emerging fields of research (review in Defeo and McLachlan, 2005; 2011; McLachlan and Brown, 2006; Scapini, 2006; Schlacher *et al.*, 2007, among others).

In 2012, South Africa hosted again the VIth International Sandy Beach Symposium, under the theme: *“Sandy beaches 2012: A new paradigm in the face of global change”*. Ecologists discussed and evaluated the current paradigms of sandy beach ecology and newly questions emerged, in response to the scientific knowledge at that time: *“What are the research gaps (with reference to patterns and processes) that should be addressed in future sandy beach research; What are the threats to sandy beach ecosystems; What are our research priorities with reference to threats and conservation of beaches?”*

Despite the advances achieved regarding knowledge on beach ecology, little is known about sandy beaches' ecosystems responses to pressures, contrary to other coastal ecosystems (e.g., estuaries, lagoons). Nevertheless, beaches are said to be among the most threatened ecosystems worldwide, and face unprecedented challenges and uncertain future (Brown and McLachlan, 2002; Schlacher *et al.*, 2007; Halpern *et al.*, 2008; Defeo *et al.*, 2009). Sandy beaches are increasingly affected by coastal squeeze, due to pressures arising

from human activities on the terrestrial side and alterations engendered by climate change in the marine side (Dugan *et al.*, 2010).

Defeo *et al.* (2009) presented a comprehensive review of threats to sandy beach ecosystems that encompass a wide range of pressures. From recreational activities (trampling, beach grooming, off-road vehicles), to engineering solutions to counteract erosion (beach nourishment, seawalls, breakwaters and groynes), different pressures are well documented as a cause of ecological damage to sandy beach habitats (Brown and McLachlan, 2002; Schlacher *et al.*, 2007; 2008b; Defeo *et al.*, 2009). However, it is broadly recognised that one of the main concerns with respect to sandy beaches is the implications when superimposing predicted climate change impacts on the already established threat of coastal squeeze (Dugan *et al.*, 2010). The combined cumulative impacts of the effects of climate change and the increasing human pressures on coastal areas may affect the physical, biological and biogeochemical characteristics of the beaches, modifying their ecological structure, their functions, and the goods and services they provide as ecosystems.

In addition, the current vulnerability of exposed sandy beaches is also due to a synergistic consequence of still being a system with low public and scientific profile (Schlacher *et al.*, 2006; Dugan *et al.*, 2010). Some regions have considerably advanced in this research field and have demonstrated the ecological relevance and vulnerability of beach environments. Such knowledge is mainly concentrated in the southern hemisphere (McLachlan and Dorvlo, 2005) essentially in South Africa (*e.g.*, Schoeman *et al.*, 2000; Harris *et al.*, 2011; 2013), Australia (*e.g.* Jones *et al.*, 2008; Schlacher *et al.* 2011; 2012; 2013) and South America (*e.g.*, Veloso *et al.*, 2008; Jaramillo *et al.*, 2012). Actually, in South Africa, scientists are now proposing a first attempt to design an ecosystem-based spatial conservation plan for sandy beaches (Harris L., personal communication), and recently Harris *et al.* (2013) proposed the first methodology for setting conservation targets for sandy beaches. In contrast, other coastal regions still have a long way to go, as the Portuguese Atlantic coast.

The key message that emerged from the Sandy Beach Symposium of 2012 was that there is an urgent need for baseline information about the ecological status of sandy beaches, and effective science-based management to maintain both biodiversity and life-support for the society, in the form of goods and services provided by these valuable ecosystems (*e.g.*, Costanza *et al.*, 1997; Barbier *et al.*, 2011).

In this first part of the present thesis, a general overview of the current paradigms of beach ecology and emergent research needs will be addressed.

Sandy beach ecosystems: goods and services

In addition to the intrinsic scenic value, the potential for recreational activities and the life-supporting characteristic, sandy beaches as ecosystems provide a plethora of goods and services that are highly valuable and unique to support human life in the coastal zone (Costanza *et al.* 1997; Schlacher *et al.*, 2008b; Defeo *et al.*, 2009; Barbier *et al.*, 2011). Among the most important services provided by sandy beaches are the coastal protection, in the form of wave dissipation and associated buffering against extreme events (*e.g.*, storms, tsunamis), the sediment storage and transport, and water filtration and purification. Beach ecosystems are also important in processing large quantities of organic material and recycling nutrients back to coastal waters (*e.g.* Barreiro *et al.*, 2013). Certain features and processes are related with the functional links between the terrestrial and the marine environments such as the flow of nutrients and energy through the food web (Dugan *et al.*, 2003), the maintenance of biodiversity and genetic resources, nursery areas for juvenile fishes and nesting sites for turtles and shorebirds (McLachlan and Brown, 2006).

The unprecedented natural and anthropogenic pressures have resulted on modified sandy beach ecosystems worldwide and therefore it has become critical to understand how the entire system and the goods and services it provides, will respond to further predicted natural and anthropogenic threats (Brown and McLachlan, 2002).

The physical environment

Sandy beaches are the world's single largest dynamic type of open shorelines and are found at the boundary between land and sea (McLachlan and Brown, 2006). The simplest physical definition of a beach is "*a wave-deposited accumulation of sediment lying between modal wave base and the upper swash limit, where wave base is the maximum depth at which waves can transport material shoreward, and the swash limit, the landward limit of wave action and sediment transport*" (Short, 1999). However, additional variables can influence a particular beach ecosystem, namely tidal currents, wind, sediment type, temperature and biotic interactions (McLachlan and Brown, 2006).

The general physical-control paradigm accepted for sandy beach ecology emphasises that these dynamic environments are defined by three main factors: tidal regimes, wave climates and sand particle size (Defeo and McLachlan, 2005). The interplay among these factors produces a wide range of morphodynamic types (review in Short, 1999), and determines the instability and complexity of the beach environments. However, the ability to absorb wave energy makes sandy beaches one of the most stable and resilient types of dynamic coastlines (McLachlan and Brown, 2006).

In terms of scientific knowledge, sandy beaches have historically been regarded as important sand deposits, and much is known about beach morphology and sedimentology (McLachlan and Brown, 2006 for a review). In addition, beach management often focuses

only on the physical attributes and processes of beaches, particularly those related to managing sand budgets and shoreline stability. This knowledge is used by coastal managers to protect both recreational amenity and societal assets, but beaches are not just sand. They provide habitats for many plant and animal species, including iconic species such as marine turtles and birds (McLachlan and Brown, 2006). Accordingly, beaches have irreplaceable anthropocentric and ecocentric values that need to be preserved.

It is well recognised the primacy of environmental control as drivers of faunal patterns and a number of studies report this strict relationship between environmental factors and biota (Defeo and McLachlan 2005, 2011; Rodil *et al.*, 2012, and references therein). Nevertheless, human activities such as beach nourishment and coastal armouring (*e.g.*, Dugan and Hubbard, 2006; Walker *et al.*, 2008; Fanini *et al.*, 2009; Schlacher *et al.*, 2012), usually used to prevent erosion, can substantially affect the prevalence and morphology of various coastal habitats and the physical properties of sandy beaches, which in turn can promote biological changes (*e.g.* abundance and diversity of communities) and alter the ecological functions of the entire ecosystem.

The biological environment

In spite of seeming as abiotic deserts, sandy beaches provide habitats for several species, ranging from buried microbes and phytoplankton, to macrofauna and vertebrates, which can include nesting sea turtles, migratory or resident shorebirds and surf-zone fishes (McLachlan and Brown, 2006). All of these components may interact in a trophic network to create the open ecosystem of sandy beaches, which exchanges sand, organic matter and nutrients with adjacent habitats, especially the sand dunes and the surf zone (*e.g.*, Colombini *et al.*, 2003a; Mellbrand *et al.*, 2011).

A major service provided by sandy beach ecosystems is the decomposition of organic matter, and the re-mineralisation of nutrients by small benthic organisms (Dugan *et al.*, 2011), which can also play an additional functioning role in filtering seawater (McLachlan and Brown, 2006). Consequently, the beach fauna can potentially contribute to the productivity of near-shore waters and the stocks of higher-profile species such as fish and shorebirds (McLachlan and Brown, 2006).

The invertebrate resident beach macrofauna, which includes crustaceans, molluscs and polychaetes, is usually dominant in exposed sandy beaches, making up more than 90 % of species abundance and biomass (McLachlan and Brown, 2006). Macrofauna has been the most well studied component of these ecosystems and much of the ecological theory on sandy beaches has been developed from the patterns and environmental responses displayed in this group of organisms (Defeo and McLachlan, 2005 for a review). Consequently, the relevance of macrofauna in sandy beaches will be specifically discussed with more detail below.

Macrofaunal assemblages and the assessment of beach conditions

Often dominated by crustaceans (amphipods, isopods, decapods), the macrofaunal assemblages of exposed sandy beaches play a relevant ecological role on the equilibrium of this ecosystem, with some species being frequently identified as key species (see for instance, Gonçalves *et al.*, 2009; 2013).

The adaptations of animals to beach environment are peculiar with respect to those of animals from other marine habitats and result from the instability of the substratum coupled with dynamic environmental conditions (waves, winds, temperature, etc.). Because beach environments lack biological structures and are extremely dynamic in space and time, their macrofaunal communities have been considered physically controlled. Indeed, the primacy of physical control on biological communities living on sandy beaches is a well-established paradigm in sandy beach ecology (Defeo and McLachlan 2005 and references therein). Several studies examined the strict relationship between physical characteristics (latitude, exposure, wave climate, tides, sediment, among others) and community features (*e.g.*, McLachlan and Dorvlo, 2005; Defeo and McLachlan, 2011; Rodil *et al.*, 2012).

Sandy-beach dwellers are very well adapted to this highly dynamic, three-dimensional environment, exhibiting remarkable physiological, morphological and behavioural adaptations to this unstable environment (Brown, 1996; McLachlan and Brown, 2006; Scapini, 2006). The range of unique adaptations displayed by these species includes mobility, rhythmicity in their behaviour and advanced mechanics of orientation displaying a high level of phenotypic plasticity (Brown, 1996; Scapini 2006). Particularly, due to their direct dependency on the physical environment, these species attributes (adaptations) should correlate closely with the physical environmental parameters of sandy beaches and change when disturbance occurs. In this sense, sandy beach macrofauna species became a valuable fraction of the ecosystem and have been proposed for ecological assessments (*e.g.*, Gonçalves *et al.*, 2013). Indeed, several studies have demonstrated that macrobenthic communities and/or individual species can respond negatively to anthropogenic disturbances (Jones *et al.*, 2008; Fanini *et al.*, 2009; Leewis *et al.*, 2012; Schlacher *et al.*, 2012). In this sense, some species (sandhoppers, isopods, crabs) have been widely proposed as suitable bioindicators of beach disturbances (*e.g.*, Ugolini *et al.*, 2008; Scapini and Ottaviano, 2010, Bessa *et al.*, 2013a). For these reasons, macrobenthic communities can be effective in assessing sandy beaches' ecological quality conditions and constitute the main theme of this thesis.

An overview on the Portuguese Atlantic coast

The coast of mainland Portugal extends for more than 900 km. Of these, 60% are beaches of which 28% is currently facing severe problems of erosion (Eurosion, 2004). The current situation has been identified as a coastal response to the reduction of river sediment sources, mean sea-level rise and human occupation of sandy beaches (reviewed in Veloso-Gomes and Taveira-Pinto, 2003). Some of the management strategies to face erosion, which have been applied in the Portuguese coastal zone have been breakwaters, dune stabilisation projects and beach nourishment, but the erosive processes still persist and in some cases the situation is getting worse (*in situ* personal observations).

Portuguese sandy beaches are highly valuable for socio-economic reasons, constituting iconic assets to society, but are less known and valued for their ecological significance (but see Marques *et al.*, 2003 and Gonçalves *et al.*, 2009; 2013). In fact, the national plans for the Management of Coastal Zone in the past (Veloso-Gomes and Taveira-Pinto, 2003) and the new proposal for the next years (Plan for the Protection and Enhancement of Littoral of Portugal for the period 2012–2015) devoted little attention to the ecological value of sandy beaches as ecosystems, which was probably due to the relatively limited biological scientific research data available to inform policy and decision making. Contrarily to other geographic zones of the Globe, the amount of scientific research carried out on the ecology of the Portuguese sandy shores has been extremely low, with the exception of the central coastal region of Portugal (Anastácio *et al.*, 2003; Marques *et al.*, 2003; Gonçalves *et al.*, 2003; 2005; 2009; 2013; Gonçalves and Marques, 2011).

Gonçalves *et al.* (2009; 2013) and Gonçalves and Marques (2011) provided a comprehensive work on the structure, composition and functioning of the macrofaunal communities in two sandy shores (Cabedelo and Quiaios) in the western Atlantic Coast of central Portugal in 1999–2000. In addition, these authors detailed the biology, population dynamics and secondary production of the following key species: the isopod *Tylos europaeus* (Gonçalves *et al.*, 2005), and the talitrid amphipods *Talorchestia brito* (Gonçalves *et al.*, 2003) and *Talitrus saltator* (Marques *et al.*, 2003) and compared the population dynamics of the last two species with Mediterranean populations. These authors opened a series of questions that were worthy of further research, in particular regarding the ecological status and functioning of sandy shores, to gather fundamental information to deal with the increasing pressures that have been threatening this coast. In Portugal, there is a need to conduct baseline research on the ecology of sandy beaches and make these results available to decision-making authorities that may be unaware of the ecological importance of these ecosystems.

General aims and thesis outline

The present PhD thesis has the general objective of contributing to a comprehensive pool of information regarding sandy beaches on the Atlantic coast of Portugal, expanding our knowledge on their ecological status and functioning, and identifying critical areas to guide future research.

To achieve this goal specific topics were addressed based on the use of macrofaunal assemblages with the following specific objectives:

- To assess the ecological status of sandy beaches subjected to potential long-term human pressures;
- To analyse the niche segregation strategies of sympatric species when dealing with contrasting food availabilities;
- To investigate the behavioural adaptations (sun orientation and endogenous rhythms) of talitrid amphipods in distinct environmental scenarios (undisturbed and after dune restoration);
- To propose a reliable indicator of anthropogenic impacts on beach-dune ecosystems.

Each chapter has been conceived as a stand-alone scientific paper either published or submitted for publication, with the exception of the Chapter III, which is based on two scientific papers. To allow separate reading of each chapter, some content overlap was unavoidable. The final part of the thesis uses the outcomes of these chapters to build up a general discussion and recommend future challenges regarding sandy beach ecology-based management on the Portuguese Atlantic coast.

The thesis is based on the following scientific papers:

Chapter I

Bessa F, Gonçalves SC, Franco JN, André JN, Cunha PP, Marques JC. Changes in macrofaunal assemblages as response indicator to potential long-term human pressures on exposed sandy beaches. *Submitted to Ecological Indicators*.

Chapter II

Bessa F, Baeta A, Marques JC. Niche segregation amongst sympatric species at exposed sandy beaches with contrasting wrack availabilities illustrated by stable isotopic analysis. *Ecological Indicators (accepted for publication)*.

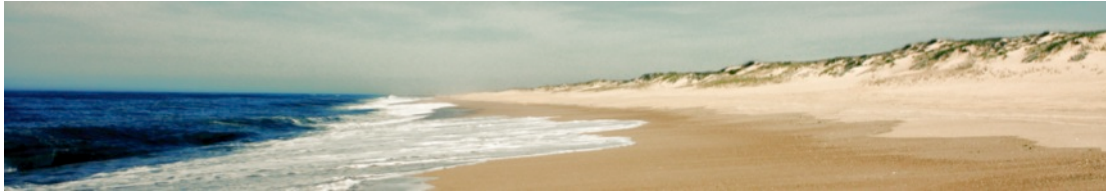
Chapter III

Bessa F, Marques JC, Scapini F, Behavioural adaptations of two sympatric sandhoppers living on a mesotidal European Atlantic sandy beach. *Submitted to Behavioural Ecology and Sociobiology*.

Bessa F, Rossano C, Nourisson D, Gambineri S, Marques JC, Scapini F, 2013. Behaviour of *Talitrus saltator* (Crustacea: Amphipoda) on a rehabilitated sandy beach on the European Atlantic Coast (Portugal). *Estuarine, Coastal and Shelf Science* 117: 168–177.

Chapter IV

Bessa F, Cunha D, Gonçalves SC, Marques JC, 2013. Sandy beach macrofaunal assemblages as indicators of anthropogenic impacts on coastal dunes. *Ecological Indicators* 30: 196–204.



Chapter I

Changes in macrofaunal assemblages as response indicator to potential long-term human pressures on exposed sandy beaches

Abstract

Sandy beaches are natural dynamic ecosystems but worldwide are becoming increasingly disturbed by intensive human direct use, coastal development and erosive evolution. In this study we examined whether ten years of potential increased human pressures have resulted in significant changes in the macrofaunal assemblages' structure and composition of two mesotidal sandy beaches (Cabedelo – urban beach, and Quiaios – rural beach) on the European Atlantic coast (Portugal). Seasonal macrofaunal collections were performed in two periods, one in 1999–2000 and another in 2010–2011, at both beaches. The physical environment did not change significantly in both beaches throughout the studied periods, however, the Cabedelo beach was subject to an increase of human pressures (tourism activities and engineering actions) in the last ten years, when compared with Quiaios beach. The univariate community descriptors (total density, species richness and diversity) did not differ significantly between the two periods at Quiaios beach. In contrast, at Cabedelo, temporal differences were found principally regarding the abundances of the amphipod *Talitrus saltator* and the isopod *Tylos europaeus*, two of the most abundant species at both beaches. PERMANOVA tests highlighted these temporal variations and the SIMPER analysis attributed to these species the main differences found between periods at the Cabedelo beach. Since the physical environment was similar in both periods, the ecological changes were most likely attributed to the increased human pressures, in particular the increase in the number of tourists observed in the urban area in the last ten years. However, our study emphasises the need of further robust and effective impact assessments and long-term studies to better discern between natural and human induced changes on sandy beaches.

Keywords: sandy beaches, macrofaunal assemblages, talitrids, human pressures.

Introduction

Human activities have been recognised to place heavy pressures on coastal ecosystems and are predicted to be exacerbated as the growth in both economies and population continue (Millennium Ecosystem Assessment, 2005; Halpern *et al.*, 2008). Exposed sandy beaches are not an exception and during the last decades, in most countries, sandy beaches have been subject to severe and increasing pressures as a result of conflicting uses such as coastal development, environmental pollution, and intensive human use of beaches, among other coastal disturbances (review in Defeo *et al.*, 2009) as those documented in the Portuguese coast (Velo-Gomes and Taveira-Pinto, 2003).

Globally, coastal tourism started in the 19th century but has clearly increased in a non-linear fashion ever since. Despite the economic benefits to the society, there are also intrinsic negative environmental costs (reviewed in Davenport and Davenport 2006).

In addition, on urbanised coasts (*sensu* Velo-Gomes *et al.*, 2008), several management practices such as nourishment and coastal armouring have been intensively used to counteract the erosive evolution of coasts, but the ecological implications of these actions have been less considered (Schlacher *et al.*, 2006; Defeo *et al.*, 2009). Superimposed on these trends of coastal impacts is a predicted increased frequency and intensity of extreme natural phenomena (*e.g.*, storms) due to the effects of global climate change that can affect the fauna and flora of ocean-exposed sandy shores dramatically (IPCC, 2007; Schlacher *et al.*, 2007; Defeo *et al.* 2009; Dugan *et al.*, 2010).

Since exposed sandy beaches are physically dynamic habitats inhabited by specific macrofauna communities (mainly crustaceans, molluscs and polychaetes), that are known to be structured mainly by physical factors (reviewed in Defeo and McLachlan, 2005), documenting the biotic responses to particular modifications of the physical environment can be used for the assessment of the ecological conditions of beaches. In particular, the upper zones of sandy beaches typically support macroinvertebrates with particular adaptations and restricted dispersal mechanisms, making them particularly vulnerable to physical disturbances on the beach environment (McLachlan and Brown, 2006).

In fact, in the last years, several studies have been showing the negative effects to macrofaunal communities and/or individual species on the beach-aeolian dune environments, when facing particular human disturbances caused by coastal management practices such as beach nourishment (*e.g.*, Jones *et al.*, 2008; Fanini *et al.*, 2009; Schlacher *et al.*, 2012; Bessa *et al.*, 2013a) and coastal armouring (Dugan and Hubbard, 2006; Dugan *et al.*, 2008, Walker *et al.*, 2008). Yet, human recreational activities have been recognised to negatively affect beach fauna caused by the intensive use of beaches (Comor *et al.*, 2008; Ugolini *et al.*, 2008; Velo-Gomes *et al.*, 2008; Schlacher and Thompson, 2012) and by the impact of road vehicles on sandy shores (Schlacher *et al.*, 2008a; 2008c, Sheppard *et al.*, 2009; Schlacher and Lucrezi, 2010; Walker and Schlacher, 2011).

Overall, the main consequences of these physical disturbances and consequent habitat fragmentation on sandy beaches are the occurrence of significantly fewer species and/or reduced densities (Colombini *et al.*, 2011), or even the disappearance of once abundant species from urbanised coasts (e.g., Hubbard *et al.*, 2013). Since these species play a pivotal role in the food web of sandy beaches (McLachlan and Brown, 2006), the effects of eventual species losses can propagate to the entire trophic web and have the potential to jeopardise the ecological functioning and integrity of the entire ecosystem.

Despite the long history of human activities on sandy shores, very little is known about their potential ecological implications along time. The main difficulty for sandy beach ecologists to address this issue is the fact that apart from the adverse effects of human activities, exposed sandy beaches show wide variations in environmental conditions, making difficult to discern natural from human-induced changes. In addition, the knowledge about the ecological conditions of beaches before the increase of the above-mentioned human pressures is still scarce.

The Portuguese Atlantic coast extends for more than 900km, of which 60% are beaches and 28% of this coast is currently facing severe problems of erosion (EuroSION, 2004). Hence, a set of coastal management practices (in particular beach nourishment and breakwaters) had been applied on the mainland coast of Portugal over the last decades (Veloso-Gomes and Taveira-Pinto 2003), and was already proposed in the Plan for the Protection and Enhancement of Littoral of Portugal (PAPVL) for the period of 2012–2015, in order to counteract the erosion process and the “coastal squeeze” phenomenon on this coastline (Veloso-Gomes and Taveira-Pinto 2003).

On the other hand, in the last years, the increase in the number of tourists recorded in the country (INE - National Institute of Statistics) has potentiated the improvement of sandy beaches’ facilities and the conditions to promote the aesthetical and economic value of beaches along the entire coast (Veloso-Gomes and Taveira-Pinto 2003). However, the ecological implications of these actions have received only a subsidiary consideration.

Gonçalves *et al.* (2009) studied the macrofauna communities’ structure at two similar undisturbed exposed sandy beaches in the central coast of Portugal during the period of 1999 and 2000, and identified three supralittoral crustaceans as key elements on these beaches. In addition, Gonçalves *et al.* (2013) proposed the use of the bioecology of these species as potential assessment tools to evaluate environmental disturbances.

Given the vital role of macroinvertebrates in the ecological functioning of sandy beach ecosystems, the goals of this study were: (1) to investigate possible temporal changes in the structure and composition of the macrofaunal assemblages on two sandy beaches that differed in the intensity of human pressures during two periods (1999–2000 and 2010–2011), and (2) to assess whether possible ecological changes reflected the effects of potential long-term human pressures. This assessment should be seen as one first step towards more comprehensive and robust evaluation of cumulative human pressures and their impacts on sandy beaches.

Methodology

Study area

Our study areas, Cabedelo and Quiaios beaches, are located in central Portugal, on the western coast of the Iberian Peninsula (Figure 1). This coastal area, presents a warm temperate Atlantic-Mediterranean climate and semidiurnal tides with maximum amplitude of about 3.5 m (Gonçalves *et al.*, 2009). The studied beaches are affected by a wave regime that has an average significant wave height of 1.8 m (with wave directions of N242–297°), reaching 5.0–6.5 m (1% of the total, with N262–282° directions) (Santos *et al.*, 1991). The Cabedelo sandy beach is located in the vicinity of the Mondego estuary, while the Quiaios beach is located further north, about 8 km south of the Cape Mondego (Figure 1). In addition, the littoral dynamics of this coastal sector and the historic beach evolution is provided by Cunha and Dinis (1998; 2002).

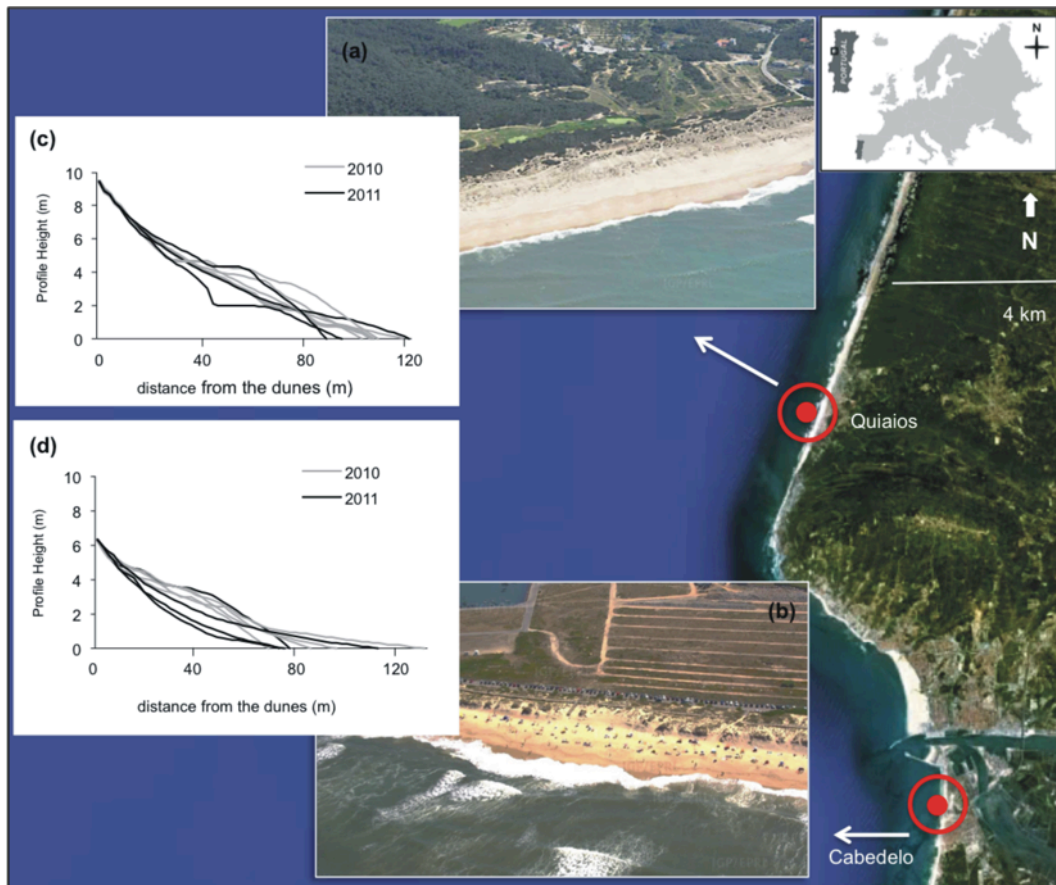


Figure 1. Location of the two sampling areas, the Quiaios (a), and the Cabedelo (b) beaches on the western coast of mainland Portugal, and the respective transversal topographic profiles (c) for Quiaios and (d) for Cabedelo drawn in 2010–2011.

During the 1999–2000 period, Gonçalves *et al.* (2009) analysed the structure and composition of macrofaunal assemblages of both sandy beaches because of their relatively undisturbed nature. However, particularly regarding the Cabedelo beach, the concept of “*undisturbed*” has been changing since this beach became increasingly popular as a tourism area in the last years (Figure 2). Cabedelo beach is widely recognised as an urban beach since it is closer to the important tourist centre town of Figueira da Foz, and have a high potential for human recreational use. In fact, in order to improve the human use of beaches, the entire urban area, including this beach, has been subjected to diverse transformation actions along time, i.e. improvement of tourist facilities (2008, local authorities), beach grooming (the removal of organic beach-cast material along the strandline that occur in particular during the spring-summer seasons) and consequently leading to an increase of human use of the beach (e.g., recreational activities such as surf championships, *in situ* observations). In addition, the aeolian dunes immediately adjacent to the beach are backed by infrastructures (boardwalks and parking areas, see Figure 1b).

In addition, the coastal zone management plans approved for the entire coast of mainland Portugal to counteract the erosive evolution (Velo-Gomes and Taveira-Pinto 2003), has been also implemented in the Cabedelo beach, in particular, the extension of a breakwater (400 m) and related adjustment with rocks on the beach (2008 and again in 2010, local authorities).

In contrast, at Quiaios beach, which is located in a rural area, the sampling point was chosen on an undisturbed zone, with limited access (no physical facilities placed on this zone of the beach, see Figure 1) and for this reason have a lower human beach use. This beach is about 3 km wide and rises to nearly 100 m and is backed by an extensive and well-developed dune sheet with varied vegetation and biota. This very exposed beach is of high-energy intermediate type, mesotidal, with moderately rich macrofauna (see Gonçalves *et al.*, 2009; Gonçalves and Marques, 2011).

In the last years, the entire coast of mainland Portugal has received an increase in the touristic occupation, especially during the warmer spring and summer months. The information about this increase in the central region of Portugal during the last ten years was provided by the national authorities of tourism (INE and Tourism of Portugal), in particular for the urban (city of Figueira da Foz) and rural (Quiaios) areas. This data was used to obtain a quantification (a proxy) of the potential increase in the human use of these sandy beaches in the last ten years.

Sampling design

Between 1999 and 2000, Gonçalves *et al.* (2009) investigated the macrofaunal communities' structure at Quiaios and Cabedelo beaches. Quantitative samples were taken monthly, during low neap tides by using a wooden square of 0.25m² to a depth of 20cm and were sieved through a 1 mm mesh. The sampling design comprised 2 transects arranged at regular intervals (10 levels, 3 replicates per level) between the low-water mark and the

foredune. For each transect, the first five sampling levels from the waterline to the high low tide watermark were considered the intertidal zone and the subsequent five levels until the base of the dunes, the supralittoral zone. The animals collected were separated in the laboratory and preserved in 70% alcohol for identification. Sediment samples were collected to determine the sediment grain size, water and organic contents in the sediment following the protocol described in Marques *et al.* (2003).

During 2010 and 2011, Cabedelo and Quiaios beaches were sampled once on each season (January for winter, May for spring, August for summer and November for autumn), comprising a total of eight sampling events. Fauna sampling design and beach surveys followed standard techniques described in Gonçalves *et al.* (2009). Topographic transverse profiles of the two beaches were obtained from a fixed point in the foredune to the swash zone at every sampling occasion.

Data analyses

For each period (1999–2000 and 2010–2011) species abundance data are expressed as ind.m². The 1999–2000 data were used for a qualitative comparison of the community structure and composition with the recent period in 2010–2011. The physical features (sediment properties, beach width) of the beaches were compared using paired-*t* tests.

The potential effects on the macrofaunal assemblages from the increased human pressures were assessed using Before-After Control-Impact (BACI) design as outlined in Underwood (1992; 1993), contrasting data from the “*impact*” location (Cabedelo beach) with a “control” reference location (Quiaios beach). The two periods were considered as “*Before*” (1999–2000) and “*After*” (2010–2011).

