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Assessment of various etho-ecological indicators for the monitoring of human impacts on Tunisian, Italian and Portuguese coastal areas

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SUMMARY

The aim of this thesis was to assess the suitability of various etho-ecological indicators for the monitoring of impacts on coastal environments, which are worldwide subject to increasing threats, due to the direct and indirect effects of human activities. Two kinds of ecosystems where considered from Mediterranean and eastern Atlantic shores, shallow coastal lagoons and sandy beaches, which are of primary relevance both for the numerous human activities they support and for the valuable ecological services they provide. According to the European Water Framework Directive (WFD, 2000/60/EC) the ecological assessment of aquatic environments has to focus on biotic communities, which are considered high-profile indicators (by integrating environmental changes over the time), while the chemico-physical variables were downgraded to secondary indicators (giving a snapshot of a specific moment).

In coastal lagoons, classed as transitional water bodies, the ecological assessment based on bioindicators is often challenging to achieve; this is due to the high variability of their living communities, adapted to a changing diversified environment, which does not allow to easily distinguish natural from human-induced environmental changes. A specific Ecological Evaluation Index (EEI) based on macrophyte community already exists for shallow coastal lagoons and we applied it on a Tunisian lagoon system, the Ghar El Melh lagoon complex, in parallel with an optical water classification, which was assessed here for the first time in a coastal lagoon in the Mediterranean basin. Water optical features are among the main driving forces in these shallow water bodies, where light availability significantly affects biotic communities' features. The Ghar El Melh lagoon is suffering an increasing eutrophication, which was actually detected by the macrophyte-based index, indicating a moderate ecological status. The same frame was depicted by the optical classification, which hence resulted to be suitable also in the Mediterranean region. The application of this method, based on water Apparent Optical Properties (AOPs) and laboratory measures of Optically Active Substances (OASs), highlighted different degrees of water turbidity and light absorption in the different lagoon sectors, thus also indicating the possible impact sources. The good agreement between the ecological and the optical classifications represents already a notable finding, but what is more noteworthy is the great amount of information that was obtained analysing the spectral features of AOPs, particularly water reflectance (R). Five kind of spectra, corresponding to five optical classes, were found, and the evaluation of their shapes permitted also to identify which OAss were more active in the

light attenuation process. Further studies are needed to validate this method as indicator of water quality for transitional water bodies, but these findings already indicate the spectral analysis of AOPs as an effective tool for the monitoring of coastal lagoon systems, to integrate the biotic characterization. The optical measures may be easily taken *in situ* by using a Portable Underwater Mini-Spectroradiometer (PUMS) and the spectral investigation permits to avoid water laboratory analyses, which are often expensive and time-consuming. Based on our results, the parallel assessment of macrophyte-coverage and spectral optical properties might represent the best monitoring procedure for Mediterranean coastal lagoons.

About sandy beaches, the use of bioindicators to monitor their ecological status was further motivated by the unique adaptations of the community inhabiting these underestimated ecosystems. Previous studies on the key-species *Talitrus saltator* (Montagu, 1808) highlighted that these sandhoppers (widespread on the Mediterranean an eastern Atlantic sandy beaches and often dominant in terms of abundance) modify their behaviour to cope with rapid environmental changes, thus overcoming their physiological limitations to life in dry environments. In particular, being subject to a dehydration risk, sandhoppers evolved a nocturnal activity (allowing to avoid the hottest daily hours) and the ability to orientate towards the moist sand near the shoreline to burrow into (zonal recovery, based on both an innate solar compass and learned references). Their circadian activity rhythm may vary depending on the habitat features, becoming more defined in harsh conditions, and their solar orientation for zonal recovery may be influenced by the shoreline stability, resulting more scattered on beaches with a changing shoreline direction. On these bases, we assessed behavioural variations of *T. saltator* as bioindicators of impact on two beaches that had been subjected to anti-erosive interventions. Together with sandhoppers' behaviour, also the macrofaunal community was analysed (already considered a reliable bioindicator of ecosystem stability), to have a control of behaviour-based bioindicators. Two case study were considered, a microtidal beach on the Thyrrenian coast of Italy, where a hard-engineering intervention was carried out in 2010, and a mesotidal beach on the Atlantic shore of Portugal, which was subject to a soft-engineering dune rehabilitation about ten years ago. In both the cases the circadian activity rhythm resulted more linked to the climatic/tidal regimes than to the changes induced by the interventions, making this adaptation unsuitable as an impact bioindicator. Instead, the sun orientation gave interesting findings for the Italian case study, for which data were available from before the seawall construction both for *T. saltator* orientation and for macrofaunal diversity. Here three sites were considered, with different coastal dynamics, at increasing distances from the seawall; the 4000 site was under erosion, the 6000 one was accreting and the 5000 one was in a dynamic equilibrium. The comparison highlighted that the best sun orientation was no more displayed by the 5000 subpopulation, but by the 4000 one. Looking at macrofaunal diversity, an increase was observed at the 5000 and 6000 stations, and both the PERMANOVA and the MDS analyses on the community composition showed a separation of these stations from the sectors closer to the seawall. These results indicated that an accretion was engendered at the 5000 and 6000 stations, favouring the diversification of invertebrates by furnishing additional habitats; at the same time, the shoreline seems to have been stabilized at the 4000 site, due to the seawall effect. Both the phenomena were clearly detected by the orientation experiments, which hence were confirmed as a possible monitoring tool for short-term impacts on shoreline dynamics, considering that behavioural adaptations may represent the first responses to environmental variations. On the other hand, the orientation tests made on the Portuguese beach, where the intervention to stabilize the dune goes back ten years ago, did not show any differences between the impacted and the control site, located at about 500 m of distance. Also the community of invertebrates did not differ between the two sites, but a significant difference was found concerning the abundance of *T. saltator*, which is likely affected by the presence of geotextiles backing the beach, preventing the sandhoppers to burrow into the sand. Hence, though sandhoppers' behaviour has resulted not suitable for the long-term monitoring of effects due to engineering interventions on sandy beaches, *T. saltator* has been confirmed to be a keystone species, the abundance of which may detect impacts that macrofaunal analyses do not point out. Moreover, this was one of the first studies assessing the long-term impacts of soft-engineering interventions on sandy beaches; the results highlighted that, though less impacting than groynes, seawalls or dykes, also geotextiles may produce long-term environmental effects, which have to be monitored for a correct beach management.

The scale of pressures threatening coastal environments require global syntheses of research and interdisciplinary approaches, to design management strategies incorporating the conservation of their key ecological attributes. Several critical gaps in our current scientific knowledge prevent us from accurately measuring and predicting the impacts affecting coastal areas; in this thesis the task was to fill some of them, with the aim to be instrumental in the development of conservation and management strategies to maintain the ecological integrity of these irreplaceable ecosystems.

1. GENERAL INTRODUCTION

1.1 Importance of coastal areas and main impacts

The coastal zone is an interactive environment subjected to major human impacts. The coastal areas (the region ±200 m elevation from current sea level), representing just 20% of the Earth's surface, hold over half of the global human population, with 37% being within 100 km of the coastline (Dennison 2008). About 70% of the world's megacities (>1.6 million) are in this zone (LOICZ 2002), making human densities about three times higher than the global mean (Small and Nicholls 2003). Moreover, this proportion is increasing due to both population growth and migration to coastal regions (Crossland et al. 2005). Some estimates point that one third of the world's population will live within 100 km of the shoreline by 2020 (GESAMP 2001), others speak of three quarter within 60 kilometres of the sea (Povh 2000). The main reason making coastal areas highly attractive for human activities and settlements is their very high productivity, due to the presence of strong gradients, both vertical and horizontal, produced by the transitions between sediments, water and air, both on land and at sea; these result in dynamic chemical and physical processes(Gönenç and Wolfin 2005). The coastal zone is an area of major sediment and nutrient deposition from both terrestrial and marine (microalgae, macroalgae, coral) sources as well as a region of intense biogeochemical cycling. The transformations of major elements are particularly active in coastal zones, which makes them ecologically very important, as they provide a number of environmental goods and services (Dennison 2008). Their dynamic nature, resulting from the transfer of matter, energy and living organisms between land and sea systems, is driven by forces that include short-term weather, long-term climate, secular changes in sea level and tides (McLachlan and Brown 2006). Moreover, coastal areas frequently encompass critical terrestrial and aquatic habitats, like estuarine areas, coral reefs, coastal mangrove forests, coastal lagoons and other wetlands, tidal flats and seagrass beds, providing essential nursery and feeding areas for many species. It was estimated that over 75% of global fisheries, feeding about one billion people, are derived from the coastal zones (LOICZ 2002). In addition, these areas support large numbers of migratory and non-migratory waterfowl and shorebirds and endangered reptiles, such as turtles and alligators. In terms of global biological productivity, the coastal zones contribute fully one-quarter to the global productivity (LOICZ 2002).

On a global scale, the dominating issue in coastal zones is definitely the human population pressure. Human alterations to hydrology (e.g., dams in rivers and coastal structures such as wharves and marinas) to maintain water supply and resources to expanding populations have a cumulative effect at a global scale. Moreover, the global climate change, influencing sea level and large-scale weather patterns, has a large impact on coastal regions (Dennison 2008). Today about 10 million people experience coastal flooding each year due to storm surges and landfall typhoons and 50 million could be at risk by 2080 because of climate change and increasing population densities (Nicholls 2004). Two-thirds of the coastal disasters recorded each year are associated with extreme weather events that are likely to become more pervasive threats because of anthropogenically driven shifts in Earth’s climate and sea level rise (Aadger et al. 2005). More and more, adaptive responses will be required in coastal zones to cope with the hazards arising as a result of global environmental change (McClean and Tsyban 2001). But these are not the only threats affecting coastal areas: other factors of continuing degradation include habitat alteration and loss, eutrophication, altered sedimentation, altered salinity, invasive species, toxic pollution, aerosol contaminants, ocean acidification, emerging diseases, overfishing and overexploitation (Sherman and Duda 1999; NRC 2000; FAO 2003; Millenium Ecosystem Assessment 2005; Crain et al. 2009). Here two kinds of coastal ecosystems will be considered in more detail, the coastal lagoon and the sandy beaches, both having very specific features to be preserved and strong human pressures threatening them.

1.2 Coastal lagoons, features and main threats

Coastal lagoons are particular ecosystems, the importance of which has not always been well understood; their ecological functioning must be analyzed and evaluated in the context of a specific type of water body, with its own characteristics (Pérez-Ruzafa et al. 2010). Coastal lagoons occupy 13% of the world’s coastlines (Barnes 1980); traditionally, they are considered as transitional systems between continental and marine domains, defined as areas of salt or brackish water separated from the adjacent sea by a low-lying sand or shingle barrier (Barnes 1980). From a physical perspective, lagoon systems are characterised by the presence of boundaries and transitions between land and water, between the water column and the sediment layer and the atmosphere, between the lagoon and the sea and, frequently, between lagoon waters and freshwater inputs. Each boundary involves physical and ecological gradients (Gönenç and Wolfin 2005), which make these dynamic systems characterised by frequent environmental fluctuations (Barnes 1980; Kjerfve 1994; Zaldivar et al. 2008). Here external perturbations, like raised temperatures, differences in precipitation and freshwater inputs, also producing gradual changes, generally result in radical, sudden variations of biotic communities, after the overtaking of a threshold point (de Wit et al. 2001; Scheffer et al. 2001). Also, in most coastal lagoons, the mean depth is rarely higher than 2 m; due to this shallowness, lagoon bottoms are usually well irradiated, currents and hydrodynamics are closely conditioned by the bottom topography and the wind affects the entire water column, resuspending materials, nutrients and small organisms from the sediment surface layer (Pérez-Ruzafa et al. 2007). Small depths also make the primary production dominated not by phytoplankton, as in oceanic waters, but by benthonic macrophytes that here receive enough light to form the structural basis of the nutrient cycle (McGlathery et al. 2001, 2007). Moreover, coastal lagoons receive chemical contaminants via riverine input, atmospheric deposition, and direct or indirect discharge of agricultural, municipal and industrial wastes. Some of the wastes are transported to the sea, but the rest is degraded on place or stored in the sediments, which can act as sources or sinks for chemical contaminants, depending on external inputs, *in situ* processes in the water column and post-depositional alterations in sediments (De Backer et al. 2011; Chapman et al. 2013). Because of all these characteristics, these environments house a notably high biodiversity, provide habitat for many species and serve as nursery areas and feeding grounds for marine estuarine opportunistic fishes (Pérez-Ruzafa et al. 2010). The coastal lagoons also provide goods and services: they support important fisheries, are used for aquaculture exploitation, provide key tourist and recreational services (like nautical sports, swimming and health care) and also offer harbour and navigation facilities. At present, these ecosystems are considered a key factor in regional development plans (Pérez-Ruzafa et al. 2007, 2010) but, while acting as important engines for the economy, the close relation of coastal lagoons with terrestrial ecosystems make them especially vulnerable to human impact, generally in the form of terrestrial and freshwater inputs increasing the sedimentation rates and the risk of eutrophication and pollution (Gönenç and Wolfin 2005; Pérez-Ruzafa et al. 2007, 2010). Coastal lagoons are among the water bodies with a more developed human and historical dimension; this long-term anthropogenic influence already produced environmental degradation in most of the lagoon ecosystems, and the social awareness on the need to protect and manage them is increasing. As they receive continental freshwaters from their catchment area and have scarce exchanges with the sea (especially the chocked and restricted coastal lagoons, see Kjerfve 1986), in most of the cases a severe degradation of water quality has occurred in the form of pollution and/or eutrophication (Lacaze 1996; GESAMP 2001; Benrjeb and Romdhane 2002). This makes the definition of an effective monitoring procedure a priority in order to achieve the sustainable management of coastal lagoon systems.

1.3 Sandy beaches, peculiarities and main impacts

Sandy beaches make up two thirds of the world's ice-free coastlines (McLachlan and Brown 2006), which make them among the most important environments for human activities (Schlacher et al. 2008). The traditional view of sandy beaches largely underestimates their value as ecosystems, their biodiversity and the critical ecosystem services which they provide (Dugan et al. 2010; Schlacher et al. 2006). Actually, a single beach can harbour several hundred species of invertebrates (Armonies and Reise 2000). The porous sand body harbours interstitial organisms such as bacteria, protozoans and small metazoans, forming a distinct food web, while larger macrobenthic invertebrates burrow actively and include representatives of many phyla (mainly polychaete worms, clams, whelks and crustaceans), which can be scavengers, predators, filter- or deposit feeders (Defeo et al. 2009). These invertebrates can reach notable abundances (ca. 100,000 ind m^{-1}) and biomasses (>1000 g m^{-1}), thanks to their unique adaptations, including mobility, burrowing ability, protective exoskeletons, rhythmic (e.g. tidal, circadian, semilunar, lunar, seasonal) behaviour, orientation mechanisms and behavioural plasticity (Chelazzi and Vannini 1988; Scapini et al. 1995; Brown 1996; Soares 1999; Scapini 2006). Beach species include marine forms (below the drift line) and air-breathing forms (around and above the drift line), most of which cannot be observed in any other environments (Schlacher et al. 2008). Supralittoral zones are important nesting areas for turtles and shorebirds, while the fauna of the lower beach may extend its distribution seawards into the surf zone, thus making it an important nursery and foraging area for fishes that, together with birds, represent the top consumers in sandy beach food webs (Defeo et al. 2009). On sandy beaches the composition and abundance of invertebrate assemblages are controlled primarily by the physical environment, particularly sand, wave and tide regimes. These features permit to class beaches as dissipative beaches (accretional, wide and flat), reflective beaches (narrow and steep with an erosional nature) or intermediate between these two extremes (McLachlan and Brown 2006). Generally, more species can colonise dissipative beaches, while fewer, mainly robust crustaceans, can establish populations in the harsh conditions of reflective beaches (McLachlan et al. 2013). As well as supporting biodiversity, sandy beaches provide unique ecological services, many of which are essential to sustain human uses of coastal areas. Among these, there are the filtration of large volumes of seawater through the porous sands, processing organic materials and recycling nutrients back to coastal waters (Kotwicki et al. 2005; Schlacher et al. 2007; McLachlan et al. 2013), the functional links between terrestrial and marine environments, the sediment storage and transport, the wave dissipation and associated buffering against extreme events (storms, tsunamis), the dynamic response to sea-level rise (within limits), the water storage in dune aquifers and groundwater discharge through beaches, the scenic vistas and recreational opportunities (Defeo et al. 2009) The ecological functions of beaches are often perceived as secondary to their economic value, which is alarming when considering the strong human presence on them, especially in the last two centuries (Nordstrom 2000). Globally, burgeoning population growth in coastal areas is placing escalating pressures on sandy beaches, which attract more tourists and recreational users than any other coastal ecosystem (Brown and McLachlan 2002; Maguire et al. 2011). The physical environment of beaches is being impacted both by immediate human activities and by the long-term effects of climate change (Nordstrom 2000; Defeo et al. 2009). A non-exhaustive directory of the main direct impacts includes recreational seashore activities (producing effects mainly as trampling, camping or car driving on the beach), related beach cleaning and grooming (often with heavy mechanical equipments), many kind of pollutions (solid waste, wastewaters and sewage, oil spills), overfishing, accidental introduction of invasive species, mining (sand for building, heavy minerals, diamonds, etc.), beach nourishments and coastal armouring against erosion (a review in Defeo et al. 2009). On this last point, a strict connection exists with the major issue of the long-term climate change effects; while unconstrained beaches are resilient, as they change shape and extend naturally in response to storms and variations in wave climate and currents, human modifications of the coastal zone has severely limited this flexibility (Nordstrom 2000), trapping beaches in a ‘coastal squeeze’ between the impacts of urbanization on the terrestrial side and manifestations of climate change at sea (Schlacher et al. 2007, Defeo et al. 2009, Doney et al. 2012). Approximately 50 m of beach retreat are expected for every 1 m rise of the sea level (Schlachler et al. 2007) and the worst scenario projects a sea-level rise of 95 cm by the year 2100 (IPCC

2007) which, together with the already observed increase of storm activity and/or intensity, has as a main result that coastlines are generally migrating inland. Even more significant than the loss of land are the indirect consequences, including damage to coastal infrastructure, salinization of wells, suboptimal functioning of the sewage system of coastal cities (with resulting health impacts), and loss of littoral ecosystems and loss of biotic resources (Slott et al. 2006). As eroding beaches become narrower, habitat disposability decreases, thus lowering the diversity and abundance of the biotic community (Dugan and Hubbard 2006; Dugan et al. 2008; Defeo et al. 2009). An effective management has become necessary in most of the cases, and worldwide coastal authorities have intervened using either 'hard' or 'soft' engineering solutions. The last interventions have often resulted in negative ecological effects, as the loss of biodiversity, productivity and critical habitats, as well as modifications of the subtidal zone, which is an important recruitment zone for many sandy beach animals (Dugan and Hubbard 2006; Peterson et al. 2006; Speybroeck et al. 2006). Hard engineering structures such as seawalls, groynes, piers, etc. have traditionally been used for the protection of developments within the coastal zone, but they are expensive and tend to promote erosion, possibly by modifying the wave and sediment processes (Phillips and Jones 2006; Dugan et al. 2011). They will generally change wave direction at the shore and interrupt or stop longshore and/or shore-normal sediment transport. Structures built on and at the back of the beach, particularly seawalls, will exacerbate beach erosion when exposed to waves and prevent the backing dune store of sand from being utilized during erosion events (McLachlan et al. 2013). Because of the problems associated with hard engineering, not least cost and maintenance, alternative soft engineering techniques working in conjunction with natural processes are increasingly being used, including beach nourishment, dune restoration, 'afforestation' or reforestation and approaches such as the construction of artificial reefs or seaweeds. These soft measures seek to protect the shore zones while recovering beach characteristics and till now have resulted to be long-lasting and ecologically sound options (Phillips and Jones 2006; Cisneros Linares 2013). However actually, the ecological effects of some recently developed methods are not still totally understood, which makes the monitoring of such interventions an important field of study in view of a correct management of sandy beaches all over the world.

1.4 Aims and objectives of the thesis

The need for an approach implementing the management of coastal ecosystems in support of resource development and sustainability around the globe has been recognized from a strategic perspective (Taylor and Groom 1989; Sherman and Duda 1999). A combination of weak governance, globalization of markets, fishing pressure and climate change has exacerbated unsustainable practices in many coastal areas; under such pressing conditions of change and uncertainty, adaptive management systems and effective governance need to be implemented (McLachlan et al. 2013).The United Nations Conference on Environment and Development

(UNCED) encouraged coastal Countries to establish programs for assessing and monitoring coastal ecosystems so as to enhance the ability of national and regional management organizations to develop and implement remedial programs for improving the quality of degraded ecosystems. In its declaration on oceans, UNCED recommended that coastal nations: (1) prevent, reduce, and control degradation of the marine environment; (2) develop the potential of marine living resources to meet human nutritional needs, as well as social and economic goals; and (3) promote the integrated management and sustainable development of coastal areas and the marine environment. In the European Union the Directive 2000/60/EC (European Community 2000), known as Water Framework Directive (WFD), established a framework for community action in water policy and management concerns, which applies to groundwater, inland surface waters, coastal and transitional waters. WFD assumes as an essential starting point the thorough understanding of aquatic ecosystems, through a targeted monitoring, based on one or more specific indicators. Aquatic (particularly coastal) systems are complex and there are many problems associated with monitoring their ecological quality, due to a great variability both on a temporal and geographical scale, making difficult any inference (Crossland et al. 2005). In coastal systems, the interactions between waves and currents, climate, geomorphological processes and fluxes of chemicals and nutrients from/to land, atmosphere and oceans are still imperfectly understood; hence, WFD does not mandate the use of a particular set of monitoring methods, as in some countries and ecosystems single stressors may predominate (e.g. organic pollution or eutrophication), while in other cases a complex mixture of stressors may affect water bodies (e.g. nutrient enrichment, hydromorphological degradation, toxic substances, overfishing). WFD aims at establishing an adequate monitoring programme based on various ‘quality elements’, including physico-chemical, hydro-morphological, biological and chemical parameters (Allan et al. 2006). The real novelty is that aquatic ecology has been put at the base of the management decision; biotic communities have become the primary focus to assess the status of aquatic ecosystems (Hering et al. 2010). The Directive thus has changed management objectives from merely pollution and/or erosion control to ensuring ecosystem integrity as a whole, and the response of the biota has been recognised as an integrative way to measure ecological quality (high-profile indicator), before changes in physical or chemical variables that are considered as secondary indicators (Ferreira et al. 2006). Thus, different assessment systems have evolved across Europe, reflecting the diversity of water bodies types and pressures. But, in the context of the Mediterranean basin, the monitoring and management efforts of the EU countries should be coordinated with the actions of the non-EU countries, countries, to undertake consistent supranational management decisions incorporating both the small (ecosystem) and the large (Mediterranean Sea) spatial scale. The importance of governance of water resources was already politically recognized in the Mediterranean area, but an acute water crisis has widely impeded the sustainable development for most of the arid and semiarid southern Countries of the region (AQUASTAT 2012; Hamdy and Choukr-Allah 2012). The Mediterranean Countries are also vulnerable to global climate change, as several studies pointed out that the region is expected to deal with more forest fires, loss of agricultural land and with more frequent and severe water shortages than any other region in the world (IPCC 2007; Senatore et al. 2011; García-Ruiz et al. 2011). Hence, the management of water resources in the Mediterranean region is a challenge of primary relevance; governments, civil societies and private sector have to participate in and promote interaction and dialogue, starting from the monitoring procedures to adopt for the correct management of coastal ecosystems across the Mediterranean shores. In this work, the monitoring of two kind of coastal ecosystems has been considered, shallow lagoons (section 2) and sandy beaches (section 3) from Tunisian, Italian and Portuguese coasts.

Concerning the coastal lagoon ecosystems, on one side their high productivity makes difficult to distinguish between natural and cultural eutrophication (Nixon1995; Alongi 1998) and, on the other side, the variable water characteristics prevent to distinguish chemical stressor effects on biotic communities from natural variability (Veríssimo et al. 2013). This is the 'Estuarine Quality Paradox' described by Elliott and Quintino (2007), who noted that estuarine biota (and all the transitional ones, including coastal lagoons), being exposed to diversified and changing water quality conditions, have a relatively high degree of tolerance/adaptation. This poses an additional constraint in determining ecosystem status from biological responses and in assessing cause–effect relationships between contaminants and biological endpoints. For these reasons, a monitoring based on biological surveys in coastal lagoons needs to account for natural changes over time and space, which makes reference comparisons typically not realistically possible (Barbone et al. 2012). The recognition of the uncertainty due to multiple confounding factors in transitional waters (Chapman et al. 2013) make preferable the measurement of multiple variables, using different methodologies; the integration of different measures to provide the best information for decision-making is essential, given the natural complexity of these ecosystems (Chapman et al 2013). Hence, it is not easy to identify the best monitoring procedure for shallow coastal lagoons. Some methods developed in Europe were considered too complex (Moss et al. 2003); Peeters et al. (2009) proved that water transparency was enough for determining the trophic status of lakes, but only a restricted set of very shallow, lowland lakes were analysed. On the other side, Reinart et al. (2003) found a not-perfect overlapping between optical and trophic properties of coastal waters and lakes in Estonia and south Finland, particularly concerning the most turbid waters. Of course, water transparency (or its counterpart, water turbidity) represents a key-feature in shallow coastal lagoons, but it cannot encompass all the range of impacts and their biotic effects. For these reasons, several efforts have still to be made to find an adequate monitoring procedure, suitable for a wild range of lagoon environments. In this work, a procedure was assessed where a biotic index, derived from EEI by Orfanidis et al. (2001), based on benthic macrophytes, was coupled with an optical classification method (Reinart et al. 2003), considering water optical properties and contents of optically active substances. The method and the results are discussed in two papers, the first one already published (section 2.2), the second currently in preparation (section 2.3).

About sandy beach ecosystems, coastal biologists are now recognising their high ecological significance (Schlacher et al. 2006, 2007), but beach management still often focuses only on physical attributes and processes, particularly those related to managing sand budgets and the stability of the shoreline (Schlacher 2008). In contrast, impacts on ecosystems are rarely included in beach routine management (McLachlan et al. 2013). One of the reasons is that, in many situations, there are critical gaps in basic ecological information required for conservation planning on beaches, and coastal managers are not enough engaged with ecologists (Schlacher et al. 2008). In the long term, robust and efficacious management interventions must be founded in a more comprehensive ecological understanding of beaches. Pressure disturbances are increasing and many of them produced impacts at the scale of tens to hundreds of kilometres, which can last from months to years (Defeo et al. 2009. Their cumulative effects may extend to regional and global scales for developed and urbanised coasts, intensively used for recreation (Cooper and McKenna 2008; Defeo al. 2009). However, long-term data sets describing either the natural dynamics of beach systems or the magnitude of human impacts on them are scarce and fragmentary. Thus, the research priority is to implement long-term monitoring programs that quantify the dynamics of key ecological attributes of sandy beaches at different levels, from individuals to ecosystems, as well as to compile regional and global databases of empirical measurements of ecosystem condition. Multiyear, large-scale data sets might help to assess the extent of range contractions or expansions and even the potential extinction of some species and to detect regime shifts and their corresponding drivers (Carpenter 2008; Schlacher et al. 2008; Defeo et al. 2009). The latter have been increasingly documented for some terrestrial, freshwater and marine systems but not for sandy beaches yet (de Young et al. 2008). Another pressing issue is to better distinguish between natural variability and human impacts (Peterson and Bishop 2005). It can be extremely difficult to separate the individual effects of different impact sources, or to find unaffected beaches that could act as truly independent controls in experiments. Because good control sites are very difficult to find, distinguishing natural variability from anthropogenic impacts can only be attempted by modelling gradients away from the impact sources, as shown by long-term and large-scale field studies (Defeo et al. 2009). As already said (section 1.3), one of the most immediate and severe threats for sandy beachesis the accelerated erosion rate (Slott et al. 2006), but often the interventions which were realized to counteract it had negative ecological consequences (Schlacher et al. 2008). Adaptive responses to erosion should instead incorporate ecologically sustainable environmental outcomes, which will require close cooperation among scientists, managers and policy makers at local, regional, national and international levels, starting from the methods used in the monitoring phase. In this study, we analysed further the possibility of using a behavioural bioindicator that had been already proposed to monitor the impacts of interventions affecting shoreline stability (Scapini et al. 2005, Fanini et al. 2009; Ketmaier et al. 2010). Hence a first published paper is presented (section 3.1.1) that summarizes the framework in which this research was developed. Here the motivations are described why the behavioural adaptations of the sandhopper *Talitrus saltator* may be proposed as indicators of impact in the temperate coastal areas. Then, a first case study was considered, of a microtidal sandy beach in Italy (Collelungo beach, Maremma Natural Park), where a gradient from erosion to accretion is observable from one extremity to the other of the 6 km extended beach. In section 3.2.1 we analysed the circadian rhythms of locomotor activity of three sub-populations of sandhoppers subject to different morphodynamics (paper in preparation), to propose rhythm variation as a possible indicator of shoreline stability. We also analysed, along the same beach, the sun orientation of sandhoppers. In section 3.2.2 a paper (under review) considered the seasonal variations of this behaviour in an ecological perspective, focusing on the role of behavioural adaptations in harsh environments like sandy beaches. In the paper of section 3.2.3, which is still in preparation, the possibility of using sun orientation of *T. saltator* as an early impact indicator was analysed, by comparing data obtained on this beach before and after the construction of a seawall against erosion. Close to the sandhoppers' behaviour, also macrofaunal diversity was compared, as this was recognized as a suitable bioindicator of beach ecosystem stability (Mattoni et al. 2000; Longcore 2003), thus furnishing a medium-long term control. Finally, a second case study was considered, a mesotidal beach on the Portuguese Atlantic coast (Leirosa beach), where a soft engineering intervention with geotextile tubes had been carried out, more than four years ago, to recover an eroded dune-system. In the first published paper (section 3.3.1) both circadian rhythmicity and sun orientation of sandhoppers were compared between a site close to the impact and a site further from it, focussing on the effectiveness of the recovery intervention and on the adaptive meaning of the observed behaviours. In the second published paper (section 3.3.2) both sandhoppers' orientation and macrofaunal diversity were compared at the two sites (impacted vs. not impacted), to check the suitability of sun orientation as a bioindicator also for long-term effects on beach stability.

2. OPTICAL CHARACTERIZATION OF MEDITERRANEAN COASTAL WATER BODIES

2.1 Introduction. The Ghar El Melh coastal lagoon

The research on lagoon ecological processes in the Mediterranean has suffered its ups and downs in the last few decades. After beginning in the 1970–1980s (see for example, Lasserre and Postma, 1982; Kapetsky and Lasserre 1984; UNESCO 1986; Carrada et al. 1988), it is only in the last 10 years that the number of publications appearing in high impact journals has increased significantly, with more than 50 Mediterranean lagoons for which some data have been published in the recent scientific literature (Pérez-Ruzafa et al. 2011). The Mediterranean coastal zone is one of the world's most densely populated areas (Papayannis and Salathé 1999), with over 130 million inhabitants concentrated in more than 50 coastal cities with populations above 100,000. In 1921 23% of Tunisian total population were classified as urban. By 2004 this had increased to 64% of the total population (INS 2005), while more than 5 million tourists visit the country each year (INS 2003; Guillaume and Comeau 2005), the majority being accommodated in coastal resorts (Ghali 2002). Similar pressures are escalating through all the south Mediterranean countries, where the population doubled in the last three decades of the twentieth century and is expected to increase by a further 96 million by 2025 (Guillaume and Comeau 2005). This rapid growth engendered a detrimental impact also on the structure of lagoons' specific biological assemblages and dynamics: some of the changes have been induced by coastal work for tourism facilities, while others relate to changes in agricultural practices in the watershed, increasing agricultural wastes and nutrient input into lagoons (Pérez-Ruzafa et al. 2011).The degradation of water quality overlaps, in Tunisia and in the other southern Mediterranean countries, with water scarcity issues (AQUASTAT 2012). Here the renewable water resources are less than 1000 m^3 water/inhabitant/year (Hamdy and Lacirignola 2005), irregularly distributed in time and space, while 76% of precipitations and 85 % of renewable resources of the whole basin are concentrated in the northern Mediterranean countries including Turkey. Due to the described changes, the demand for freshwater is rapidly growing (Thomson et al. 2009) and this fact, together with the expected effects of global climate change ((IPCC 2007; Senatore et al. 2011; García-Ruiz et al. 2011), make Tunisian shallow lagoons among the most sensitive areas to environmental stresses (Romdhane 2001; Turki and Hamza 2001).Understanding ecosystems' functioning through monitoring and modelling is an important prerequisite for the development of common Mediterranean management policy for coastal lagoons (Thompson et al 2009).