The biological data from the BACI design were analysed with Permutational Analysis of Variance (PERMANOVA) (Anderson 2001) with four factors: (1) Beach (fixed: Cabedelo and Quiaios), (2) Time (fixed; 1999–2000 and 2010–2011); (3) Zone of the beach (fixed and nested in Beach factor, supralittoral and intertidal) and (4) season (fixed: nested in Zone and Beach: spring, summer, autumn and winter). Under this approach, an impact is indicated by statistically significant Beach x Time interaction (i.e. temporal trajectories of Cabedelo and Quiaios from before and after the potential increase in the human pressures), and pairwise tests were performed to discriminate the differences within the levels of each factor. The response variables chosen for univariate descriptors were: mean total density, species richness (number of taxa) and diversity (Shannon-Wiener's Index) and were fourth root transformed. Euclidean distance similarity matrices were used for univariate data based on 9999 permutations of residuals under a reduced model.

The same statistical framework (BACI and PERMANOVA) was used to analyse spatial and temporal patterns in multivariate macrofaunal assemblages and pairwise comparisons were performed whenever significant differences were detected among the interaction term or the main factors (Anderson, 2001). Based on fourth root transformed

species abundance, data similarities were calculated using the Bray-Curtis coefficient. The statistical significance of variance components was tested using 9999 permutations (if the number of permutation was lower than 150, the Monte Carlo permutation p was used) and was set at a threshold of 0.05. Differences in macrofaunal assemblages' structure were visualised using nonmetric multidimensional scaling ordinations of the average values from each Beach x Time interaction. Where significant differences were found in the PERMANOVA tests, similarity percentage routine (SIMPER) was used to verify which taxa contributed the most to the dissimilarities. All analyses were performed using the PRIMER v.6 with the PERMANOVA + add-on (PRIMER-E Ltd., Plymouth, UK).

The potential relationships between the changes in the macrofauna communities and the increase in the number of tourists were analysed using Pearson's correlations.

Results

Habitat: what changed in 10 years?

The specific beach physical environment was highly similar between the two time periods (1999–2000 and 2010–2011) for both Cabedelo and Quiaios beaches (Table 1). The topographic transverse profiles drawn in 2010 and 2011 for both beaches revealed a stable shoreline slope with no clear differences between the years (Figs. 1c and 1d). In addition, the physical measurements (mean width and extension of the intertidal area) were highly similar between the periods at both locations ($p > 0.05$), being Quiaios wider than Cabedelo (Table 1). Furthermore, paired t -tests applied to the overall environmental variables on both beaches did not detect any significant temporal variations (Table 1). According to the Wentworth grain size scale described in McLachlan and Brown (2006) the Quiaios beach had coarser sediments, while the Cabedelo beach had medium sands; a similar pattern was registered among periods (Table 1).

Table 1. Comparison of physical features of Cabedelo and Quiaios beaches between the 1999–2000 and 2010–2011 periods. Mean values (\pm SE) are presented, and p values refer to paired t -test between periods.

Feature	Cabedelo			Quiaios		
	1999–2000	2010–2011	p	1999–2000	2010–2011	p
Width of the beach (m)	60 (20)	74 (18)	0.217	100 (10)	93 (15)	0.3791
Intertidal area (m)	30 (10)	40 (18)	0.284	50 (9)	40 (10)	0.0964
Sediment grain-size (μ m)	Medium sand (250-500)	Medium sand (250-500)	-	Coarse sand (500-1000)	Coarse sand (500-1000)	-
Sediment moisture (%)	3.24 (1.22)	7.47 (6.68)	0.0608	2.64 (0.95)	4.55 (4.26)	0.1784
Supralittoral	2.53 (1.44)	2.17 (1.37)	0.5286	2.08 (1.30)	1.36 (1.02)	0.1281
Intertidal	4.11 (1.88)	6.77 (5.45)	0.152	3.07 (0.88)	4.76 (3.83)	0.1855
Organic matter content (%)	0.15 (0.05)	0.16 (0.13)	0.9594	0.19 (0.35)	0.45 (0.62)	0.2397
Supralittoral	0.13 (0.03)	0.12 (0.07)	0.9249	0.19 (0.35)	0.61 (0.87)	0.161
Intertidal	0.15 (0.04)	0.14 (0.14)	0.962	0.21 (0.38)	0.29 (0.13)	0.4429

The precise human trampling effect on the beach on both periods was not possible to assess (there is no available data on the number of tourists on the beach itself), in this sense, we analysed the number of tourists staying in the central region of Portugal during the period of 1999 to 2011 (Figure 2). In these last ten years there was a clear increase in the number of tourists in the central region (Figure 2a), with a higher number being recorded in the urban zone when compared with the rural area of Quiaios (Figure 2c). A seasonal pattern was visible in both periods, with higher number of tourists being recorded during the spring-summer months (from May to September, Figure 2c).

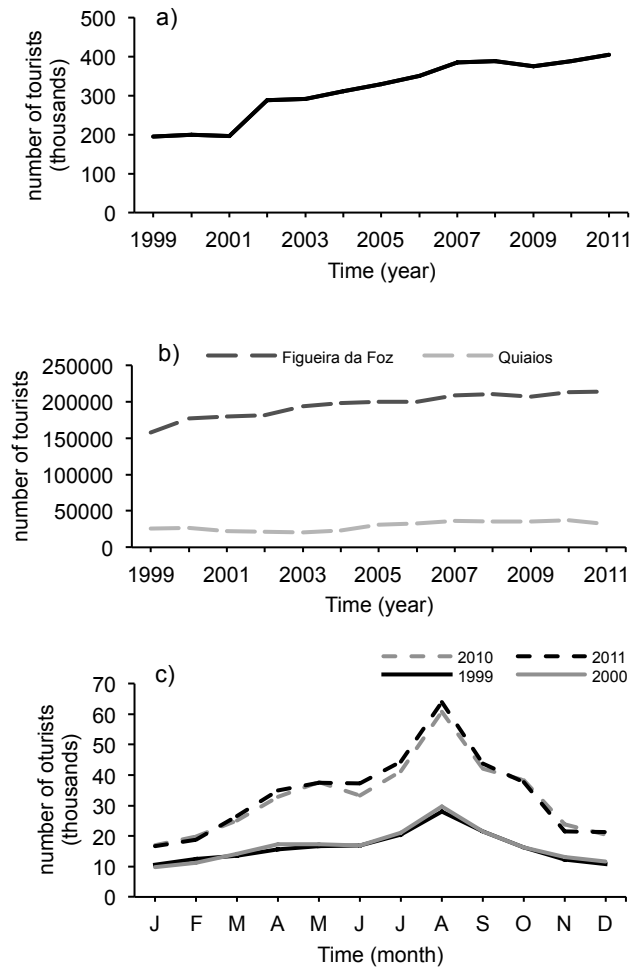


Figure 2. Number of tourists from 1999 to 2011 (a) in the central region of Portugal, (b) at Quiaios and Figueira da Foz, and (c) distributed by month in the central region (indicator: occupation rate, source: INE).

Macrofaunal assemblages: what changed in 10 years?

A total of 33 different taxa were recorded in Quiaios and Cabedelo beaches over the studied period and the taxonomic structure was similar for both beaches during the two periods (1999–2000 and 2010–2011, Table 2). In particular, the three most abundant taxa (adding up to 90% of the total abundance) were crustaceans, namely, the isopod *Tylos europaeus* and two talitrid amphipods, *Talitrus saltator* and *Talorchestia brito*. However, a different pattern of dominance of these species in the structure of the macrofauna community was recorded for each beach. In the Cabedelo beach, the most abundant species were *T. saltator* (54%), followed by *T. europaeus* (11%) and *T. brito* (8%), while in Quiaios the most abundant species were *T. europaeus* (67%), followed by *T. brito* (20%) and *T. saltator* (9%). Still, this pattern was highly similar in both periods. Resident species such as the isopod *Eurydice pulchra* and *Eurydice naylori*, the amphipods *Pontocrates arenarius* and *Haustorius arenarius* and the coleopteran *Phaleria cadaverina* were also common on both beaches

during the two periods considered. The mysids *Gastrosaccus sanctus*, and the coleopterans *Callicnemis latreillei* and *Aegiala arenaria* occurred only at Cabedelo, whilst the coleopteran *Gonioctena olivacea* were only observed at Quiaios beach.

Univariate patterns

Regarding species composition based on density data of macrofaunal assemblages, at Cabedelo beach was recorded a decrease in the average total density of macrofauna, in particular in the supralittoral zone (Figure 3a, $72 \pm 17 \text{ ind.m}^2$ in 1999–2000 and $18 \pm 4 \text{ ind.m}^2$ during 2010–2011). Nevertheless, these differences were not detected in the PERMANOVA outputs (Table 2, p (perm) > 0.05 for all factors with interaction with time) where the mean total density of macrofauna, the number of taxa, and diversity (Shannon-Wiener Index) of both beaches did not register significant different temporal trajectories (interaction term B x T: p (perm) > 0.05 for these univariate descriptors, Table 2).

Table 2 Results of PERMANOVA tests performed on the community structure measures (Total density, Species richness, Shannon-Wiener Index, *Talitrus saltator*, *Tylos europaeus* and *Talorchestia brito* densities) between beaches (B – Cabedelo, Quiaios), time periods (T – 1999–2000, 2010–2011), and zones on the beach (Z – supralittoral, intertidal).

Factor	df	Total density		Species richness		Shannon-Wiener Index		<i>Talitrus saltator</i>		<i>Tylos europaeus</i>		<i>Talorchestia brito</i>	
		MS	F	MS	F	MS	F	MS	F	MS	F	MS	F
B	1	0.74	0.04	0.23	0.41	2.03	4.91	59.29	5.94***	105.35	5.78**	13.56	3.75
T	1	11.39	2.61	0.04	0.02	1.38	6.19	19.87	9.43***	7.57	2.52	4.21	3.91
Z (B)	2	0.92	0.04	0.06	0.11	0.11	0.28	12.79	1.28	3.02	0.17	16.48	4.56**
B x T	1	7.65	1.76	1.26	5.54	0.61	2.75	24.81	11.77***	4.99	1.66*	1.66	1.54
S x (Z (B))	12	21.93	7.95***	0.58	2.42*	0.42	1.94	10.15	11.19***	18.55	12.31***	3.66	3.39***
Z (B) x T	2	9.64	2.22	0.45	1.96	0.49	2.18	4.72	2.24*	1.59	0.53	4.05	3.76
S (Z (B)) x T	12	4.38	1.59	0.23	0.95	0.22	1.03	2.13	2.35	3.02	2.01	1.08	0.99
Res	20	2.76		0.24		0.21		0.91		1.51		1.08	
Total	51												

Bold values indicate significant results: * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$

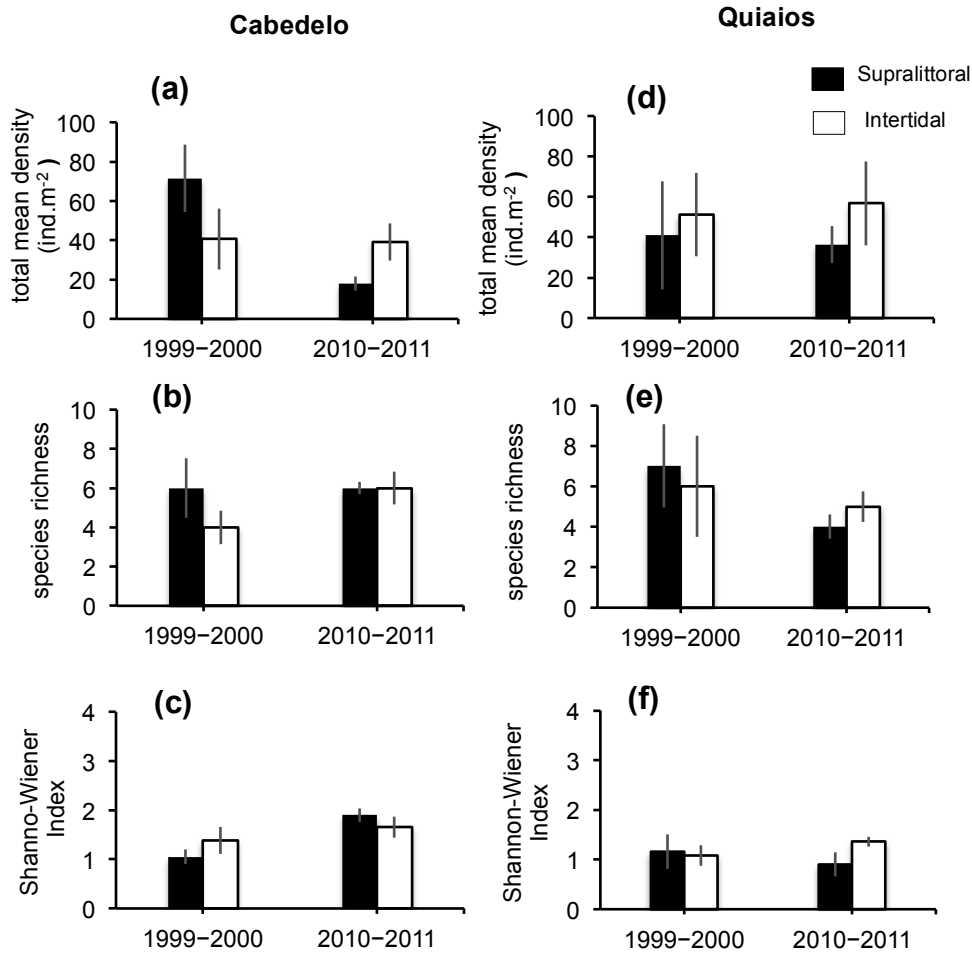


Figure 3. Spatio-temporal variation of the univariate descriptors of the faunal assemblages: Total mean density (a and d), Species richness (b and e), and Shannon-Wiener Index (c and f), for Cabedelo and Quiaios beaches among periods. Mean values and standard errors are presented for the zones (supralittoral and intertidal) of each studied beach.

However, concerning the individual average density of two of the most abundant species (the talitrid *T. saltator* and the tyliid *T. europaeus*), PERMANOVA tests showed a clear different temporal trajectory for each beach considered (Table 2 and Figure 4), with a significant interaction Beach x Time found for both species (Table 2, *T. saltator* p (perm) = 0.0046, and *T. europaeus* p (perm) = 0.0053). The posterior pairwise comparisons for this significant interaction term, showed that the temporal differences between 1999-2000 and 2010-2011 were only detected for these species on the Cabedelo beach (Pairwise test: *T. saltator* p (perm) = 0.0132, and *T. europaeus* p (perm) = 0.0109), with both species showing a clear decrease in their abundances between the two periods considered (Figure 4a and 4b).

On the other hand, at Quiaios beach no significant temporal variability was shown (Pairwise comparisons all p (perm) > 0.05) for both *T. saltator* and *T. europaeus* (Figure 4c and 4d).

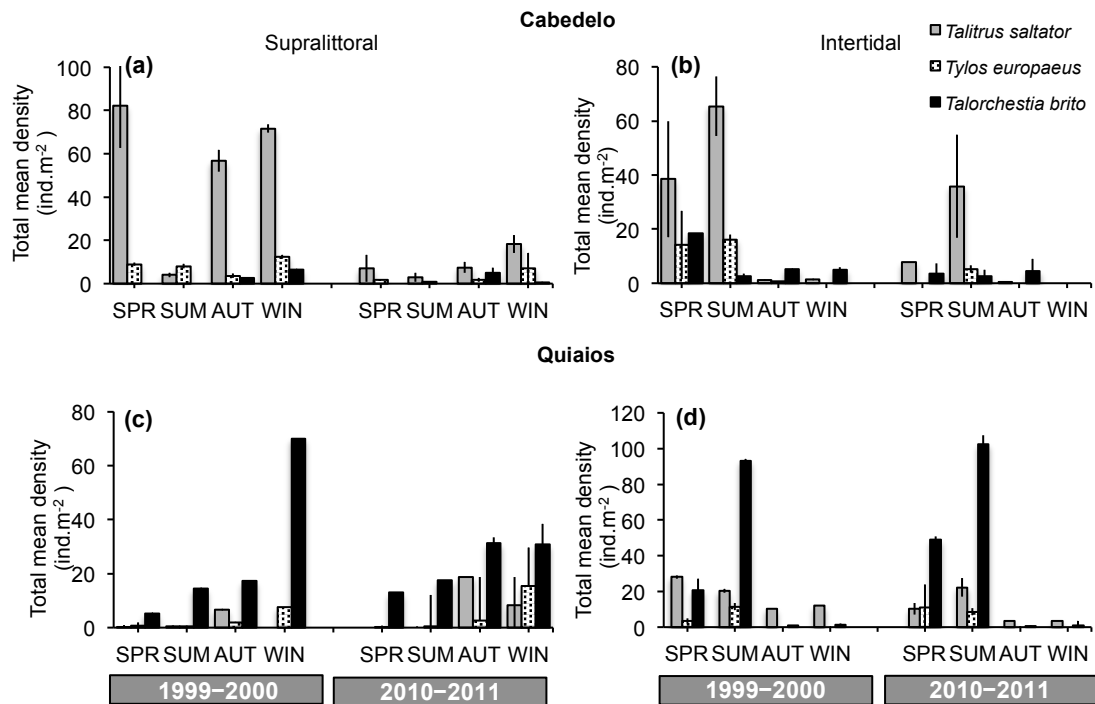


Figure 4. Mean total density (\pm SE) of the three most abundant species on Cabedelo (a and b) and Quiaios (c and d) beaches between the two time periods for each beach zone: Supralittoral (a and c) and Intertidal (b and d). SPR – spring; SUM – summer; AUT – autumn and WIN – winter.

The density of the sympatric talitrid *T. brito*, varied only between the zone of each beach and along the seasons (Table 2) but did not register a temporal variation among the periods for each beach (Table 2, B x T interaction: p (perm) = 0.2459).

In terms of variation in the spatial scale, in the case of the talitrid *T. saltator*, the decrease in their abundances between the two periods at Cabedelo beach occurred in both supralittoral and intertidal zones of the beach (pairwise tests resulted > 0.05 on all terms from the interaction term Z (B) x T, Table 2). Additionally, PERMANOVA tests showed also a borderline significant interaction in the term S (Z (B)) x T (Table 2 p (perm) = 0.05) for *T. saltator*, and the investigation of the pairwise comparisons showed a significant decrease in the species density between 1999–2000 and 2010–2011 in the supralittoral zone of Cabedelo beach, during the spring (Fig 4a, and p (MC) = 0.05), and in the intertidal zone during the summer (Figure 4b, p (MC) = 0.02). A similar spatio-temporal pattern was detected for *T. europaeus*, with a significant temporal decrease in their abundances being recorded in the supralittoral zone of Cabedelo beach during the springtime (Pairwise: p (MC) = 0.0036).

In order to investigate the changes in the abundance of these two species between the two periods with the potential effects of increasing human presence at Cabedelo beach,

we performed a Pearson correlation between the density of *T. saltator* and *T. europaeus* and the number of tourists in the same period. For both species, a negative and significant correlation was found between these two variables (*T. saltator*: $r = -0.879$, $p = 0.004$; *T. europaeus*: $r = -0.49$, $p = 0.05$).

In contrast, at Quiaios, no significant spatial or temporal differences over the studied periods were found for all the univariate faunal descriptors (Table 2, p (perm) pairwise comparisons for all terms was > 0.05 , Figure 3d, 3e and 3f). In this beach, the average value for the total macrofauna was highly similar for both periods (46 ± 5 ind.m² in 1999–2000 and 48 ± 3 ind.m² during 2010–2011) and a similar pattern was observed for the species richness (Figure 3e) and diversity (Figure 3f).

Multivariate patterns

The multivariate structure of macrofaunal assemblages showed the effect of time (T), beach (B) and zone of the beach considered (Z (B)) (Table 3). Additionally, the PERMANOVA tests revealed a significant Beach x Time interaction (p (perm) = 0.0077, Table 3), implying that the temporal trends in the macrofaunal communities' structure differ between the two beaches (Table 3).

Table 3. PERMANOVA results and pairwise comparisons performed to compare faunal assemblages between beaches (B – Cabedelo, Quiaios), periods (T1: 1999–2000, T2: 2010–2011), and zones of the beach (Z: Sup – supralittoral, Int – intertidal). Bold values correspond to significant results.

Factor	df	MS	Pseudo-F	P (Perm)
B	1	12533	47.12	0.0004
T	1	5373.5	8.59	0.0006
Z (B)	2	8378.6	3.15	0.0083
B x T	1	2624.3	4.19	0.0077
S x (Z (B))	12	2697.3	4.28	0.0001
Z (B) x T	2	2223	3.55	0.0039
S (Z (B)) x T	12	625.9	0.99	0.4962
Res	20	630.6		

Pairwise Test	Condition	P (MC)
B x T	Cabedelo x T1 vs. T2	0.0028
	Quiaios x T1 vs. T2	0.2903
Z (B) x T	Cabedelo x T1 vs. T2 x Sup	0.0335
	Cabedelo x T1 vs. T2 x Int	0.0347
	Quiaios x T1 vs. T2 x Sup	0.7466
	Quiaios x T1 vs. T2 x Int	0.0765

Analyses of pairwise comparisons on this interaction term confirmed the temporal stability of the community structure at Quiaios beach (p (perm) = 0.2903, Table 3), with significant differences in community structure detected only in Cabedelo between 1999–2000 and 2010–2011 (p (perm) = 0.0028, Table 3 and Figure 5). At this beach, the temporal variation in the macrofauna structure among periods was also recorded in both supralittoral (p (perm) = 0.0335) and intertidal (p (perm) = 0.0347) zones of the beach (Table 3, Figure 5). The most important taxa contributing to dissimilarities among periods at Cabedelo (SIMPER analysis), were the amphipod *T. saltator* and the isopod *T. europaeus*. These two species were responsible by 46% of the 63% dissimilarities found between periods in this beach.

Together, these results mirror the univariate analyses (mean total density for two of the most abundant species, *T. saltator* and *T. europaeus*) by suggesting a gradual change in the abundances of these species at Cabedelo beach observed over the 10–years interval.

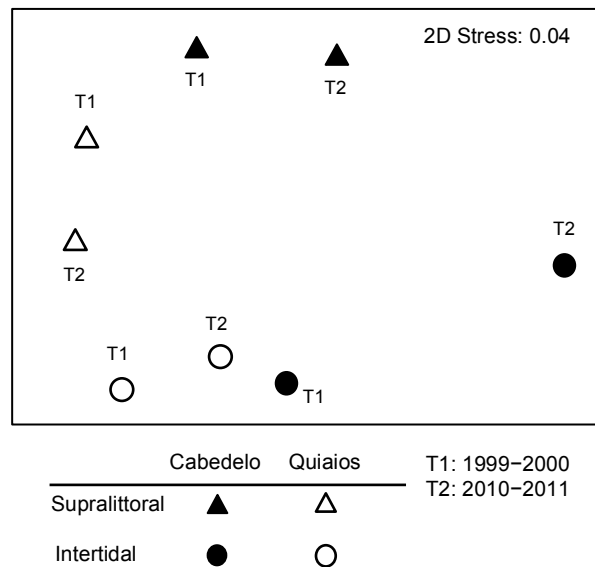


Figure 5. Ordination (NMSD) of centroids derived from Principal Coordinate Analysis (PCO) for each combination of Beach (Cabedelo vs. Quiaios) and zones of the beach (supralittoral vs. intertidal) for the two studied periods.

Discussion

The current work allowed us to compare the macrofaunal assemblages of rural (Quiaios) and urban (Cabedelo) beaches on the Atlantic coast of mainland Portugal (central region), that have received different intensities of human pressures in the last 10 years, and also to compare this information with a former study carried out on the same exposed sandy beaches in 1999–2000 (Gonçalves *et al.*, 2009).

Concerning the environmental conditions analysed, our data showed a highly similar physical environment between the two study occasions (1999–2000 and 2010–2011) at both beaches (Table 1), and since both surveys were performed following the same sampling design, changes in the macrofaunal assemblages are most likely to be an indicator of long-term potential human pressures recorded. Additionally, both beaches are exposed to similar meteorological conditions because they are located close to each other (distanced only by 8 km) and had similar wave exposure rates (see more details in Gonçalves *et al.*, 2009). The human pressures recorded at Cabedelo in the last ten years were the frequent adjustment of the local breakwaters, the construction of recreational facilities, the increase of tourism in the region, and the related human activities derived from the use of the beach (beach grooming and pollution).

Regarding the composition and structure of the macrofaunal assemblages, the analyses on both univariate and multivariate data revealed a different temporal trajectory for Cabedelo and Quiaios beaches. At Quiaios beach, the mean total density of the macrofauna sampled, the number of species and diversity (Shannon-Wiener Index), and the overall structure of the macrofauna was highly similar and stable for both time periods considered which appears to reflect the steady environmental conditions recorded in this beach. This trend was also reported for the most abundant species (*T. europaeus*, *T. brito* and *T. saltator*), which is fully coherent with the fact that in this beach, no major physical changes (coastal engineering) or intensive increase in the recreational activities were detected in the last ten years. Therefore, the undisturbed character of this beach described in the 1999–2000 by Gonçalves *et al.* (2009) seems to persist in the recent 2010–2011 period.

In contrast, a different scenario was described for the Cabedelo beach. Our study identified major temporal significant declines in the abundance of two invertebrate species, the amphipod *T. saltator* and the isopod *T. europaeus*, two key species in these exposed sandy beaches (Gonçalves *et al.*, 2009; 2013). Multivariate techniques (SIMPER) also discriminate these species as responsible for the main differences in the structure of the macrofaunal assemblages between the two periods at this beach. The lower densities of the talitrid *T. saltator* in 2010–2011 at Cabedelo when compared with the first period analysed, were recorded in both supralittoral and intertidal zones of this beach. Still, these differences were driven primarily by shifts in the relative densities of this talitrid in the supralittoral zone of the beach during the spring (also recorded for *T. europaeus*), and in the intertidal zone during the summer, which is eventually reflected by the highest intensity of human use of beaches in the hottest and more touristic seasons, and by the different distribution of these species along the shore, in order to avoid the dry conditions that occur in the supralittoral zone of the beach during the summer months. The pattern of distribution of these species in the shoreline, and their habitat requirements were already described for mesotidal conditions in temperate regions, where *T. saltator* tends to occupy a lower position on the shore during the hottest months of the year (e.g., Colombini *et al.*, 2002; Bouslama *et al.*, 2009; Lastra *et al.*, 2010).

In this study, even if the increase in the number of tourists occupying the central region was used as a measure (proxy) of the quantification of human use of beaches, the negative correlation found with the decrease in the number of *T. saltator* and *T. europaeus* can sustain the hypothesis of a potential impact that stems from recreational use of the beach during the touristic seasons (spring-summer). These results also support our prediction that the cumulative disturbances on the upper zone of the beach due to the construction of facilities, shoreline development and grooming over time, can potentially be also associated with the declines of abundance of these species that relied mainly on the supralittoral zone of the beach. For instance, the presence of breakwaters *per se* can limit the physical communication with adjacent beaches and consequently may not allow the connectivity of populations of these and other upper beach species, which in turn along time may compromise the viability of these macroinvertebrates on this beach.

Despite sandy beaches are considered resilient environments, intensive human modifications of the coastal zone can severely limit the ability of beaches to adjust to changes in shoreline stability and limit its flexibility (Nordstrom, 2000). Still, supralittoral species, such as talitrids and tylics are known to be well adapted to the highly dynamic and variable conditions acting on beaches but their limited dispersal and habitat dependencies makes them also sensitive indicators of beach disturbances (Defeo and Gomez, 2005; McLachlan and Brown, 2006). For instance, some studies have been illustrated an overall remarkable negative impact on supralittoral species as a result of human beach use (*e.g.*, Fanini *et al.*, 2005; Veloso *et al.*, 2006; 2008; Ugolini *et al.*, 2008) and shoreline modifications such as the construction of seawalls or beach nourishment actions (*e.g.*, Dugan and Hubbard, 2006; Fanini *et al.*, 2007; Bessa *et al.*, 2013a).

Both talitrid and tylic species represent the bulk of the secondary production and the energy flow from temperate systems (*e.g.*, Colombini *et al.*, 2003b; Lastra *et al.*, 2010; Gonçalves and Marques, 2011), acting as important key elements in ecological processes that take place on sandy beaches (Dugan *et al.*, 2003; Gonçalves and Marques, 2011). Furthermore, recreational beaches are usually affected by regular mechanical beach cleaning (in particular in the supralittoral zone), which is responsible for a considerable reduction of the organic matter available for the fauna on the beach, but also for the disturbance of the sediment, leading to the absence of suitable niches to support these communities, creating a resource-limited environment (McLachlan and Brown 2006). The reduction of these important trophic groups at Cabedelo may produce important negative consequences on the ecosystem energy balance.

Overall, our results revealed ecological changes in the beach ecosystem at Cabedelo. However, the assessment of these changes in the system as a result of long-term human pressures is here only considered potential, because our estimates rely on an approximate quantification of the human use of beaches and on information about the physical constructions on the beach, and the potential cumulative human pressures can be reduced or increased by natural variability and/or other stochastic events.

As mentioned previously, wide interpretation of our results must be done with caution as we were limited by only one replicate of each shoreline type and based only on two periods with an interval of ten years, therefore general conclusions about the consistency of the changes observed are forcibly partial. However, we arguably agree that the increase of human pressures recorded on Cabedelo beach may be a contribution factor for the lower number of the two key species, but further detailed and robust data are undoubtedly be needed to fully provide an assessment of cumulative human impacts on sandy beaches and determine its ecological effects. Indeed, the nature of changes and especially the long-term monitoring might be the next challenge for sandy beach ecologists in particular on the European Atlantic coast that has being increasingly disturbed (Veloso-Gomes and Taveira-Pinto 2003). Herein, sandy beach ecologists have been enhancing the need to protect and restore the ecological structures, functions and values of beaches, however, the efficacy of any active management in this regard in the Portuguese coast is hindered by the lack of baseline information about potential impacts acting on the system and the ecological responses of beach environment and biota. Since the ecological relevance of exposed sandy beaches and ecosystem is not considered in the priority of these actions, we think that this work can be one first attempt to provide valuable information of the potential effects of the increase of human pressures that can be translated into potential ecological impacts and jeopardise the entire ecosystem and the goods and services that humans directly derive from it (Defeo *et al.*, 2009).

Accordingly, multi-year assessments of macrofauna data may provide essential information about the drivers of change, and the duration of the events can anticipate future scenarios for vulnerable areas and help in guiding decision-makers with regard to coastal areas. Considering that recreational activities and extreme climate events (*e.g.*, storms) are predicted to become stronger and more frequent (Brown and McLachlan 2002; Defeo *et al.*, 2009), consequences for the coastal ecosystems are inevitable. Globally, it is generally accepted that beach recreation is the major service provided by ocean beaches to society in urbanised coasts. However, as Jaramillo (2012) said "*sandy beaches are not only important for recreation but also need conservation*".