The 34 km^2 Ghar El Melh lagoon is located on the north-western side of the Gulf of Tunis (37°06'-37°10'N, 10°08'-10°15'E), between Ras Tarf (Cape Farina), and the estuary of Majerda River. Maximum depth in the lagoon is 3.8 m, with an average of 0.8 m; the bottom is relatively flat and muddy (Rasmussen et al. 2009). A 10–70 m wide channel (1.5 - 2.5 m deep), named El Boughaz, connects the water body to the sea. The water body consists of a complex of three wetlands: the main lagoon or El

Bhira (26.7 km^2), Sebkhet El Ouafi (5.2 km^2) in the southeast and Sebkhet Sidi Ali El Mekki (3.7 km^2) in the northeast. These two minor basins are still shallower (0.5 m). The Jbel Nadhour (maximum altitude 325 m), a hill with east-west orientation scored by several streams (ruz), delimits the northern boundary of the lagoon whilst the Utica floodplain lies inland to the west, almost all constituted by marshlands and swamps. A ridge created as a levee of the Mejerda River defines the southern margin of the lagoon. The small town of Ghar El Melh on the northern shores accommodates a number of small fishing boats, concentrating their activities within the lagoon. Formerly, the Ghar El Melh lagoon was fed directly by Oued Majerda, which at the moment has its mouth displaced southward, while now the main supplies come from streams drained by the catchment area, extended on about 131 km^2, together with atmospheric precipitations falling down directly at the surface (Oueslati et al. 2010). The mean rainfall of about 540 mm/year is irregular, occurring mainly between September and April. At the same time there is a strong evaporation, increased by winds and high temperatures, for an average value of some 1450 mm/year. The mean temperature of the winter season is 11°C whereas in summer the average is of 27°C, with peaks over 30°C, reaching also 40°C when the southern wind blows from the desert. Mean water salinity is 36 and 51‰ in winter and summer, respectively, which is much more than Mediterranean water values, found between 36 and 39‰ (Moussa et al. 2005). Also nitrogen and phosphorous concentrations seem quite elevated, compared to oligotrophic waters of Mediterranean Sea, being the former between 60 and 1080 mg/m^3 (in winter and summer, repectively) and the latter between 30 and 960 mg/m^3 in the same seasons (Oueslati et al. 2010). A high spatial heterogeneity was described regarding the lagoon trophic status, with a worse water quality in western and southern sectors, far from the opening toward the sea, and a better situation nearer to the sandbar (Moussa et al. 2005). Data on macrophyte densities also indicated a reduction of sea influence and a worsening of water quality in the last years (Romdhane 1985, Shili et al. 2002). The lagoon suffers of water contamination and circulation problems associated with human activities: industry, agriculture and population increases have all impacted the water body, especially an excess of nutrients from sewage effluent (Ayache et al 2009). In particular, drainage water derives from intensively cultivated land, and some industrial development within the Utica floodplain also ultimately drains to the lagoon. Pollution impacts have been noted (Beyrem 2002, Flower et al. 2009; Ayache et al. 2009), mainly due to the discharge of raw sewage from human settlements. Romdhane (1985) reported that in the mid-1980s 100 m^3/day of sewage was discharged directly into the lagoon from the Ghar El Melh town, which has probably increased as the population has grown (Chouba et al. 2007).Waste from Aousja, a town to the northwest with a municipal population of nearly 4000 in 2004, is also discharged into the northwest corner of the lagoon during periods of heavy rainfall. In the next two paragraphs a monitoring procedure is described and assessed, and in the final section 2.4 the outcomes are discussed.

2.2 Optical characterisation of a coastal lagoons in Tunisia: ecological assessment to underpin conservation

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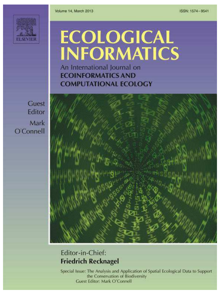

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Key words: Coastal lagoon; Optical Properties; Macrophytes; Ecological assessment; Monitoring procedure.

ABSTRACT

Ghar El Melh is a shallow lagoon (average depth of 0.8 m) that has undergone an eutrophication process due to growing human pressures. To obtain a global frame of the ecosystem functioning, an optical and an ecological classification were used in parallel. Downwelling and upwelling spectral irradiances were measured in situ in 22 sampling stations across the water body; then Apparent Optical Properties (AOPs), namely reflectance R(λ) and vertical attenuation coefficient $K_d(\lambda)$ were calculated for each wavelength of visible spectrum, furnishing typical spectra from turbid waters, rich in dissolved and suspended matter. From water samples of the same stations the concentrations of OASs (Optically Active Substances), i.e. Chromophoric Dissolved Organic Matter (CDOM), Non-Algal Particulate (NAP) and Phytoplankton, were assessed. The use of an optical classification for water bodies rich in TSM and CDOM, integrating AOPs and OASs, highlighted a great spatial heterogeneity, well overlapping with hydrology and human impacts patterns. A modified version of the Ecological Evaluation Index (EEI), considering the macrophytes distribution (based on a visual assessment of macrophyte coverage, without quantitative sampling) was then used, highlighting an intermediate ecological condition, despite high water turbidities. The integrated use of both systems thus furnished a complete characterization, rapidly detecting the most impacted sectors and the possible primary causes. The method might be applied as a monitoring procedure in other Mediterranean coastal lagoons, with the aim to adopt a common conservation strategy for these important transitional water bodies.

1. Introduction

Coastal lagoons have been exploited by humans because of their high productivity and biodiversity. In these areas, many ecotones occur (water/sediments, fresh/brackish/sea waters, atmospheric/water circulation, pelagic/benthonic communities), which, together with supplies from the catchment area and the sea, may cause the establishment of strong gradients. This results in a higher capacity for producing energy in comparison to marine environments (Gönenç & Wolfin, 2005). Coastal lagoons have been used by humans for settlements, fishing, aquaculture and agriculture, putting pressures on these ephemeral and dynamic systems. This is especially true for Mediterranean coastal lagoons because of their shallow waters and low volumes, which make them vulnerable to global climate changes more than inland lagoons. In the Mediterranean sea, temperatures are expected to rise from 0.2 to 0.6°C each decade (IPCC, 2007), engendering a rise of the sea level that seriously threatens the integrity of such transitional ecosystems (Eisenreich, 2005). In Tunisia, lagoon environments are important both from an ecological and economic point of view, covering an area of about 1,100 km2 from the northern to the southern coasts of the country. Currently, almost all these transitional water bodies undergo environmental degradation, due to pollution (domestic and industrial waste waters, organic and mineral nutrients rich waters from catchments exploited for farming, industries, etc.) and recent increases of sea-tourism activities. The latter result in the building of hotels, roads, ports, marinas, etc., almost never planning for the possible impacts on ecosystems. One of the main risks from these human stressors is the eutrophication of the Tunisian coastal lagoons, which is also the case for the Ghar El Melh lagoon, situated in Tunisia N-W. In 2008 an optical and ecological classification was carried out, to evaluate whether the existing methodologies for coastal waters investigation were suitable for transitional water bodies, and how these methodologies should be improved, with the main goal to provide a practical, repeatable monitoring procedure, useful for the management and conservation of Mediterranean coastal lagoons.

2. Materials and methods

2.1. The study area

The Ghar El Melh lagoon complex is located on the north-western side of the Gulf of Tunis (10°08’- 10°15’E; 37°06’- 37°10’N) between Tunis and Bizerte. Currently the lagoon complex has an about elliptical form, 7 km long and 4.5 km wide, with an average depth of 0.8 m, (0.2 m and 3.8 m minimum and maximum, respectively) (SCET_ERI, 2000). In the past, Ghar El Melh lagoon was fed by Oued Majerda, a river that currently has its mouth displaced southward. Now the main water supplies come from streams drained by the catchment area, together with atmospheric precipitations falling down directly on the surface.

The main threat to the stability of the ecosystems here is the sediment drift modification due to the construction of an harbour close to the opening channel, together with the issues linked to the water quality, caused by a poor regulation and management of recent touristic and agricultural developments. In this part of Tunisia a consistent rise of tourism is responsible for an augmented fresh water demand, mainly during the summer season, associated with increased sewage waters production, not subject to any kind of sanitization treatment before returning to the catchment area. Also, agricultural methods are being altered, and there is no regulation of the types and amount of pesticides, fertilizers and other organic and mineral substances used by local farmers to increase their crops.

2.2. Optical Properties and Classifications

In the paper we define irradiance (E) as the luminous radiant flux per surface unit (in W/m^2); $E = d\Phi/dS$. To calculate water optical properties, the main physical quantities to be measured are downwelling irradiance (E_d) on a plane horizontal surface, due to the radiation coming from the superior semi space, and upwelling irradiance (E_u), the same measure due to the radiant flux rising from the inferior semi space (Kirk, 1994). By taking measurements of upwelling and downwelling irradiance, in the entire spectrum of visible light at different depths, it is possible to calculate two important optical properties of waters:

Reflectance (R): the upwelling on downwelling irradiance ratio, for any specific wavelength, roughly indicating the backscattering/absorption ratio, calculated as follows:

$$R(\lambda) = E_u(\lambda)/E_d(\lambda) \tag{1}$$

Vertical attenuation coefficient for downwelling irradiance (K_d), which expresses the Ed variation with depth z; that for each considered wavelength can be calculated as:

$$K_d(\lambda) = -[\ln E_{d1}(\lambda) - \ln E_{d2}(\lambda)]/(z_2 - z_1) \tag{2}$$

Such characteristics are called the Apparent Optical Properties (AOPs) because they are not only properties of the radiant field, but also of the water body. They are closely associated with the so-called Inherent Optical Properties (IOPs) of water, allowing to use AOPs instead of IOPs, which are more difficult to estimate (Gordon et al, 1975; Gordon and Morel, 1983; Kirk, 1984). Therefore, if measured at high solar elevation, the spectral variations of R and K_d can be used to classify natural waters based on the different Optically Active Substances (OASs) that contribute to light attenuation. These include Chromophoric Dissolved Organic Matter (CDOM, also called “yellow substance”), Non-Algal Particulate (NAP, or non-phytoplanktonic fraction of TSM, or tripton) and phytoplankton. Previous studies have investigated methods to measure the optical properties of natural waters and create optical classifications. One of the first methods, based on K_d, was created by Jerlov (1976), which proposed a scale from 1 to 9 types for coastal waters. As this method was created mainly to analyse oceanic waters, normally poor in organic matter; it is not uncommon that coastal basins display attenuation values exceeding up to eight times those of the most turbid Jerlov’s class, type 9 (Reinart et al., 2003). Morel & Prieur (1977) elaborated an optical classification based on the reflectance spectra R(λ), separating the so called case 1 and case 2 waters. In the former case, phytoplankton is optically dominant (pelagic waters), whereas in the latter one the main role is played by NAP or CDOM (inland and coastal waters). This classification was re-examined by Prieur and Sathyendranath (1981) , who considered absorption spectra. Another optical classification applicable mainly to inland waters was proposed by Kirk (1980), distinguishing waters on their prevalent absorption components. With the aim to focus on case 2 waters and consider the high diversity existing among them, an alternative classification was proposed by Reinart et al. (2003), planned for lakes, but suitable for all coastal waters in small and shallow bays, influenced by river contributions and affected by sediments suspension, i.e. all kind of basins comparable to lakes. The method is based on K_d, R and Secchi depth, but also employs OASs concentrations; the criterion for including a particular type of water in a particular optical class is found by the K-means clustering technique. Waters belonging to class C (Clear) show a relatively small amount of OASs, are transparent, have the smallest K_d and their R is about 2%, with the optical properties determined mainly by phytoplankton pigments. In M (Moderate) waters the colour is modified mainly by CDOM. Class T waters are turbid but not highly eutrophic and have suspended particles (both organic and mineral) causing high scattering and high R values. Such water bodies are shallow and their suspended matter may contain a rather large amount of mineral particles from the bottom. The V (Very turbid) waters present a large amount of Chl_a (chlorophyll a, > 60 mg m^{-3}), generally during phytoplankton blooms. Type V is typical of shallow eutrophic water bodies, as already described by Kirk (1981). Class B (Brown) are brownish-water humic basins, having high levels of CDOM, in particular of humic acids; K_d, is also very high, while R is extremely low (less than 0.2%). We chose to use this method to obtain a fine spatial classification of the Ghar El Melh waters, dominated by dissolved and suspended matter.

2.3 Sampling Protocol

In April 2008, we sampled a set of 22 stations in the lagoon, chosen to cover a range of environmental conditions, in which measures of spectral E_d and E_u (between 400 and 730 nm) were taken just above the water surface, at 10 cm and 50 cm of depth (when this was allowed by the depth of the basin) or otherwise at the maximum possible depth. Irradiances were measured by means of a portable diode-array spectroradiometer (AvaSpec-2048, Avantes), to which a 50 μm fiber optic was connected, with a cosine collector (CC3-UV); the measured spectra were acquired and visualized through a laptop. Irradiance measures were taken near noon with calm waters and reduced cloudiness conditions to avoid strong fluctuations of the underwater radiant flux (Kirk, 1994). Secchi disk measures were not taken because of the limited basin depth and an indirect estimate was calculated from the measured K_d values: $Z_{Secchi} = 2/K_d$ (Shifrin, 1988). At the same time, for each station superficial water samples were collected to measure their main components in the laboratory. TSM (Total Suspended Matter, as a proxy of NAP) was determined according Strickland and Parsons (1972) modified by Van der Linde (1998) for the salt wash procedure. To determine CDOM absorption the protocol of Bricaud et al. (1981) was applied, using the interpolation method of Stedmon et al. (2000) and Twardowski et al. (2004); the concentration was finally obtained by the approximation of Nyquist (1979). Chl_a was extracted and analysed following the procedure of Lazzara et al. (2010). A visual survey was also carried out in the whole basin, by defining ecologically uniform, non-overlapping permanent-polygons (PPs), in which the percentage cover of benthic macrophytes (angiosperms, epiphytic algae and macroalgae) was assessed. A modified version of the Ecological Evaluation Index (EEI) by Orfanidis et al. (2001) was then applied, integrating these snapshot data collected during spring with those described by Rasmussen et al. (2009), referring to February 2003 and September 2004, with the aim to have a more complete characterisation of the ecosystem features. The index assumes that shifts in the quality of structure and functioning of transitional water bodies can be detected by separating benthic macrophytes into two Ecological State Groups (ESGs I, II) typical of a pristine or degraded ecological state, respectively (see Viaroli et al., 2008). In all the Permanent-Polygons, the percentage cover was visually evaluated to establish abundance (%) of each ESG, and the EEI of each PP was determined. Scores were then multiplied by the relative surface area of each PP and then summed, giving the final weighted EEI.

3. Results

3.1. Optical Properties

The main results on OASs concentrations are summarized in table 1. TSM (proportional to NAP contents) was high and its organic fraction was around 20%, making NAP more likely to produce scattering than absorption during the light attenuation process. Chl_a concentrations were low in the whole basin, indicating a reduced phytoplankton presence, whereas CDOM contents resulted relatively high, in the range of coastal and estuarine waters (Kirk, 1994) and of inland waters (Binding et al., 2008).

In table 1 are also shown some important summaries relative to the lagoon optical properties. The values of R at 10 cm from the surface (R_{10}) represent each station, due to the small bottom influence at this depth.

Table 1 Main summaries for Ghar El Melh waters in April 2008. For Optical Active Substances: Total Suspended Matter (TSM), Chlorophyll a (Chl_a) and Chromophoric Dissolved Organic Matter (CDOM). For Apparent Optical Properties: mean vertical attenuation coefficient in the visible domain (K_d), reflectance (R10) at 555 nm and blue/green reflectance ratio.

	Mean	Min.	Max.	St. Dev.	N
TSM (mg/l)	30.25	13.19	46.84	12.73	11
Chl_a (mg/m³)	1.69	0.41	6.43	1.50	17
CDOM (mg/l)	1.50	0.67	2.21	0.43	13
K_d *vis*(m^{-1})	2.81	1.23	5.29	1.30	13
R_{10} 555	8.8%	0.9%	19.1%	0.05	13
R_{10} ratio (443/555)	0.32	0.19	0.52	0.09	13

The values in the green band (R_{555}) were quite high (1% - 19%), and the blue/green reflectance ratio (443/555) was smaller than 1, indicating a strong absorption of the blue light (Ouillon and Petrenko, 2005). The analysis of reflectance spectra confirmed that the whole basin was constituted of case 2 waters, with a typical peak at about 570 nm (where absorption of CDOM, water and pigments is smaller) and a minimum at 670 nm, due to the chlorophyll a absorption peak. Analysing the K_d spectra, calculated between 10 and 50 cm of depth, a great variability of the light attenuation was observed (table 1). Based on the features described by Prieur and Sathyendranath (1981), two patterns were recognized: 1) in almost all stations CDOM strongly absorbed light, with a reduced contribute of phytoplankton, but 2) in few cases the scattering, ascribable mainly to particulate matter (Morel & Prieur, 1977; Gordon & Morel, 1983), prevailed on the absorption of photons. To apply the optical classification of Reinart et al. (2003), based both on AOPs and on OASs contents, once calculated the average R and K_d in the visible spectrum, we obtained Secchi depth for each sampling station, by the approximation of Shifrin (1988). It was surprising to discover that all the five optical classes were present in the lagoon, giving rise to a consistent spatial heterogeneity, not detected by classical optical methods. The spatial distribution of classes was consistent with the results from OASs analysis and with previous data on lagoon hydrodynamics (Moussa et al., 2005; Thompson et al., 2009). As shown in Fig. 1, waters were clear close to the channel and moderate in the northern sector, becoming very turbid in the central and southern areas. Also the Sidi Ali El Mekki waters were very turbid, even brown, due to the minimal depths and sediments suspension by wind.