Chapter II

Niche segregation amongst sympatric species at exposed sandy shores with contrasting wrack availabilities illustrated by stable isotopic analysis

Abstract

Wrack supplies (macroalgae, carrion) are a common feature of sandy beaches worldwide. These allochthonous inputs are a potential high-quality food subsidy for beach fauna, but little is known about the feeding ecology and niche segregation strategies of these species in beaches with limited wrack availabilities. We used stable isotopic ratios of nitrogen and carbon to examine the diets and niche segregation among three sympatric crustaceans, the amphipods *Talitrus saltator* and *Talorchestia brito*, and the isopod *Tylos europaeus*, in two temperate beaches, Cabedelo and Quiaios, on the Portuguese Atlantic coast, with contrasting wrack availabilities, over winter and summer. In the beach with limited wrack (Quiaios), consumers showed significantly different interspecific and temporal isotopic signatures, suggesting a distinct foraging habitat and niche differentiation. Mixing models outputs (SIAR) revealed in this case a seasonal shift in the diet of consumers from terrestrial sources in winter towards marine-based sources (phytoplankton) in the summer. In contrast, at Cabedelo, consumers showed clear overlap in isotopic signatures, with similar contributions of the available wrack to their diets. As a whole, an opportunistic and generalist feeding behaviour was described for these species, namely for *T. saltator*. Isotopic profiles support the occurrence of spatial overlap during summer but different foraging strategies of the three species in these beaches during winter. Our results show that on temperate beaches with ephemeral wrack supplies, consumers might need to adopt different foraging strategies and niche segregation for their subsistence, and additionally illustrate the viability of using stable isotopes to capture subtle changes in trophic niches.

Keywords: sandy beach, primary consumers, Peracarid crustaceans, diet, stable isotopes, niche segregation.

Introduction

Exposed sandy beaches form a highly dynamic interface between marine and terrestrial ecosystems that are generally characterised by little *in situ* primary productivity (McLachlan and Brown, 2006). These transitional habitats are mainly subsidised by allochthonous inputs of organic material from the coastal ocean (Griffiths *et al.*, 1983; Colombini *et al.*, 2003a; Dugan *et al.*, 2003), that vary from phytoplankton to beach-cast wrack, consisting of seagrasses, macroalgae, and carrion (Griffiths *et al.*, 1983; Rossi and Underwood, 2002). This marine organic material is transported onshore by physical forces (*e.g.*, wind, waves and currents), and accumulated in large volumes in the surf zones, being an important food resource to beach consumers – a trophic subsidy (*sensu* Polis *et al.*, 1997). The allochthonous inputs of nutrients for beach fauna are ubiquitous on exposed sandy shores worldwide and the pulse subsidies have multiple effects on food web dynamics on coastal areas (McLachlan and Brown, 2006; Heck *et al.*, 2008).

One of the most important ecological roles of the invertebrates (as primary consumers) inhabiting sandy shores is the fragmentation and decomposition of beach-cast wrack as sources of energy and nutrients (organic matter) (Colombini *et al.*, 2003a; Catenazzi and Donnelly, 2007; Lastra *et al.*, 2008; Salathé and Riera, 2012). In addition, the availability of wrack would influence the structure of these primary consumers (mainly crustaceans), which in turn supports the growth of consumers in higher trophic levels (*e.g.*, shorebirds) resulting in the transfer of energy across coastal ecotones (review in Colombini *et al.*, 2003a).

The wrack deposits exported from the surrounding ecosystems such as rocky shores or seagrass beds are highly variable in space and time, and are also dependent on the beach type and the nearshore hydrodynamics (Orr *et al.*, 2005; Gonçalves and Marques, 2011; Gómez *et al.*, 2013). Beach fauna, commonly dominated by air-breathing scavenging crustaceans, face many challenges locating food resources and have likely evolved behavioural and physiological adaptations to live in this nutrient-limited environment (McLachlan and Brown, 2006). In general, most of these scavengers (namely talitrid amphipods and tylid isopods) live buried between the drift line and the base of the foredunes during the day, emerging at night to feed and move across the shoreline (Scapini *et al.*, 1997). Although these species have generally been regarded as essentially herbivorous, an opportunistic behaviour (detritivorous) has been described for these taxa (Adin and Riera, 2003; Porri *et al.*, 2011).

The vertical distribution of available resources on sandy shores has been identified as a main factor driving population abundance and zonation of co-occurring mobile fauna (Jaramillo *et al.*, 2006; Ince *et al.*, 2007; Rodil *et al.*, 2008; MacMillan and Quijón, 2012). Therefore, sympatric crustaceans need to partition both *in situ* and allochthonous marine resources to avoid direct competition for food resources on these systems (Huxel *et al.*, 2002). Competition may, however, be lowered if species partition their niches by separating their surface activity (*i.e.*, feeding in different zones), being active during different times of the

day, or a combination of both spatial and temporal foraging strategies. In fact, differences in space and time partitioning of locomotor activities over the beach surface, have been reported for several species of these brooding crustaceans (e.g., Scapini *et al.*, 1992; Jaramillo *et al.*, 2003; Lastra *et al.*, 2010). Understanding foraging strategies of consumers inhabiting the same system is essential to understand their trophic relationships and ecological roles.

On exposed sandy beaches of temperate region, the abundant talitrid amphipods and oniscoidean isopods, are considered important wrack-deposit consumers (Lastra *et al.*, 2010; Rossi *et al.*, 2010; Gonçalves and Marques, 2011). Several studies highlighted the influence of beach wrack on the shore face as food resources for primary consumers (e.g., Hyndes and Lavery, 2005; Heck *et al.*, 2008; Crawley *et al.*, 2009; Rossi *et al.*, 2010) and its role as an habitat for abundant beach invertebrates (e.g., Colombini *et al.*, 2000; Olabarria *et al.*, 2007; Duong and Fairweather, 2010; MacMillan and Quijón, 2012). The dietary wrack preferences of particular taxa (mainly amphipods) were also extensively studied, and while some studies showed similarly strong preferences for the brown algae (Adin and Riera, 2003; Crawley and Hyndes, 2007; Olabarria *et al.*, 2009; Duarte *et al.*, 2010), others showed that they can be indiscriminate feeders (McLeod *et al.*, 2013) and even not related with specific wrack species (Colombini *et al.*, 2009; Porri *et al.*, 2011).

In recent years, food web ecologists have been taken advantage of natural variation in isotope composition of food items to reconstruct the importance of primary consumers and diet patterns of several species to the food web (Peterson and Fry, 1987; Post, 2002; Boecklen *et al.*, 2011). Stable isotopes ratios of carbon and nitrogen have been proved to be able to discriminate among various primary producers that did support the food web in several coastal regions (DeNiro and Epstein, 1978; 1980; Baeta *et al.*, 2009; Bergamino *et al.*, 2011). Stable isotope analysis (SIA) is currently among the most powerful tool for the study of trophic relationships and estimation of niche segregation among species in the marine environment (Cummings *et al.*, 2012), and its use in characterising the food web structure of macrofaunal communities or consumers diets is relatively common for the sandy beach ecosystems (e.g., Olabarria *et al.*, 2009; Rossi *et al.*, 2010; Bergamino *et al.*, 2011; Colombini *et al.*, 2011). However, on sandy beaches with a low contribution of wrack, where organic material is typically composed of ephemeral algal fragments and vascular dune plants detritus, the feeding ecology and the niche segregation strategies of beach consumers are poorly understood.

Exposed sandy beaches are among the most common coastal ecosystems along the Western Portuguese coast (European Atlantic coast), but some beaches can differ in the wrack availabilities (Gonçalves *et al.*, 2011). Some of the most conspicuous representative species of these beaches are the talitrid amphipods *Talitrus saltator* and *Talorchestia brito*, and the isopod *Tylos europaeus*, widely studied in the central coast of Portugal (Marques *et al.*, 2003; Gonçalves *et al.*, 2005; 2009; 2013).

The aim of the present study was to determine if there is isotopic evidence for significant niche segregation among sympatric crustaceans living on exposed sandy shores

with contracting wrack availabilities. We compared therefore the temporal isotopic signatures of primary consumers and potential food sources, the diets of consumers, and the pattern of distribution along the shore at two highly dynamic beaches on the European Atlantic coast (Portugal). Finally, we examined whether our findings based on stable isotope ratio analyses agree with previous descriptions of spatial surface activity segregation for these sympatric crustaceans.

Methodology

Study area

The study was carried out on two high energetic mesotidal sandy beaches: Cabedelo (40°07'32" N, 8°51'49" W) and Quiaios (40°12'21" N, 8°53'48" W) beaches, located on the European Atlantic coast (central Portugal) during the winter (January) and summer (July) 2011 (Figure 1).

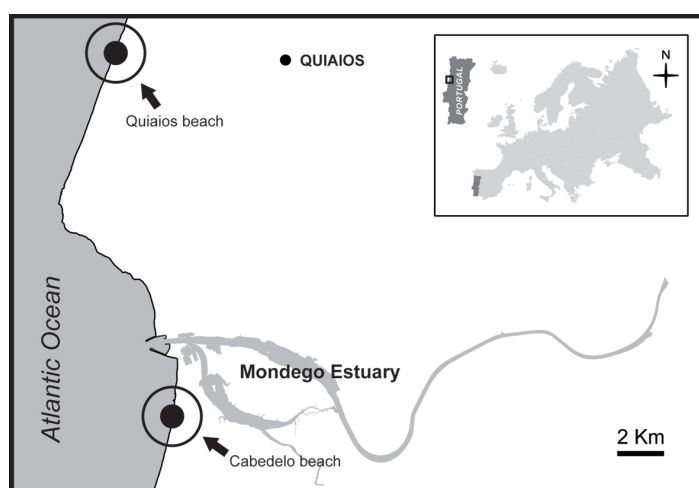


Figure 1. Map of the study area showing the sampling beaches: Cabedelo and Quiaios, on the European Atlantic coast (Portugal).

This area is characterised by a maritime climate with mild winters and cool summers with the minimum average temperature of the coldest month of 4.8 °C and the maximum average of the warmest month of 24.2 °C (Danielsen, 2008). Both beaches present a high wave exposure environment and a mesotidal regime, with a mean spring tidal range of ca. 3.5 m (Gonçalves *et al.*, 2009).

The Cabedelo sandy beach have a high recreational potential since it is located in a urban area while the Quiaios beach is located further north, about 8 km south of the Cape Mondego and more preserved from anthropogenic disturbances. The former beach is narrow (width approximately 80m) and contains medium sediments, whereas Quiaios is wider (width approximately 100m) and consists mainly of coarse sands (for more detailed description of

the area, see Gonçalves *et al.*, 2009). Quiaios beach is backed by a well-developed foredune, up to 2m in height, which is vegetated with the dominant dune plants: *Ammophila arenaria* (L.), *Euphorbia paralias* (L.), *Elymus farctus* subsp. *farctus* (L.) and *Otanthus maritimus*.

At Cabedelo beach (touristic beach), the local facilities located in the vicinity of the foredunes have partially damaged it and limited the local dune plants abundance and diversity (*in situ* personal observations). However, this beach received large amounts of drift wrack (mainly macroalgae) that accumulate in the surf zone coming from rocky shores near the beach, while Quiaios is clearly more limited (Gonçalves and Marques, 2011).

A full characterisation of the main physical features and macrofaunal communities of both beaches is provided by Marques *et al.* (2003) and Gonçalves *et al.* (2009; 2013).

Sampling design

Crustaceans (*T. brito*, *T. saltator* and *T. europaeus*) and potential food sources: beach wrack (*Fucus* sp., *Ulva* sp., carrion) and dune plants detritus were collected (depending on their availability) along the sea-dune axis from Quiaios and Cabedelo beaches during both wet (winter, January) and dry (summer, July) seasons. In addition, seawater was collected from the swash zone of the beach for determination of suspended particulate organic matter (POM) as a proxy of phytoplankton.

Animals were collected by hand and were kept alive in local sand and then transported to the laboratory for stable isotopic analysis. Wrack quantity (g. m⁻²) and the organic matter content in the sediment were determined according to the procedures described in Marques *et al.* (2003). A second sub-sample was taken during neap tide from each sampling season in order to assess the natural zonation pattern of the sympatric crustaceans. To reduce the risk of disturbing the animals, a distance of at least 10 m was kept between the samples. At each beach, samples (cores 0.05 m², 30cm deep) were collected with PVC cylinders at 10 equally spaced levels along two separate transects on the sea-dune axis. Animals were sieved through a 1mm mesh bags, preserved in 70% alcohol and taken to the laboratory for species identification. Simultaneous to biological sampling, water temperature (°C) and salinity were measured *in situ* using a YSI (Professional plus) field probe.

Laboratory procedures and stable isotope analysis (SIA)

Water samples (5l of seawater *per* season and *per* beach) collected for phytoplankton (chlorophyll *a*) measurements were filtered through Whatman GF/F glass-fibre filters. Particulate organic matter (POM) was measured by filtering 1.5l of seawater collected, onto precombusted (450°C, 4h) Whatman GF/F filters (0.45µm pore size) with a low-pressure vacuum pump within 2h of collection.

In the laboratory, animals for SIA were sorted alive and held in filtered seawater for 8 h to allow their stomach to clear. Subsequently, organisms were identified and separated by species. Crustaceans, beach wrack and dune plants were rinsed with Milli-Q water to remove

potential detritus and oven dried (60°C, 48h). Once dried, samples were ground into fine powder using mortar and pestle, weighted, and loaded into tin capsules.

The carbon and nitrogen isotopic composition of the samples was determined using a Flash EA 1112 Series elemental analyser coupled on line via Finningan conflo II interface to a Thermo delta V S mass spectrometer. The carbon and nitrogen isotope ratios are expressed in delta (δ) notation, defined as the parts per thousand (‰) deviation from a standard material (PDB limestone for $\delta^{13}\text{C}$ and atmospheric nitrogen for $\delta^{15}\text{N}$; $\delta^{13}\text{C}$ or $\delta^{15}\text{N} = [(R_{\text{sample}}/S_{\text{standard}}) - 1] \times 1000$, where $R = {}^{13}\text{C}/{}^{12}\text{C}$ or ${}^{15}\text{N}/{}^{14}\text{N}$). Precision in the overall preparation and analysis was better than 0.2 ‰ for both $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$.

Data analyses

At both beaches and for each sampling occasion, the mean zonation (corresponding to relative abundance along the transects from the sea-line to the back of the dunes) was calculated for all individuals collected. The frequency of each species found was calculated.

To determine if isotopic signatures varied among consumers (and food sources), beaches and seasons, to account for data heteroscedasticity, we used a three-way Permutational Analysis of Variance (PERMANOVA) (Anderson, 2001). The dependent variables $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ signatures were compared independently among the fixed factors beach (Cabedelo and Quiaios), seasons (winter and summer), and consumers (*Talitrus saltator*, *Talorchestia brito* and *Tylos europaeus*). We considered seasons as fixed factor because sampling dates were not chosen randomly from the possible dates over a year, being instead selected using the criterion that they should include the most extreme periods of the year in this region. When appropriate, *a posteriori* multiple comparisons were used to test for differences between/within groups for pairs of levels of factors, and Monte Carlo tests were included where insufficient unique permutations existed for meaningful tests (< 150). The tests were based on Euclidean distance matrices using 9999 unrestricted permutations of raw data and calculated from normalised, untransformed data. Statistical analyses were conducted using Primer v.6 and PERMANOVA (Primer-E Ltd., Plymouth, UK).

In order to obtain the relative contribution of the different food sources to the diets of consumers we used a Bayesian stable-isotope mixing model (SIAR; Parnell *et al.*, 2010) which allows the inclusion of isotopic signatures, elemental concentrations and fractionation together with the uncertainty of these values within the model. Although fractionation is usually accepted to be relatively constant at each trophic level, amphipods and herbivores in general may show great variability (Vander Zanden and Rasmussen 2001). To investigate the role of the different wrack species as food sources we applied the traditional mean trophic enrichment of 1 ‰ for $\delta^{13}\text{C}$ and 3.4 ‰ for $\delta^{15}\text{N}$ (DeNiro and Epstein; 1979; 1981; Minagawa and Wada, 1984; Adin and Riera, 2003), to allow for comparisons with similar studies based on the trophic ecology of the same or congeneric species (e.g., Adin and Riera, 2003; Olabarria *et al.*, 2009; Rossi *et al.*, 2010; Porri *et al.*, 2011). In addition, these values are known to better identify wrack species as food sources for amphipod species than other

values recently proposed in the literature, as demonstrated by Rossi *et al.* (2010). Data were graphically represented using dual-isotope graphs.

The estimated proportional contributions of each source item to the diet of consumers were plotted by SIAR using 95%, 75% and 25% credibility intervals (Parnell *et al.*, 2010).

Results

Environmental variables and food sources

Water temperature and salinity were similar at Quiaios and Cabedelo beaches in sampling occasions (Table 1). Both beaches were organically enriched, although Cabedelo presented higher wrack quantity and more organic matter content in the sediment (Table 1), while Quiaios showed the highest concentration of chlorophyll *a* during the warmer season (Table 1). The wrack quantity collected was higher during the winter at both beaches (31.06 g. m⁻² in Cabedelo and 9.76 g. m⁻² in Quiaios).

Table 1. Physicochemical parameters and autotrophic variables recorded each season (winter and summer 2011) at Quiaios and Cabedelo beaches, Portugal.

	Quiaios		Cabedelo	
	Winter	Summer	Winter	Summer
Water temperature (°C)	12.7	18	12	18.2
Salinity	35.5	35	33	34
Total phytoplankton (mg. Chl <i>a</i> m ⁻³)	2.51	5.91	1.59	2.68
Organic matter content (%)	0.16	0.34	0.32	0.37
Wrack quantity (g. m ⁻²)	9.76	0.81	31.06	15.21

Consumers' zonation

The distribution of sympatric crustaceans from the lower to the upper zone of the beach exhibited clear seasonal patterns of variation (Figure 2).

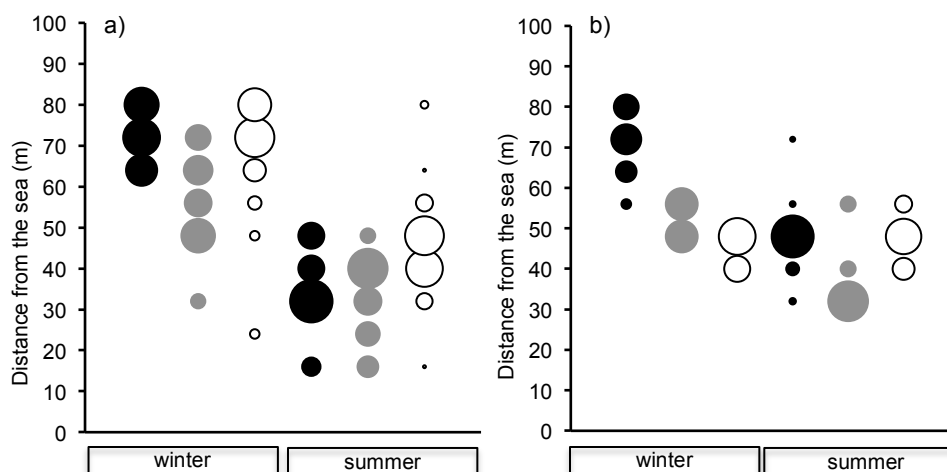


Figure 2. Mean zonation of crustaceans sampled during winter and summer 2011 on the Quiaios (a) and Cabedelo (b) beaches, Portugal. The y-axis shows the distance from the sea (m). Bubble size indicates the frequencies recorded of crustaceans. Black bubbles: *Talitrus saltator*; grey bubbles: *Talorchestia brito* and white bubbles: *Tylos europaeus*.

During the winter at Quiaios, consumers occurred mainly in the supralittoral zone (the zone above the drift line; Figure 2a), while in Cabedelo only *T. saltator* was limited to the supralittoral zone being the other two species found near the drift line (Figure 2b). On the other hand, at Cabedelo, during the warmer season all consumers remained restricted to the drift line and 78 % of *T. brito* individuals were found near the swash zone (about 30 m from the sea). An identical summery pattern was observed at Quiaios, with all crustaceans being distributed in the lower shore on the beach (Figure 2a).

Stable isotopes

Consumers

The mean $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values obtained for the sympatric crustaceans (*T. saltator*, *T. brito* and *T. europaeus*) varied significantly between the beaches and seasons (Table 2; Table 3; PERMANOVA interaction for all factors p (perm) < 0.05). The examination of PERMANOVA interaction term (Specie x Beaches x Date) showed that in both seasons all consumers from Cabedelo showed significantly higher isotope ratios than their counterparts in Quiaios (Table 2; PERMANOVA, p (perm) < 0.05). The only two exceptions were observed during the winter, for *T. brito* (p (perm) = 0.7327), which had similar $\delta^{15}\text{N}$ values (12.82 ± 0.51 ‰ in Cabedelo and 12.75 ± 0.14 ‰ in Quiaios), and for *T. europaeus*, which presented higher $\delta^{13}\text{C}$ values in Quiaios (Table 2). $\delta^{13}\text{C}$ values of all consumers were significantly depleted during winter in both beaches (PERMANOVA pairwise: p (perm) < 0.05), except for *T. brito* in Cabedelo which had similar values in both seasons (Table 2).

In contrast, $\delta^{15}\text{N}$ values have not shown significant temporal differences for *T. europaeus* in Quiaios (PERMANOVA: p (perm) = 0.2459), and for both talitrids in Cabedelo (Table 2; PERMANOVA: *T. saltator* p (perm) = 0.5910, *T. brito* p (perm) = 0.3877).

There was also inter-specific significant segregation in both $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of consumers at Quiaios during winter (Figure 3a; PERMANOVA: p (perm) < 0.05), suggesting differences in foraging behaviour between consumers (diet and/or feeding area). However, during summer, talitrids showed similar $\delta^{13}\text{C}$ values (*T. saltator* = - 18.67 ‰ and *T. brito* - 18.67 ‰), and *T. saltator* exhibited relatively $\delta^{15}\text{N}$ depleted values compared to the other two species (Table 2).

Table 2. Average $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values (‰, \pm SD) of beach consumers and potential food sources during the winter and summer 2011, at Quiaios and Cabedelo and beaches, Portugal.

	Quiaios				Cabedelo				
	n	Winter		Summer		Winter		Summer	
		$\delta^{13}\text{C}$	$\delta^{15}\text{N}$	$\delta^{13}\text{C}$	$\delta^{15}\text{N}$	$\delta^{13}\text{C}$	$\delta^{15}\text{N}$	$\delta^{13}\text{C}$	$\delta^{15}\text{N}$
Consumers									
<i>Talitrus saltator</i>	6	- 20.69 (0.18)	8.99 (0.13)	- 18.67 (0.18)	9.58 (0.27)	- 19.29 (0.41)	12.11 (1.15)	- 18.02 (0.38)	11.86 (0.39)
<i>Talorchestia brito</i>	6	- 19.25 (0.41)	12.75 (0.14)	- 18.56 (0.54)	11.36 (0.29)	- 16.23 (0.27)	12.82 (0.51)	- 16.53 (0.39)	12.57 (0.48)
<i>Tylos europaeus</i>	6	- 16.81 (0.23)	11.73 (0.65)	- 15.75 (0.52)	11.31 (0.52)	- 19.31 (0.21)	12.53 (0.44)	- 14.73 (0.61)	11.86 (0.34)
Sources									
POM	3	- 16.59 (0.18)	7.16 (0.24)	- 18.55 (0.27)	7.03 (0.18)	- 17.89 (0.46)	6.43 (0.38)	- 18.43 (0.31)	8.79 (0.03)
Dune plants	3	- 26.40 (0.41)	2.57 (0.24)	- 26.14 (0.21)	2.66 (1.24)	- 26.06 (0.08)	8.06 (0.08)	- 28.17 (0.24)	4.48 (0.68)
<i>Fucus</i> sp.	3	- 20.05 (0.03)	8.54 (0.02)	- 19.39 (0.54)	9.05 (0.07)	- 17.67 (0.11)	10.78 (0.97)	- 18.55 (0.14)	9.70 (0.02)
<i>Ulva</i> sp.	3	- 13.37 (0.11)	9.77 (0.07)	- 14.29 (0.21)	14.06 (0.07)	- 14.28 (0.03)	12.56 (0.09)	- 16.35 (0.04)	12.78 (0.21)
Carrion	3	-	-	-	-	- 18.38 (0.03)	11.05 (0.06)	- 19.06 (0.93)	9.05 (0.03)

In contrast, in Cabedelo, there was ample overlap in $\delta^{15}\text{N}$ values of consumers in winter (p (perm) > 0.05), while in the summer, *T. brito* was significantly more $\delta^{15}\text{N}$ enriched than the other co-occurring crustaceans (Table 2; Figure 3c and 3d). *Talorchestia brito* was also significantly more $\delta^{13}\text{C}$ enriched in winter, and *T. europaeus* during the summer (PERMANOVA: p (perm) < 0.05; Table 2).

Table 3 Results of three-way PERMANOVA testing for differences in $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of beach consumers (*Talitrus saltator*, *Talorchestia brito* and *Tylos europaeus*) and food sources (POM, dune plants, *Fucus* sp., *Ulva* sp. and carrion) between beaches (Quiaios and Cabedelo, Portugal) and seasons (winter and summer 2011).

Consumers	$\delta^{13}\text{C}$				$\delta^{15}\text{N}$		
	df	MS	F	P (perm)	MS	F	P (perm)
S – Species	2	12.53	256.63	0.0001	19.81	74.99	0.0001
B – Beach	1	5.13	105.14	0.0001	33.14	125.56	0.0001
Se – Season	1	14.06	287.95	0.0001	3.11	11.8	0.0012
S x B	2	5.18	106.16	0.0001	8.81	33.37	0.0001
S x Se	2	5.18	68.64	0.0001	1.42	5.42	0.0078
B x Se	1	0.53	10.32	0.002	0.01	0.01	0.0069
S x B x Se	2	3.11	63.82	0.0001	1.42	5.39	0.0079
Res	60	0.04			0.26		
Total	71						
Food sources	$\delta^{13}\text{C}$				$\delta^{15}\text{N}$		
	df	MS	F	P (perm)	MS	F	P (perm)
S – Species	3	9.78	2160.5	0.0001	5.27	209.02	0.0001
B – Beach	1	0.01	0.08	0.7799	0.42	16.612	0.0011
Se – Season	1	0.08	17.22	0.0013	0.13	5.18	0.0372
S x B	3	0.02	5.41	0.01	0.78	30.78	0.0001
S x Se	3	0.31	67.85	0.0001	0.44	17.61	0.0001
B x Se	1	1.35	298.67	0.0001	5.31	210.81	0.0001
S x B x Se	3	1.19	88.32	0.0001	2.49	98.79	0.0001
Res	18	0.01			0.02		
Total	33						

Food sources

The dune plant signatures of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ were more depleted on average than the other food items at both beaches (Table 2, Figure 3).

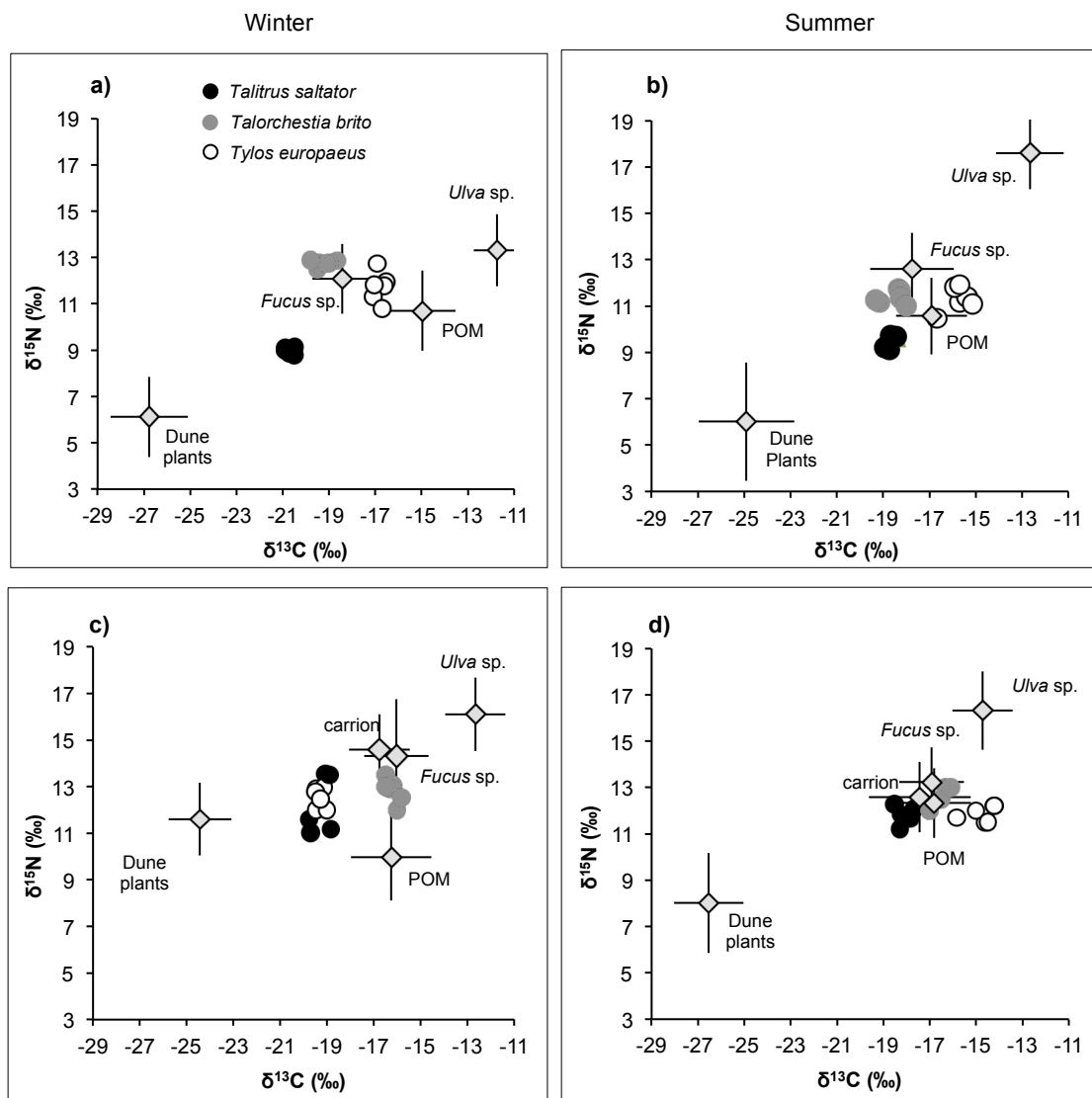


Figure 3. $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values (mean \pm SD) of beach consumers (*Talitrus saltator*, *Talorchestia brito* and *Tylos europaeus*) and their potential food sources collected at Quiaios beach during winter (a) and summer (b) 2011, and at Cabedelo beach during winter (c) and summer (d) 2011. Values for food sources were corrected by the appropriate trophic fractionation values (1 ‰ for $\delta^{13}\text{C}$ and 3.4 ‰ for $\delta^{15}\text{N}$).

The $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of all potential food sources showed significant differences between beaches in the same season (Table 2; Table 3: PERMANOVA all interaction terms: p (perm) $<$ 0.05).

The available food sources in Quiaios showed statistically temporal distinct isotopic signals (p (MC) $<$ 0.05) except for the dune plants (Table 2; PERMANOVA: p (MC) $>$ 0.05). The isotopic ratios of all sources at Quiaios varied also significantly among them in both seasons (p (perm) $>$ 0.05), and ranged from -26.40 to -13.37 ‰ ($\delta^{13}\text{C}$), and from 2.66 to 14.06 ‰ ($\delta^{15}\text{N}$; Table 2; Figure 3a; 3b).