3.2 Ecological Assessment

For the Ghar El Melh lagoon we also used

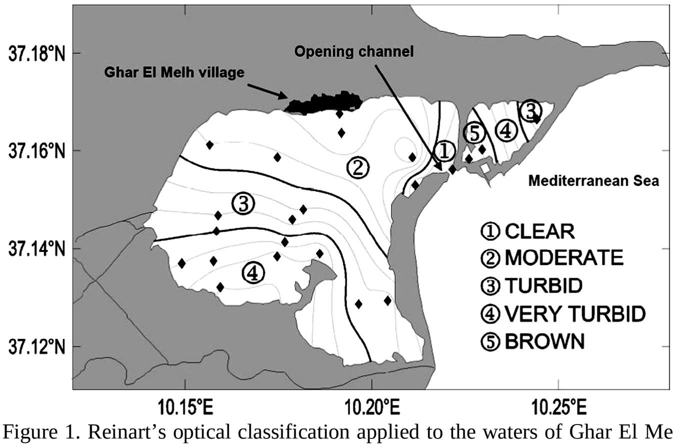

Figure 1. Reinart's optical classification applied to the waters of Ghar El Melh and Sidi Ali El Mekki lagoon system (north-west Tunisia) in April 2008.

data from Rasmussen et al. (2009), not considering the small Sidi Ali El Mekki lagoon, so the modified version of EEI was not applied to this part of the system. Based on the results from spatial analysis of OASs and AOPs, we divided the main basin into three PPs,:

1. The part near the opening in the sandbar and at N-E of it, with moderate turbidity and smaller contents of substances, which represents about the 10% of total surface;
2. The central and N-W sectors, more turbid, with higher contents of OASs and slower rates of exchange with the sea, covering approximately 60% of the basin;
3. The S-W area, which showed a higher turbidity and higher contents of OASs, due to the slow water turnover, including about 30% of the lagoon.

In the first sector we could observe, in agreement with the literature (Shili et al., 2002), a considerable cover of *Ruppia cirrhosa*, a perennial seagrass classed in ESG I; this species decreased toward the central part of the lagoon, where it was about equivalent to the covering of *Cladophora* algae, filamentous opportunistic species classed in ESG II. These latter species became highly dominant in the S-W zone, where *R. cirrhosa* almost disappeared. Therefore, in the first PP the EEI was 8 (good ecological status), in the second one it was 6 (moderate) ecological class, while in the third one we obtained a value of 2 (bad status). Hence the weighted EEI for the entire lagoon was 6.8, i.e. a good, almost moderate ecological condition.

4. Discussion and Conclusions

Although a single spring survey cannot offer an exhaustive frame of such a complex ecosystem as a coastal lagoon, the results indicated some interesting possibilities for future monitoring strategies.

First of all, it was stressed the utility of taking in situ light measures, easily performed by means of a portable diode-array spectrometer. AOPs analysis proved to be a valid tool for the rapid assessment of water contents of phytoplankton, CDOM and NAP, as the laboratory analyses of water samples confirmed.

Moreover, the use of the optical classification by Reinart el al. (2003) offered, in a rapid and repeatable way, a detailed picture of the actual conditions of the Ghar El Melh lagoon and the connected Sidi Ali El Mekki lagoon, revealing a considerable spatial heterogeneity, that resulted to be consistent with the human impacts acting on these ecosystems. Waters were classified as M in the North of the basin, where marine influence was still acting and the small fishermen’s village produced reduced impacts, quickly turning to V where the depths decreased, permitting to wind to raise sediments from the bottom (Moussa et al., 2005; Thompson et al., 2009), and where inputs from cultivated lands were stronger. Briefly, a relation was observed between the types of human activities and the achieved optical classification. Therefore water optical classification may represent a valid tool for conservation purposes, permitting a rapid identification of the sectors more affected by human impacts. Moreover, the graphical representation of the optical results over time, with special reference to seasonal trends, may permit to managers and other stakeholders to follow the basin evolution and to understand the effect of meteorological variables on its waters chemistry, furnishing useful indications for planning the best conservation strategy.

However, to assess the global ecological status of the lagoon, the need rose for a tool based on ecosystem biotic components, not considering OASs contents, thus permitting to integrate the optical characterization. A modified version of the EEI by Orfanidis et al. (2001) was applied, using just a visual survey of the macrophytes cover, that may be proposed for other ecosystems too, where the sampling of vegetation or fauna may be restricted. Being specific for shallow coastal lagoons, EEI avoids some common errors of classic limnological techniques, based on water chemical composition; here we found that, despite high contents of solved and suspended matter, the lagoon actually had a quite good ecological status, closer to mesotrophy than to the eutrophication that was supposed in the introduction of this paper. The lack of any linear relations between optical and trophic classification of coastal water bodies was highlighted: as already stressed by Arst and Reinart (2009), oligotrophic waters are usually clear, while eutrophic or dyseutrophic ones tend to be very turbid or brown, but turbidity is not necessarily an index of bad ecological quality, so it would be incorrect to infer one feature from the other one. The best practice should be, as we did, the parallel assessment of both the aspects. To conclude, we know that the main threats to the Ghar El Melh system equilibrium are two. First of all, a reduced contribution of marine water in most of the basin, which favours the accumulation of suspended and dissolved matter far from the channel; secondly, uncontrolled inputs of fertilizer compounds from agricultural lands at S-W, that could rapidly produce a worsening of the lagoon water quality, leading to the clearing of a threshold-value, after which a return could be impossible (de Wit et al., 2001; Muradian, 2001). Both the phenomena were clearly detected by the described optical classification, while their effects on the biotic component were highlighted using the ecological index. Thus, in this study a monitoring procedure is suggested, based on in situ light penetration and benthic macrophytes cover measures, which is highly informative, not invasive or destructive, easy and cost-effective to perform. Moreover, the proposed method can be easily understood by stakeholders, a necessary requirement for its correct and continuous application. We hope that this work will be of some utility not just for this specific case-study, but also for other transitional water bodies endangered by human pressures along Mediterranean coasts, having a great value, both economic and touristic, but first of all for environmental conservation.

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2.3 Characterisation of a Tunisian coastal lagoon through hyperspectral underwater irradiance.

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ABSTRACT

Mediterranean coastal lagoons are among the most biodiverse and threatened ecosystems; along the European coasts many of them are protected areas, while others underwent to various degradation processes as a consequence of human activities in the lagoons themselves or of water management in the inlets and outlets. On North African shores a degradation process is still starting, but is rapidly increasing, due to the recent touristic and agricultural development; monitoring procedures are thus required, to identify the main factors acting on the lagoon ecosystems' equilibrium and manage them properly. We developed an optical monitoring procedure that was applied in the Ghar El Melh lagoon (North Tunisia) recently subject to management changes, from fisheries (now displaced to the open sea) to leisure place and sink for agricultural wastes. In situ irradiance was measured, furnishing hyperspectral Apparent Optical Properties (AOPs) of each sampling station in the lagoon. Water samples were also taken, to assess by routine laboratory analyses the concentrations of Optically Active Substances (OASs). We applied an optical classification, not yet used in the Mediterranean environment, specific for shallow coastal water bodies rich in dissolved and suspended materials, obtaining a detailed characterization of the lagoon optical features. The results were consistent with findings from hydrological and ecological studies in the lagoon. The hyperspectral optical characterization appeared to be a useful monitoring tool for Mediterranean coastal lagoons, as it easily indicated the threatened sectors and possible impact sources. On the other side, the method should be adapted to local specificities in order to make it suitable for water and environmental managers. A more exhaustive research should be made, to validate the procedure also for other coastal water bodies around the Mediterranean, the sustainable use of which represents an increasing challenge for the society.

***Key words**: Mediterranean Coastal Lagoons, Shallow Water Bodies, Optically Active Substances, Hyperspectral Optical Properties, Monitoring Procedure.*

1. Introduction

Because of their importance for human activities and vulnerability, there is a worldwide increasing awareness of the need to protect and manage coastal lagoons in view of the sustainability of the water resource as well as of their unique ecosystems. These water bodies, due to their high productivity and biodiversity (Gönenç and Wolfin, 2005), have always been heavily exploited, but the rate of change has dramatically grown over recent decades, with a negative impact on their biological communities and ecosystem dynamics, particularly along the coasts of European, developed countries (Viaroli et al., 2005; de Wit et al., 2012). These changes relate mainly to coastal works for tourism facilities and infrastructures, or to changes in agricultural practices in the watershed, with increasing wastes and nutrients input into the lagoons, which in the Mediterranean region is aggravated by scarce and unpredictable water supply (Scapini and Ciampi, 2010). These threats may be aggravated by the issue of the sea level rise that was predicted for the next years (Eisenreich, 2005). Hence, a scientifically sound water and ecosystem management is required for coastal lagoons, including a continuous monitoring of their abiotic and biotic components, especially along the southern coasts of the Mediterranean Sea, where the human development is now rapidly increasing. However, to manage an ecosystem, it has to be deeply understood, which means to know its components, the way they are linked, the processes involved in their functioning and the cause–effect relationships between the human activities and the alteration of ecological processes. That is a prior step to construct bio-economic models and decision support tools for coastal lagoon management (Pérez-Ruzafa et al., 2011).

The optical characterization of natural waters has been widely recognized as a useful way to evaluate the main biological, chemical and physical features of water bodies. Particularly OASs, Optically Active Substances, interact with photons by absorption and scattering processes producing the solar light attenuation, i.e. modifying the radiation both in intensity and colour, in a specific manner depending on their composition and relative concentrations in the water body (Kirk, 1994). In natural water bodies, the main substances altering the radiant field are water itself, Chromophoric Dissolved Organic Matter (CDOM), Non-Algal Particulate (NAP) and phytoplankton (Prieur and Sathyendranath, 1981; Carder et al., 1991; Babin et al., 2003). CDOM, also said yellow substance or gilvin (Kirk, 1976), is the full amount of dissolved humic and fulvic compounds (mainly derived from the microbial decomposition of vegetal tissues), while NAP, the particulate material having a non-phytoplanktonic nature, includes mineral particles and organic suspended solids. Considering just the ‘visible light’ (i.e. the fraction of the electromagnetic radiation between 400 and 700 nm), water molecules mainly absorb the red, longest wavelengths (λ), while CDOM shows a stronger absorption for the shortest λ, in the violet and blue sector of the visible spectrum. NAP can act in two ways; when its composition is mainly inorganic, it typically scatters solar light at all the visible wavelengths (white scattering), whereas absorption dominates on scattering and its spectrum is analogous to CDOM, when it includes a large fraction of organic particulate substances (Kirk, 1994). Finally, phytoplankton cells contain several absorbing pigments (chlorophylls, carotenoids and biliproteins), the concentration and composition of which depend from environmental variables, such as nitrogen concentration and light availability, both as intensity and spectral distribution (Hoepffner and Sathyendranath, 1991). Phytoplankton cells also differ in size, shape and pigment packaging, so that a general absorption spectrum cannot be determined for natural populations (Bricaud et al., 1998; Babin et al., 2003); anyway, all phytoplankton species contain chlorophyll a (Chl_a), generating recognizable absorption features in vivo, with maxima at about 440 nm and at 678 nm. Absorption (a) and scattering (b), together with the global attenuation of light ($c = a + b$) are Inherent Optical Properties (IOPs) of natural waters, as they depend just on the substances in the water, not on the geometric structure of the light field (Kirk, 1994). As IOPs cannot be easily measured under natural conditions, the studies on natural water bodies are more frequently based on the so-called AOPs, Apparent Optical Properties (Gordon et al., 1975; Gordon and Morel, 1983; Kirk, 1984). The latter depend also from the luminous field spatial configuration, but their strict relation with IOPs and the easier measurement make them a good proxy of IOPs in most natural situations (Kirk, 1994). The measure of the radiant flux per surface (quanta m^{-2} sec^{-1}) on a horizontal plane, both from the superior semi-space (downwelling irradiance, E_d) and from the inferior one (upwelling irradiance, E_u), is the starting point to calculate sea water AOPs, its diffuse attenuation coefficient (K_d) for downwelling irradiance and its reflectance (R). Based on these properties, different optical classifications were elaborated for natural water bodies (Jerlov, 1976; Morel and Prieur, 1977; Kirk, 1980; Prieur and Sathyendranath, 1981; Baker and Smith, 1982; Vertucci and

Likens, 1989; Kaczmarek and Wozniak, 1995; Reinart et al., 2003), with the aim to quantify relationships among OASs in each class. According to the classification of Morel and Prieur (1977), still used in remote sensing studies, Case 1 represents the phytoplankton-dominated waters, typically oceanic and off-shores ones, and Case 2 represents all other possible water bodies, i.e. lakes, lagoons, transitional, estuarine and coastal waters. Here NAP and CDOM, carried by the rivers or re-suspended from the bottom, play an important role in attenuating the light, while their variable optical dominance complicates the simple relationships between OASs observed for the Case 1 waters. For these reasons, the optical classifications specific for Case 2 waters not only are less numerous with respect to Case 1, but were often optimized for local conditions (Eloranta, 1978; Tshekhin, 1987; Vertucci and Likens, 1989; Koenings and Edmundson, 1991; Kaczmarek and Wozniak, 1995). Reinart et al. (2003) proposed an optical classification for the Case 2 waters of Estonian and southern Finnish lakes, which the authors claim to be applicable to all coastal waters in shallow bays, influenced by river contributions and affected by sediments' re-suspension, i.e. all kind of water bodies optically comparable to lakes. This classification relies on AOPs and water parameters normally included in the routine monitoring (Secchi depth, concentration of Chl_a, CDOM and Total Suspended Matter, TSM, as a proxy of NAP).

We applied the classification of Reinart et al. (2003) on a coastal lagoon located on the north coast of Tunisia, the Ghar El Melh lagoon, subject to various impacts that are common and increasing in the Mediterranean region (Scapini and Ciampi, 2010). Yet, we chose to take hyperspectral optical measures; in this way, together with applying the Reinart et al. (2003) classification as a control, we focussed on the spectral features of optical data, more explicit and complementary with respect to laboratory analyses of OASs, to obtain a new, cheap and synthetic tool to for the assessment of natural waters quality. The spectral analysis of AOPs, particularly R, could represent a rapid monitoring tool for shallow coastal water bodies on the Mediterranean shores, useful for their management, complementing the use of optical remote sensing data for monitoring phytoplankton blooms, primary production, optical components and thus eutrophication (Ferreira et al., 2011, Novoa et al., 2012, Caroppo et al., 2013). In this paper the results of a single survey are described, to furnish a starting point for future, desirable applications in the management of Mediterranean coastal water bodies.

2. Methods

2.1. The study-site

The Ghar El Melh lagoon is a system made of three minor basins (main lagoon, El Ouafi Lagoon, Sidi ali El Mekki Lagoon, Fig. 1), located on the coast of the Gulf of Tunis (10°08'-10°15' E; 37°06'- 37°10' N), between Tunis and Bizerte. It has an average depth of 0.8 m (0.2 m and 3.8 m minimum and maximum) (Moussa et al., 2005). Until the 19th Century, the main water supplies were provided by the Majerda River (Paskoff, 1985), of which the mouth was then displaced southward; nowadays water comes mainly from the small streams of the catchment area and from the atmospheric precipitations falling down directly at the lagoon surface or running down from the hills located at the northern side of the lagoon. While the west and south shores are constituted by marshlands and swamps, at east a quite thick sandbar separates the lagoon from the sea, with one single channel joining the two water environments, 85 m wide and 2.5 m deep (Moussa et al., 2005). Hence, water exchanges with the sea are very scarce, resulting in long retention times. Furthermore, the shallowness of the lagoon produces an overlap between the superficial productive (trophogenous) layer and the beneath one (tropholitic layer); in other words, the bottom is almost all illuminated and the

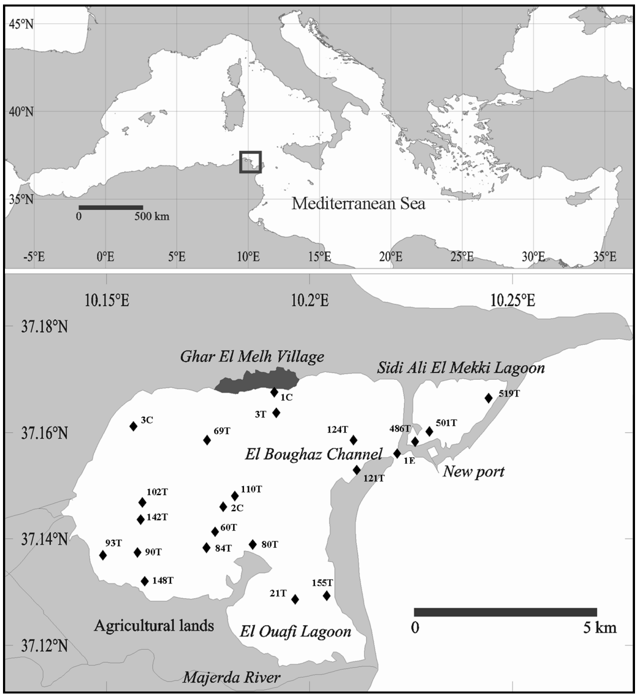

Fig. 1. The study-site. In the upper panel: geographical localization of the Ghar El Melh lagoon, North Tunisia (37°06’- 37°10’ N; 10°08’- 10°15’ E) in the Mediterranean Basin. In the lower panel: map of the Ghar El Melh lagoon, with the sampling stations (diamonds) and indication of the main interest points.

wind can mix the whole water column, causing a remarkable acceleration of the biogeochemical processes (Carrada et al., 1988). For the shallowness of the lagoon and its limited opening to the sea, the fishery activities in the lagoon have considerably decreased in the last years, after the construction of a new fishery port on the sea side. The lagoon is now included in a larger plan of coastal protection, for its ecological and landscape value (Scapini, 2010). Considering also the semi-arid climate, with few and unpredictable rainfalls concentrated in spring and autumn, the Ghar El Melh lagoon results to be particularly sensitive to changes in its delicate equilibrium. One of the threats to the ecosystem stability is the sediment drift modification, started in 1974 by the building of the New Port close to the lagoon opening to the sea (Moussa et al., 2005).