The dominant food sources found at Cabedelo showed less segregation in the isotopic signatures in both seasons (Table 2, Figure 3c and 3d). The $\delta^{13}\text{C}$ values of POM, *Fucus* sp. and carrion showed seasonal stability (Table 2; p (MC) > 0.05), while $\delta^{15}\text{N}$ values for *Fucus* sp., *Ulva* sp. and carrion exhibited a slight variation (Table 2).

Mixing model outputs

Statistical comparisons between potential food resources contribution to the diet of consumers estimated by SIAR were performed for each beach and *per* season (Figure 4 and Figure 5).

Regarding the Quiaios beach, bayesian mixing model outcomes showed a high variability in the relative contribution of the three major organic matter sources to the diet of consumers (Figure 4). During winter, *T. saltator* selected mainly dune plants as food source with a mean contribution of 47% (Figure 4a), with diet switching in the summer, with POMs contributing 75% to its diet (Figure 4a). In contrast, SIAR results showed that the co-occurring talitrid *T. brito*, also during winter, relied mainly on the brown algae *Fucus* sp. as basal source (on average 41%; mean min = 5%; mean max = 84%; Figure 4b), with a mixed diet with similar contributions of sources in the summer (Figure 4b). *Fucus* sp. showed also the highest mean relative contribution to the diet of *T. europaeus* (40%), followed by *Ulva* sp. (28%) and POM (20%) while dune plants represented a minor contribution in winter (Figure 4c). By contrast, during summer, POM showed the highest contribution to the diet of *T. europaeus* (56%), displaying proportion values from 21 to 87% (Figure 4c).

Our model for Cabedelo beach indicated that in general all food sources contributed to the diet of consumers, since no more than 40 % of individual contributions were found in both seasons (Figure 5). In winter, dune plants appeared as the most important food source for *T. saltator* and *T. europaeus* diets (40% each), while *T. brito* seems to had a mixed diet composed mainly by POM (35%) followed by similar proportions of *Fucus* sp. (20%), *Ulva* sp. (19%) and carrion (18%) (Figure 5b). In summer, *Fucus* sp. was the main food assimilated by *T. brito* (40%) and *T. europaeus* (33%), while *T. saltator* depended on similar proportions of carrion (26%), *Fucus* sp. (25%) and POM (24%). *Ulva* sp. and dune plants provided minor contributions (\approx 15% each) during the warmer season at Cabedelo (Figure 5).

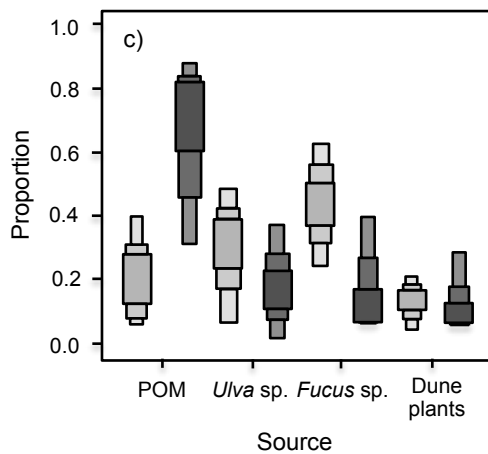
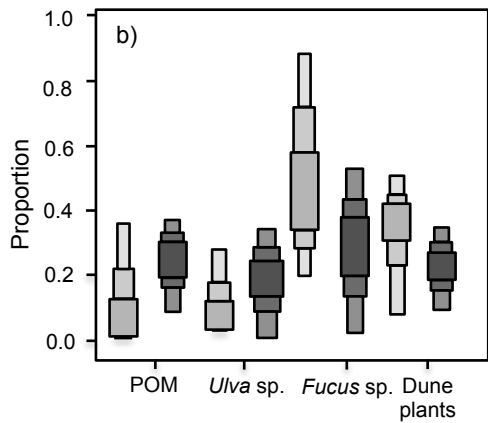
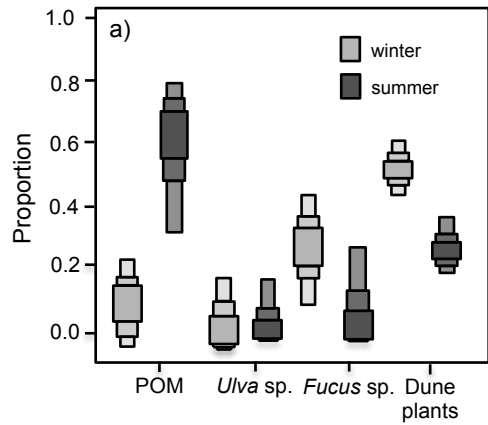


Figure 4. Results of SIAR (95, 75 and 25% credibility intervals) for Quiaios beach showing estimated food sources contribution to (a) *Talitrus saltator*, (b) *Talorchestia brito*, and (c) *Tylos europaeus* diets during both winter (light boxes) and summer (dark boxes) 2011.

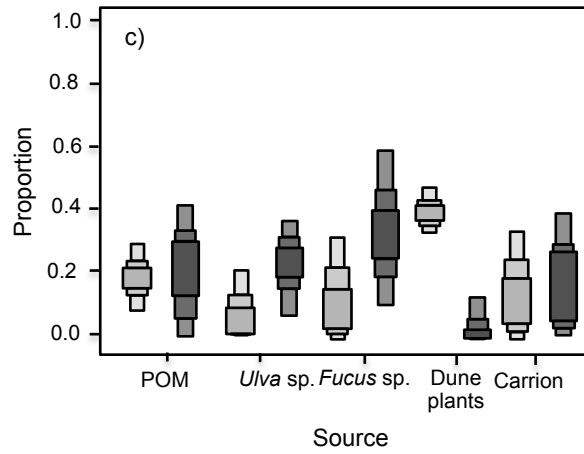
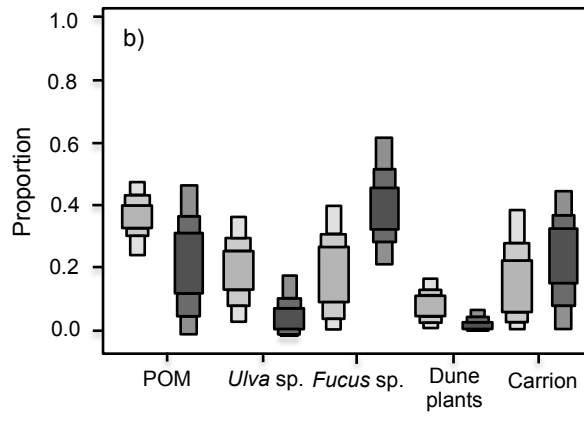
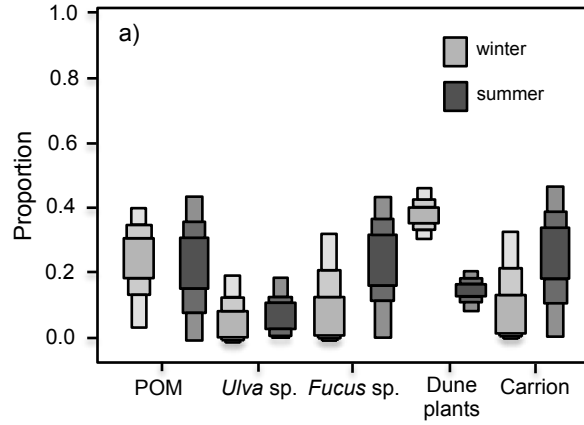


Figure 5. Results of SIAR (95, 75 and 25% credibility intervals) for Cabedelo beach showing estimated food sources contribution to (a) *Talitrus saltator*, (b) *Talorchestia brito*, and (c) *Tylos europaeus* diets during both winter (light boxes) and summer (dark boxes) 2011.

Discussion

The transfer of organic material from allochthonous sources to primary consumers varies widely among beaches with different morphodynamics (Bergamino *et al.*, 2013), but little is known about the ecological feeding strategies of these consumers when dealing with environmental and/or physical fluctuations.

The use of stable isotope analysis and zonation patterns of primary consumers in this study revealed several important aspects of the foraging ecology of three sympatric crustaceans - the talitrid amphipods *T. saltator*, *T. brito* and the isopod *T. europaeus* - from two sandy beaches with contrasting wrack availabilities.

Both beaches are highly dynamic and subject to similar environmental conditions, since they are relatively close to each other (8km apart, Figure 1). The main differences between these mesotidal beaches were related with the allochthonous inputs from the sea. The highest deposits of wrack species (*Fucus* sp., *Ulva* sp., carrion) were recorded mainly on the drift line of Cabedelo, whereas at Quiaios, the major allochthonous source arrived in the form of phytoplankton (Table 1). Gonçalves and Marques (2011) highlighted the clear dependence of macrofauna of these beaches on the wrack subsidies, namely their positive influence on the density.

The stable isotopic analysis revealed significant differences in both carbon and nitrogen values of primary consumers and the potential food sources between beaches. In particular, the isotopic signals of all sympatric crustaceans were significantly higher in Cabedelo beach as compared to Quiaios. The higher $\delta^{15}\text{N}$ signatures recorded in the consumers living at Cabedelo, which is adjacent to the Mondego estuary, can be related to the available nitrogen released from the river mouth to the coastal area. Nitrogen values of marine consumers are thought to reflect nutrient enriched zones, such as estuaries, and several studies have linked nutrient loadings with high biotic $\delta^{15}\text{N}$ (Vander Zanden *et al.*, 2005).

As inputs of distinct food sources may be seasonally variable, isotopic signatures of primary consumers associated with those sources are known to present also seasonal variations (Rossi *et al.*, 2010; Bergamino *et al.*, 2011). Our study has shown a striking temporal difference in carbon and nitrogen isotopes among all consumers for the beach with low accumulations of wrack.

At Quiaios beach, during winter (Figure 3a), the significant differences found in the isotopic signatures of both primary consumers and food sources might suggest different foraging areas for these crustaceans. Since arrival of macrophyte material were stochastic at best in Quiaios, and presented seasonal and spatial fluctuations, this variability is likely to affect the diet of consumers and the need to forage on different available sources for its requirements. Here, the amphipod *T. saltator* had the most terrestrial isotopic signal with lower $\delta^{13}\text{C}$ signatures, i.e., signatures closer to those dune plants, while *T. brito* and *T. europaeus* signatures were similar to those of the allochthonous resources (*Fucus* sp. and

POM). This pattern was clearly consistent with the zonation observed during the coolest season, when *T. saltator* was exclusively found in the supralittoral zone, and the other two-occurring species showed a lower position on the beach (Figure 2a). This suggests some spatial segregation along sea-dune axis, with *T. saltator* foraging higher on the beach and hence, closer to the foredunes. Moreover, SIAR outputs were in accordance with the position occupied by the sympatric crustaceans in winter in this beach. Dune plants were the main contributor to the diet of *T. saltator* (47%) and *Fucus* sp. the main contributor to the other two species diets (about 40%). Consequently, the ecological niche of these species might differ during winter, with a spatial partitioning allowing the coexistence of beach primary consumers at Quiaios. In this situation, a shortage in wrack detritus availability seemed to promote a supralittoral foraging area for *T. saltator* offering additional potential food resources (such as dune plant detritus).

The behavioural plasticity of *T. saltator* had been widely recognised, as a species capable to move across the shoreline in order to maintain an optimal position on the beach (Scapini *et al.*, 1997). In addition, this species is considered to be an opportunistic feeder and a poor swimmer, thus in order to avoid the risk of being swept in this dynamic beach during spring tides, this species can adopt the strategy of remaining at the base of the foredunes where can find also a safe refuge.

In contrast, the isotopic signatures of consumers varied markedly in summer, suggesting a temporal change in the foraging area, with consumers occurring mainly in the lower zone of the beach, as close as 10 meters from the sea in the case of *T. brito* and *T. europaeus* (Figure 2a). The SIAR results provided evidence of the temporary diet switching, in the case of *T. saltator* from dune plants detritus in winter to POM-dominated diet in summer. A relatively similar trend was also observed for *T. europaeus* and, although to a less extent, for *T. brito* (Figure 4). Diet switching clearly indicates an opportunistic foraging strategy in which a consumer may change its diet to take advantage of temporary available food resource or to replace a preferred resource that has become unavailable. Here, both patterns emerged, since these species (especially during the summer), needed to find a moist sand stripe along the shoreline to avoid desiccation, and a food resource available, in this case represented by higher phytoplankton concentrations (Table 1). The coastal foraging habitat preferences showed by these species are confirmed by their higher $\delta^{13}\text{C}$ values in summer as compared to winter, which indicates a benthic carbon source that is primarily available in the intertidal zone of the beach. The habitat available in this beach favoured the distribution of sympatric species along the shoreline, but with a different vertical position depending on the season and resources availability.

Our results are in accordance with those described by Lastra *et al.* (2010), which found clear differences in the surface activity in the same species analysed here, with evident niche differentiation along the spatial axis. These authors also highlighted the lower position on the beach for *T. brito*, a pattern also recorded in our study in both beaches. To this regard, the estimation of stable isotopes of *T. brito* (specifically nitrogen values) was similar between

beaches and seasons (Table 2), which is also illustrated by the main contribution of the same single food type (mainly *Fucus* sp.) to its diet. Our results emphasise therefore the intertidal affinity and limited foraging habitat of this amphipod species, which was also recognised by Fallaci *et al.* (1999).

Contrasting to Quiaios, the same sympatric primary consumers showed at Cabedelo had identical mean $\delta^{15}\text{N}$ values during the winter, which suggests substantially similar trophic niches. The little variation in $\delta^{13}\text{C}$ values found between *T. saltator* and *T. europaeus* (Table 1) indicate that these species might consume the same food resources. Actually, SIAR results reflected this prediction, dune plants appearing as the main contributor to the diet of *T. saltator* (39%) and *T. europaeus* (40%), while nearshore POM represents the main food source to *T. brito* (mean 35%). The vertical pattern of distribution also revealed a higher position of *T. saltator* on the shore, whereas the other two occurring species were found closer to the drift line. Therefore, at Cabedelo, some spatial segregation seems to occur amongst crustaceans, although in a less extent as compared to Quiaios.

During summer, consumers exhibited a relatively narrow range of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ signatures, which indicates similar and rather narrow niches (Figure 3b). This interpretation is reinforced by the overlap in summer zonation of these species in the drift line. Mixing models revealed that these consumers feed on mix food resources since any item contributed more than 40% to its diets (Figure 5). Nevertheless, the similar contributions of POM, *Fucus* sp. and carrion (about 20% each) therefore suggest an opportunistic and generalistic feeding behaviour of these species at Cabedelo.

Previous studies with amphipods (and *T. saltator*) consuming wrack have reported feeding preferences for macroalgae over other food items (*e.g.*, Adin and Riera, 2003; Crawley and Hyndes, 2007; Duarte *et al.*, 2010; Poore and Gallagher, 2012), however, Rossi *et al.* (2010) described an opportunistic behaviour for amphipods that relied on seagrasses (*Sargassum muticum*) when this resource was deposited in large quantities on the shore, and Porri *et al.* (2011) found an ephemeral relationship between amphipods and specific wrack subsidies in South Africa. Herein, we also describe an opportunistic feeding behaviour, mainly of the consumers at Cabedelo beach, which might utilise the various sources indiscriminately as a result of the higher availability of wrack deposited on the shore. Such small-scale differences in food accessibility allow consumers to forage on the same location without the need to move across the beach, a pattern also consistent with the concentration of animals observed near the wrack deposits. Furthermore, these results agree with the hypothesis reported by Gonçalves and Marques (2011) that there might be considerable overlap between *T. europaeus* and *T. saltator* foraging areas in this beach.

An important feeding aspect emerged also from our results, which illustrates that the green algae *Ulva* sp. had minor contribution to the diet of consumers at both beaches, where it is only occasionally found (personal observations). This is consistent with previous studies that reported that supratidal invertebrates had little effects on the decomposition of *Ulva* sp. (Catenazzi and Donnelly, 2007), as well as with the findings of Adin and Riera (2003) and

Olabarria *et al.* (2009), which claimed that *Ulva* sp. was the less preferential food source for the sandhopper *T. saltator*.

Overall, instead of elucidating unambiguously interspecific differences in diets, our results provide evidence of the influence of wrack availability on the foraging strategy of primary consumers, and illustrate how temporal fluctuations may affect niche segregation.

The spatial winter niche differentiation found at Quiaios clearly showed how sympatric crustaceans in this beach were distributed along the marine-terrestrial gradient to avoid physiological stress on this beach and potential interspecific competition. Given the level of resource partitioning observed in winter in both beaches, although to a less extent at Cabedelo, it is likely that these crustaceans have adopted specific feeding strategies to maintain an optimal position along the shore. Such strategies may function to reduce interspecific competition allowing congeneric species to successfully co-occur in similar systems (Huxel *et al.*, 2002). On the other hand, at Cabedelo beach, where wrack subsidies are abundant, crustaceans were able to be more concentrated on the drift line, exhibiting opportunistic and generalist feeding behaviour, and consequently an overlap in the foraging habitat.

Our study illustrates the viability of using stable isotopes to capture subtle changes in trophic niches, in this case seasonal and corresponding winter foraging individual specialisation in beach primary consumers. Even generalist species in poor-environments can cohabit exhibiting different spatial and temporal feeding strategies, and our findings agree with Rodríguez and Herrera, (2012) paradigm that isotopic niche might mirrors trophic niche of sympatric species with similar feeding diets. The food partitioning may allow the coexistence of interacting species and provides evidence that biological interactions are indeed important in the community structure of sandy beach macrofauna.



Chapter III

Behavioural adaptations of two sympatric sandhoppers living on a mesotidal European Atlantic sandy beach

Behaviour of *Talitrus saltator* (Crustacea: Amphipoda) in a rehabilitated sandy beach on the eastern Atlantic Coast (Portugal)

Behavioural adaptations of two sympatric sandhoppers living on a mesotidal European Atlantic sandy beach

Abstract

Behavioural plasticity of supralittoral species on sandy beaches constitutes a key factor in their survival and evolution. Two sympatric talitrid amphipods (*Talitrus saltator* and *Talorchestia brito*) from a mesotidal exposed sandy beach on the European Atlantic coast (Portugal) were compared as regards behavioural adaptations of orientation and zonation patterns in natural conditions. Several sets of orientation experiments were carried out during the hottest seasons (spring and summer 2011 and 2012) at Quiaios beach, a highly dynamic sandy beach. Multiple regression models were fitted to the angular data and the environmental effects on orientation were investigated for each species. Both talitrids resulted well orientated towards the shoreline and finely tuned to the small-scale mesotidal environment but a different use of local cues and climatic features in the two species was apparent. *T. saltator* revealed a less precision in the orientation performance (bimodal distribution sea- and land- wards), especially during the spring, with less dependence to the sun cues and higher dependence on climatic conditions. In addition, the mean zonation of *T. saltator* was across the whole beach (the land-sea axis) during both sampling seasons. For *T. brito* the landscape vision, sun visibility and the tidal range enhanced the orientation to the shoreline. On this beach, *T. saltator* appeared to have a more flexible orientation with respect to *T. brito*, which appeared to be more dependent on the conditions offered by the intertidal zone, a behaviour confirmed by its restricted zonation below the high tide mark. Consequently, *T. saltator* showed a more flexible behaviour that may be considered an important adaptation to dynamic and tidal sandy beaches.

Keywords: behavioural adaptations; mesotidal; sandy beach; sympatric species; talitrid amphipods.

Introduction

Exposed sandy beaches are extremely dynamic and unstable habitats in which resident faunal patterns were well correlated with the physical environment such as sediment properties, beach slope and intertidal swash climate (McLachlan and Brown, 2006). This concept has been widely reported and proposed as a general paradigm in sandy beach ecology (Defeo and McLachlan, 2005; McLachlan and Brown, 2006). Therefore, the sandy beach macrofauna that inhabit these systems exhibit specific adaptations including mobility, rhythmic locomotor activity and sun orientation which are not rigid, but become adjusted according to the physical and environmental variations, being adaptive traits of invertebrates on sandy shores (Defeo and Gomez, 2005; McLachlan and Brown, 2006). In fact, behavioural plasticity plays a crucial role in the survival and evolution of the sandy beach macrofauna since these animals need to display a range of strategies to successfully colonise this environment (Brown, 1996; McLachlan and Brown, 2006). Consequently, behavioural measurements may constitute a suitable indicator of species responses to environmental changes since behaviour is correlated with the physiological changes exhibited by the animals under different environmental conditions.

For decades, crustaceans were one of the most studied Invertebrata taxa in behavioural research on sandy beaches (Papi and Pardi, 1953; Hazlett, 1995; Scapini, 2006).

In Italy, Papi and Pardi (1953) demonstrated for the first time the sun orientation capabilities of sandhoppers to find and maintain the optimal direction towards a safe zone in the beach. In these particular species, a safe zone in which to burrow is usually the wet sand stretch found at the drift line, which offers protection from both dehydration and immersion (reviewed in Scapini, 2006).

The sun is known to be the main cue to orientate on the beach, however, landscape vision, beach slope and environmental conditions, such as habitat dynamics (*e.g.*, tidal range changes), and seasonality of climatic conditions, may influence the orientation performance of these talitrid amphipods on sandy shores (Scapini *et al.*, 1997; Scapini *et al.*, 2006; Fanini and Scapini 2008).

Scapini (2006) postulated that sandy-beach talitrids inherit a set of possible behavioural responses, of which selective pressures act on the survival of individuals adopting the most suitable behavioural response in the particular conditions they live. Thus, inherited behavioural traits are coupled with the learning ability to modify the responses according to contexts. Besides the need to cope with a highly variable physical environment, sandhoppers need to adjust their behaviour also to avoid intra- and inter- specific competition for space and food (Colombini *et al.*, 2002; Lastra *et al.*, 2010). Since niche partitioning increases resource exploitation by diverse species, differences in time and space zonation in supralittoral species are the strategy used to allow the coexistence of sympatric species (Fallaci *et al.*, 1999; Bouzlama *et al.*, 2009; Lastra *et al.*, 2010). Individuals often vary in their

degree of behavioural adaptation and species in which behavioural patterns are more flexible, have individuals more responsive to the variation of environmental stimuli (Brown, 1996).

The role of talitrid amphipods is crucial for the sandy beach ecological functioning, as they take part in the trophic link that supports the food web in sandy-dune systems (Dugan *et al.*, 2003; Brown and McLachlan, 2006), moreover, talitrids bioecology was one of the most well-studied research area in these environments (*e.g.*, Marques *et al.*, 2003; Scapini, 2006; Lastra *et al.*, 2010).

Among the supralittoral species on temperate sandy shores, are the abundant sympatric talitrids *Talitrus saltator* (Montagu 1808), which are widely distributed throughout the northern temperate zone (European Atlantic coast and the Mediterranean Sea) (Marques *et al.*, 2003), and *Talorchestia brito* (Stebbing 1891) which often occurs in sympatry with the former species on Atlantic beaches though rarely on Mediterranean ones (Vader, 1970; Scapini *et al.*, 2002; Gonçalves *et al.*, 2003; Lastra *et al.*, 2010). On an exposed Atlantic beach in France, these two sympatric species showed different zonation patterns of activity, being *T. brito* generally found near the waterline (intertidal zone), while *T. saltator* ranged between the waterline to the back of the foredunes (supralittoral and intertidal zones) (Fallaci *et al.*, 1999).

In particular, the ecological relationships between environmental features and behavioural adaptations of *T. saltator* were widely studied, especially on the Mediterranean coasts (Scapini *et al.*, 2005a; Scapini, 2006; Fanini *et al.*, 2007; Colombini *et al.*, 2012). Additionally, behavioural traits were also correlated with environmental and physical disturbances on sandy shores (*e.g.*, Scapini *et al.*, 2005a; Fanini *et al.*, 2007; Bessa *et al.*, 2013b) and hence this species was considered as a potential bioindicator of environmental stress (Scapini and Ottaviano, 2010).

In contrast, on the European Atlantic coast, research studies were mainly focused on the ecology and dynamics of *T. saltator* populations (Anastácio *et al.*, 2003; Marques *et al.*, 2003; Gonçalves *et al.*, 2009) with behavioural studies being performed only on the French coast (Gambineri *et al.*, 2008; Rossano *et al.*, 2009). For *T. brito*, population dynamics was also studied on the Portuguese Atlantic coast (Gonçalves *et al.*, 2003) but the behavioural adaptations of both species to this coastal environment were never recorded.

The overall outcomes from these behavioural studies are in accordance with the fact that behavioural responses of talitrids may vary according to the habitat and physical conditions (exposure and tidal ranges) from specific environmental contexts. Therefore, we predict that sympatric talitrids from highly dynamic mesotidal sandy shores such as the case of some beaches in the European Atlantic coast may need to adopt specific mechanisms of spatial and temporal variations in their behavioural strategies to cohabit in this natural dynamic environment. In particular, we test the hypothesis that a better orientation is expected for the species that is zoned more seawards and are less tolerant to temperature fluctuations (Colombini *et al.*, 2002), and that a higher behavioural flexibility is expected for the more robust and resistant species (Morrit, 1987). The aim of this study was then to

compare the behavioural adaptations of two sympatric sandhoppers (*T. saltator* and *T. brito*) inhabiting a dynamic mesotidal Atlantic sandy shore (Portugal) namely their orientation and zonation patterns on the beach. The influence of environmental factors on talitrids orientation performance was also assessed.

Methodology

Study site

Field studies were carried out at Quiaios beach (40°12'21" N, 8°53'48" W) on the European Atlantic coast in Portugal (Figure 1). This relatively undisturbed beach had a mean width of about 100 m and was backed by small dunes of about 2m height. According to the McLachlan's (1980) scheme, Quiaios is classified as a very exposed beach (exposure rate: 16). This coastal area, located in the central region of Portugal presents a warm temperate Atlantic-Mediterranean climate and semidiurnal tides with maximum amplitude of about 3.5 m (Gonçalves *et al.*, 2009; Gonçalves and Marques, 2011). The investigated area is characterised by a maritime climate with mild winters and cool summers. The thermal amplitude is low and advection fog is frequent throughout the year, even during summer mornings (Danielsen, 2008).

Previous studies performed at Quiaios beach showed that the macrofauna community diversity inhabiting this area was relatively scarce (about 14 species) and that crustaceans were the most abundant taxon (Gonçalves *et al.*, 2009). Among them, the dominant species were the talitrid amphipods *Talitrus saltator* and *Talorchestia brito* and the isopod *Tylos europaeus* (Gonçalves *et al.*, 2009; Gonçalves and Marques, 2011).

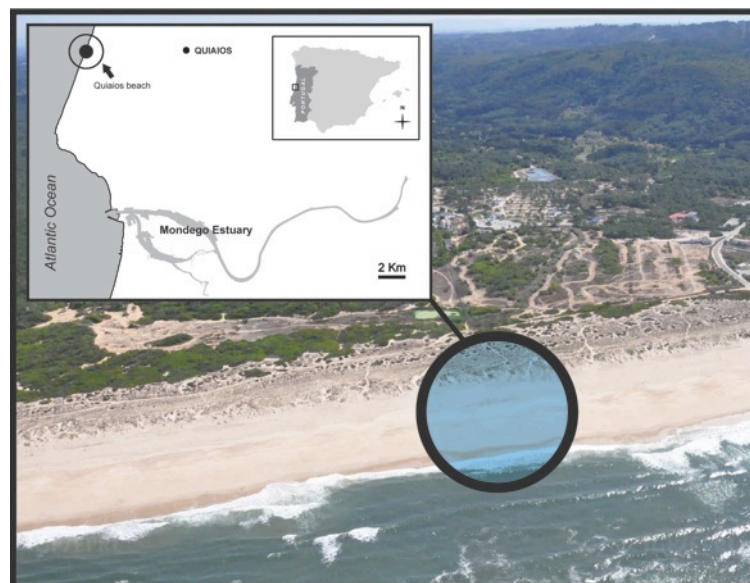


Figure 1. Location of the experimental area, the Quiaios beach (40°12'21" N, 8°53'48" W), on the European Atlantic coast (Portugal).

Orientation experiments

The orientation of the two sympatric talitrids *T. saltator* and *T. brito* were tested on Quiaios beach during spring (May) and summer (July) 2011 and during the same months in 2012 (four experimental sessions). Sandhoppers were collected by hand from the sand during neap tide on the day of the experiments. Two experimental arenas were placed in the supralittoral zone of the beach and the animals collected were tested following the standard protocol described by Scapini *et al.* (2005a). The Plexiglas arena (70 cm diameter) had 72 pitfall traps at the circumference, each subtending 5°, and was positioned 1 m above the beach surface. The trap number 1 was oriented towards the North using a magnetic compass. The direction perpendicular to the shoreline at the capture point, TED (theoretical escape direction), was 300°. In each experiment, one starting at 9:00 am and another at 3:00 pm (both solar time), four successive releases of 10 sandhoppers were performed, testing a total of about 80 individuals each session. Sandhoppers were inserted via a transparent Plexiglas tube in the centre of the arena and then released. The directional choice of each individual was registered via the trap number, and the tested specimens were preserved in alcohol (75%) for later morphometric analyses in the laboratory. The experimental design comprised tests with rising and ebbing tide in the same day. Additionally, the experiments were performed alternating releases of individuals with and without landscape view, by using or taking out a screen around the rim of the arena.

The following environmental variables were measured along with the orientation experiments: air temperature (°C) and air relative humidity (%) using a digital thermohygrometer; and solar global radiation (lux) using a lux meter. The sky features were assessed by two factors, sun visibility (three levels: visible, shaped or not visible) and sky cover (0-8/8 scale). The sun azimuth was estimated from the geographic coordinates of the site, the dates of the experiments and the time of the day of each release. In the laboratory, the individuals tested were identified and sexed on the basis of their sexual features visible under the stereomicroscope (magnification 20x). The number of articles of the flagellum of the second right antenna was counted as a proxy of age and the cephalic length (mm) was measured as a proxy of size (Marques *et al.*, 2003).

Mean Zonation

The zonation of burrowed amphipods was studied during spring (May) and summer (July) 2011 and 2012 (four sessions), the same months of the orientation experiments. In those surveys, during neap tide, cores (0.05 m², 30 cm deep) were collected with PVC cylinders at 10 equally spaced levels along two transects (separated by 30 meters) extending from the back of the dunes to the swash zone. Each level consisted of three sediment cores replicates, the sediment was sieved through a 1 mm mesh bags and the amphipods were sorted and stored in alcohol for further morphological analyses in the laboratory. The species were identified (*T. saltator* or *T. brito*) and the sex determined by the presence of sexual characteristics (oostegites or penes) (Marques *et al.*, 2003).

Data analyses

The distributions of orientation choices (angles) of sandhoppers obtained at each experimental session were analysed using the statistics of circular distributions (Fisher, 1993). The circular distributions were plotted and the following statistics were calculated: the mean direction (with 95% confidence intervals) and the precision of orientation (indicated by the mean vector length r , ranging from 0 - random distribution - to 1 - maximum precision around the mean direction) (Batschelet, 1981). Additionally, the homogeneity of each distribution was tested with the Rayleigh's test and the probability density functions were fitted with the kernel method (Fisher, 1993). Axial orientation was checked for the distributions in order to detect an eventual cross-shore escape (sea-dune axis), using the method of doubling the angles and calculating the orientation axes (Fisher, 1993).

The contemporaneous effects of variables and factors on the orientation distributions were estimated through multiple regression analysis adapted to angular data (Spherically Projected Linear Models, SPLMs) (Marchetti and Scapini, 2003). The best model was evaluated by the highest likelihood with the minimum number of parameters, using the Akaike Information Criterion (AIC). The significance of each factor was estimated by means of the Likelihood Ratio Test (LRT) on the difference between the best model and the model without the specific factor. The overall circular distributions and statistical analyses were performed using S-Plus 6 Insightful software with a library developed *ad hoc* (Marchetti and Scapini, 2003).

The seasonal mean zonation pattern of the amphipod populations was obtained by calculating the distance (m) from the sea-line (0 m) to the back of the dunes of each amphipod found in the samples collected from both transects. The frequency of each species found was calculated.

Results

Interspecific differences in orientation

During the orientation experiments, the highest air temperature, humidity and solar radiation were recorded during the summer days (Table 1). A total of 447 individuals of *Talorchestia brito* were tested under the same conditions as 231 individuals of *Talitrus saltator* with a general female biased ratio similar for both species (Figure 2).

Table 1. Mean and standard deviation of the environmental variables recorded during the orientation tests at Quiaios beach.

Variable	spring	summer
Air temperature (°C)	24.20 ± 1.33	27.81 ± 4.49
Air relative humidity (%)	60.75 ± 9.15	52.30 ± 15.43
Solar global radiation (lux)	94.39 ± 25.52	98.93 ± 25.07

The circular plots for the distribution of orientation angles for both talitrid species tested at Quiaios beach showed a general seaward distribution, with the mean direction including the TED (300°) in its confidence interval (Figure 3 and Table 2). The maximum orientation precision was obtained for *T. brito*, with a higher mean resultant length ($r = 0.408$) when compared to the *T. saltator* performance ($r = 0.219$) (Table 2). In both cases the Rayleigh's tests for uniformity resulted significant ($p < 0.0001$, Table 2).

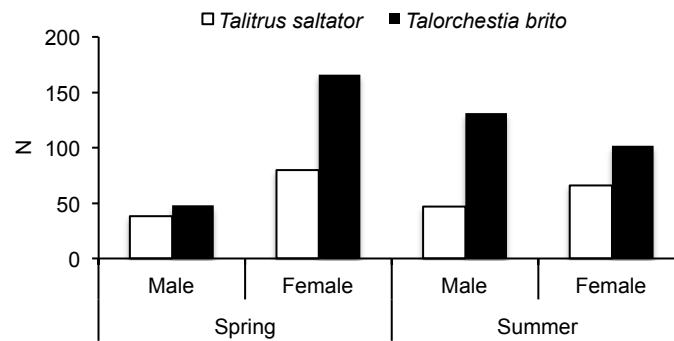


Figure 2. Total number (N) of individuals within the samples tested for orientation by species and seasons (spring and summer).

The *T. saltator* graphs detected a main seaward direction, but also a second peak towards the opposite direction (kernel distribution, Figure 3b).

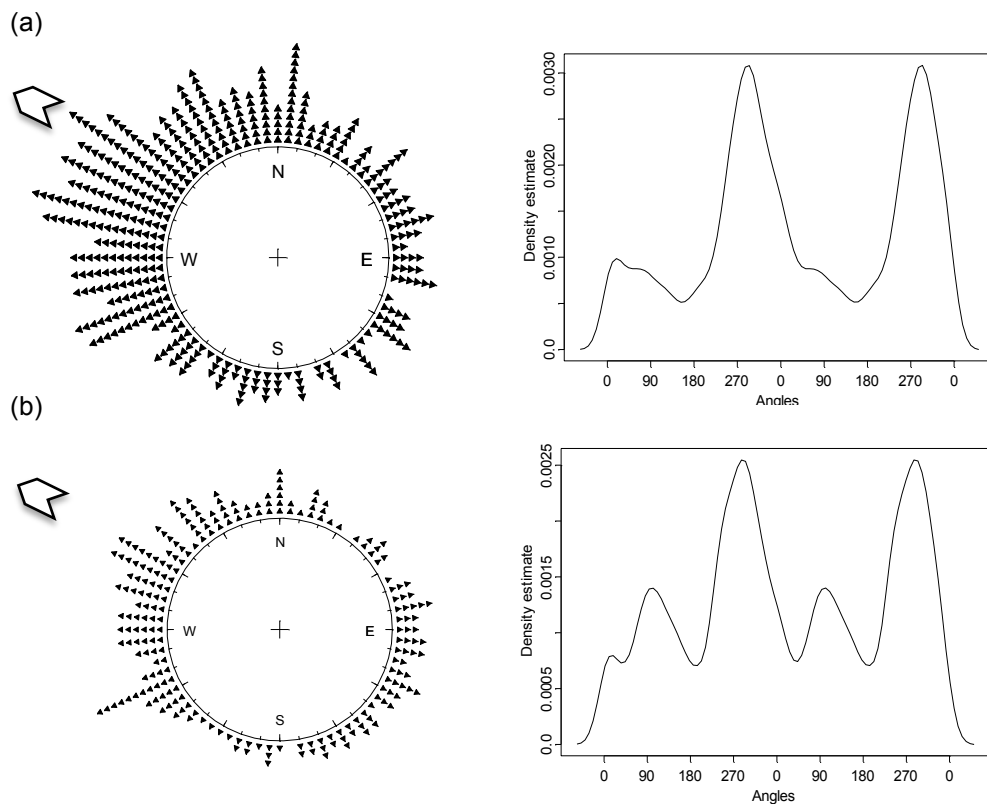


Figure 3. Circular plots of orientation angles for *Talorchestia brito* (a) and *Talitrus saltator* (b) tested under natural conditions at Quiaios beach and the respective density estimates shown in the Cartesian graphs (kernel method) plotted on the right. White arrows show the TED, theoretical seaward direction. The circular statistics are summarised in Table 2.

In fact, the bimodal orientation was checked and resulted significant, with a higher mean resultant length ($r = 0.250$, when doubling the angles) revealing the orientation tendency of *T. saltator* to both seaward and duneward directions with a total mean axial direction of 215° which is included in the confidence interval (Table 2).

Table 2. Circular summaries statistics for orientation distributions by species (*Talitrus saltator* and *Talorchestia brito*) tested at Quiaios beach. The numbers in parenthesis represent the results from the axial orientation of *T. saltator*.

Species	<i>Talitrus saltator</i>	<i>Talorchestia brito</i>
Mean angle ($^\circ$)	292 (215)	302
Confidence Interval 95% ($^\circ$)	± 12	± 7
Mean resultant length (r)	0.219 (0.250)	0.408
Sample circular dispersion	7.761 (7.164)	2.405
Rayleigh test (p)	< 0.001 (< 0.001)	< 0.001

We developed additive linear models and tested the possible interactions of species with all other variables and factors. The best model (selected via the lowest AIC) included the species interaction and retained few variables, namely, season (spring or summer), the landscape vision and the size of the individuals (Table 3).

Two separate models were consequently developed for each species, and the best models calculated were the followings:

Talitrus saltator: Orientation ~ Season*** + Size*** + Solar radiation* + Sky Cover* + Air Temperature* + Sun azimuth*; Likelihood = 752.743; AIC = 780.743; Degrees of freedom = 217; (***) $p < 0.001$; (**) $p < 0.01$; (*) $p < 0.05$; Likelihood Ratio Test).

Talorchestia brito: Orientation ~ Landscape Vision*** + Season*** + Air Temperature* + Tide* + Air Humidity* + Sun visibility; Likelihood = 1393.912; AIC = 1421.912; Degrees of freedom = 433; (***) $p < 0.001$; (**) $p < 0.01$; (*) $p < 0.05$; Likelihood Ratio Test).

The factors and variables are displayed in a decreasing order of significance according to the LRT (Likelihood Ratio Test); those factors not included in the model did not significantly contribute to improve the likelihood of the model.

Table 3. Baseline and best additive model with all variables and factors, and the best model calculated with the species interaction selected through the Akaike Information Criterion (AIC).

Model	Score
Baseline model	Orientation ~ year + season + time of the day + sun azimuth + landscape vision + tide + air temperature + air humidity + sky cover + sun visibility + solar radiation + species + sex + size + age AIC 2225.13 df 646
Additive best model	Orientation ~ landscape vision + Season + Species + Size + air temperature + solar radiation + tide + sky cover AIC 2208.96 df 660
Best model with interaction	Orientation ~ species* (season + landscape vision + air temperature + size) AIC 2207.41 df 658

The chosen models for the two sympatric talitrids have some factors in common (season and air temperature) in the same order of significance.

The orientation plots by season for each species indicated a significant better orientation during the summer sessions than in spring (the circular dispersion was lower, the mean resultant length was higher and the confidence interval was narrower, for both species) (Figure 4 and Table 4). In fact, while *T. brito* showed a unimodal distribution for both seasons, *T. saltator* orientation during spring was axial (axial mean resultant length = 0.2134) towards the sea and towards the dune (Figure 4).

For *T. saltator*, the sun orientation was more related with environmental conditions and the best model retained air temperature and solar radiation as important factors in orientation. Along with the environmental conditions, the cephalic length (as a proxy of size) was also significant for *T. saltator*, with bigger individuals showing higher orientation precision ($r = 0.3347$) when compared with individuals having a cephalic length smaller than 1 mm, which had a lower orientation precision ($r = 0.1457$).

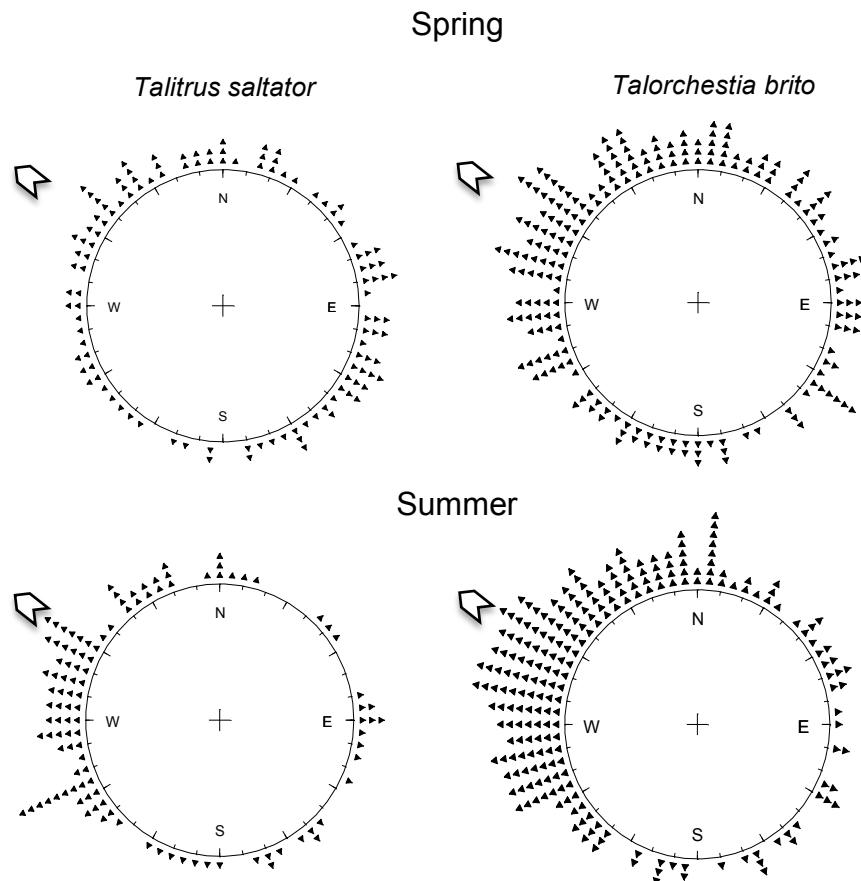


Figure 4. Orientation distribution plots of the sandhoppers tested at Quiaios beach. Distributions are reported by season (spring and summer) and species (*Talorchestia brito* and *Talitrus saltator*). White arrows indicate the Theoretical Escape Direction seawards: 300°. The summary statistics of the distributions are reported in Table 4.

For *T. brito*, the landscape vision was the most important factor influencing orientation (Figure 4, Table 4), this species was better orientated when landscape vision was permitted (TED was within the confidence interval and the mean resultant length was higher) (Table 4).

Table 4. Circular summaries statistics by season (spring and summer), landscape (visible and screened) and tide (ebbing and rising) for the two sympatric species tested. The Theoretical Escape Direction (TED) is 300°. Asterisks beside the mean vector length indicate the significance of the Rayleigh's test for homogeneity of distributions (* $p < 0.001$).

Factor	Level	<i>Talitrus saltator</i>			<i>Talorchestia brito</i>		
		Mean Direction (°) ± CI 95%	Mean Vector length (r)	N	Mean Direction (°) ± CI 95%	Mean Vector length (r)	N
Season	Spring	19.43 ± 21	0.118	118	314 ± 12	0.291*	214
	Summer	276 ± 13	0.460*	113	297 ± 8	0.522*	233
Landscape	Visible	280 ± 16	0.276*	123	284 ± 29	0.537*	219
	Screened	313 ± 20	0.174	108	330 ± 11	0.351*	228
Tide	Ebbing	293 ± 16	0.264*	118	297 ± 11	0.334*	222
	Rising	290 ± 19	0.174	113	306 ± 9	0.483*	225

During the experimental sessions, the tidal cycle changed from the morning to the afternoon, but only for *T. brito* the tide factor had a significant influence on orientation, with the highest precision occurring during the rising tide ($r = 0.483$ and circular dispersion = 1.627), whereas a more scattered response was observed during ebbing tide hours ($r = 0.333$ and circular dispersion = 3.756), however significant (Rayleigh test < 0.001) (Table 4).

Talitrids zonation

The zonation pattern of both species in this mesotidal beach showed that talitrids relied mainly on the intertidal area during the hottest seasons (spring and summer) (Figure 5). While *T. brito* occurred only in the intertidal area (the first 40 meters), the sympatric *T. saltator* shifted its mean zonation pattern along the sea-land axis reaching the highest distance from the sea (76 m) during the summer months (Figure 5). Throughout the seasons of the study, the highest mean densities were recorded for *T. brito* (148 ind.m⁻²) as compared to *T. saltator* (86 ind.m⁻²), a pattern also visible in the talitrids collected for the orientation tests (Figure 2).

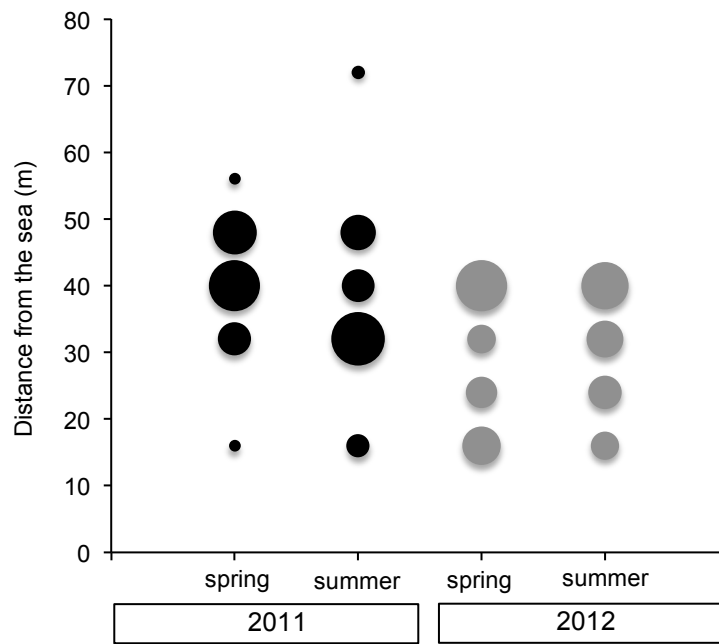


Figure 5. Talitrids mean zonation sampled during the orientation experiments (spring and summer 2011, 2012) at Quiaios beach. On y-axis is the distance from the sea (m). Bubble size indicates talitrids' frequencies recorded: black bubbles: *Talitrus saltator* and grey bubbles *Talorchestia brito*.

Discussion

The supralittoral zone of coastal ecosystems is an important environment for studying potential natural and/or human-induced disturbances, because the macrofauna of this zone live close to their thermal tolerance limits and exhibit particular behavioural traits to face the environmental fluctuations (McLachlan and Brown, 2006). Consequently, behavioural plasticity is quite often beneficial for marine species and in some cases accounts for most of the observed phenotypic responses to environmental variation (Scapini *et al.*, 1988; West-Eberhardt, 1989; Brown, 1996). Individuals may differ in their plasticity or responsiveness and hence in their degree of behavioural adjustment (Brown, 1996).

In this study, we analysed the differences in behavioural adaptations between two sympatric talitrid amphipods (*Talitrus saltator* and *Talorchestia brito*), which are the most ecologically significant taxa in exposed sandy beach habitats from the temperate areas (Marques *et al.*, 2003; Scapini, 2006). The behavioural traits of these talitrids were investigated by means of orientation performances and zonation patterns in the natural context. This study was undertaken on a mesotidal beach on the European Atlantic coast (Quiaios beach, Portugal), during the hottest seasons (spring and summer), since it is known that in this beach sandhoppers decreased in number during the winter due to the death of older individuals after the breeding season (Marques *et al.*, 2003; Gonçalves *et al.*, 2003). In

addition, the highest temperatures in this area are always reached during the spring-summer seasons (Gonçalves *et al.*, 2009) and high temperatures are suitable to test orientation in these species (Scapini, 2006). Moreover, the meteorological conditions characterising the environmental features at Quiaios beach during the experimental sessions followed the usual seasonal pattern, with higher air temperatures, solar radiation and less humidity during the summer (Table 1).

Both species showed a finely tuned to the environment, with a more precise orientation towards the sea during the summer conditions than during spring, clearly indicating that the meteorological changes that occur seasonally (in this case, higher temperatures in summer) are likely to induce a high precision of orientation towards a safe zone, as a local behavioural adaptation.

Behavioural studies on talitrids were mainly performed in the Mediterranean beaches (reviewed in Scapini 2006), however the differences in exposure and tidal ranges on the European Atlantic coast are suggested to cause variations in talitrids' behaviour, in particular the influence of tidal regime (in this case the tide can reach about 3.5 m) in the orientation performance of talitrids (Gambineri *et al.*, 2008; Rossano *et al.*, 2009). Therefore, different environmental conditions are expected to act both in Atlantic and Mediterranean sandy beaches, which may require different degrees of adaptation in local populations.

In fact, in our study the regression models revealed that different environmental variables affected the sun orientation performance of the two sympatric species in different ways. In general, *T. saltator* orientated to both seaward and landwards direction (axial distribution), and showed a greater scatter response with respect to *T. brito* (Figure 3). Scapini *et al.* (2002) recorded a similar pattern for conspecific individuals but from a different habitat, a non-tidal Mediterranean beach (North-western Tunisia).

Herein, the best model described for *T. brito* population showed a clear use of sun visibility and landscape vision as references in orientation (landscape was the most significant factor retained in the best model). *T. brito* was better oriented when landscape vision was permitted (Table 4), thus confirming the importance of other references besides the sun for sandhoppers orientation, a trend also described for a talitrid population (*T. saltator*) in the Atlantic beaches in France (Rossano *et al.*, 2009). Meteorological variables such as air temperature and humidity also affected the orientation of *T. brito*. Gonçalves *et al.* (2003) studied the population dynamics of the talitrid *T. brito* in the same beach studied here, and reported significant correlations between their dynamics and air temperature and humidity, which can affect the need of *T. brito* to find a higher moisture content in the sediment, which in turn is available in the lower zone of the beach. In addition, these authors recorded significant morphological differences among these species, *T. brito* had smaller body sizes when compared to *T. saltator* (Gonçalves *et al.* 2003), which may imply that the former species may have higher transpiration rates and consequently lower desiccation tolerances like those reported from congeneric species (Morrit, 1987).

In the present study, the model that explained *T. brito* orientation retained also the tidal range as an important factor determining orientation, since the risk of being swept away would be prevalent on this mesotidal beach, which supports the previous information and is also in agreement with Fallaci *et al.* (1999) findings, for the French coast and Lastra *et al.* (2010) in north-western Spain, where a concentrated activity of *T. brito* was observed during the ebbing tide. Such higher concentration during ebbing tide was also evident by the lower shore position recorded for *T. brito* in our study (Figure 5). In fact, both species showed a partially overlapping zonation but *T. saltator* was spread along the sea-dune axis and *T. brito* was restricted to the intertidal area (Figure 5). These differences likely reflect talitrids adaptations to the strikingly different thermal conditions of the respective zone on this dynamic beach.

A similar zonation pattern was exhibited by talitrid populations in the north-west of Tunisia, showing a main distribution of talitrids in the dunes in winter and near the waterline in summer, with dispersion along the transect in the intermediate seasons (autumn and spring) (Colombini *et al.*, 2002; Bouzlama *et al.*, 2009).

In our results, for *T. saltator*, the SPLM model for orientation described a different response when compared to *T. brito* at Quiaios beach. Meteorological variables such as air temperature, solar radiation and sky cloudiness were the main cues influencing the orientation of *T. saltator* at this beach. This species revealed a less precision in the orientation performance, a less dependence on the sun cues and high influence of the climatic conditions. In fact, Quiaios beach is located on a coastal area with a special climate, where air humidity is relatively high even in the hottest seasons, with a constant fog in the morning (*in situ* personal observations), which may allow *T. saltator* to maintain its optimal conditions above the tidemark without the need to perform daily precise migrations across the beach. This idea is supported by the high behavioural flexibility showed by *T. saltator*, which displayed different behaviours under natural conditions in both seasons, choosing different strategies according to the different situations. This result also supports the hypotheses formulated by Borgioli *et al.* (1999) and Nardi *et al.* (2003), which suggested that when sand moisture is sufficiently high on the beach, there is no need for a highly precision in sun compass for talitrids to reach a safe zone. Additionally, since *T. saltator* is known to be a poor swimmer (Dahl, 1964), this response is suggested to be a strategy to reach the base of the dune in order to avoid the risk of being washed away by the high tides occurring in this mesotidal beach. This hypothesis may also support the fact that the intrinsic features (size of the individuals) related to the animal morphology were also significant factors on *T. saltator* orientation. The size of individuals (measured as cephalic length) was significant for *T. saltator* population (but not for *T. brito*), with a significant lower precision of orientation being observed in the individuals with less than 1mm cephalic length, compared with bigger individuals (> 1mm). The smaller individuals may avoid the risky conditions of long migrations across the shore due to the tidal regime. This trend was also recorded for a *T. saltator*

population at Leirosa beach, located on the same Atlantic coastal stretch (Bessa et al., 2013b).

Accordingly, in our study, *T. saltator* had a more flexible orientation mechanism with respect to *T. brito*, which showed to be more dependent to the conditions offered by the intertidal zone, clearly supporting our previous hypotheses about the potential different behavioural flexibility of each species considered. We interpret the flexible behaviour observed in *T. saltator* as an evolutionary important adaptation to the dynamic tidal sandy shores characterised by an extreme variability in physical features especially in the supralittoral zone.

Colombini et al. (2013) also observed different strategies in two sympatric talitrids on a Tyrrhenian non-tidal sandy beach (Italy). These authors found that while *T. saltator* was capable of a precise orientation, when tested in natural conditions, *Orchestia gammarellus* was more adapted to a terrestrial environmental and was less efficient during the surface movements, by remaining restricted to the dune slack areas. In this case, different spatial strategies were found in these species, which allow for niche segregation since the zonation patterns did not overlap. A similar strategy of spatial segregation was found in our study on the Quiaios beach. *T. brito* showed a lower behavioural flexibility and precise orientation as compared to *T. saltator* as it remained confined to the intertidal zone, and was more dependent on tides and air temperature conditions than the latter species. Apparently, *T. brito* may face more risky conditions when dealing with a possibly change in the beach environment.

A precise orientation was demonstrated for *T. saltator* on the Tyrrhenian sandy beach, when compared with the sympatric species found in the back-dune (*O. gammarellus*) (Colombini et al., 2013). In our study, in turn we found a precise orientation for *T. brito* than for the *T. saltator* population. Tidal rhythms, appropriate orientation and stricter habitat requirements may play a key role in the maintenance of these species in different sandy beaches.

Another study carried out on an Atlantic shore of Morocco showed similar behavioural strategies for three sympatric talitrid species (*T. brito*, *T. saltator* and *Africorchestia spinifera*) to cope with environmental changes (Fanini et al., 2012a). These authors obtained the same model for the orientation distributions of these talitrids, disregarding the species, and the same variables were retained in the best model to explain the orientation of the three species tested. The opposite was shown in our study, as we found a different model for each species, which suggests that different populations of talitrids do not behave all in the same way, and each species may have been adapted to the ecological constraints prevailing on the coasts where they live.

Therefore, behaviour of sympatric talitrids seems to be a complex and dynamic process constantly updating with the environment. Our current findings warrant further investigation with congeneric species from contrasting environments such as macrotidal sandy beaches. Such comparisons may provide valuable information on the adaptability of a

given taxon to changing environmental conditions and we strongly believe that species with higher behavioural flexibility are hardened by long-term potential stress scenarios in a more frequently altered open-coast sandy beaches.

Behaviour of *Talitrus saltator* (Crustacea: Amphipoda) in a rehabilitated sandy beach on the European Atlantic Coast (Portugal)

Abstract

Environmental and human controls are widely accepted as the main structuring forces of the macrofauna communities on sandy beaches. A population of the talitrid amphipod *Talitrus saltator* (Montagu, 1808) was investigated on an exposed sandy beach on the Atlantic coast of Portugal (Leirosa beach) to estimate orientation capabilities and endogenous rhythms in conditions of recent changes in the landscape (artificial reconstruction of the foredune) and beach morphodynamics (stabilisation against erosion from the sea). We tested sun orientation of talitrids on the beach and recorded their locomotor activity rhythms under constant conditions in the laboratory. The orientation data were analysed with circular statistics and multiple regression models adapted to angular distributions, to highlight the main factors and variables influencing the variation of orientation. The talitrids used the sun compass, visual cues (landscape and sun visibility) to orient and the precision of orientation varied according to the tidal regime (rising or ebbing tides). A well-defined free-running rhythm (circadian with in addition a bimodal rhythmicity, likely tidal) was highlighted in this population. This showed a stable behavioural adaptation on a beach that has experienced a process of artificial stabilisation of the dune through nourishment actions over a decade. Monitoring the conditions of such dynamic environments and the resilience capacity of the inhabiting macroinfauna is a main challenge for sandy beach ecologists.

Keywords: beaches, rehabilitation process, *Talitrus saltator*, orientation behaviour, endogenous rhythms, tides

Introduction

Ocean sandy beaches are dynamic environments that make up two-thirds of the coastlines (Bird, 1996; McLachlan and Brown, 2006). These unique ecosystems are iconic assets to society and are facing escalating pressures worldwide (Brown and McLachlan, 2002; Schlacher *et al.*, 2007; 2008b; Defeo *et al.*, 2009). The significant loss of beach-dune habitats as a result of global warming, sea level rise and marine erosion processes caused by both natural and human induced impacts is increasing, and beaches are identified as threatened ecosystems (Defeo *et al.*, 2009; Dugan *et al.*, 2010) especially as > 70 % of the world's beaches are experiencing erosion (Bird, 1996). In addition, a plethora of anthropogenic impacts from coastal engineering, industrial activities, urbanisation, pollution and activities linked to tourism, are ubiquitous in marine communities (Schlacher *et al.*, 2007; Defeo *et al.*, 2009; Browne and Chapman, 2011).

In the recent decades, coastal management has been challenged to develop and implement measures that may safeguard the ecological and functional values of beach and dune ecosystems. So-called "soft engineering" interventions (nourishment processes) seek to mitigate beach erosion, but may cause ecological damage to sandy beach habitats (Nordstrom *et al.*, 2005; Peterson and Bishop, 2005; Speybroeck *et al.*, 2006; Bezzi *et al.*, 2009). Sand dunes are common features of shore areas, at the interface between the sea and land. Dune environments have significant values, forming natural barriers that protect coastal communities against the effects of severe weather events, providing habitat for several species and acting as buffer against eroding wave action (Short, 1999). Human pressures on dune systems are diverse, and these systems have been severely modified (Nordstrom, 2000).

Artificial beach nourishment and stabilisation of shorelines have been intensely used worldwide as opportunities to restore natural beach and dune habitats (Nordstrom 2005; Bezzi *et al.*, 2009; Bakker *et al.*, 2012). The mitigation of the (natural and human) threats to beaches and of the ecological impacts on macrofaunal communities is becoming a challenge for managers, who more and more frequently ask the advice of researchers (*e.g.*, Peterson and Bishop, 2005; Fanini *et al.*, 2009; Walker and Schlacher, 2011; Schlacher and Thompson, 2012; Leewis *et al.*, 2012). However, due to limited data about the life history of the affected species, the absence of previous data (before restoration process) and on recovery rates, as well as the possibility of cumulative effects of repeated damaging events may compromise the scientific inference about the fauna responses to environmental threats. The benthic organisms inhabiting the littoral zone of sandy beaches are limited to species with a high tolerance towards several forms of environmental stress, however, this high tolerance is not unlimited (Jaramillo *et al.*, 1996; McLachlan and Brown, 2006).

The resident mobile fauna has to cope with this harsh and dynamic system and may use integrated behavioural adaptations in order to reach and maintain the optimal safe zone on the beach (Pardi and Ercolini, 1986; McLachlan and Dorvlo 2005; Fanini *et al.*, 2008). The

beach populations display a range of unique adaptations to these environments, including mobility and burrowing abilities, rhythmicity in their behaviour and orientation plasticity (Brown, 1996; Scapini, 1997; Nardi *et al.*, 2003; Defeo and Gómez, 2005; McLachlan and Brown, 2006; Scapini, 2006; Rossano *et al.*, 2009; Fanini *et al.*, 2012a; 2012b).

The sandhopper *Talitrus saltator* (Montagu, 1808) is common and widespread (Mediterranean, Baltic and Eastern Atlantic coasts) inhabitant of sandy beaches, which lives on beaches with granulometry ranging from medium to coarse sand (Marques *et al.*, 2003). This species spends its whole cycle on the supralittoral zone with incursions into the intertidal area during low tides or to the sand dunes. Therefore, talitrid amphipods developed behavioural adaptations to cope with a wide range of environmental variation to maintain their position across the beach, avoiding dry conditions and inundation by waves (reviewed by Scapini, 2006).

Behaviour in talitrids represents an integration of environmental features experienced by individuals during their life span, which is expressed in a continuous updated process of adaptation to the environment and may become inherited through generations (Hartwick 1976, Scapini, 2006; Fanini and Scapini, 2008). The environmental features, such as landscape cues, seasonality of climatic conditions and habitat dynamics (*e.g.*, tidal regime), may influence the orientation performance of talitrids on sandy shores (Hartwick 1976; Scapini *et al.*, 2005a; Fanini and Scapini 2008). Seasonal variations of climatic conditions are known to affect the life cycle and the behaviour of *T. saltator* (Scapini *et al.*, 2005a). In this sense, orientation performance may rapidly change on unstable beaches, whereas under stable conditions, sandhoppers tend to develop a sun compass mechanism to recover the optimal zone in the beach (reviewed by Scapini, 2006).