Moreover, in the last years touristic and agricultural development, still poorly regulated and managed, has generated several issues linked to the lagoon water quality. On one side, the intensification of tourism activities has led to an increase of freshwater demand (mainly during summer) and to a greater production of sewage waters that are not sanitized before returning to the catchment area. On the other side, also the diffusion of more productive agricultural methods, even more based on pesticides, fertilizers and other organic and mineral compounds, being not yet subject to a severe regulation, have started an enrichment of the lagoon waters, the extension of which is not yet well known.

2.2. Sampling Protocol

In April 2008, 22 stations (Fig. 1) were chosen along significant transects in the lagoon, to cover a large range of environmental conditions. In each station hyperspectral E_d and E_u (between 400 and 730 nm, with frequency of 1 nm) were measured just above the water surface, at 10 cm and at 50 cm of depth (when this was possible) or otherwise at the maximum possible depth. The measures were taken with a small, portable instrument assembled ad hoc that was named PUMS (Portable Underwater Mini-Spectroradiometer), i.e. a diode-array spectrometer (AvaSpec-2048, Avantes), having 350-900 nm grating, a 50 µm fiber optic SMA connection, provided with a cosine collector (CC3-UV). Spectra were immediately visualized and saved through a laptop, where the software AvaSoft 6.1 had been set up. Irradiance measures were taken around noon, with calm waters and reduced cloudiness conditions, as quickly as possible, to avoid strong fluctuations of the underwater radiant flux (Kirk, 1994). To minimize shade effects by the boat, PUMS was always used on the boat side in front of sun. At the same time, surface water samples were collected from each station and put in thermally insulated containers. They were filtered as soon as possible by means of two apparatuses, for 25 mm and 47 mm GF/F glass fiber filters (Whatman), connected to a vacuum pump. For the extraction of pigments water volumes ranging from 100 to 300 ml were filtered and the25 mm filters were then stored in 3 ml of 100% acetone at 5°C for laboratory analyses. To determine TSM concentrations water volumes ranging from 500 ml to 1000 ml (according to the amount of suspended substances) were filtered, using 47 mm filters that were then preserved at a temperature of -18°C. A sample of 100 ml of water from each station was also filtered with 47 mm filters and fixed with sodium-azide NaN_3, to obtain a final concentration 0.0002 mg/l, to later calculate CDOM concentrations. Due to unsuitable meteorological conditions, we could not take water samples from the minor basin Sidi Ali El Mekki; hence OASs contents were not determined for this sector of the lagoon complex.

2.3. Laboratory procedures

To estimate phytoplankton concentrations, chlorophylls extraction and analysis were carried out, according the procedure of Lazzara et al. (2010). Acetonic extracts absorbances were read by a spectrophotometer (Shimadzu UV 2501 PC) against 90% acetone. From the absorbance values, chloro-phaeopigments contents were calculated, through a monochromatic equation (Jeffrey and Humphrey, 1975; Lazzara et al., 2010).
To evaluate TSM content and its organic fraction, the filters had been put in a muffle for 24 hours and weighted, before sampling; after filtrations they were oven-dried and weighted again, permitting to establish TSM concentrations (mg/l) according Strickland and Parsons (1977), modified by Van der Linde (1998) for the salt wash procedure. A successive passage in a muffle at 450°C for 24 hours followed by a last weighting permitted to obtain the organic fraction too. To calculate CDOM concentrations, water samples fixed with NaN3 were re-filtered in 0.2 µm PC (Nucleopore) membrane and put in quartz cuvettes with an optical path of 10 cm. Absorbance measurements were carried out by a Shimadzu UV 2501 PC spectrophotometer against distilled water and NaN_3 at the same concentration of the sample; the resulting values were converted in absorption according to Bricaud et al. (1981). Spectra were then interpolated in the whole range (300-700 nm) by an exponential function and a nonlinear least squared method (Stedmon et al., 2000; Twardowski et al., 2004). The interpolated CDOM absorption value (m-1) at 440 nm was utilized as an estimate of their concentration; we also calculated a mg/l value by the approximation: $[CDOM](mg/l) \approx a(CDOM_{450})/0.212$ (Nyquist, 1979).

2.4. Optical properties

Underwater irradiance spectra were smoothed by a simple moving average (3-7 nm window), to reduce the effects of natural fluctuations in the radiant field, and AOPs were calculated from downwelling and upwelling irradiance as:

- Spectral diffuse reflectance (R): the upwelling on downwelling irradiance ratio, for any λ in the visible band, roughly indicating the ratio backscattering/absorption:

$$R(\lambda) = E_u(\lambda)/E_d(\lambda) \qquad (1);$$

- Spectral diffuse attenuation coefficient for downwelling irradiance (K_d), expressing the $\ln E_d$ variation with depth z, for each considered wavelength:

$$K_d(\lambda) = - [\ln E_{d1}(\lambda) - \ln E_{d2}(\lambda)]/(z_2 - z_1) \qquad (2).$$

Secchi depth measures could not be taken, due to the limited water depth; an indirect estimate was calculated from the mean K_d values: $Z_{Secchi} = 2/ K_d$ (Shifrin, 1988).

3. Results

3.1. OASs concentrations and patterns

The results from laboratory analyses (detailed in Nourisson et al., 2013) showed that CDOM concentrations were relatively high (0.4-2.2 mg/l), in the range of coastal and estuarine waters (Kirk, 1994) and of inland waters (Binding et al., 2008).TSM concentrations (proportional to NAP contents) were high (13-47 mg/l), with an organic fraction of about 20%, indicating a dominance of mineral sandy sediments in TSM composition, whereas pigments concentrations (Chl_a) resulted to be low in the whole basin (0.4-6.4 mg/m3), due to a reduced phytoplankton presence. Then OASs spatial patterns were analysed.

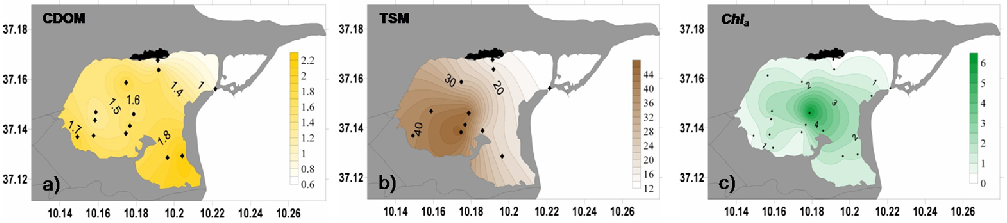

Fig. 2. Spatial patterns of Optically Active Substances as resulting from the laboratory analyses of water samples. a) CDOM (mg/l); b) Total Suspended Matter (mg/l), as a proxy of NAP; c) Chloro-phaeopigments (mg/m^3), as an index of phytoplankton distribution. The intensity of the colour is proportional to the quantities of OAS observed.

CDOM was more concentrated in the southern area of the water body (Fig. 2a); TSM and therefore NAP as well were higher in the western/central part of the main lagoon (Fig. 2b), while Chl_a resulted more abundant in the central part of the basin (Fig. 2c), displaying just a partial overlapping with NAP.

3.2. Optical Properties

The K_d spectra, calculated between 10 and 50 cm of depth, showed a great variability in the light attenuation (Fig. 3a), with values at 440 nm ranging between 1.59 m^{-1} and 5.39 m^{-1} .

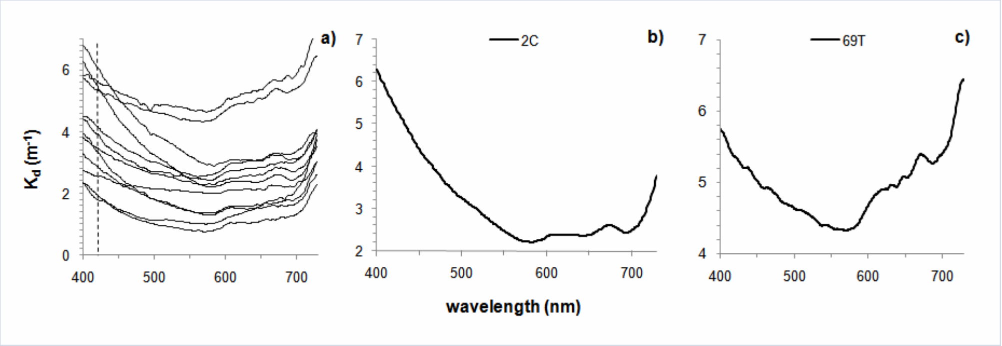

Fig. 3. Diffuse attenuation spectra from the sampling stations in the Ghar El Melh lagoon. a) global vision of all the K_d spectra, showing the variability of light attenuation. Dashed line = 440 nm, the reference wavelength used to compare attenuation values; b) the most recurring shape, called “reversed-J”, indicating a strong absorption by CDOM; c) the so called “U-shape”, found in some stations, typical of waters with valuable contents of NAP.

Two patterns, typical of Case 2 waters, were recognized: almost all stations showed a K_d shape so-called “reversed-J” (Fig. 3b), but in few cases a “U-form” was observed (Fig. 3c) (Prieur and Sathyendranath, 1981). About spectral reflectances, the measure made at 10 cm of depth (R_{10}) was chosen to represent each station (Fig. 4), being less influenced by the bottom colour. Also in this case a great variability of values and shapes was visible (Fig. 4a), but some common features were identified.

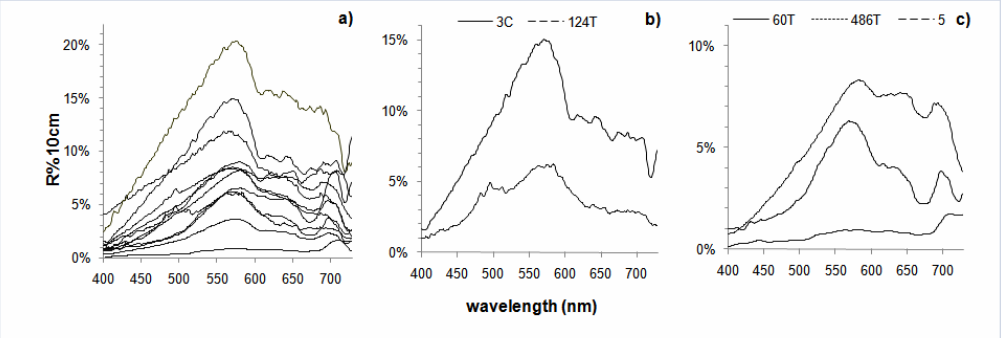

Fig. 4. Spectral reflectances, at 10 cm depth, from the sampling stations in the Ghar El Melh lagoon, well fitting the Reinart et al. (2003) optical types (see discussion). a) global vision of all the R spectra, showing their variability. b) Two typical R spectra, solid line: spectrum from a “Clear” station; dashed line: spectral R from a “Moderate” station; c) Three typical R spectra, solid line: R spectrum from a “Turbid” station; dashed line: spectrum from a “Very turbid station”; dotted line: spectral R from a “Brown” station.

The values of R_{10} in the green band (R_{555}) were quite high (1-19%), indicating a conspicuous light backscattering (see the discussion in Nourisson et al., 2013). The blue/green reflectance ratio (R_{443}/R_{555}) was always smaller than 1, i.e. R was higher in the green with respect to the blue band, due to a major absorption at short wavelengths by dissolved and suspended material, while backscattering was similar (Fig. 4a; Ouillon and Petrenko, 2005). Most of the reflectance spectra had the smallest values in the red band, indicating a dominance of absorption on scattering at these λ (Fig. 4a). From a more detailed observation the variability of the spectral feature among the stations can be organized in five groups, whose spectral characteristic are reported in Fi.g 4b and 4c. In addition this spectral classification also corresponds with the geographical distribution: stations in the same area have similar spectral characteristics. The ones closest to the channel connecting the lagoon with the sea showed a maximum at 550- 580 nm, rapidly decreasing at both sides, with values at 500 nm higher than at 650 nm, e.g. st. 3C (Fig.4b, solid line). In some stations not far from the opening channel, the same maximum at 550-580 nm was observable, but with higher values of reflectance at 650 nm than at 500 nm, and hence the 650/500 ratio higher than 1 (Fig.4b dashed line, st. 124T). In the central part of the main lagoon, stations were found with a R maximum between 580 and 600 nm and a minimum at 675-680 nm (Fig. 4c solid line, st. 60T). Also, another spectrum was observed, quite similar to this one, but with a second, substantial maximum at 690-710 nm (Fig. 4c, dashed line, st. 519T) in the smallest lagoon of Sidi Ali El Mekki. Here some stations also displayed the last kind of spectrum, having its maximum shifted to the red part of the spectrum, at about 700 nm, with considerably smaller values (Fig. 4c, dotted line, st. 486T). To note that in the three kinds of spectra of Fig. 4c high values were visible at 690-700 nm, not observable in those of Fig. 4b; moreover the last spectrum (st. 486T) showed a substantially higher plateau beyond 700 nm, not entirely visible in the pictures . This kind of spectrum was observed at the the Sidi Ali El Mekki lagoon, with the shallowest waters, where the seagrasses on the bottom were well visible; their waxy cuticle reflecting red and IR electromagnetic radiation may have generated the so-called red-edge effect, a strong increase of R for the longest λ (Miller et al., 1991; Gitelson et al., 1996).

3.3. Optical classification

For each sampling station, we calculated the mean R and K_d in the whole visible spectrum and from the diffuse attenuation coefficient we obtained also Secchi depth (Shifrin, 1988). Then, we applied the optical classification of Reinart et al. (2003) that is based on a K-means clustering technique (Reinart et al., 2003; StatSoft, 1995), in which these AOPs are considered together with OASs contents (Chl_a , TSM and absorption values of filtered seawaters at 380 nm, indicating CDOM concentration). A score Si allows to group waters in five optical classes, namely clear (C), moderate (M), turbid (T), very turbid (V) and brown (B). The spatial distribution of optical classes in the Ghar El Melh lagoon complex was expressed in Fig. 5, through an interpolating software (Surfer 7, Golden Software) from the numeric values Si obtained using the above mentioned classification procedure (Reinart et al., 2003). The dominating optical classes were T and V and the whole distribution was quite consistent with the lagoon hydrodynamics (Moussa et al., 2005; Thompson et al., 2009) and with OASs patterns (see paragraph 3.1 above). Waters were clear (C) only in the immediate proximities of the opening channel, moderate (M) in the northern sector (still influenced by marine inputs), turbid (T) and very turbid (V) in the central and southern zones, the shallowest ones, receiving stronger inputs from the cultivated lands. The Sidi Ali El Mekki waters, having the smallest depths, resulted to be very turbid (V) and even brown (B), due to the sediments suspension by the wind.

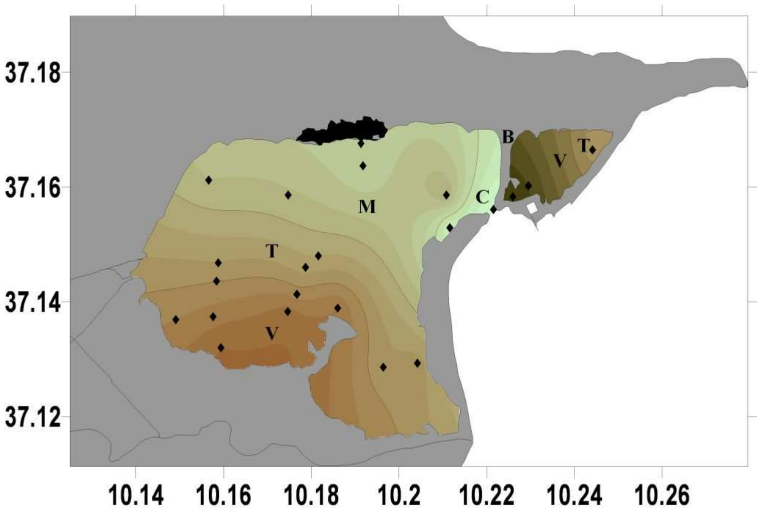

Fig. 5. Final optical classification, obtained for the Ghar El Melh Lagoon system, according to the Reinart et al. (2003) methodology, from the integration of mean AOPs in the visible wavelengths and OASs concentrations measured in each sampling station (diamonds). C: clear water; M: moderate; T: turbid; V: very turbid; B: brown.