Orientation in these species may be affected also by human impacts on beaches such as trampling, beach erosion and groyne constructions (Scapini, 1997, Scapini *et al.*, 2005a; Fanini *et al.*, 2007). Thus, sandhoppers orientation could be considered as a suitable bioindicator of beach changes and may be proposed to assess beach conditions (Fanini and Scapini, 2008). Sandhoppers also present rhythmic adaptation to cyclic phenomena as night-day alternation and tides. Differences were observed with latitude and ecological conditions in the rhythmic locomotor behaviour, namely the expression of circadian periodicity (Nardi *et al.*, 2003, Scapini *et al.*, 2005b; Rossano *et al.*, 2008; 2009).

The ecological importance of tidal cycles and related orientation behaviour in supralittoral species has highlighted the importance of rhythmic behaviour to avoid predation, desiccation and immersion, and maintain the suitable optimal zone when tides recede (Rossano *et al.*, 2009). A precise endogenous clock and navigational ability has allowed talitrid amphipods to become a model for investigations on behavioural adaptations in sandy beach environments (Naylor, 1972; Scapini *et al.*, 1992; Scapini *et al.*, 1996; Scapini *et al.*, 2005b; Rossano *et al.*, 2008). Several studies have attempted to establish the suitability of supralittoral amphipods as bioindicators of the impacts on beaches, such as nourishment processes, mechanical beach cleaning, constructions and activities linked to tourism, which

may alter the population structure and in some cases also the behavioural responses (Scapini 2006; Fanini *et al.*, 2007; 2009; Ugolini *et al.*, 2008; Veloso *et al.*, 2008; Scapini and Ottaviano, 2010).

In the Leirosa beach (European Atlantic coast, Portugal), a project of stabilisation of the dune system through geotextile incorporation was carried out in the past decade (Reis *et al.*, 2008). The main goal of our study was to describe the behavioural features of sandhoppers in this reconstructed beach-dune system and provide baseline information about the pattern used by this species to cope with the physical constraints in this beach. The lack of previous information regarding the dynamics of the resident species did not allow us to pose the question on the direct effects of these perturbations on *T. saltator* behaviour, through a pre- and post-action experimental design. However, our study is needed to propose hypotheses on the population responses to ongoing changes. Our hypothesis was that to an effective dune restoration measure might correspond behavioural features (orientation precision and sharp activity rhythms) fitted to the actual environment. The ultimate goal was to test whether a new artificial stabilised environment (foredune) may lead to a stable behavioural performance.

Methodology

The Study site

The Leirosa sand dune system ($40^{\circ} 02' 57.33''$ N, $08^{\circ} 53' 35.01''$ W) is located south of Figueira da Foz, on the European Atlantic Coast (Portugal) and has an extension of about 10km (Figure 1).



Figure 1 Location of the study area, the Leirosa beach ($40^{\circ} 02' 57.33''$ N, $08^{\circ} 53' 35.01''$ O) on the eastern Atlantic coast of Portugal (Photo credits for the right image: Google Earth, Version 6, accessed January 2012).

The beach is characterised by a well-developed sand dune system with low pressure from tourism. The beach is mesotidal with semidiurnal tides, with waves that frequently reach amplitudes of about 3m. The sea direction, the Theoretical Escape Direction for seaward orientation (TED, the perpendicular to the waterline measured at the collection site), was 310°. In the Leirosa beach, an underwater effluent discharge system was constructed in 1995 and the dunes were damaged and completely destroyed (Reis *et al.*, 2008). After three years, a rehabilitation action was conducted, which began with the reconstruction of the foredune, followed by its re-vegetation, in order to rapidly re-establish the desired height and slope of the beach-dune system (Reis *et al.*, 2008; Carmo *et al.*, 2010).

This coastal stretch is currently characterised by strong active marine erosion, mainly due to littoral drift retention caused by the construction of a local port and reduced sediment deposition as a consequence of engineering interventions in the watershed (Carmo *et al.*, 2010). From 2001 to 2009 beach erosion problems did not permit the maintenance of the frontal dune and several proposals for a rehabilitation of the Leirosa sand dunes were analysed. The adopted solution consisted of dune reconstruction using beach nourishment with layers of geotextiles filled with local sand and a monitoring programme is under way (Reis *et al.*, 2008; Carmo *et al.*, 2010). The orientation experiments took place on the beach in front of the artificial foredune that had been recovered.

Orientation experiments

Orientation experiments were performed in the reconstructed site of the beach in May and September 2011. Sandhoppers were collected on the beach short before each experiment and kept in a container with moist sand. The experiments were performed using a Plexiglas arena with a 40cm diameter, having 72 pitfall traps of 5° each on its rim. The arena was positioned on a tripod at about 1 m above the beach surface, with the pitfall 72 oriented to the North. The amphipods were placed in the centre of the arena and released after being kept 1/2 minute in a Plexiglas tube. Tests were carried out in the morning, starting at 9:00 am solar time, and in the afternoon, starting at 3:00 pm solar time, alternating two conditions regarding the landscape vision, with and without screen, using a white cardboard positioned around the arena to screen off the landscape view (this protocol was the same as that of Scapini *et al.*, 2005a). During the experiments, the following environmental variables were recorded: air temperature, air humidity, sun visibility and sky cover. The sun azimuth was estimated from the geographic coordinates of the site and the time of the day of each orientation release. After the release of a samples of 8 groups of 10 individuals each, amphipods were collected from the pitfall traps and stored in 75% alcohol for later morphometric analyses under the microscope to estimate the sex, cephalic length and number of articles of the second antenna, following Marques *et al.* (2003). During each experiment, at low tide, the extension of the intertidal and supratidal zones and the beach slopes were measured and beach profiles were drawn. Sediment grain size composition was determined and classified according to the Wentworth scale described in McLachlan and

Brown (2006). Meteorological data, water temperature and salinity, wave height and period and wind velocity were obtained from the nearest meteorological station (Figueira da Foz harbour, <http://www.meteo.pt/pt/maritima/costeira>).

Endogenous activity rhythms

In May and September 2011 live samples of freshly collected adult *T. saltator* were air-transported within a sealed aerated box under dark conditions from Leirosa beach to the Department of Evolutionary Biology, University of Florence (Italy). For each experiment, a total of 55 talitrids were placed individually in cylindrical recording chambers (12cm in diameter, 16cm high), provided with an infrared ray recording system, within a temperature controlled room (Scapini *et al.*, 2005b; Rossano *et al.*, 2008; 2009). Individuals were kept in the humid substratum collected on the shore of origin and “Tetramin” dry fish food was provided *ad libitum* on a piece of filtering paper. Each recording chamber was connected to a logger, downloading every 20 minutes the number of the infrared ray interruptions caused by the animals surface activity. Recordings were performed in constant dark and at a constant temperature of $18\text{ }^{\circ}\text{C} \pm 1^{\circ}\text{C}$ for 21 days.

Data Analysis

Orientation

Individual data of orientation expressed as angles to the North ($^{\circ}$) were plotted in circular diagrams and the statistics of circular distributions were estimated using a library developed ad hoc working within S-Plus Insightful software (Marchetti and Scapini, 2003). For each circular distribution the following statistics were calculated: the mean angle, the mean resultant length, the confidence interval of the mean direction and the Rayleigh test for uniformity (Fisher, 1993). The density distribution curve, smoothed with the kernel method, was also estimated for each distribution. The effects of environmental variables and factors were analysed using multiple regression analysis adapted to the angular distributions (SPLM, Spherically Projected Linear Models, Marchetti and Scapini, 2003). The best model (maximum likelihood with the least number of parameters) was chosen using the AIC (Akaike Information Criterion) and the effects of each individual variable were estimated using the LTR (Likelihood Ratio Test), by comparing the best model with the nested one without the variable tested.

Endogenous activity rhythms

The results of the recording sessions were presented for analysis in the form of actograms, drawn with the software Chart 35 (developed by D.D. Green, University of Birmingham, UK). The activity was plotted showing 48h in the two adjacent columns and the 21 days of recording in the rows. The dashed bars at the top of the actograms represent the dark in the natural light-dark cycle at the day of collection and the arrows the high tide peaks during the recording period. The times of the high tides were calculated using the Instituto

Hidrográfico tide tables (<http://www.hidrografico.pt/>). Periodogram analysis was performed on the time series, based upon the Whittaker periodogram and modified by Harris and Morgan (1983), using the Time Series software package (Gerard Harris Computing, Bristol, UK). The percentages of survival, number of active animals (animals active for at least 2/3 of the recording session), and the number of periodic animals (circadian or bimodal) were determined. Periods were calculated in a range from 10h20' to 30h20'. The period definition was reported as correlation ratio and the 99.9%, 99% and 95% probability lines were drawn. All periods calculated by periodogram analysis were considered significant at the 95% probability line. When present, bimodality periods were checked. MESA analysis (Maximum Entropy Spectral Analysis) was also conducted to check signal-to noise ratio (SNR).

Results

According to the McLachlan's (1980) rating scheme, Leirosa is classified as an exposed beach (exposure rate: 15). During both experimental months (May and September) the environmental measurements were taken in the beach at the ebbing tides, and a supralittoral zone of about 30m of extension with average slope of 6% resulted (Table 1). According to Wentworth scale, Leirosa beach had medium sand grains (Table 1). The water temperatures ranged from 14.5°C to 19.1°C and the water salinity had a mean value of 36.1 ± 0.5 during both experimental months (Table 1).

Table 1. Beach physical environmental features: mean values ± standard deviations for the experimental period of study are given.

Characteristic	Leirosa
Width of the beach (m)	46 ± 8
Extension of the supralittoral area (m)	29 ± 6
Average slope (%)	6 ± 0.4
Foredune height (m)	8 ± 1
Sediment granulometry (mm)	Medium sand (0.250 - 0.500)
Tidal range (m)	0.36 - 3.20
Wave height (m)	1.5 ± 0.5
Wave period (s)	9 ± 1
Water salinity	36.1 ± 0.5
Water temperature (°C)	16.8 ± 2.3
Wind velocity (m/s)	14.6 ± 1.4

For the orientation experiments, a total of 277 amphipods were analysed (161 in May and 116 in September), and the sex ratio showed a higher percentage of females (72% in May and 62% in September) with respect to males. The sandhoppers population had a mean cephalic length of about 1mm and the number of antenna articles ranged from 6 to 39 (Table 2). During the orientation experiments the majority of individuals had conditions of visible sun and the mean sky cloudiness was about 1 in the ranged 0-8 scale, indicating a clear sky (Table 2). Air conditions (temperature and humidity) showed clear seasonal trends, which is the typical pattern found in this temperate region (Table 2).

Table 2. Environmental and population variables observed during the orientation experiments in Leirosa beach (May and September). Mean values \pm standard errors, median and range (given by the minimum and maximum reached during the experiments) and N, the number of individuals used in the orientation tests.

	May		September	
	Mean \pm SE	Median (Range)	Mean \pm SE	Median (Range)
N	161		116	
Females (%)	72		62	
Sex ratio (m/f)	0.4		0.6	
Number of antenna article		23 (6 - 34)		20 (10 - 39)
Cephalon (mm)	0.8 \pm 0.1	0.8 (0.2 - 1.3)	1.0 \pm 0.1	1 (0.4 - 1.9)
Air temperature ($^{\circ}$ C)	28.1 \pm 0.4	26 (22 - 35)	24.4 \pm 0.2	24 (22 - 28)
Air humidity (%)	54.3 \pm 1.3	66 (26- 75)	75.3 \pm 0.9	77 (60 - 92)
Sky Cover		1 (0-8)		1 (0- 8)
Sun visible (n)	134		99	
Sun shaped (n)	27		17	

Orientation tests

The circular distributions of the orientation angles of *Talitrus saltator* in both months (May and September) are shown in Figure 2 with summary statistics information. The general trend of orientation for this population was to the north-western quadrant with a unimodal distribution of orientation angles and a mean direction of $312^{\circ} \pm 10^{\circ}$ that included the TED (310°) in its confidence interval. The Rayleigh test for uniformity was significant ($p < 0.001$).

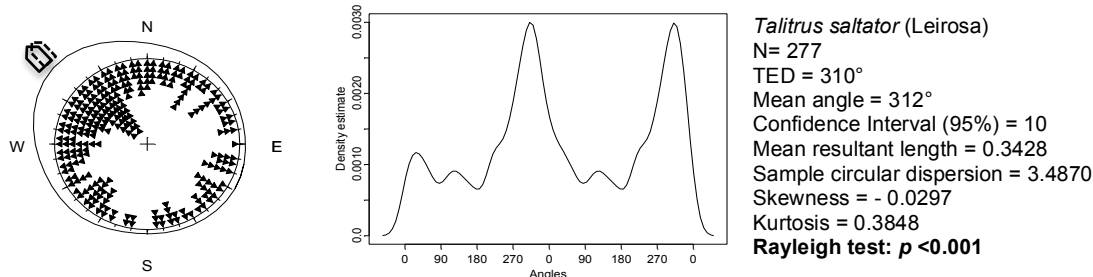


Figure 2 Circular plot and summary statistics of orientation angles of *Talitrus saltator* (Montagu, 1808) tested on Leirosa beach. Density estimates (kernel method) are double plotted on the Cartesian graphs (on the right). Arrow with continuous line: Theoretical Escape Direction seawards (TED); Arrow with punctuated line: Mean Angle; N: number of sampled individuals.

The variables considered in the regression SPLM analysis were: month (May/September), sun azimuth, landscape vision (landscape/no landscape), tide (rising/ebbing), air temperature, air humidity, sky cover (0-8), sun visibility (visible, veiled, shaped, not visible), sex (m/f), cephalic length and number of antenna articles.

We started from the additive model including all the variables and factors (11) that could affect orientation:

Orientation ~ month + sun azimuth + landscape vision + tide + sun visibility + sky cover + air temperature + air humidity + antenna articles + sex + cephalic length; Likelihood = 848.1351; AIC = 896.1351; Degrees of freedom = 253.

We developed a number of models by reducing the number of variables. The best additive model chosen (according to the Akaike information criterion, AIC) was the following:

Orientation ~ landscape vision*** + month** + tide** + air temperature* + air humidity* + antenna articles* + cephalic length; Likelihood = 853.8913; AIC = 889.8913; Degrees of freedom = 259 (** $p < 0.001$; ** $p < 0.01$; * $p < 0.05$; Likelihood Ratio Test).

The factors and variables are displayed in a decreasing order of significance according to the LRT; factors not included in the model did not significantly contribute to improve the likelihood of the model.

The landscape vision was found to be the most influencing factor, followed by the month. In order to check the importance of the seasonality on orientation, a model with the interaction of the month with all the other variables and factors was developed. In this model, the month factor displays a significant interaction with all other factors, (LRT, likelihood ratio test, $p < 0.05$) thus, the best model (with the maximum likelihood and minimum number of parameters) was described with the interaction of month:

Orientation ~ month (landscape vision^{***} + sun azimuth^{***} + sun visibility^{***} + tide^{***} + cephalic length^{**}; Likelihood = 813.3399; AIC = 861.3399; Degrees of freedom = 253 (^{***} $p < 0.001$; ^{**} $p < 0.01$; ^{*} $p < 0.05$; LRT).

Two separated models were consequently calculated for each month:

May: Orientation ~ Landscape vision^{***} + sun visibility^{***} + antenna articles^{***} + sun azimuth^{**} + tide^{*}, Likelihood = 487.03; AIC = 511.03; Degrees of freedom = 148 (^{***} $p < 0.001$; ^{**} $p < 0.01$; ^{*} $p < 0.05$; LRT).

September: Orientation ~ Sun azimuth^{*} + tide^{*} + air humidity^{*} + cephalic length^{*} Likelihood = 315.5825; AIC = 335.5825; Degrees of freedom = 106 (^{***} $p < 0.001$; ^{**} $p < 0.01$; ^{*} $p < 0.05$; LRT).

The two models obtained have some factors in common (sun azimuth, tide and morphological measures regarding the size of the individuals, namely, antenna articles and cephalic length). To highlight the effects of the significant factors, we analysed the circular distributions separately (Figure 3 with the respective statistical information summarised in Table 3).

The distribution of angles revealed that amphipods had a more precise orientation in September than in May (Figure 3). In September, sandhoppers were less dispersed as compared to May (the circular dispersion was lower, the mean resultant length was higher and the confidence interval was narrower, Table 3). The distribution of orientation angles in May revealed that amphipods were better oriented when landscape vision was permitted, while with the landscape screened off a scattered response was observed (Figure 3). In fact, without landscape vision significant deviations from the TED were confirmed by the confidence intervals of the mean direction ($70^\circ \pm 22^\circ$, not including the TED, Table 3).

The best model for May described also sun visibility as a highly significant factor (Table 3). A better orientation was shown by animals tested with visible sun as compared to sun not visible. This was confirmed by the Rayleigh test that was significant with sun visible only (Table 3). The experiments were carried out in the morning and in the afternoon (with respectively different sun azimuths) and the SPLM analysis confirmed the sun azimuth as a significant factor for both months ($p < 0.01$). The distribution obtained in September was unimodal when the tide was ebbing, but with the rising tide, some smaller peaks appeared to different directions (see Figure 3).

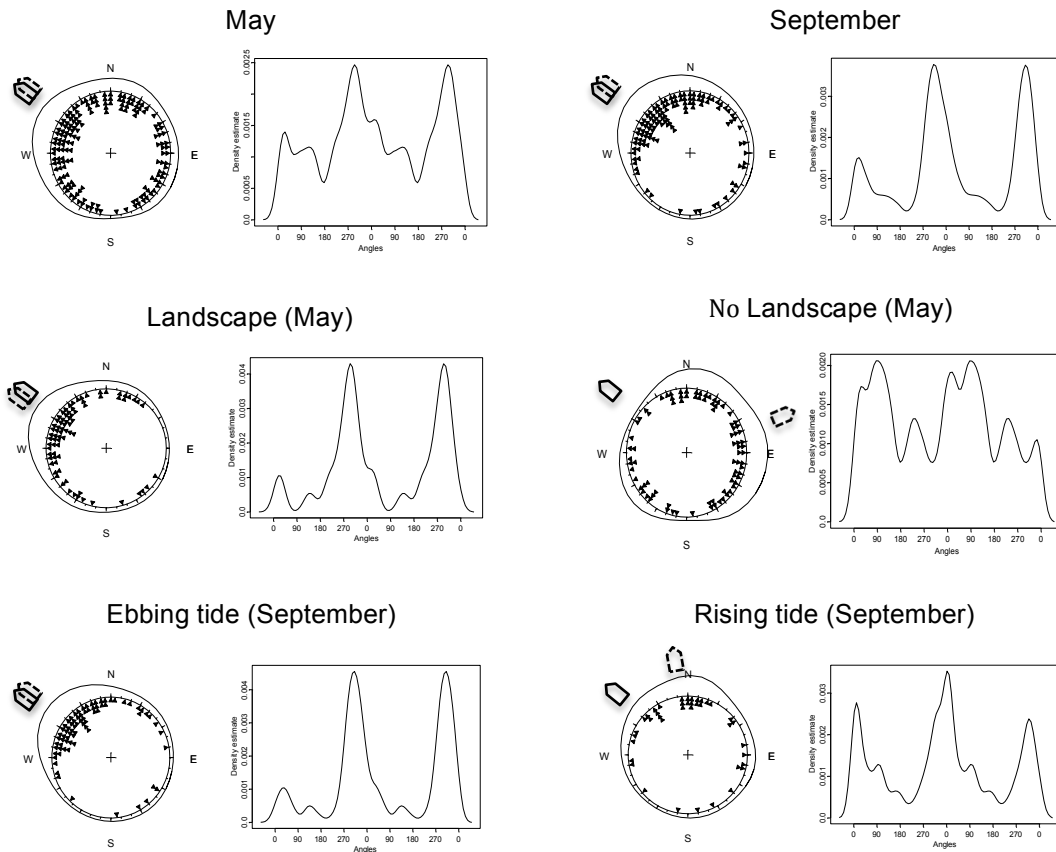


Figure 3. Circular plots for angular distributions considering some significant factors resulted from the best orientation model for both months. Arrow with continuous line: Theoretical Escape Direction seawards (TED); Arrow with punctuated line: Mean Angle. The respective summary statistics are reported in Table 3.

This scattered response was confirmed by the Rayleigh test (not significant, Table 3). For both months a better orientation was observed for bigger individuals, which had more than 15 antenna articles (May) and a cephalic length higher than 1 mm (September, Figure 3). However, the Rayleigh tests for both distributions of the small and big individuals were significant ($p < 0.001$), with the exception of the distribution obtained with animals with less than 15 antenna articles (Table 3).

Table 3 Summary statistics for orientation distributions. CI: Confidence Interval (95%), N: number of individuals in the distribution. TED = Theoretical (expected) Escape Direction Seawards.

Month	Test Condition	N	Mean	Mean vector	Circular	p-value
			direction \pm CI (TED = 310°)	length	dispersion	(Rayleigh test)
May	All	161	312° \pm 15°	0.2313	7.913	<0.001
September	All	116	320° \pm 12°	0.5544	1.104	<0.001
May	Landscape vision	80	296° \pm 13°	0.604	0.9748	<0.001
May	No landscape vision	81	70° \pm 22°	0.1978	11.73	n.s.
May	Sun visible	133	302° \pm 17°	0.2331	7.32	<0.001
May	Sun shaped	27	239° \pm 26°	0.4747	1.141	n.s.
May	Ebbing tide	79	290° \pm 18°	0.3476	4.032	<0.001
May	Rising tide	81	284° \pm 25°	0.1372	20.04	n.s.
May	Antenna articles > 15	118	292° \pm 17°	0.2892	5.662	<0.001
May	Antenna articles < 15	36	330° \pm 30°	0.258	6.553	n.s.
September	Ebbing tide	76	319° \pm 12°	0.6514	0.6752	<0.001
September	Rising tide	40	350° \pm 23°	0.4451	1.981	<0.001
September	Landscape vision	60	320° \pm 15°	0.5738	1.131	<0.001
September	No landscape vision	56	320° \pm 16°	0.5337	1.056	<0.001
September	Cephalic length < 1 mm	57	334° \pm 17°	0.5331	1.343	<0.001
September	Cephalic length > 1 mm	55	326° \pm 15°	0.5973	0.8354	<0.001

Endogenous activity rhythms

More than 90% of the individuals tested (all were adults) survived in the recording chambers during both test periods (May and September) and were healthy at the end of the experiments. Due to sand accumulation in front of the sensors, some data were discarded (not recorded) and only the animals active (and recorded) for more than 15 days were analysed (49 individuals). The percentages of survival, activity and rhythmicity were not significantly different between the two months (Fisher Exact Test, $p > 0.05$). Some slight differences in the mean periods and SNR were observed between the two months, with no significant differences (Wilcoxon Rank-Sum Test, $p > 0.05$), thus we analysed the total samples of both recording sessions together.

The inspection of the actograms (Figure 4a and 4b) indicated that talitrids were mostly active during the nocturnal subjective hours, however, some of them showed a second peak of locomotor activity. The periodogram analysis showed that about 96% of the considered animals had a clear locomotor activity pattern throughout the experiment, with a basic circadian rhythm significant for 85% of the active amphipods. The activity corresponded to the time of subjective darkness, with a mean period of 24h 15'. A second activity peak

(bimodal pattern) was significant at 12h 8' for the 26% of the active animals, but with a minor definition ($\text{SNR} = 0.06 \pm 0.04$) in comparison with the circadian peak ($\text{SNR} = 0.22 \pm 0.04$).

During both months, the activity of several adults peaked also just before the high tide hours during the spring tide (arrows, Figure 4a). The same pattern of activity was found with the periodogram analysis (Figure 4c and 4d), which represents the free-running periods of the individuals, estimated over the entire time series (21 days), showing a circadian period and a bimodal period peak recorded in some individuals (e.g., Figure 4c).

In order to check the bimodality, periodogram and MESA analyses were performed on 5 individuals that showed clear bimodality (Figure 5). These times series confirmed a clear circadian periodicity around 24h 20' (periodogram) and 24h 50' (MESA) for the five individuals with a second (bimodal) period of 12h 20' (periodogram) and 12h 50' (MESA). The difference of about 30 minutes between the periodogram and MESA analyses is known and was explained by Palmer (1995). For the bimodal actograms (Figure 5a) a higher definition was shown for the circadian rhythm than for the bimodal one, and a clear coincidence of this activity pattern with the diurnal high tide time was visible (Figure 5a, see arrows).

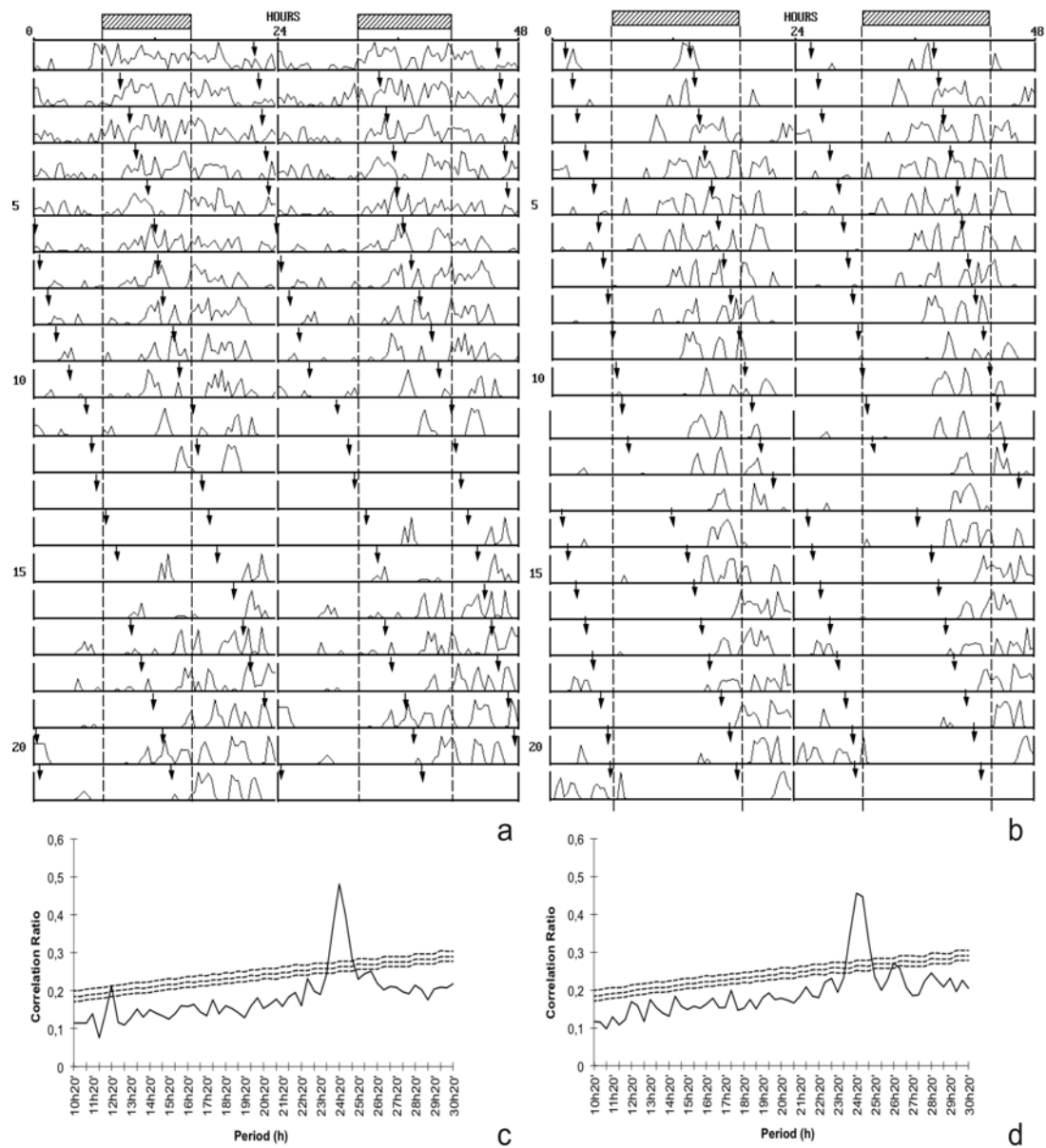


Figure 4. Actograms and periodograms of two individuals *Talitrus saltator* (Montagu, 1808) recorded singly in May and September. Dashed bars at the top of actograms indicate times of natural darkness at the day of sample collection; arrows represent high tide peaks during the recording period. Activity is double plotted on the right of and below first 24 h. Probability lines in the periodogram are, from the top to bottom, the 99.9%, 99% and 95% probabilities for peak significance. **a)** Experiment of May, animal 5 and respective periodogram **c)** τ_c (circadian period) = 24h 20', SNR = 0.912, τ_b (bimodal period) = 12h 20', SNR = 0.013. **b)** Experiment of September, animal 7 with respective periodogram **d)** τ_c = 24h 20'; SNR = 0.816.

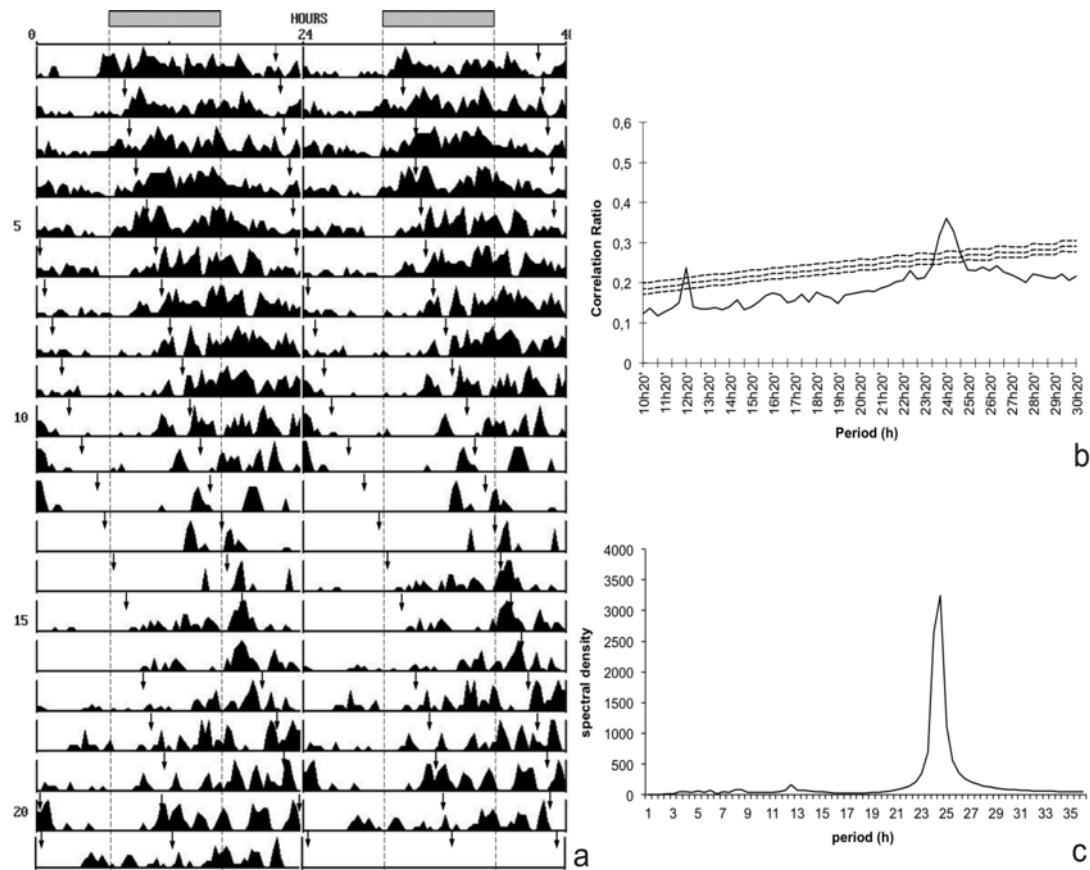


Figure 5. Mean of activity of 5 bimodal individuals **(a)** and respective periodogram and MESA analysis. Dashed bars at the top of actograms indicate times of natural darkness on the collection day and arrows represent high tide peaks during the recording period. Probability lines in the periodogram are, from the top to bottom, the 99.9%, 99% and 95% probabilities for peak significance. **(b)** Periodogram: τ_c (circadian period) = 24h 20', SNR = 0.431, τ_b (bimodal period) = 12h 20', SNR = 0.103; **(c)** MESA analysis: τ_c = 24h 50', spectral density = 3238, τ_b = 12h 50', spectral density = 136.