4. Discussion

The abundance of CDOM and the low Chl_a contents highlighted by the laboratory analyses may be explained by two hypotheses, not mutually exclusive. Firstly, most of the organic substance may reach the lagoon from its drainage basin, particularly from the surrounding cultivated lands. Secondly, CDOM may originate in the water body from the decomposition of macrophytes, i.e. vascular plants and macroalgae, abundant in this lagoon (Ramzi Sghaier et al., 2011), as generally in coastal lagoons (Castel et al., 1996; Orfanidis et al., 2001), often representing the structural basis in these productive ecosystems (Mann, 1973; McRoy and Lloyd, 1981, Zaldivar et al., 2008). The CDOM high concentration in the southern sector of the main lagoon and the El Ouafi Lagoon may indicate that most of the organic matter was carried there from the Majerda River and its channels after flowing through intensively cultivated lands. The high TSM contents, together with their mainly inorganic composition, highlight that NAP was involved in the high reflectance values, more than in the strong absorptions, which is consistent with the results from AOPs measurements. TSM concentrations were higher in the western/central part of the lagoon, where particles from both the bottom and the agricultural lands may have contributed to increase the turbidity. Finally, the low phytoplankton contents were consistent with the major role of macrophytes in shallow coastal water bodies, as it has been said above. The non-overlapping distributions of Chl_a and CDOM may confirm that the latter matter originated outside the lagoon.
About spectral K_d, , the “reversed-J” shape of most spectrum curves was consistent with high concentrations of CDOM and small contents of phytoplankton. In the stations displaying a “U-form”, which were less numerous, K_d was high in the whole range, indicating a general strong diffusion, to ascribe to particulate matter (Morel and Prieur, 1977; Gordon and Morel, 1983). Hence the analysis of the K_d spectra may discriminate stations where NAP was more concentrated with respect to the absorbing substances (pigments and CDOM). Concerning the R spectra (Fig.4), they showed a good agreement with those described by Reinart et al. (2003) in their optical classification method, corresponding to the five suggested water classes. The shapes observed in the stations closest to the channel connecting the lagoon with the Mediterranean Sea corresponded to the spectra described for clear waters (C), while in the central stations the shape was typical of moderate waters (M). In the central zone we

observed R spectra corresponding to turbid waters (T), while those observed in the minor lagoon named Sidi Ali El Mekki had the features described for very turbid (V) or also brown waters (B). Actually, the R% values observed in the Ghar El Melh Lagoon were similar to those given for Estonian and Finnish lakes (Reinart et al., 2003), even not always exactly the same, but this is not astonishing, as here a Mediterranean coastal lagoon was considered, not an inland water body of northern Europe, with a very different climate and rain regimen. Also, the categories found looking at R spectra did not always exactly fit those obtained applying the full classification procedure. This imperfect overlap was already highlighted by Reinart and Herlevi (1999), particularly concerning classes C and M, and can be explained considering that the clustering of information from both AOPs and OASs provides a more complete frame with respect to just the R spectral analysis. Anyway, the global frame resulted almost unvaried, confirming that the spectral analysis of reflectance can represent an effective way to monitor water optical properties, providing a good approximation also without laboratory analyses of water samples, which is often time-consuming and expensive.

5. Conclusions

Considering the increasing threats to coastal environments, a comprehensive monitoring of their evolution, including coastal lagoons, is of primary relevance for their correct management. The United Nations Conference on Environment and Development (UNCED) Declaration on Oceans recommended that coastal nations: (1) prevent, reduce, and control degradation of the marine environment; (2) develop and increase the potential of marine living resources to meet human nutritional needs, as well as social, economic, and development goals; and (3) promote the integrated management and sustainable development of coastal areas and the marine environment. In accordance with this, the European Union adopted the Water Framework Directive (WFD, Directive 2000/60/EC) (European Union, 2000) establishing the basic principles of sustainable water policy in the European Union to improve the sustainability of water resource in its member states. The Directive aims to provide a basis for the development of shared strategies towards the further integration of policy areas; hence, in the Mediterranean region context, coordination between EU (under the WFD regulations) and non-EU countries represents an important challenge. Concerning Mediterranean coastal lagoons, their management requires concerted efforts and close coordination between scientists and managers of different compartments at international level, both to improve our knowledge of the involved ecological processes and to design common policies and management actions (Pérez-Ruzafa et al., 2011). The first step must be the establishment of the ecological status of the considered systems; however inter and intra-lagoon variability and homeostatic responses may preclude distinguishing human impacts and ecosystem deterioration from the natural variability of the systems under concern; there is a need to design adequate indicators for ecological quality assessment of coastal water bodies (Elliott and Quintino, 2007; Pérez-Ruzafa et al., 2007, Tagliapietra et al., 2009). Lagoon processes show heterogeneous patterns at different spatial and temporal scales, which are not always well known. To distinguish human-induced changes from natural variability in coastal lagoons, an in-depth knowledge of processes leading to the degradation of such ecosystems is required, which can be obtained only by applying detailed monitoring programmes (Pérez-Ruzafa et al., 2011). Till today, methods developed on European coasts to fulfil the WFD criteria often resulted complex for a long-term and large-scale application (Moss et al., 2003; Peeters et al., 2009); moreover, not necessarily the same indicators are the best ones at the whole Mediterranean region level. The goal of this study was to evaluate whether hyperspectral optical measurements of AOPs may be suitable to define the ecological quality status and the main impacts acting on Mediterranean coastal lagoons. The global frame observed in the campaign of April 2008 in the Ghar El Melh lagoon (Fig. 5) was consistent with the results from the control optical classification (Reinart et al., 2003) and with the known hydrodynamic patterns of the lagoon system (Moussa et al., 2005; Thompson et al., 2009). Therefore this procedure may highlight ecological trends even more quickly than outflows and inflows measurements (Thompson et al., 2009). Moreover, the proposed hyperspectral methodology may provide a synthetic ecological indicator of water quality. Also, the in situ optical characterization of lagoon waters resulted suitable to show the effects of the two main factors supposed to influence the lagoon equilibrium, i.e. a reduced supply of marine water in most of the lagoon and the uncontrolled inputs of pesticide and fertilizer compounds from agricultural lands into the lagoon south-western sectors, apparently suffering for an excessive turbidity. Other patterns may be found in different seasons, and perhaps some annual trend may be identified, helping managers and other stakeholders to understand the lagoon evolution which, in turn, should promptly suggest ways of controlling the nature and evolution of such processes.

However, the difficulties in obtaining water samples and Secchi depth measures from the shallowest sectors of the lagoon and the imperfect overlap between the spectral analyses and the final classification keeps open the question about the suitability of this procedure for all Mediterranean coastal water bodies. Considering that the analysis of R spectra already furnished the main information on water turbidity in each station, an easier classification method, specific for the shallow water bodies of most Mediterranean coastal lagoons, should be developed, based mainly on the AOPs spectral analysis. About this matter, other surveys using the same methods have been recently carried out in the Mediterranean Sea, covering a wide range of environments (e.g., in touristic ports in the framework of MAPMED project; CBC-MED programme, 2011-2014) and seasons, and different optical properties are being analysed, to furnish clear classification criteria for coastal water bodies of various nature. This process, together with the large amount of information compiled over recent years on these water bodies (hydrological or ecological data have been published in the scientific literature on more than 50 Mediterranean lagoons, representing just the best known) should allow to establish general criteria to identify cause–effect relationships and, hence, to draw up conceptual models (Pérez-Ruzafa et al., 2011). These in turn should contribute to make the assignment of priorities regarding management actions and possible research lines.

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2.4 Discussion and conclusions

As already stressed in the discussion sections of the two papers above presented, the monitoring of coastal lagoons evolution is of primary relevance for their correct management, which requires to establish their effective ecological status. There is a need for adequate indicators for quality assessment of coastal water bodies (Elliott and Quintino 2007; Pérez-Ruzafa et al. 2007, Tagliapietra et al. 2009); lagoon processes show heterogeneous patterns at different spatial and temporal scales, which makes challenging the identification of the best monitoring procedure. Until today, various methods to fulfil the WFD criteria, but all of them seemed too complex or not general enough to be applied for the long-term monitoring of a large set of Mediterranean lagoon environments (Moss et al. 2003; Peeters et al. 2009).

Although the single survey on which this research is based could not photograph the actual ecological state of the Ghar El Melh lagoon, some interesting possibilities for developing a monitoring strategy were highlighted. Firstly, we stressed the utility of taking *in situ* light measures, easily performed with a small, portable instrument that was named PUMS (Portable Underwater Mini-Spectroradiometer), permitting to take measures also in very shallow waters. Together with the water samples analyses, the irradiance measures permitted to apply an optical classification (Reinart el al. 2003) for the first time in the Mediterranean basin, obtaining a detailed picture of the lagoon optical features, consistent with the main human stressors that are known to act on this ecosystem. However, this method should involve the measurement of also the Secchi depth, which was not feasible in the shallowest sectors of the water body, and an imperfect overlap was observed of the measured reflectance (R) spectra with those described in the classification method, developed for north-European water bodies. Actually, the differences concerned only the most turbid sectors of the water body, yet globally the findings from the spectral analysis were consistent with the classification of Reinart et al. (2003), and also with the known hydrodynamic patterns of the lagoon system (Moussa et al. 2005; Thompson et al. 2009). In other words, the spectral analysis of the water optical properties already resulted quite effective in detecting the two main impacts affecting the lagoon equilibrium, i.e. a reduced supply of marine water (due to the sedimentary drift engendered by the port construction close to the channel) and the increasing nutrients inputs from agricultural lands in the south-western sectors. Hence, the spectral analysis of R (or other optical properties too) may provide a synthetic ecological indicator of water quality permitting to develop a simple classification method, specific for the Mediterranean shallow coastal lagoons. To evaluate this possibility, a further set of surveys has been performed, using the same methods, in a wide range of environments along the Mediterranean shores (e.g., in touristic ports in the framework of MAPMED project; CBC-MED programme, 2011-2014), and several optical properties are being analysed, to establish more robust classification criteria, coupled with the analysis of hydrological and ecological data. This process should help to identify cause–effect relationships, then permitting to draw up suitable conceptual models for the Mediterranean coastal water bodies. Until then, an effective ecological characterization cannot leave aside the biotic components, which integrates several kind of ecosystem changes and shifts. For this purpose, we recommend to apply our modified version of the EEI by Orfanidis et al. (2001), based on a visual survey of the macrophytes cover, instead of a more invasive sampling procedure. This procedure might be suitable for other ecosystems too, where the sampling of vegetation or fauna may be restricted. By its application we found that, despite high water turbidity, the ecological status of the lagoon was moderate, highlighting that no linear relations can be found between optical and trophic classification in the case of shallow coastal water bodies, where turbidity is not necessarily an index of bad ecological quality. Thus, the best practice should be, following our example, a parallel assessment of both the aspects. A monitoring procedure based on *in situ* light penetration and benthic macrophytes cover assessment seems to be a good compromise permitting to account for the several confounding factors described for transitional waters (Elliott and Quintino 2007; Barbone et al. 2012; Chapman et al. 2013; Verissimo et al. 2013). Furthermore, the combination of the two methods is easy and cost-effective to perform, not invasive or destructive, and easy to understand for stakeholders, which is an important requirement for its correct and continuous application. Through a long-lasting monitoring, seasonal or annual trends would be identified, contributing to make the assignment of priorities in management actions and possible research lines.

3. BEHAVIOURAL ADAPTATIONS OF *TALITRUS SALTATOR* AND MACROFAUNAL ANALYSES AS IMPACT INDICATORS

3.1 Introduction

The sandhopper *Talitrus saltator* (Montagu, 1808) (Crustacea, Amphipoda) is widespread on the Mediterranean and eastern Atlantic sandy coasts. These crustaceans breath by means of gills and have a cuticle without waxes, which makes them not adapted to the life in dry environments, as the upper zones of beaches often are. On sandy beaches, physical constraints continuously produce conditions of risk or stress (McLachlan and Brown 2006); to overcome their physiological limitations individuals rely on behavioural adaptations, common to most of the invertebrates of sandy beaches (Williams 1995; Defeo and Gómez 2005; Schlacher et al. 2008). Many of them, including sandhoppers, display a burrowing attitude, as the wet sediments near the water's edge offer moist conditions; another fundamental adaptation is the nightly activity rhythm (Bregazzi and Naylor 1972; Williams 1980, 1983), allowing to avoid dehydration during the hottest daily hours. The studies on locomotor activity rhythms highlighted a high variability between and within populations, driven by the meteorological factors (Nasri-Ammar and Morgan 2006; Ugolini et al. 2007) and also wrack presence and distribution, as they can offer a shelter during daytime (Nardi et al. 2003). Also motility and orientation towards the shoreline are basic behaviours for sandhoppers' survival. Their ability to recover the optimal zone is called 'zonal recovery' and is based on universal (sun, moon, Earth's magnetic field) and local cues (landscape, beach slope, winds), which are used in an opportunistic way (Scapini 2006). Orientation ability becomes even more crucial after displacements occurring during daytime (e.g, to escape from a predator), when the dehydration risk is higher. In this case the sun compass represents the most reliable mechanism for a rapid recovery (Pardi and Ercolini 1986; Walsh et al. 2010). Different populations may show innate directional tendencies, adjusted on the direction of the shoreline of origin, which may be fixed in the long term by a process of natural selection (Scapini and Pardi 1979; Pardi and Scapini 1983; Scapini et al. 1988). However, sandhoppers may also rely on their individual experience to cope with changes acting faster than in evolutionary times (Ugolini and Scapini 1988; Fanini et al. 2009). This behavioural plasticity in orientation permits sandhoppers to face immediate environmental variations (Brown 1996; Soares et al. 1999; Price et al. 2003), like changes of air temperature and humidity (Williams 1995), exposure to waves and tidal range (Williams 1995; Rossano et al. 2009), wrack abundance and distribution (Nardi et al. 2003). Also the shoreline stability may affect the orientation of *T. saltator*: on beaches with a stable shoreline sandhoppers orient consistently seawards, showing statistically significant distributions, and are more scattered when shoreline direction is continuously changing (Scapini et al. 1995). On these bases, Scapini et al. (2005) and Fanini et al. (2007a) have proposed sun orientation of *T. saltator* as a bioindicator of shoreline instability on sandy beaches. On the other hand, the coastal morphological dynamics, by acting on the habitat suitability and safeness, can be responsible for changes also in the activity rhythm expression. Bouslama et al. (2011) studied the behavioural response of *T. saltator* in two beaches differing in physical, geomorphological, and landscape characteristics and observed significant differences in the circadian period and rhythmicity rate between the two populations, with more defined circadian rhythms in the less impacted beach.

Hence, in this thesis we deepened the links between human impacts on coastal dynamics (due to both hard and soft-engineering interventions) and the adaptive behaviours of *T. saltator*, with the specific aim to assess their suitability as bioindicators of shoreline stability. A framework has been described in the first paper (section 3.1.1) presented here, in which several authors contributed for the different case-studies. The findings on the Italian one an the results described at section 3.2.1, on activity rhythms, originated from this thesis work. A seasonal trend emerged concerning orientation performances, which was described at section 3.2.2. In the sections 3.2.3 and 3.3.2 we also reported macrofaunal analyses as a control for the proposed behavioural bioindicators. The results are discussed at section 3.4. In the following table 1, the papers/manuscripts presented in this chapter are summarized, with the study site and the main topics analysed in each work.

Table 1. Papers/manuscripts on *Talitrus saltator* behaviour reported in this thesis.

Section	Study site	*Title*	Main topics
3.1.1	Collelungo beach (Italy), Oued Laou beach (Morocco)	*Monitoring changes of sandy beaches in temperate areas through sandhoppers' adaptations.*	Circadian rhythms and sun orientation of sandhoppers with respect to local impacts as monitoring tools.
3.2.1	Collelungo beach, Italy	*Circadian rhythms in three Talitrus saltator (Crustacea, Amphipoda) sub-populations from an Italian beach with varying morphodynamics. A possible monitoring tool of shoreline stability.*	Circadian rhythms of *T. saltator* with respect to the stablilty of the local shoreline, assessment of the method as a possible monitoring tool.
3.2.2	Collelungo beach, Italy	*Seasonal variation in the orientation of Talitrus saltator: an ecological interpretation.*	Sun orientation of *T. saltator* with respect to periodical and sudden changes in the beach environment.
3.2.3	Collelungo beach, Italy	*Orientation of Talitrus saltator and macrofaunal analyses on a dynamic sandy shore before and after the construction of an anti-erosive stone bank.*	Comparison of *T. saltator* orientation and macrofaunal community indices before and after an hard engineering intervention to evaluate the use of orientation as an early impact bioindicator
3.3.1	Leirosa beach, Portugal	*Behaviour of Talitrus saltator (Crustacea: Amphipoda) on a rehabilitated sandy beach on the European Atlantic Coast (Portugal).*	Circadian rhythms and sun orientation of *T. saltator* with respect to the local environment for the evaluation of the impact of a dune recovery intervention.
3.3.2	Leirosa beach, Portugal	*Macrofaunal community abundance and diversity and talitrid orientation as potential indicators of ecological long-term effects of a sand-dune recovery intervention.*	Comparison of *T. saltator* orientation and macrofaunal indices close to a natural dune and to a rehabilited one, to evaluate the orientation as a long-term impact bioindicator for soft-engineering interventions.

3.1.1 Monitoring changes in sandy beaches in temperate areas through sandhoppers' adaptations

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ABSTRACT

Sandhoppers (Amphipoda, Talitridae) are keystone species of sandy beach ecosystems in temperate areas. Several traits have been studied and proposed as bioindicators of impacts on sandy beaches, at individual (physiology and behaviour), population (life cycle, abundance, population dynamics and structure, morphometry and fluctuating asymmetry) and species (genetic structure and variation) levels. Sandy beaches, where these species spend their whole life cycle, are dynamic environments, subject to periodic changes (night-day, tides and seasons), as well as to impacts that may cause erosion or accretion of the littoral zone. The first response of an animal to potentially injuring factors is shown by its avoidance behaviour regarding the potential stress. This behaviour may be a rapid escape reaction, shown by single individuals, the whole or part of the population. Activity rhythms, changes in zonation, burrowing, escape reactions, orientation towards the optimal zone on the beach, are all adaptations to environmental changes, increasing the survival chances of the individuals and populations that express such behaviours. In this paper we present case studies of the talitrid species' behavioural adaptations related to natural and human impacts on sandy beaches in the Mediterranean: (1) erosion/accretion dynamics of a Tyrrhenian beach in central Italy (Maremma Regional Park), and (2) increasing urbanisation on a beach in north-western Morocco (Oued Laou river mouth). The best behavioural adaptation was shown by the populations from more stable coastlines. For the survival value of rapid and possibly anticipatory responses to stressful factors, behavioural variation may be proposed as an early-warning indicator of environmental changes. The adaptation at higher levels (population and species) may reflect, on the one hand, the evolvability of the species and, on the other hand, the changeability of the environment. Adaptations at population level may be used as bioindicators of past changes over generations and evolutionary times.

INTRODUCTION

Sandy beaches are ecotones between land and sea and host a variety of macroinfauna, supported by nutrients from the sea (Dugan et al., 2003; McLachlan & Brown, 2006). For their abundance and ecological role sandhoppers and beachhoppers (Talitridae) are keystone species in these ecosystems: mainly scavengers, but also herbivorous and predators (Colombini et al., 2003; Serrano et al., 2008). Cannibalism is a phenomenon that could also naturally occur when subsides are scarce (authors' unpublished observations); adult cannibalism against juveniles was observed in the laboratory by Duarte et al. (2010). Sandhoppers spend their whole life cycle on beaches and different cohorts are found together, in some cases in different beach zones (Kennedy et al., 2000; Fallaci et al., 2003; Marques et al., 2003; Scapini & Dugan, 2008). The life-span was modelled on beaches at different latitudes and lasted for 9-18 months (Williams, 1978; Marques et al., 2003). Thus, individuals are subject to various environmental factors throughout the seasons. This is particularly relevant in temperate climates, where seasonality can be considered a natural, periodical occurrence of higher dehydration risk in summer, habitat loss in winter, etc. Human-driven impacts on sandy beaches overlap with natural seasonal changes: touristic seasons are well-defined in time, in which natural features for sandy beaches, like warm summers, merge with social features (holiday periods) (Butler, 1998). This leads to a marked seasonality of human presence on sandy beaches. However, human-driven impacts can also be continuous, affecting the beach-dune ecosystem as a whole. This is the case for coastal development, resulting in long- term impacts that go beyond the mere supralittoral zone (Nordstrom, 2000). Are sandhopper populations able to cope with such dynamics, acting faster than in evolutionary times? We focus here on physical impacts recognised as the main driving forces on beach fauna McLachlan & Brown, 2006). The physiology and behaviour of talitrids may reflect environmental features when these are cyclic (tidal, diel or seasonal) and predictable. Populations living at increasing distances from the coastline show an increasing variation in their circadian rhythmicity (Rossano et al., 2008). Plasticity, particularly behavioural plasticity, was interpreted as an adaptation to unpredictable changes in sandy beach environments (Scapini et al., 1988; Brown, 1996; Fanini et al., 2009). On the other hand, morphological and physiological constraints may prevent the colonisation of some beach zones under stressful climatic conditions (dry air, waves and strong winds, etc.). Air breathing through gills may be a constraint for the risk of dehydration and submersion; moreover, anoxic sediments are not suitable to air breathing. The sandhopper cuticle is relatively resistant to mechanical damage, but not impermeable. The osmotic stress of immersion in fresh water (rainfall, surface runoffs and coastal water bodies during the wet season) is only partially compensated physiologically (Morrit, 1989) and active avoidance behaviour is needed for survival. High sand and air temperatures during summer are avoided by zonation and burrowing into the wet sand near the water's edge, while at temperatures below 10°C sandhoppers remain buried in the sand (Scapini et al., 1992). Burrowing behaviour is a key adaptation to sandy beaches (Williams, 1995). The incoherent sediments offer protection to buried animals as sand grains are periodically wetted and oxygenated by sea water. The superficial sand layers are subject to dehydration, and the burrowing animals have to adjust their position both vertically (through sand layers) and horizontally (across the beach). A choice for specific sand humidity, salinity, pH and grain size characterizes sandhoppers' behaviour. Differences related to the variation of environmental features were observed among individuals of different size, age and sex, populations and species (Williams, 1995; Colombini et al., 2005). Zonation is related to the community as well: different species are found in different beach zones. However, most species living in sandy beaches change their zonation periodically according to environmental changes driven by tides, nightday alternation and seasons (Scapini et al., 1992; Fallaci et al., 2003; Colombini et al., 2005). Different species of sandhoppers can co-occur on a beach maintaining different zonation (Lastra et al., 2010), but zonation changes were also observed in different age stages within the same species (Kennedy et al., 2000; Scapini & Dugan, 2008). Sandhopper zonation into the dunes, observed in winter, may reflect the risk of being displaced into the sea by waves and storms (Scapini et al., 1992; Charfi-Cheikhrouha et al., 2000). Moreover, in addition to zonation along the y-axis, the distance from a fresh water source (i.e., a river mouth or a coastal lagoon) may also influence talitrid distribution on the beach. Rivers transport sediments of various granulometry, depending on the quality of sediments and the currents' velocity, and near river mouths sediments are distributed along the coast according to a gradient of distance from the river mouth. A changeable distribution according to sand granulometry and moisture was observed in the arthropod community along the coast (Colombini et al., 2005). As a consequence, the most suitable zone for sandhopper survival is not fixed in time and space. Sandhoppers track changes in the position of the zones, and are always found in the most suitable location according to the time of day (night and day changes) and season. Locomotor activity rhythm is a widespread adaptive behaviour observed in talitrid species from coastal habitats (Bregazzi & Naylor, 1972; Williams, 1980). Activity is mainly nocturnal to avoid dehydration during the driest and hottest hours of the day in summer. *Talitrus saltator* (Montagu, 1808) populations from the British Isles and the Mediterranean showed high variability in the expression of the locomotor endogenous rhythm, with relation to latitude and local ecological conditions (Bregazzi & Naylor, 1972; Williams, 1980; Edwards & Naylor, 1987; Williams, 1995; Nardi et al., 2003; Rossano et al., 2008, 2009). Variation was observed between and within populations and within single individuals in the different life-stages and seasons. Populations may express different periodicity according to their ecology (Rossano et al., 2008). Moreover, within the same population, a higher circadian periodicity was observed in spring-summer and inter-individual variation in autumn-winter, thus revealing a seasonality in the expression of rhythmicity (Nardi et al., 2003). The seasonal and climatic factors were found to be the main driving forces. Comparing Italian and Tunisian populations from beaches with similar morphology, a better definition of the period was observed in spring-summer and winter respectively, due to the extreme conditions of the North-African shores in summer, inducing a shift in the talitrids' activity to the milder winter season (Scapini et al., 2005a; Nasri-Ammar & Morgan, 2006; Ugolini et al., 2007). The presence and distribution of wrack may also influence the circadian rhythm expression, as wrack can become a shelter during daytime hours, so that on beaches where wrack and debris are abundant, talitrids' activity needs not be perfectly synchronized with the night (Nardi et al., 2003). In addition, beach morphodynamics may be relevant for rhythm expression, as they may impact on habitat suitability and safety (Cardoso 2002). However, evidence of a strict relation between rhythm expression and beach morphodynamics is still lacking. Motility and orientation are also considered key adaptations to sandy beaches, where physical constraints are continuously shaping the habitat (McLachlan & Brown, 2006). In highly changeable environments, motility is an effective way to escape from stressful or risky conditions. Moreover, rapid movements in all (unpredictable) directions may be suitable to avoid predation (Ugolini, 1996). Zonal orientation is instead the best strategy for recovering the optimal zone on the beach, if the animals have been displaced. Zonal orientation occurs across the y-axis, i.e., that perpendicular to the shoreline, which is the shortest way to recover the optimal zone, and not a return to a specific point. The orientation mechanisms used by talitrids to recover the optimal zone have been studied since the 1950s (Williamson, 1951; Pardi & Papi, 1953; for a review, see Scapini, 2006). On a dry substrate sandhoppers are directed seawards, while they head landwards when wetted by sea water. The cues used in orientation are both local (landscape, beach slope and/or wind) and universal (sun, moon, sky light and/or the Earth's magnetic field); the animals use the available cues in an opportunistic way, depending on the prevailing beach features. The hierarchical use of orientation cues was studied by Walsh et al. (2010) in *Talorchestia longicornis* (Say, 1818). According to Pardi & Papi (1953) the sun-compass represents the most reliable mechanism used by *T. saltator* on a beach to perform a rapid zonal recovery. A precise clock is needed to compensate for sun movement throughout the day (Scapini et al., 2005a). At night, searching movements represent a minor risk, due to the lower probability of dehydration and predation; in any case, the moon may be available for orientation most nights (Papi & Pardi, 1953; Ugolini et al., 2003). The question is still open whether sun and moon orientation rely on one, two or even three clocks (Meschini et al., 2008). Scapini (1997, 2006) interpreted the differences in sandhoppers' and beach-hoppers' orientation observed by different research groups as adaptations to different environmental features. The use of landscape vision is most suitable to recover the preferred zone at the base of the dune after the nocturnal foraging migration on tidal beaches (Edwards & Naylor, 1987), while sun orientation is best used to recover the zone at water's edge to avoid the risk of dehydration on micro-tidal reflective and intermediate beaches (Scapini, 2006). The precision of orientation with respect to the y-axis is a measure of the adaptation to the beach's features: all individuals adopt the same strategy, as they have learned to find the most suitable direction (in the short term) or have been selected for the most suitable orientation mechanism (in the long term). A scatter of the samples may reflect poor motivation to orient (air/substrate humidity) or lack of adaptation depending on a frequently changing shoreline, recent colonisation or stressed population. In this latter case, individuals would probably use other mechanisms simpler than a sun compass, such as phototaxis or scototaxis (Gambineri & Scapini, 2008).

The aim of this paper was to show that understanding an organism's adaptation (here the sandhopper *T. saltator* and the beach-hopper *Orchestia gammarellus* (Pallas, 1766) from Mediterranean micro-tidal coasts) may contribute to understanding the environmental features and their changes occurring at the temporal and spatial scales of the populations' life-cycle. Case studies will be shown in which talitrids' behavioural adaptations were related to the changing features of sandy beaches. The considerations developed may also be extended to most talitrid species (Williams, 1995; Scapini & Dugan, 2008; Fanini et al., 2009). The rationale behind such an approach is that organisms integrate the features of the environment in which they live in their behaviour, and thus a good knowledge of the species may reveal environmental features and their trends of change.

MATERIAL AND METHODS

The capture of the samples

The following species were considered in the case studies presented here: *Talitrus saltator* and *Orchestia gammarellus*. Sandhoppers and beach-hoppers are night active; so searching for them during the day may be a challenge because of their changing zonation. Transects covering the whole beach from waterline to the base of the dune were searched for sandhoppers by removing the surface layers of sand before the experiments. In those beach sectors where sandhoppers appeared scarce, pit-fall traps were positioned across transects from the sea to the dune and left active throughout the night, in consideration of the nocturnal activity of the animals.

Description of the sampling sites

In Italy, a case study was represented by the Collelungo beach, in the Maremma Natural Park (Tyrrhenian coast of Grosseto, central Italy; 42°38′N 11°4′E), a coast with limited tidal excursions, measurable in centimeters, although not irrelevant for animals living near the waterline. Here an erosion gradient was observed, with the strongest erosion found at the mouth of the Ombrone River, in the north-western sector of the beach, gradually lessening with distance and finally producing an accretion in the beach segment located 6 km in the south-eastern sector (Pranzini, 2001). Such dynamics were accelerated in the last decades by several careless interventions along the river (Ciampi, 2007), for which a 1200 m stone bank on the left side of the river mouth was built on the littoral in 2009. For the activity rhythm study, adult individuals of the two sympatric species *T. saltator* and *O. gammarellus* were collected on the sandy beach of Collelungo at about 6 km from the Ombrone River mouth during the months of June, July and August 2007. There, the presence of a dune-slack with seasonal salt marshes favoured the beach-hoppers' and sandhoppers' colonisation of an area extending about 100 m inland. In particular, during the summer *O. gammarellus* colonised the dune-slack and *Juncus acutus* bushes, whereas *T. saltator* colonised both the beach and the dune, marginally sharing the habitat with *O. gammarellus*; however, during the winter the two species' areas overlapped displaying their maximum level of sympatry (Gagnarli, 2009). Orientation experiments were performed on *T. saltator* at three beach points, covering the spectrum of erosive dynamics: the first was located at 4 km from the river mouth, where the erosion was strong, another was at 5 km, where the coastline was apparently under equilibrium and slightly accreting, while the last was at 6 km, in a littoral stretch where a stronger accretion was observed. *O. gammarellus* was not found at the 4 and 5 km beach stretches. In each point, the main beach and meteorological features were measured for each replicate: slope and width of the beach, air temperature, relative humidity and sky cover. In May (spring), July (summer) and September (autumn) 2011, on a total of 1351 specimens, 407 of which from the eroded beach stretch (4 km), 461 from the stable beach stretch (5 km) and 483 from the accreting one (6 km), tests were repeated simultaneously in the three points.

Orientation experiments in Morocco were carried out on two beaches, one on the left side of the mouth of Oued Laou River and the other on the right, at 1 km distance (35°27′N 5°6′ W). This area was also characterized by small tidal excursions. In the area there were two villages: Oued Laou, the larger one, to the left and Ka'asrass to the right. The area of Oued Laou (8431 inhabitants in 2004) was characterized by a higher urbanization aimed at developing tourism, while the activities of the Ka'asrass area (860 inhabitants) were basically traditional. There was a different use of the two beaches, with higher impacts on the left side of the river mouth (Fanini, 2006). In particular, a progressive increase in building construction in the area of Oued Laou has been observed in the recent years, with the risk of compromising the stability of this beach (Oueslati, 2006). In each beach stretch, beach slope and width were measured and the meteorological features were recorded for each replicate: air temperature, relative humidity and sky

cover. The orientation tests were performed on these two beach points simultaneously on two subsequent days in May 2004. We tested a total of 620 *T. saltator* individuals, 314 on the left river side beach (Oued Laou) and 306 on the right river side beach
(Ka'asrass).

Recording of activity rhythms in the laboratory

The recording of activity was performed by way of an automatic electronic system located at the Department of Biology (Florence): 61 *T. saltator* individuals and 56 *O. gammarellus* individuals were singly placed in cylindrical recording chambers 10 cm of diameter and 13 cm high, filled with a few centimeters of substratum from the collection area and food ad libitum provided on a piece of untreated filter paper. Each recording chamber was provided with an infrared beam and interruptions of the beam were recorded by a logger every 20 min for 21 days. The recording apparatus was placed under controlled conditions in a room with the temperature kept at 18 ± 1°C. The recordings were made in continuous darkness to permit the expression of the endogenous rhythms. At the end of the recordings, the data were downloaded and analysed with specific software: the locomotor activity patterns were represented using the Chart software package 35, which produced actogram plots. In the actograms the activity of each individual was expressed as a daily percentage over the daily maximum and plotted in rows of 48 h and two columns. In the actograms each row reports the second day (0-24 h) represented in the previous row (24-48 h) so that across the whole plot the visualization of data gives continuity. Periodogram analysis based on the Dorscheidt & Beck method (Harris & Morgan, 1983) was performed using the Time Series package that worked on the Excel platform and calculated the circadian period τ (when present) and its definition for each individual time series. The definition of the period was expressed as signal-to-noise ratio (SNR) with $p = 0.05$, i.e., the ratio between the height of the correlation ratio peak and the 95% probability line.

Orientation experiments on the beach

The orientation was measured on the beach by releasing *T. saltator* in the centre of a standard arena device, made of a cylindrical box of Plexiglas with a 40 cm diameter, having 72 pitfall traps of 5° each at its rim; pitfall trap 72 was oriented to the North. The arena was positioned horizontally on a tripod at 1 m above the beach surface. A white cardboard was placed around the arena's circumference to screen off the landscape view, and tests were alternated with and without the screen. The animals were placed in the centre of the arena by means of a transparent Plexiglas tube (3 cm diameter) inserted vertically through the cover of the arena; after 1 min the tube was removed and the animals were free to hop or run in the chosen direction. At each release we registered the following variables: time of the release, traps where the animals had fallen, air temperature (°C; electronic thermo-hygrometer), air humidity (%; electronic thermo-hygrometer), sky cloudiness (0-8; visual appreciation), sun vision (visible, covered, shape, not visible; visual appreciation). Every experimental series was repeated twice: in the morning around 9:00 (solar time) and in the afternoon around 15:00 (solar time). In each series of releases 8 samples of 10 individuals each were tested successively. The orientation of individuals tested in small groups can be considered independent (Scapini et al., 1981). All tested individuals were kept in separate tubes filled with 75% alcohol for later morphometric measurements and check of the species and sex. The statistical analysis of circular distributions was applied to analyse orientation (Fisher, 1993; Marchetti & Scapini, 2003). The Rayleigh Test for uniformity was applied to the circular distributions, to test the concentration of the directional choices around the mean; this test is based on the length of the mean vector (Batschelet, 1981). A multiple regression analysis adapted to circular distributions (Spherically Projected Linear Models, SPLM) was applied to the datasets (Scapini et al., 2002; Marchetti & Scapini, 2003). These are linear models with the angles of orientation as dependent variables, and environmental variables as independent ones. The Likelihood Ratio Test was used to test the significance of the influencing factors on orientation.