Discussion

Previous studies on talitrids reported significant relationships among behavioural performances of individuals, population structure, genetic variability of populations and the morphodynamics of the shorelines (Borgioli *et al.*, 1999; Scapini *et al.*, 2005a; Fanini and Scapini, 2008; Ketmaier *et al.*, 2010). In our case we focused on a particular sandy beach that had been subjected to human induced changes in the dune system, with nourishment processes along a decade (Reis *et al.*, 2008; Carmo *et al.*, 2010).

In order to assess the stability of the actual artificial foredune we investigated the *T. saltator* behaviour by means of field observations (orientation) and laboratory experiments (endogenous rhythms). Despite the long duration of the nourishment process and the difficulty to maintain a stable foredune in this beach, the environmental variables presented small fluctuations throughout the study period (May and September 2011), thus indicating a relative stability of the environmental physical conditions. The results from the orientation

tests revealed that the talitrids have good orientation capabilities towards the TED and use both sun and landscape cues to recover the safe zone in this beach, near water's edge. This capacity is in agreement with the literature available for *T. saltator* regarding orientation, characteristic for stable shorelines (Borgioli *et al.*, 1999; D'elia *et al.*, 2001; Scapini *et al.*, 2005a; Scapini, 2006). We confirmed also for this population the importance of landscape vision in orientation, since talitrids allowed to see the landscape features performed more concentrated seaward orientation as compared to those, which had the landscape screened off from view. In fact, for this population, the multiple regression analysis (SPLM) highlighted visual cues (landscape vision, sun azimuth and sun visibility) as the main factors influencing orientation. The response to landscape vision suggests that this feature represents a mechanism other than sun compass used by sandhoppers to orient seawards, responding to immediate physical changes, such as dehydration. This is in accordance with the findings on the Mediterranean coasts (Borgioli *et al.*, 1999; Scapini, 1997; Scapini, 2006), where populations from flat beaches relied more to the sun compass in contrast to populations with a well-developed dune landscape, which rely more on landscape vision. The high foredune resulted from the rehabilitation process in the Leirosa beach might provide a prominent landscape cue, particularly after the final restoration process (May 2011). The risks that sandhoppers may face in dynamic environments led them to include landscape features (such as the silhouette of the dune) into their behaviour to maintain their safe zone on the beach (Scapini *et al.*, 1992).

It is known that the temporal factor may strongly affect the motivation of sandhoppers to express zonal recovery, determining different physiological conditions with respect to surface moisture (reviewed by Scapini, 2006). In fact, in the Leirosa site, in September 2011 we registered a dry late summer season, with higher temperatures when compared to the standard recorded in the previous year (for more details visit <http://www.meteo.pt/pt/oclima/acompanhamento/>). The SPLM analysis emphasised the importance of the interaction of month as a factor influencing orientation, and the significant factors differed between the two months, with a high dependence on the landscape vision in May, while in September the meteorological (air humidity) conditions were recorded as the main factor affecting sandhoppers orientation.

An important factor responsible for orientation in talitrids on the Leirosa beach was the tide, as sandhoppers were better oriented with ebbing tides than with rising tides. According to previous findings on talitrids from Atlantic beaches (reported in Naylor 2010), when the tide was ebbing the animals tended to follow the water that moved away and directed seawards to search for food and shelter against dehydration near the waterline; instead, when tide was rising talitrids were more scattered, as if they tended to escape from the advancing water to avoid being submerged and recover the supralittoral zone.

The multiple regression analysis of orientation distributions emphasised the importance of tidal influence on the precision of sandhoppers orientation in this mesotidal beach. Our results are in agreement with the studies performed on other Atlantic sandy beaches in

Brittany (France), where talitrids also relied on tidal cycles (Gambineri *et al.*, 2008; Rossano *et al.*, 2009).

In general, our results confirm older findings, throwing new light on the ecological meaning of sandhoppers orientation and the conditions in which a learning of direction finding may occur in nature (Scapini, 1997; Scapini, 2006). From these results we infer that dunes stabilisation, despite the changes in the landscape, favour a high precision of orientation towards a safe zone, which suggests a local behavioural adaptation in the short time, as Fanini *et al.* (2007) proposed regarding nourishment processes on Mediterranean beaches.

The inhabitants of coastal habitats are exposed to several environmental cycles, such as the light-dark cycle, such as the light-dark cycle, the ebb and flow of tidal waters and the alternation of seasons (Palmer, 2000; Naylor, 2010). These cyclic environmental variations result in the need for selection of biological timing systems (endogenous clocks) that can oscillate within the range of environmental periodicities. Free-running endogenous rhythms of circatidal, circadian and circalunar periodicity were demonstrated for marine coastal species (*e.g.*, *Carcinus maenas*) in constant laboratory conditions (reviewed in Naylor, 2010). Similarly, in mesotidal beaches, as Leirosa, mechanisms that allow a supratidal species to anticipate the tidal change can be of critical importance to avoid immersion, drowning or desiccation (reviewed by Tessmar, 2011). It is well known that the circadian and/or circatidal rhythms expressed in animals maintained under laboratory conditions reflect patterns of behaviour expressed by the organisms in their natural habitat (Palmer, 2000; Naylor, 2010). Here, we investigated the endogenous locomotor activity of *T. saltator* from a mesotidal beach in the eastern Atlantic coast with and artificial foredune. The periodogram analysis showed a clear endogenous rhythm in *T. saltator* close to 24h ($24\text{h } 15' \pm 6'$) throughout the registration period of 21 days and a second bimodal activity peak near 12h ($12\text{h } 8' \pm 7'$), which appeared just before the high tide hours. The high tide and night-time patterns of activity clearly suggest that *T. saltator* population from Leirosa possesses both the circadian and circatidal biological clocks. The circadian pattern of this species is a trend basically similar to that reported in earlier studies for Mediterranean and Atlantic populations (Bregazzi and Naylor, 1972; Nardi *et al.*, 2003, Scapini *et al.*, 2005b, Nasri Ammar and Morgan, 2006; Rossano *et al.*, 2008; 2009). Williams (1983), in the Isle of Man (UK) analysed the endogenous rhythms in the amphipods *T. saltator*, *Deshayesorchestia deshayesi* and *Orchestia gammarellus*, revealing that endogenous rhythms displayed a nocturnal circadian pattern, with no clear evidence of any circatidal influence. On the other hand, Craig (1973) for the Californian talitrid *Orchestoidea corniculata*, and other authors for Atlantic sandhoppers (*T. saltator* and *D. deshayesi*, Gambineri *et al.*, 2008; Rossano *et al.*, 2009) demonstrated that the migratory behaviour of these species was related to tidal periodicity. On the Chilean coast, Jaramillo *et al.* (2003) showed evidence of a circatidal component with circadian locomotor activity in the talitrid *Orchestoidea tuberculata*. In our study, *T. saltator* showed a tidal rhythm component in the locomotor activity, a pattern also found on the Atlantic French coast (Rossano *et al.*, 2009). In our case, the tidally rhythmic behaviour persisted for the

whole recording session (21 days) under constant conditions. It was assumed that the biological clock that governs tide-associated intertidal organisms' rhythms has a period of approximately 12.4h, an interval that reflects the period of the ebb and flow of the tide (Palmer, 2000). Our results confirm a similar trend. Although more than 90% amphipods survived at the end of the recording sessions, only 26% showed a clear bimodal rhythm, likely due to the stress of being in laboratory under constant conditions. Palmer (2000) hypothesised that the lack of evidence of circatidal endogenous rhythmicity in crabs may be a result of the breakdown of internal coupling processes between the circadian and tidal clocks causing the missing peaks or slitting rhythms. We agree with Palmer (2000), who poses the question of the absence of particular stimuli in the laboratory, typical of a particular shoreline, such as wave action, making the tidal rhythm difficult to persist and not easily detectable during the recording sessions. In our case, all animals that displayed bimodal rhythms showed the same pattern, allowing us to suggest a tide-associated activity rhythm for the population of our study. A better orientation occurred during the ebbing tide, while during the rising tide, talitrids tended to rapidly disperse to different directions, likely to avoid the risk of inundation by the waves. This high activity of sandhoppers was also in accordance with the rhythms observed during the 21 days of recording. The free-running (endogenous) activity rhythm of talitrids inhabiting the Leirosa beach showed that adults become active at night, move about to forage near the water, and burrow at sunrise. We may also infer that they emerge during the day to move on the surface of the beach at the high tide to avoid being submersed by waves on this exposed beach, a pattern also found in the French Atlantic coast (Fallaci *et al.*, 1999).

We emphasise that such natural pattern of behaviour was described after the geomorphological alterations suffered by the Leirosa beach. In talitrids, behavioural variation is expected to be the first reaction to environmental changes, thus suggesting a stabilisation of the beach environment under study. The actual stabilisation of the foredune in the Leirosa beach, as a result of a decade of engineering actions, has induced stable orientation behaviour and clear endogenous rhythms of *T. saltator*. A continuous stabilisation process is under way in this beach and this baseline information may be important for future management and monitoring programs.

Our results reinforce the conclusions made by Scapini and Ottaviano (2010), who proposed *T. saltator* behaviour as a bioassay to assess the conditions acting on beaches, in particular physical constrains. We encourage researchers to take into account the behavioural traits of these key species in such environments when dealing with monitoring programs and policies strategies.



Chapter IV

Sandy beach macrofaunal assemblages as indicators of anthropogenic impacts on coastal dunes

Abstract

Ocean sandy beaches are iconic recreational assets to society and have undergone rapid degradation caused by both natural and anthropogenic pressures. A major effect of urbanisation on biota stems from artificial structures placed in aquatic systems. While the installation of these structures has been widespread, our understanding of how they impact shoreline habitats and fauna is relatively limited. On the sandy Leirosa Beach, on the European Atlantic coast (Portugal), a decade of cumulative impacts was recorded, with disruption of the frontal dune, dune nourishment and the incorporation of geotextiles to reconstruct the dune and maintain it as an artificial structure. Two years after the dune rehabilitation process at Leirosa Beach, seasonal sampling campaigns (from spring 2010 to winter 2011) were carried out to assess the effects of the artificial dune (and the first signs of its eventual disruption) on macrofauna compared to a natural dune on the same beach. During the study period, the rehabilitated foredune maintained the average slope, with similar sediment characteristics (finer sediments) when compared with the natural dune system, with no physical significant differences being appreciated between the two sites. The macrofaunal assemblages were represented especially by crustaceans (amphipods and isopods), with similar mean total density, taxon richness and diversity between sites. Special attention was given to the supralittoral species, and the results revealed that the natural zone harboured a significantly higher density of sandhopper *Talitrus saltator* with respect to the artificial zone. PERMANOVA results detailed the significant difference that occurred, particularly during the warmer seasons (spring and summer 2010), the recruitment period described for this species along this coast. In fact, SIMPER analysis revealed that *T. saltator* accounted for 46% of the dissimilarities between the natural and artificial dune sites. The presence of geotextiles extending across the supralittoral zone appeared, on the one hand, to allow for the presence of talitrids, but the application of textiles around the area prevented them from burrowing, thus causing them to avoid the rehabilitated area. The results highlighted the potentially negative effects of this artificial dune system if textile disruption continues, and the usefulness of *T. saltator* as an effective and reliable ecological indicator for these ecosystems. Thus, evaluating the risks and magnitude of human interventions is a major challenge for sandy beach ecologists, and baseline information is required if we are to better understand how resident macrofaunal species deal with future impact scenarios.

Keywords: Sandy coasts, dune rehabilitation, macrofaunal assemblages, *Talitrus saltator*, bioindicator.

Introduction

Exposed sandy beaches encompass a wide and unique range of ecosystem services to humankind (e.g., water filtration, provision of habitat and coastal protection), yet they are facing rapid degradation by both natural and anthropogenic pressures (Brown and McLachlan, 2002; Schlacher *et al.*, 2007; Defeo *et al.*, 2009; Doney *et al.*, 2012). Coastal zones are physically very dynamic, unstable environments, and the erosion problem is the clearest sign of this instability (Hanson and Lindh, 1993). Beaches have been enduring the impact of urbanisation and recreation on land and manifestations of climate change at sea (; Harley *et al.*, 2006; Schlacher *et al.*, 2008b; Dugan *et al.*, 2010). As mounting human pressure has been prompted by the changes caused by global warming, such as rising sea levels and increased storminess (IPCC, 2007), the demand for engineered solutions to combat these problems (particularly beach erosion and the coastal squeeze phenomenon) is intensifying worldwide (Charlier *et al.*, 2005; Chapman and Underwood, 2011).

The development of several engineering responses designed to protect coastal areas can be divided into “hard engineering”, when artificial structures such as seawalls, groynes and breakwaters are constructed right on the beach; and the so-called “soft engineering”, which involves beach replenishment, restoration or nourishment, a tried structure-free method that consists of importing sand directly to and for the beach (Charlier *et al.*, 2005; Nordstrom, 2005; Speybroeck *et al.*, 2006; Browne and Chapman, 2011; Chapman and Underwood, 2011). Globally, most of the shoreline stabilisation projects are designed to meet engineering and financial criteria, without considering their habitat value, even though artificial shorelines can cause loss of species and the alteration of ecological natural processes that sustain natural biodiversity (Chapman and Underwood, 2011).

Dunes are an important component of the beach landscape, providing an extremely valuable habitat for nesting birds, and food and protection for fauna, but this natural coastal landform and biota have been eliminated or reduced to a large extent by common beach management practices (Nordstrom, 2000; Feagin *et al.*, 2005).

On sandy beaches, macrofaunal assemblages play a vital role in the processing of nutrients and organic matter and act as a link between higher trophic consumers through important and complex food webs (McLachlan and Dorvlo, 2005; McLachlan and Brown, 2006). Physical perturbations can not only modify the beach’s physical habitats but also alter the species number and composition, jeopardising their presence in these endangered systems.

Studies have evaluated the effects of these impacts on sandy beach macrofauna. Schlacher *et al.* (2008a) detected the physical disturbance caused by road vehicles on beaches and their negative impact on macrofauna, while Schlacher and Thompson (2012) analysed the effects of beach recreation, and Schlacher *et al.* (2012) the effects of nourishment processes on beaches in Australia. The ecological responses of coastal armouring (Dugan and Hubbard, 2006), the constructions of groynes (Fanini *et al.*, 2009;

Walker *et al.*, 2008) and seawalls (Jaramillo *et al.*, 2002) and their impacts on resident macrofauna have alarmed ecologists. Moreover, certain crustaceans have already been proposed as suitable indicators and used as monitoring tools for coastal management on beaches and dunes (Fanini *et al.*, 2009; Schlacher *et al.*, 2011; Noriega *et al.*, 2012). In particular, the amphipod talitrid *Talitrus saltator* has been used as a tool to assess beach impacts (Ugolini *et al.*, 2008; Veloso *et al.*, 2008; Scapini and Ottaviano, 2010; Bessa *et al.*, 2013a). This opportunistic species is characterised by a short life span and wide geographical distribution, making it suitable for environmental monitoring (Marques *et al.*, 2003; Scapini, 2006). This species inhabits the supra- and mid-littoral zone of the beach (Scapini, 2006) where it found optimal conditions for burrowing. In addition, the role of talitrid amphipods is crucial for the sandy beach ecosystem, as they act as a food source for most of the species in the upper levels of the sandy-dune food web (Dugan *et al.*, 2003; Brown and McLachlan, 2006). The sensitivity of sandhoppers to disturbances on sandy beaches and their responses have already been documented for a nourished beach in Italy by Fanini *et al.* (2007). On a Spanish beach, Veloso *et al.* (2008) also found that sandhoppers density is lower in urbanised sites as a response to human presence. These authors have argued that the magnitude of beach disturbance might determine the degree of negative impact on sandhoppers and jeopardise their survival in these systems.

The assessment of the direct effects of the “sympathetic” artificial method (beach nourishment) on macrofauna has received special attention among scientists (Peterson and Bishop 2005; Speybroeck *et al.*, 2006; Jones *et al.*, 2008; Bezzi *et al.*, 2009; Baker *et al.*, 2012). However, knowledge regarding the cumulative and long-term effects is still scant (Leewis *et al.*, 2012) and further investigation is needed.

Leirosa Beach is located on the European Atlantic Coast (Portugal) (Figure 1). The area’s economy depends heavily on tourism and recreation industries. In 1995 the construction of an underwater effluent damaged the continuity of the Leirosa dune system. The use of heavy machinery and ensuing erosion problems led to the launching of a rehabilitation programme (Reis *et al.*, 2008; Carmo *et al.*, 2010). Dune rehabilitation started in 2000 with a process that allowed the mechanical reestablishment of the dune (nourishment) to the desired height and slope, but the severe winter conditions thwarted the restoration plan and a new intervention was needed. In 2005, the solution implemented to stabilise the dune system consisted of placing geotextile sand containers (geocontainers), filled with local sand. Nonetheless, after two years the dune was partially damaged because the bottom layers were partially destroyed by storm events. The last renourishment episodes took place in 2008, when geotextile tubes were chosen to reinforce the bottom layers of the rehabilitated stretch of the dune system (Carmo *et al.*, 2010). These tubes gave additional stability to a dune structure that had been deformed by the loss of sand filling from the bottom geotextile layers. Revegetation was performed to ensure better fixation of the sand and stabilisation of the front of the Leirosa dune system (Carmo *et al.*, 2010). Once the restoration project was finished, in 2010, certain signs of geotextile disruption that could threaten the dune stability

were detected, due to the ongoing shoreline erosion that has been a constant along this coastline (Carmo *et al.*, 2010). Although the direct effects of this story of cumulative impacts on macrofauna were impossible to detect through a before-after control-impact (BACI) study (Underwood, 1994), we designed a programme to deduce whether or not the artificial dune stretch mimicked a natural one on the same beach.

Therefore, the main goal of this study was to deduce whether or not the rehabilitated artificial landscape could harbour the same natural macrofaunal communities when compared with a natural dune area on the same beach. Moreover, we aimed at evaluating the extent of potential ecological impact arising from the possibility of disruption of the artificial foredune.

Methodology

Study site and sampling design

The artificial dune resulting from the restoration programme (beach nourishment, geotextile incorporation and foredune reconstruction) is located on Leirosa Beach on Portugal's Atlantic coast (Figure 1).

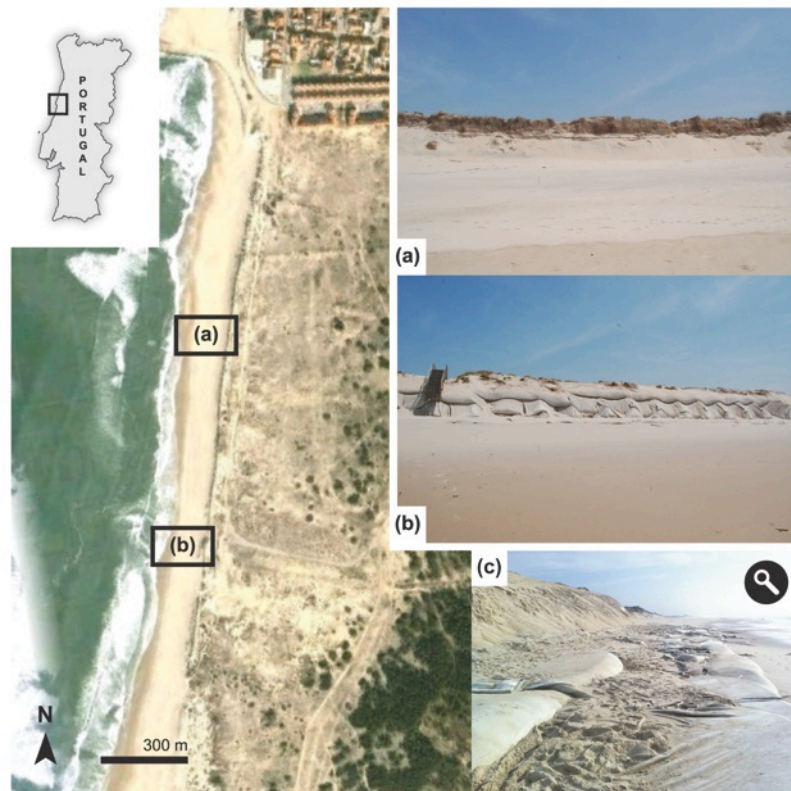


Figure 1. Location of the study area, the Leirosa Beach (40° 02' 57.33'' N, 08° 53' 35.01'' W) on the European Atlantic Coast (Portugal), and the spatial layout contrasting the natural dune zone (a) and the artificial dune zone (b) with the signs of textile disruption (c). (Photo credits for the left image: Google Earth, Version 6, accessed October 2012).

This exposed sandy beach is of the intermediate morphodynamic type, composed of medium-sized sands, and wave height typically reaches a maximum of about 3 m. For the purpose of this study, the spatial design incorporated contrasts between the beach with the artificial dune and a zone with a natural dune (reference point) with physically similar conditions. In addition, the control point was 400 m upstream (north) of the artificial area so as to minimise any treatment effects (such as increased sediment input) that might adversely affect the control site (Figure 1). Biological samples and environmental data were collected once each season (spring 2010, summer 2010, autumn 2010 and winter 2011). Sampling was conducted around low tide, and at each site (natural and artificial), biological sampling consisted of triplicate transects arranged at regular intervals (10 levels) between the low-water mark and the foredune. Benthic macrofauna was sampled by extracting sediment cores (inner diameter 250 mm, 300 mm deep). We considered ten sampling levels, taking samples at each level, per zone (rehabilitated vs. natural dune areas) and per time (four campaigns), each sample consisting of three random cores of sediment. Samples were first washed through a 1-mm sieving bag in the field, retaining macrofaunal organisms, which were fixed in 4% formalin and later sorted in the laboratory and preserved in 70% alcohol. Animals were identified to the lowest possible taxon.

Beach profiles at each of the two sites were measured from the base of the foredune to the low-water mark and at low tide. Sediment samples consisting of three replicates (cores 25 mm diameter, 300 mm deep) were taken from each site, considering the supralittoral and intertidal zones separately, to determine sediment moisture content, sediment organic matter and granulometry. In the laboratory, sediment moisture content was determined as total weight loss after being dried to a constant weight (60° C for 72 h). Sedimentary organic matter content was defined as the difference between the weight of each sample after oven-drying at 60° C for 72 h followed by combustion at 450° C for 8 h, and was expressed as the percentage of the total weight. Granulometry analyses were performed by dry-sieving with a sieve shaker with different mesh sizes, corresponding to the five classes described by Brown and McLachlan (1990): (a) gravel (> 2000 µm), (b) coarse sand (500-2000 µm), (c) mean sand (250-500 µm), (d) fine sand (63-250 µm), and (e) silt and clay (<63 µm). The relative content of the different grain-size fractions was expressed as a percentage of the total sample weight. The mean sediment was calculated using GRADISTAT software, following the Folk and Ward method (Blott and Pye, 2001).

Data analysis

The beach's physical features (beach width, beach slope, mean grain size, sediment moisture and sedimentary organic matter) were compared between sites (natural and artificial) using paired t-tests. To examine the effects of the artificial dune zone, the variation in univariate measures [i.e. mean total densities, species richness, diversity (Shannon-Wiener Index) and the abundance of common species] was tested through a series of one-way PERMANOVAs (Anderson, 2001), the design of which included three factors: (1) Time (fixed;

four sampling sessions, one per season: spring 2010, summer 2010, autumn 2010 and winter 2011), (2) Site (fixed; Natural vs. Artificial), and (3) Zone (fixed; Supralittoral vs. Intertidal zone). Euclidean distance similarity matrices were used for univariate data.

Non-parametric (permutational) multivariate analysis of variance, through three-way PERMANOVA, was used to test hypotheses about macrofauna assemblage differences between both natural and artificial dune sites. The same design used in the univariate analysis was employed, and, if appropriate, multiple comparisons were used *a posteriori* to test for differences between and/or within pairs of factor levels. The tests were based on 9999 permutations and the data were fourth-root transformed and converted to a Bray-Curtis dissimilarity matrix before the tests. The assemblage responses were visualised by non-metric multidimensional scaling (nMDS) and separate analyses of similarity between both sites were examined through ANOSIM tests (Clarke, 1993). The contribution by individual species to the dissimilarity between natural and artificial dune zones was calculated using the SIMPER (Clarke, 1993) procedure and taxa were considered important if their contribution to percentage dissimilarity was > 3%. All statistical analyses were performed using Primer v.6 and PERMANOVA (PRIMER-E Ltd., Plymouth, UK).

Results

Physical environment

A full characterisation of the physical variables of both areas (natural and artificial dune sites) on Leirosa Beach is given in Table 1.

Table 1. Comparison of beach's physical properties between the natural and artificial dune sites. *P* values refer to paired *t*-test between sites.

	Natural		Artificial		Natural	Artificial	<i>P</i>
	Min	Max	Min	Max	Mean	Mean	
Width of the beach (m)	58	80	62	70	70	62	0.3337
Average slope (%)	5.32	7.16	6	6.54	6.04	6.27	0.6097
Supralittoral width (m)	15	36	10	28	26.75	20.25	0.3465
Mean grain size (μm)							
Supralittoral	584	719	503	689	640	628	0.8297
Intertidal	966	1087	904	1047	925	912	0.9173
Sediment moisture (%)							
Supralittoral	0.51	11.81	0.45	11	4.58	4.31	0.9392
Intertidal	6.62	18.03	6	18	14.77	14.25	0.8971
Organic matter content (%)							
Supralittoral	0.21	2.44	0.22	2	0.81	0.68	0.8531
Intertidal	0.32	1.31	0.28	1.2	0.7	0.65	0.8655

Both habitats had highly similar sediment properties (grain size, moisture and organic matter content), slope, ($p > 0.05$) and their morphology (width) was not significantly different (Table 1). Both sites surveyed were moderately wide with an intermediate slope of about 6% (Table 1). The supralittoral beach face fluctuated in width between sites, proving wider in the natural dune zone (about 26 m and 20 m in the artificial zone). Mean particle size determination showed that sediment in both sites was classified as medium sized (according to the Wentworth scale described in Brown and McLachlan, 1990) and no significant differences were found over the four sampling periods. The supralittoral zone registered finer sand grains (640 μm in the natural site and 628 μm in the artificial site) becoming coarser towards the lower zone of the beach (the mean size ranged from 904 to 1047 μm in the artificial site and from 966 to 1087 μm in the natural one). During the sampling sessions, signs of textile disruption in the artificial dune were visible with the textiles extending along the supralittoral zone (details in Figure 1c). The similar physical conditions between the sites validated the premise that it is improbable that differences documented for biota between the natural dune site and the artificial dune site responded to those physical conditions, and was due instead to the artificial landscape and the eventual textile disruption.

Macrofauna: density and diversity

Regarding macrofauna, we identified and counted specimens belonging to 14 species at both habitats (see Appendix A). Crustaceans were consistently the most abundant group comprising amphipods (six species) and isopods (three species). Some insects (Coleoptera, Hymenoptera and Mecoptera) and one Mysidacea species (*Gastrosaccus sanctus*) were also collected in both sites.

Trends in univariate measures of community structure varied throughout the sampling seasons (Figure 2 and Table 2).

The total macrofauna density (Figure 2a) was significantly different between seasons and zones on the beach, but no difference was found between the sites (PERMANOVA: Pseudo- $F = 2.65$, P (perm) > 0.05 ; Table 2). The total density of macrofauna associated with the natural zone was higher than the artificial zone only during the warmer seasons (spring and summer 2010) (Figure 2a), particularly, in spring 2010 in the supralittoral zone (Figure 2b), and during summer 2010 for the intertidal zone (Figure 2c), although these differences were not detected in the PERMANOVA tests (P (perm) > 0.05 , Table 2).

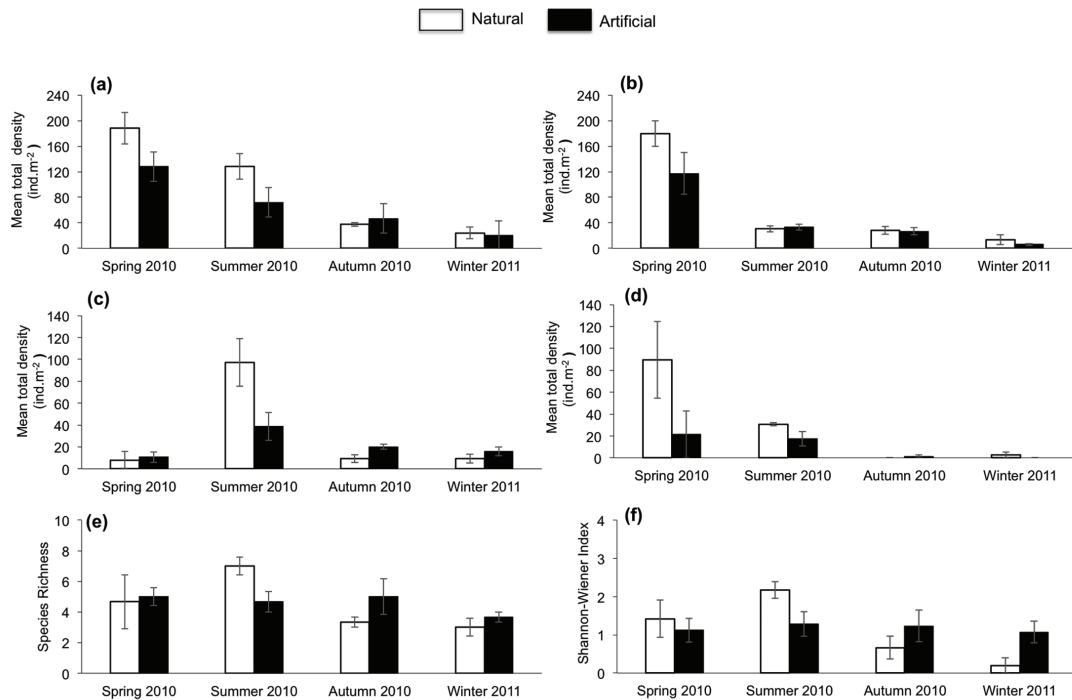


Figure 2. Variation in the mean (\pm se) of (a) total macrofauna density, (b) total density in the supralittoral zone and (c) in the intertidal zone, (d) density of *Talitrus saltator*, (e) species richness, (f) Shannon-Wiener Index, between the natural and artificial dune sites.

There were no major differences in species richness between sites on the whole (Figure 2e), although significant spatial variations occurred between sampling dates (PERMANOVA Time x Site: $P < 0.01$, Table 2). In fact, species richness was higher in summer 2010, at the natural site, and in autumn and winter at the artificial one (Figure 2e). The variation of the Shannon-Wiener index values followed a pattern entirely similar to that of species richness (Figure 2f and Table 2).

Particular attention was given to the talitrid amphipods *T. saltator* and *Talorchestia brito*, which were clearly dominant in the assemblages (Figure 2d; Table 2 and Appendix A). The artificial dune site harboured significantly fewer *T. saltator* individuals than the natural one (PERMANOVA: Pseudo- $F = 6.45$, $P < 0.01$), while no significant differences were detected with regard to *T. brito* (Table 1).

On the other hand, it is important to notice the occurrence of temporal fluctuations in the mean density of *T. saltator* (PERMANOVA: Time x Site, $P < 0.01$, Table 2), with almost no individuals being found at either site during the coolest sampling periods (autumn 2010 and winter 2011) (see Figure 2d). Regarding beach zonation, both species registered differences between the intertidal and supralittoral zones (Table 2), with *T. brito* density being particularly variable throughout the study period and within zones (PERMANOVA; Time x Zone, P (perm) < 0.001 ; Table 2).

Table 2. Summary of one-way PERMANOVA results for the univariate analysis of fauna descriptors (Total Density, Species Richness, Shannon-Wiener Index, *Talitrus saltator* density and *Talorchestia brito* density) between sites (natural vs. artificial), time (spring 2010, summer 2010, autumn 2010 and winter 2011) and zone (supralittoral and intertidal).

Factor	df	Total Density		Species Richness		Shannon-Wiener Index		<i>Talitrus saltator</i>		<i>Talorchestia brito</i>	
		MS	Pseudo-F	MS	Pseudo-F	MS	Pseudo-F	MS	Pseudo-F	MS	Pseudo-F
T - Time	3	1.90	22.78***	4.01	5.68**	0.55	4.88**	6.91	16.81***	1.01	3.29*
S - Site	1	0.22	2.65	0.55	0.77	0.02	0.002	2.65	6.45**	0.02	0.003
Z - Zone	1	0.96	11.52*	1.01	1.43	0.01	0.09	1.94	4.72*	8.34	27.45***
T x S	3	0.21	2.56	3.17	4.48**	0.53	4.81**	1.92	4.67**	0.44	1.43
T x Z	3	1.60	19.11***	2.67	3.77*	0.13	1.16	0.9	2.20	10.8	35.07***
S x Z	1	0.02	0.03	1.30	1.84	0.06	0.53	0.06	0.16	1.63	5.36
T x S x Z	3	0.22	2.65	1.46	2.06	0.24	2.21	0.38	0.94	0.22	0.74
Residual	29	0.08		0.71		0.11		0.41		0.30	

Bold values indicate significant results: * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

Macrofaunal community structure

Results of multivariate analysis showed that variability was significantly different between assemblages located in sites with different dune conditions, but this variation was not consistent over time (Table 3). Pairwise tests done *a posteriori* on these interactions (between Time and Site) showed that assemblages differed significantly between sites only during the spring 2010 sampling period (the *p*-value for the pairwise test on differences in community structure between both sites was 0.013) (Table 3). A similar pattern was also seen in the densities of the macrofaunal assemblages in spring 2010 (Figure 2b), although statistical tests did not detect significant differences in this sampling period (PERMANOVA: Time x Site (summer), *P* (perm) = 0.087).

Table 3. Summary of Permutational Multivariate Analysis of Variance (PERMANOVA), examining differences on macrofaunal assemblages between sites (natural vs. artificial), time (spring 2010, summer 2010, autumn 2010 and winter 2011) and zone (supralittoral and intertidal). Pairwise tests refer only to the interactions between Time x Site (T x S).

Main Test	df	SS	MS	Pseudo-F	<i>P</i> (perm)
T - Time	3	35462	11821	11.202	0.0001
S - Site	1	3358.5	3358.5	3.1828	0.019
Z - Zone	1	18349	18349	17.388	0.0001
T x S	3	9927.5	3309.2	3.136	0.0013
T x Z	3	25071	835.9	7.9196	0.0001
S x Z	1	2066.7	2066.7	1.9586	0.1364
T x S x Z	3	7119.7	2373.2	2.249	0.0254
Residual	29	30601	1055.2		
Pairwise Test	Condition				<i>P</i> (perm)
T x S	spring 2010 x natural vs artificial				0.0134
	summer 2010 x natural vs artificial				0.0872
	autumn 2010 x natural vs artificial				0.2890
	winter 2011 x natural vs artificial				0.1126

The nMDS bi-plot illustrates a moderate segregation of habitats over the whole study period (Figure 3a). The stress value for the two-dimensional ordination was relatively low at 0.08 but the ANOSIM test showed no significant differences between sites ($r = 0.011$, $p = 0.035$), confirming that group separations are poor when analysed for all sampling dates. Nevertheless, the noteworthy Time x Site interaction revealed by PERMANOVA showed significant differences regarding the spring 2010 sampling period (Table 3).

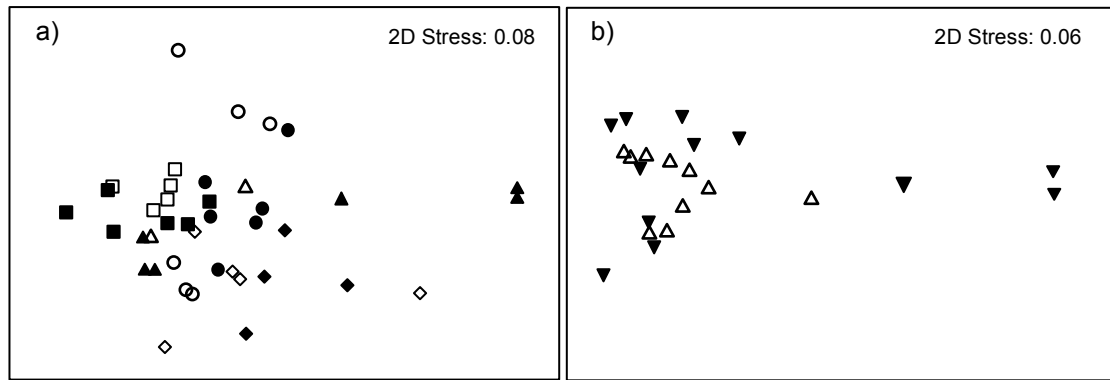


Figure 3. Two-dimensional non-metric multi-dimensional scaling ordination (nMDS) of variation in macrofaunal assemblages structure contrasting natural (open symbols) and artificial (solid symbols) dune zones during the whole sampling seasons (a) and particularly during the spring 2010 session (b). Symbols indicate each session (Triangles = spring 2010; Squares = summer 2010; Circles = autumn 2010 and Diamond = winter 2011).

When pooling the data set provided by samples taken in spring 2010, the nMDS ordination broadly reflected a significant effect of the sites on the community structure (Figure 3b). In fact, the ANOSIM test revealed key differences between sites in this sampling period ($r = 0.32$, $p < 0.01$). The percentage of the difference between sites accounted for 53% of dissimilarities (SIMPER), with *T. saltator* being the most important species shaping the differences in macrofaunal assemblages associated with both habitats. Furthermore, the PERMANOVA test showed that in spring 2010 these differences were significant (pairwise test), with the sandhopper *T. saltator* accounting for 46% of the dissimilarities between the natural and artificial dune sites (SIMPER, Table 4).

Table 4. SIMPER analysis showing the contribution of common species to the average Bray-Curtis dissimilarity between Natural and Artificial dune sites during the spring 2010 session.

Taxon	Average abundance (ind.m ⁻²)		Av.Diss	Contrib.%	Cum.%
	Natural	Artificial			
<i>Talitrus saltator</i>	2.95	0.94	11.53	48.29	48.29
<i>Eurydice pulchra</i>	0.56	0.62	4.11	17.20	65.49
<i>Gastrosaccus sanctus</i>	0.56	0.47	3.60	15.09	80.58
<i>Talorchestia brito</i>	2.82	2.80	2.10	8.77	89.35
<i>Tylos europaeus</i>	2.07	2.09	1.63	6.84	96.19
Insecta	1.68	1.50	0.91	3.81	100

Discussion

Despite the importance of engineering works on sandy beaches when it comes to making decisions aimed at protecting the coast line, the cumulative and long-term effects of artificial structures on macrofaunal assemblages and the communities' recovery times requires more attention. It is not easy to conduct an adequate impact assessment plan, due to the complexity of finding a control area with the same physical characteristics and the possibility of performing a before/after control-impact survey (Underwood, 1994), allied with the inherent environmental variability in space and time. However, not only the geomorphological information needs to be well known during the implementation of an impact assessment, but also the actual conditions faced by that particular environment.

In our case, it was impossible to detect the cumulative effects of a decade of nourishment and renourishment processes along the Leirosa Beach stretch, since they started in the early 2000s (Reis *et al.*, 2008). Yet the success of maintaining an artificial dune may be threatened due to the current erosion and coastal squeeze phenomenon being recorded along this coastline (Carmo *et al.*, 2010). Our findings nevertheless made it possible to elucidate the effects of the presence of an artificial landscape on macrofaunal assemblages two years after the end of the nourishment project, as well as the first signs of dune disruption.

It is known that species living on sandy beaches have to cope with this harsh, dynamic environment, and their behaviours are indeed variable as they respond strongly and rapidly to beach morphodynamics (McLachlan and Brown, 2006).

Leewis *et al.* (2012) performed a long-term nourishment impact assessment study, and concluded that macrofaunal recovery took place within a year of the impact, although this was different for each species. Schlacher *et al.* (2012), performed a detailed Beyond BACI design study, and showed that impact immediately after nourishment was massive for macrofauna but that signs of recovery started five months later. Species-specific negative nourishment impact was detected for the amphipod *Exoediceros fossor* in Australia (Jones *et al.*, 2008), but once again recovery began from several weeks to a year for this species. Similarly, in Spain, Herrera *et al.* (2010) found a negligible impact during the nourishment phase on the European fiddler crab (*Uca tangeri*). Macrofaunal species subsequently responded to these changes in beach morphodynamics by rearranging themselves along the shore depending on what was available to them. Thus, these studies argued the importance of knowing the autecology of the resident fauna so as to be able to mitigate the effects of these impacts on management assessment plans.

Also, the macrofauna inhabiting this dynamic environment have to cope with small- and large-scale modifications, and certain species could be recognised as potential indicators of changes in marine environments, due to their behavioural plasticity (Brown, 1996).

Taken as a whole, our results revealed that similar communities were found at the natural and rehabilitated (artificialised) sites, with no significant differences being found

regarding the species richness, diversity and mean total densities (Figure 2). Nevertheless, looking more in detail at the densities of the most abundant species, PERMANOVA tests showed significant differences regarding the sandhopper *Talitrus saltator* density between sites (Table 2).

This well-studied amphipod (Marques *et al.*, 2003; Scapini, 2006; Fanini *et al.*, 2007), which lives particularly in the supralittoral zones of beaches, migrates regularly from the shoreline to the dune, to maintain the optimal zone avoiding both desiccation in the dry zone and inundation by waves (reviewed by Scapini, 2006). Sandhoppers carry out their life cycle on the same beach throughout the year, so they may integrate the effects of environmental variability (Scapini, 2006). Since they are strictly linked to the supralittoral environment, the responses to environmental variation represent an integration of constant updating to the environmental condition (Scapini, 2006). Sandhoppers are important elements in ecological processes that occur on sandy beaches and, more specifically they play a key role in the food web structure, as important consumers of wrack and a food source for most of the upper levels of the trophic chains in sand-dune environments (Dugan *et al.*, 2003).

This enables them to act as a feasible keystone species (i.e. a species whose removal from the ecosystem is expected to consistently change the community composition, Mills *et al.* 1993).

The baseline of data on this species on sandy beaches makes sandhoppers good candidates for bioindicators of environmental stress (see revision in Scapini and Ottaviano, 2010). In fact, the behavioural plasticity of talitrids enabled them to become a special bioindicator species for human perturbations on sandy beaches (Ugolini *et al.*, 2008; Veloso *et al.*, 2008; Scapini and Ottaviano, 2010).

In our case, in the artificial dune zone, the presence of large geotubes and the amount of disrupted textiles found in the supralittoral zone (see Figure 1b) led us to hypothesise that amphipods could not burrow in this zone and might therefore avoid this area whenever they needed to recover the optimal zone on the beach. Accordingly, it is not surprising that the lowest number of individuals in the artificialised area in comparison to the reference one was found both in the spring and summer 2010 sampling periods. Indeed, statistical tests detected differences in *T. saltator* densities especially during the warmer season, when this species recruitment occurs on the Western Atlantic coast (Marques *et al.*, 2003), while during the cooler period (autumn 2010 and winter 2011) almost no individuals were recorded at either site (Figure 2d). Thus, successful employment of artificial dunes in this system could jeopardise the presence of this supralittoral species in this site.

Therefore, such behavioural changes linked to the artificial dune zone could be well suited as an early warning signal for wider negative ecological impact (as demonstrated by reduced densities of *T. saltator* in this zone). This finding makes this species suitable as a baseline indicator of habitat degradation, given its sensitivity to physical variation in the beach and dune ecological conditions as demonstrated here. Our results reinforce the notion that *T. saltator* could be used as a bioindicator of human changes on sandy beaches.

On the other hand, regarding the talitrid *Talorchestia brito*, a widely distributed species along the European Atlantic coast (Gonçalves *et al.*, 2003, Lastra *et al.*, 2010) that frequently coexists sympatrically with *T. saltator*, significant differences were detected not between the two sites but between the zones on the beach (Table 2). This can be explained by the intertidal affinity described for this species, which is more often found close to the sea (Fallaci *et al.*, 1999; Gonçalves *et al.*, 2009). This distributional pattern can explain differences between beach zones in both of the sites we found, as well as the absence of artificial dune effects on this species.

Dune nourishment projects need to be well implemented; otherwise certain residual effects could persist through time. Even if the foredune at Leirosa had lasted throughout these years without any disruption, with the supralittoral species always present, the differences found appear to support the idea of how these impacts could be intensified the longer the sand bags are in place. But, as occurred in 2006 at Leirosa, they can break apart and have very limited capacity to create a habitat for marine organisms. Our results back this notion, but conclusive evidence will require more robust long-term ecological research and replication of beaches across larger geographic areas.

Knowledge not only of the response and recovery rates of macrofaunal assemblages to human impacts, but also of the durability of artificial structures implemented on sandy beaches must be taken into account by scientists and decision makers, namely local authorities around the world (Jaramillo, 2012, McLachlan *et al.*, 2013).

Furthermore, evaluating not only the magnitude of engineering modifications to the community structure but also the changes in the ecological processes that occur in this system is a serious challenge for sandy beach ecologists.

McLachlan *et al.* (2013) have also stressed the need to assess the frequency and magnitude of both press and pulse disturbances, the importance of providing projections of future impacts and their magnitude, frequency and intensity, and the need to identify early warning indicators of disturbance.

We agree with Chapman and Underwood (2011) who ask for collaboration between ecologists and engineers to create infrastructures that will sustain biodiversity in such valuable ecosystems, but also the need for more experimental tests involving prediction about what happens when such impacts takes place. Given the current trends and predictions for climate change (Brown and McLachlan, 2002; Doney *et al.*, 2012; IPCC, 2007) and continuously rising sea level effects, shoreline stabilisation programmes should adopt an adaptive management approach that allows designs to be modified in line with changing conditions over time. Artificial structures need to be well implemented; otherwise they will jeopardise the resilience of beaches on account of more frequently disturbed scenarios in the future.

Appendix A. Mean total abundance (%) of macrofaunal assemblages from the natural and artificial dune sites during the four sampling sessions (A - spring 2010, B - summer 2010, C - autumn 2010 and D - winter 2011).

	Natural					Artificial				
	A	B	C	D	Mean	A	B	C	D	Mean
Amphipoda										
<i>Talitrus saltator</i>	47.5	24	-	13.3	28.3	16.7	24	5	-	15.2
<i>Talorchestia brito</i>	35.5	29.2	64.3	53.3	45.6	53.1	37	55	13.3	39.6
<i>Talorchestia deshayesii</i>	-	-	-	-	-	-	-	7.5	-	7.5
<i>Pontocrates arenarius</i>	-	8.3	10.7	-	9.5	-	-	-	-	-
<i>Haustorius arenarius</i>	-	-	-	-	-	4.2	-	-	13.3	8.8
<i>Urothoe elegans</i>	-	-	-	26.7	26.7	-	-	-	6.7	6.7
Total	83	61.5	75	93.3	78.2	74	61	67.5	33.3	59
Isopoda										
<i>Tylos europaeus</i>	10	30.2	3.6	-	14.6	17.7	35.2	-	-	26.4
<i>Eurydice pulchra</i>	1.4	-	-	-	1.4	3.1	-	-	46.2	24.7
<i>Eurydice naylori</i>	-	-	-	-	-	-	-	7.5	-	7.5
Total	11.4	30.2	3.6	0	11.3	20.8	35.2	7.5	46.2	27.4
Mysidacea										
<i>Gastrosaccus sanctus</i>	1.4	8.3	14.2	-	8	1	1.9	25	13.3	10.3
Total	1.4	8.3	14.2	0	6	1	1.9	25	13.3	10.3
Insecta										
Mecoptera	1.4	-	3.6	-	2.5	1	-	-	3.6	2.3
Hymenoptera	0	-	-	6.7	3.4	2.1	-	-	-	2.1
Coleoptera										
<i>Phaleria</i> sp.	2.8	-	3.6	-	3.2	1.1	1.9	-	3.6	2.2
Total	4.2	0	7.2	6.7	4.5	4.2	1.9	0	7.2	3.3

General discussion

This last section of the thesis synthesises and discusses its contribution to increase the baseline knowledge on the use of macrobenthic communities to assess the ecological status and functioning of exposed sandy beaches on the Portuguese Atlantic coast, and adds some suggestions for future research challenges.

The assessment of sandy beaches' ecological condition

As it has been highlighted throughout this thesis, sandy beaches are valuable ecosystems, from any perspective. In fact, sandy beaches support a unique collection of species that coupled with the key ecological processes they are involved, and together with other coastal ecosystems, can be vital to meet the basic needs for human survival in coastal areas worldwide (Millennium Ecosystem Assessment, 2005). Consequently, there is a compelling need for scientifically sound management if these systems are to sustain their ecological structure, functions and services to humankind (Schlacher *et al.*, 2008b; McLachlan and Defeo, 2013). However, these ecosystems are particularly vulnerable to coastal squeeze of burgeoning coastal human populations and the multiple predicted pressures of climate change (Brown and McLachlan, 2002; Defeo *et al.*, 2009; Dugan *et al.*, 2010, among others). Therefore, it is important to focus research resources to investigate compelling scientific needs.

In seeking to identify priority questions, ecologists (*e.g.*, Schlacher *et al.*, 2008b; Defeo *et al.*, 2009) have recognised that, in order to maintain the ecological processes and biodiversity on sandy beaches, it is required: (1) a deep knowledge of the biota and their adaptations to the physical environment, (2) the evaluation of the ecological status and potential stressors acting on the system, and (3) to propose systematic management and conservation plans for sandy beaches as ecosystems.

In the present thesis, the first and second requirements were addressed and an overall picture of the ecological status of mesotidal exposed sandy beaches in the Portuguese Atlantic coast was given, adding information about the adaptations of three previously identified key species, the talitrid amphipods *Talitrus saltator* and *Talorchestia brito* and the tylid isopod *Tylos europaeus* (see for instance Gonçalves *et al.* 2009; 2013).

When macroinvertebrates are used to identify possible drivers of change on sandy beach ecosystems, previous information on what we may expect to find under natural undisturbed (or less disturbed) conditions is required (Salas *et al.*, 2006). Still, the classification of ecological conditions of benthic communities is indeed a crucial step in assessment procedures. Gonçalves *et al.* (2013) proposed the bioecology of talitrids and tylics on the Portuguese Atlantic coast as a potential monitoring and assessment tool to evaluate the effects of environmental disturbances, namely anthropogenic.

In this study (Chapter I), the potential effect of increased human pressures on macrofaunal assemblages were assessed taking advantage of the baseline knowledge provided by Gonçalves and Marques (2011) and Gonçalves *et al.* (2009; 2013) on the same beaches in 1999–2000, and comparing those results with a recent sampling programme undertaken in 2010–2011. However, the effect of increased human pressures here was only considered potential because our estimates relied only on the quantification of increased number of tourists in the urban area (Cabedelo), and on a comparison with a reference beach in a near rural coastal area (Quiaios). The evaluation of the macrofaunal assemblages' structure and composition between these two periods (1999–2000 and 2010–2011) revealed a shift in the community composition in the Cabedelo beach, where the two key species (*Talitrus saltator* and *Tylos europaeus*) were found in a significantly lower number, while in Quiaios similar biological components between the two studied periods were recorded. Since potentially increased human pressures were only detected at Cabedelo beach, the decreases observed in the abundance of these species are likely attributed to these human-induced changes that can potentially be translated into ecological negative impacts on this beach. However, the estimation of this effect is a complicated issue. Even if a relation between the increased human pressures between the two periods, namely increased tourism intensity and the decline in the key species' abundance is shown here, the question as to whether these changes are natural or human-induced still remains open.

The assessment of human impacts on coastal areas requires a separate quantification of the natural and anthropogenic sources of environmental variability (Peterson and Bishop, 2005). This statement is challenging in sandy beaches because they are naturally dynamic and highly variable, with natural variability occurring in different spatial (gradients) and temporal scales (regular and/or pulse events), which reveals that this is a topic demanding further research.

In the present study, the difficulty to completely distinguish human activities from natural changes, the use of only one reference beach and the restricted temporal scale analysed (two periods with an interval of 10 years) hindered the establishment of a complete assessment of human impacts. Based on this study, it was highlighted the need to improve experimental designs to better distinguish natural variability from anthropogenic impacts and perform long-term programmes on sandy beaches in order to obtain a scientifically sound, adequate, and reliable ecological impact assessment (Defeo *et al.*, 2009) Despite the difficulty to accomplish these tasks, the ideal approach should include the identification and monitoring of previous conditions (before-impact), the use of multiple control sites around the impacted area, to minimise spatial confounding and enhance statistical power. In addition, the stressors should be identified, quantified and correlated with the responses of the biotic components, and a posterior analysis of possible recovery time evaluated, the so-called "BACI" (Before-After, Control-Impact) and "beyond-BACI" (multiple controls) approaches (Underwood, 1992; 1994).

Ecological niche of peracarid crustaceans

Understanding the manner in which various factors influence species interactions in food webs is a central goal of current ecological research. In particular, the mechanisms connecting ecosystems' boundaries constitute ecological complex networks, and for exposed sandy beaches, are poorly understood. Food webs on sandy beaches are potentially subsidised by the flux of organic matter across these system boundaries (sea and land) (McLachlan and Brown, 2006). Such subsidies include seagrasses, macroalgae and carrion of marine origin (i.e. wrack) and dune plant detritus from the terrestrial adjacent system and are known to be instrumental on sandy beaches (and their inhabitants) where *in situ* productivity is low (Dugan *et al.*, 2003, McLachlan and Brown, 2006). Some authors have illustrated the strict relationship between sandy beach consumers and these food supplies (e.g., Olabarria *et al.*, 2009; Bergamino *et al.*, 2011; Porri *et al.*, 2011), however, little is known about the niche segregation strategies of sandy beach primary consumers when dealing with contrasting food availabilities or even the consequences of a temporal or permanent lack of a given food resource to the entire food web. Despite the key role of macrobenthic communities in the trophic web of sandy beaches and the underlying ecological processes (organic matter processing) in which they are involved (Dugan *et al.*, 2003; Lastra *et al.*, 2008), it is important to understand their foraging strategies when dealing with environmental fluctuations and the consequences for the entire trophic web. In this study (Chapter II), niche segregation strategies of three sympatric peracarid crustaceans when facing scenarios of contrasting food availabilities were determined in the same beaches studied before. Results revealed that crustaceans had distinct diets and related niche segregation strategies, directly related with the temporal availability of food supplies and the environmental conditions at each beach. Knowledge of the feeding behaviour and related foraging strategies of these key species is an important issue to evaluate in a potential changing scenario, when food supplies may be reduced or suppressed by the changes occurring in the surrounding environments. Further research is necessary to evaluate the food limitation hypothesis on sandy beaches. Coastal pollution, beach grooming and dune disturbances are examples that can seriously contribute to the change of the available nutrient pools and compromise the active key vector that these species represent for the trophic web of these boundaries systems, jeopardising the ecological functioning of the entire beach ecosystem. Evaluating the impacts of these disturbances on the entire food web of exposed sandy beaches and adjacent ecosystems should be an important challenge for sandy beach ecologists.

Behavioural adaptations of talitrids and their potential role as bioindicators

Sandhoppers, e.g., the amphipod talitrids *Talitrus saltator* and *Talorchestia brito*, are widespread and common inhabitants of sandy beaches in temperate regions, as in the Portuguese coast (e.g., Marques *et al.*, 2003, Scapini, 2006; Lastra *et al.*, 2010). A rich literature is available on the biology (e.g., Marques *et al.*, 2003; Gonçalves *et al.*, 2003), and behavioural adaptations (review in Scapini, 2006) of these species over a range of sandy beaches. However, the majority of the reports on the behavioural level are from Mediterranean sandy beaches (review in Scapini, 2006, but see Gambineri *et al.*, 2008; Rossano *et al.*, 2009). Despite the well-known biology of these species in the Portuguese Atlantic coast (Marques *et al.*, 2003; Gonçalves *et al.*, 2003; 2009; 2013) there were to date no information about the behavioural adaptations of talitrids on these mesotidal beaches.

In this study, behavioural adaptations (sun orientation) of sandhoppers were studied on two different ecological scenarios and revealed in general a good sun orientation performance for the populations analysed (Chapter III).

In the relatively undisturbed beach of Quiaios, the two sympatric talitrids *T. saltator* and *T. brito* showed different behavioural strategies to face changes on this high dynamic mesotidal beach environment, leading to the main conclusion that sandhoppers do not behave all in the same way. Herein, *T. saltator* showed a more flexible behavioural response than *T. brito*, which was confined to the intertidal environment, supporting the hypotheses of a higher level of terrestrialsation of the former species and higher resistance capacity when dealing with possibly stressful conditions. In this sense, sandhoppers are able to exhibit distinct behavioural adaptations according to geography, habitat or local changes (physical disturbances) in the same environment. The behavioural adaptations (orientation) of sandhoppers studied in this beach can be also used as reference knowledge when dealing with environmental disturbances, since behavioural features have been proposed as an early warning potential indicator of beach change (Scapini and Ottaviano, 2010).

In the second case study encompassed in the Chapter III, the question addressed was if the talitrid *T. saltator* used the new artificial foredune constructed on the Leirosa beach as visual cue to help sun orientation, in order to find a safe zone on the beach. The behavioural features of *T. saltator* revealed a good integration of the new landscape into the orientation performance and a well-defined free-running rhythm for sandhoppers in this beach. This fact revealed that these animals could readapt to a physical reconfiguration of sandy beaches. In this sense, behavioural studies of talitrids can be a useful tool not only to assess disturbances acting on beaches, but also to understand the resilience capacity of these species to deal with both human and natural changes which are predicted to increase in these ecosystems (Brown and McLachlan, 2002; Dugan *et al.*, 2010).

It is however interesting to notice that, although this species exhibited a stable behaviour in the zone of the “new” landscape provided at Leirosa beach, the number of

individuals in the surrounding artificial dune site were significantly lower when compared to a natural dune zone in the same beach. This pattern was consistent throughout the year studied and was highlighted in the supralittoral zone of the beach where the textiles are placed (Chapter IV). The reduced number of *T. saltator* recorded in the artificially re-constructed zone revealed less viability to live and burrow in this zone when compared with a natural dune site in the same beach. Despite the good orientation performance of *T. saltator* recorded in front of the artificial dune, the first signs of textile degradation visible in this zone may indicate that talitrids may avoid this zone. Based on this study, the sandhopper *T. saltator* was suggested as a reliable bioindicator of anthropogenic impacts on coastal dunes-beach ecosystems.

In conclusion, regarding the results achieved in this study, the use of macrobenthic communities was a valuable tool to evaluate the ecological conditions of three sandy beaches from the Portuguese Atlantic coast subjected to different environmental conditions. In addition, the deep knowledge of particular adaptations (foraging strategies and behavioural adaptations) of key species on different ecological scenarios might be regarded as valuable information about the ecology of these key species and can be used as baseline information in future ecological integrity assessments of sandy beaches. Additionally, the studies performed in this thesis as a whole provide also important clues for the use of each studied species (or congeneric species) as potential bioindicators or biomonitoring tools in exposed sandy beaches. However, a deep background about their individual adaptations, tolerances and ecological strategies is needed to accurately depict major trends regarding their ecological relevance. The use of bioindicators, however, is not just restricted to single species, but should consider the entire community of beach inhabitants, which encompassing a broad range of environmental tolerances, can act as bioindicators and represent multiple sources of data to assess environmental conditions in a multimetric approach (Salas *et al.*, 2006).

Suggestions for future research

Beach conservation: a way forward

The limits of our scientific understanding on how sandy beach ecosystems respond to human threats are fast emerging as crucial impediments for the conservation of these ecosystems (Schlacher *et al.*, 2006; 2007). Sandy beach conservation, however, encompasses more than just “*saving the animals on the beach*”. Rather, it requires a holistic view that considers the three pillars of sustainable development: economic, social and environmental systems (Krelling *et al.*, 2008). As a consequence, there is an urgent need to make appropriate and scientifically founded decisions on how to manage our coastal areas. However, as pointed out before, there is still a lot of work to do.

Based on the findings achieved during the studies performed in the present thesis, some suggestions for future research for the Portuguese coast are given, which can be extended to other similar coastal contexts:

- Promoting long-term studies to offer a deep knowledge about patterns and processes on sandy beaches and their related biota, which can be used for reference conditions or/and to ecological modelling;
- Use of multiple ecosystem components (biological – macroinvertebrates, phytoplankton, shorebirds, and fishes – and physical-chemical variables) to assess the ecological status of sandy beaches.
- Developing appropriate ecological impact assessments, caused by human-induced disturbances (*e.g.*, beach nourishment, breakwater, seawalls) and climate change effects (*e.g.*, sea level rise, increased storminess) on beach biota;
- Conducting social studies in order to value sandy beaches as ecosystems (ecological goods and services) and setting conservation targets for these ecosystems.

The above are a few suggestions for beach research that would inform management authorities. Moreover, liaison with beach managers would enhance the effectiveness of much research on coastal areas.

In the future, comprehensive coastal management strategies must include an evaluation of the ecological state of sandy beaches and use this information to direct management and conservation decisions. In addition, when official authorities formally include sandy beaches in coastal and marine conservation planning initiatives at a national or international level, this will in turn contribute to elevate the currently poor profile beaches hold as ecosystems (Dugan *et al.*, 2010).

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