RESULTS

The study sites

At Collelungo, the beach widths clearly indicated the existence of an erosion gradient, with a width of just 15 m at the point closest to the river mouth (4 km), 30 m at 5 km and 36 m at 6 km (table I).

Table 1. Main physical and climatic variables measured at Collelungo (Italy) during the experiments

	Season	4 km	5 km	6 km
Beach width (m)	Spring	15	30	30
	Summer	15	30	36
	Autumn	15	30	36
Mean beach slope (%)	Spring	4.12	2.44	1.39
	Summer	5.22	2.27	1.67
	Autumn	3.48	1.86	1.43
Air temperature range (°C)	Spring	23.0-38.0	22.0-27.0	20.3-33.7
	Summer	31.2-38.3	29.0-34.0	29.0-36.0
	Autumn	26.0-37.0	23.0-35.0	25.2-39.6
Air relative humidity range (%)	Spring	41.0-42.0	17.0-50.0	23.0-33.0
	Summer	41.0-62.0	42.0-71.0	49.0-76.0
	Autumn	24.0-55.0	22.0-49.0	17.0-42.0

The only seasonal variation was observed at the 6 km point, showing beach accretion of 6 m from spring to summer. The different erosion rate was reflected also by the beach slopes at the three points, with slopes of 4.27°, 2.19° and 1.50°, decreasing with increasing distance from the river mouth (table I). No significant seasonal trends were recognizable in the slope variations. Air temperature and relative humidity did not differ significantly among the sites, and while temperature was almost the same during summer and autumn, with about 6° more than in spring (seasonal mean values of 26.43°, 32.87° and 32.60° in spring, summer and autumn, respectively), humidity was considerably higher in summer than in autumn (mean values of 35.04%, 57.63% and 32.52% in spring, summer and autumn, respectively) (table I). In Morocco the beach slopes were similar on both sides of the river mouth, the sand was coarse with no differences between the two river banks (table II). The beach was wider at Oued Laou (left side of the river), where a depression, communicating with the sea through a canal, was observed in front of the buildings constructed on the former dunes. Air temperature and humidity ranges were similar to those recorded during the spring experiments in Italy (table II).

Table 2. Main physical and climatic variables measured in Morocco at Oued Laou (left side of the river mouth) and Ka'asrass (right side of the river mouth) in spring during the experiments

	Oued Laou	Ka'asrass
Beach width (m)	69	45
Mean beach slope (%)	4.50	5.25
Air temperature range (°C)	19-32	19-32
Air relative humidity range (%)	30-67	30-67

The circadian rhythms of *Talitrus saltator* and *Orchestia gammarellus* from Collelungo

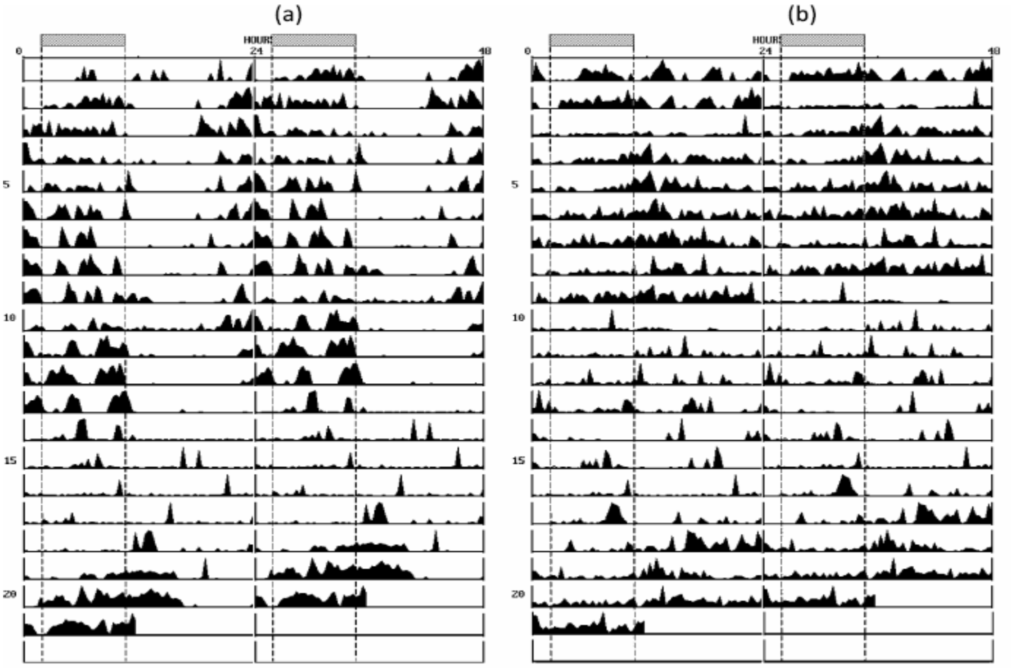

Fig. 1. Actograms of the activity under constant darkness: a, an individual *Talitrus saltator* (Montagu, 1808); b, an individual *Orchestia gammarellus* (Pallas, 1766). On the horizontal axis, the time of day is shown (1-24 h), on the vertical axis, the day (1-21 days) after the onset of the registration. The grey blocks at the top indicate the dark phase on the first day. The diagrams are doubled (each day is shown twice, i.e., on the right of the first row and on the left of the second) to show periodicity. The activity of each 20 min is indicated as a percentage of the maximum activity.

A high percentage of active individuals was recorded for both species (94% for *Talitrus saltator* and 90% for *Orchestia gammarellus*) and, among these, a significantly lower number of periodic individuals was observed for *O. gammarellus* (77%) compared to *T. saltator* (96%) (Pearson's chi square test with Yates' continuity correction = 5.3378, df = 1, *p* = 0.0209). To show the type of variation observed during the 21 days of recording, two examples of actograms are reported with the activity patterns of a *T. saltator* and an *O. gammarellus* specimen (fig. 1a and b, respectively). The former pattern of activity is more regular and a clear periodicity is observable (τ = 24 h 20 min, SNR = 0.5404) throughout the whole pattern with a limited internal variability (fig. 1a). The pattern of activity in the latter actogram is less clear and the activity is less defined (τ = 24 h 20 min, SNR = 0.0780) with a higher internal variability (fig. 1b). The periodogram analysis for the whole group of samples showed shorter periods, closer to 24 h, for *T. saltator* as compared to *O. gammarellus* (mean periods = 24 h 19 min ± 6 min and 24 h 41 min ± 12 min, respectively), but the difference was not statistically significant (Wilcoxon rank-sum test with correction, Z = 1.607, p = 0.108). Instead, the mean period definition (SNR) for *T. saltator* (0.503 ± 0.062) was significantly higher than the SNR for *O. gammarellus* (0.286 ± 0.046) being almost double (Wilcoxon rank-sum test with correction, *Z* = −0.8745, *p* = 0.004). These results are reported in fig. 2, where the definition is plotted against the period and the distributions

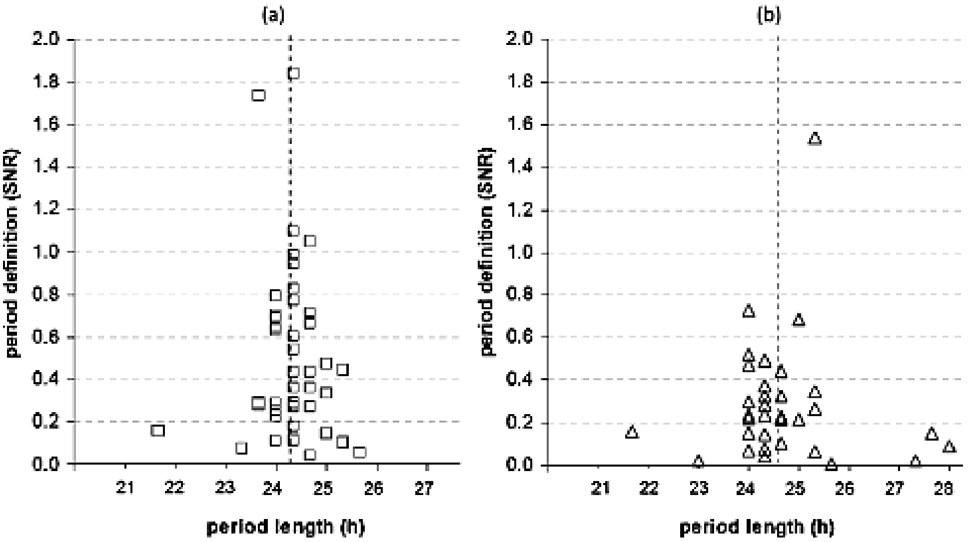

Fig. 2. Period definition (SNR) plotted against the periods (in h) of the whole samples: a,*Talitrus saltator* (Montagu, 1808); b,*Orchestia gammarellus* (Pallas, 1766).*T. saltator*: mean period=24 h 19 min±6min and SNR=0.503±0.062; N=61.*O. gammarellus*: mean period=24h41min± 12 min and SNR=0.286±0.046; N=56.

of the two species are compared, highlighting a different behaviour of the two species, with periods closer to 24 h and higher definitions in *T. saltator* (fig. 2a) than in *O. gammarellus* (fig. 2b).

Sun orientation of *Talitrus saltator* on Collelungo beach

T. saltator orientation performances resulted to be best adapted to the shoreline direction in the sample of the eroded stretch of littoral, at 4 km from the river mouth, with the highest concentration (r = 0.340, p<0.001, Rayleigh Test) and a mean angle of 191° with a mere deviation of 13° from the expected direction seawards (178°) (fig. 3a). Contrary to expectations, a more variable orientation was observed in the midway point, with a significant concentration of r = 0.225 (p<0.001, Rayleigh Test) and a mean angle of 221°, deviating 20° from the expected direction seaward (201°), but with a significant fraction of specimens that moved in the opposite direction landwards (fig. 3b). The orientation performance was intermediate in the accreting segment, furthest from the river mouth. Here the sample showed a concentration of r = 0.227 (p<0.001, Rayleigh Test) and a mean angle of 197°, deviating 15° from the seaward direction (201°) at that point (fig. 3c). Multiple regression models were developed with the environmental and intrinsic variables and factors, likely to influence orientation: beach point (4 km, 5 km, 6 km), season (spring, summer, autumn), landscape vision (landscape, no landscape), morning/afternoon, solar time, azimuth, air temperature, air humidity, sky cover (cloudiness from 0/8 to 8/8), sun visibility (4 levels: visible, veiled, shape, not visible). The intrinsic factors (sex, cephalic length and antennal articles) were not significant. The beach point factor was the most important source of variation and there was a highly significant interaction between this factor and the other variables (Likelihood Ratio Test: p<0.001). This analysis confirmed that the three distributions obtained at the three beach points were statisticallydifferent, meaning that talitrids inhabiting different beach stretches displayed different behaviour.

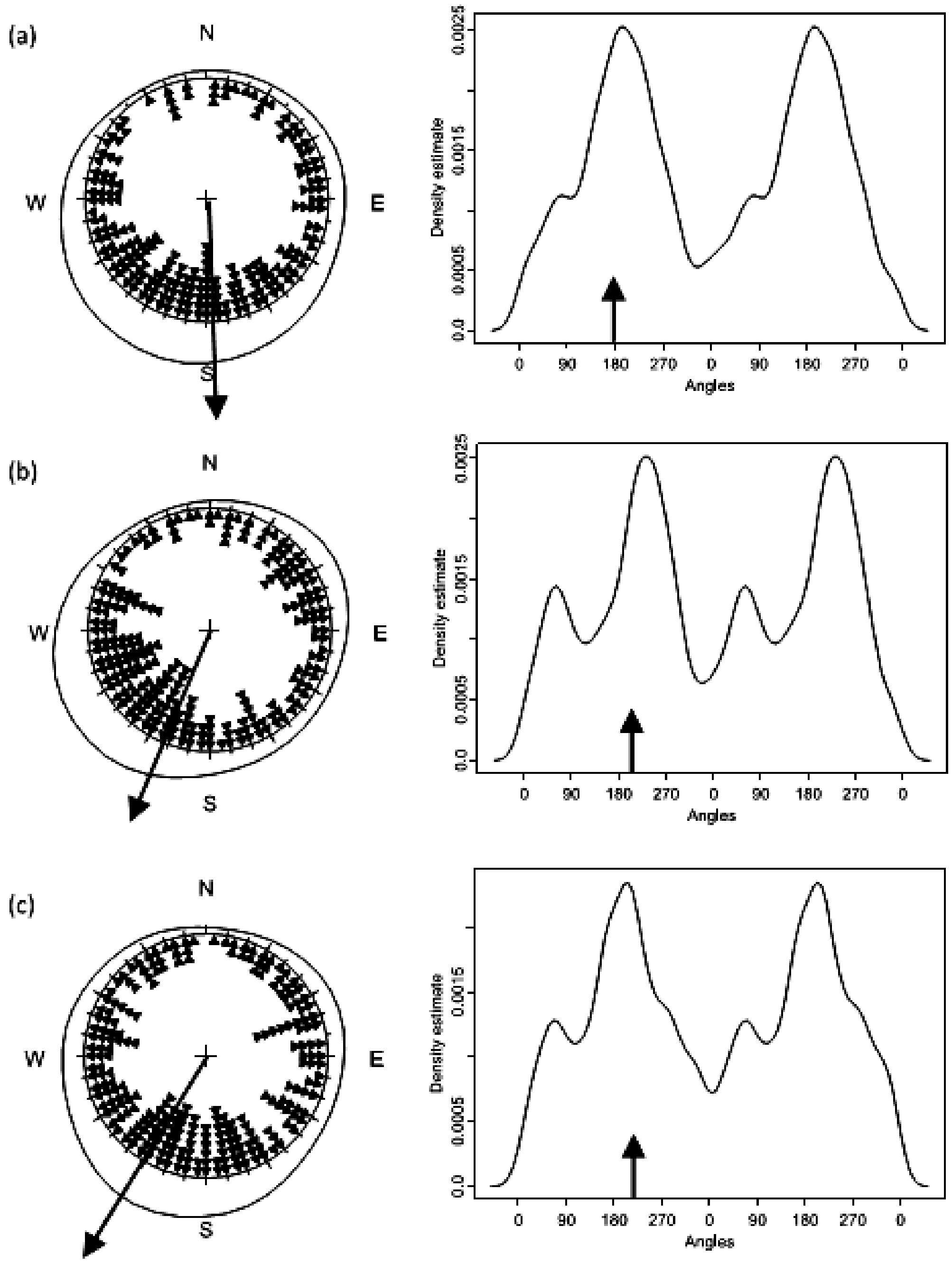

Fig. 3. Sun orientation at Collelungo beach of *Talitrus saltator* (Montagu, 1808): a, at 4000 m from the Ombrone mouth; b, at 5000 m; c, at 6000 m. The triangles show the directions of the samples; the line diagrams show the kernel density distributions, both in polar and Cartesian coordinates (in this case the diagrams are doubled to show circularity). Arrows show the direction seaward at each point: a, 178°; b, 201°; c, 212°. Summary statistics of the distributions: a, mean angle =191±15°, r =0.340,N =208; b, mean angle =221±20°,r =0.225,N =236; c, mean angle =197± 22°,r =0.227,N =240.

Sun orientation of *Talitrus saltator* at Oued Laou and Ka'asrass

The orientation of *T. saltator* showed clear differences between the population of the beach on the left side of the river mouth and that on the right side (fig. 4a, b). The samples from left side beach (fig. 4a) showed a more scattered orientation than those from the right side beach (fig. 4b) (*r* = 0.231 and r =0.627, respectively; p<0.001, Rayleigh Test for concentration to the meandirection). On the left side beach (fig. 4a), the seaward direction (60°) was not included within the interval of confidence of the mean direction, which significantly deviated to the North (14±27°). On the other hand, the samples from the right side beach (fig. 4b) showed a significant seaward direction that did not differ from the expected direction seawards (mean angle=52±9°). Multiple regression models were developed with the variables and factors, both environmental and intrinsic, influencing orientation: beach (left/right), day of experiment (1/2), morning/afternoon, time of day, air temperature, air humidity, sky cover (cloudiness from 0/8 to 8/8), sun visibility (4 levels: visible, veiled, shape, not visible), sex (male/female), cephalic length and adult/juveniles. The beach factor was the most important source of variation and there was a highly significant interaction of this factor with the other variables (Likelihood Ratio Test:p<0.001). This analysis confirmed that the two distributions obtained on the two beaches were statistically different, again indicating that the talitrids from the two beaches display behavioural differences.

DISCUSSION

According to Scapini (2002: 120), the

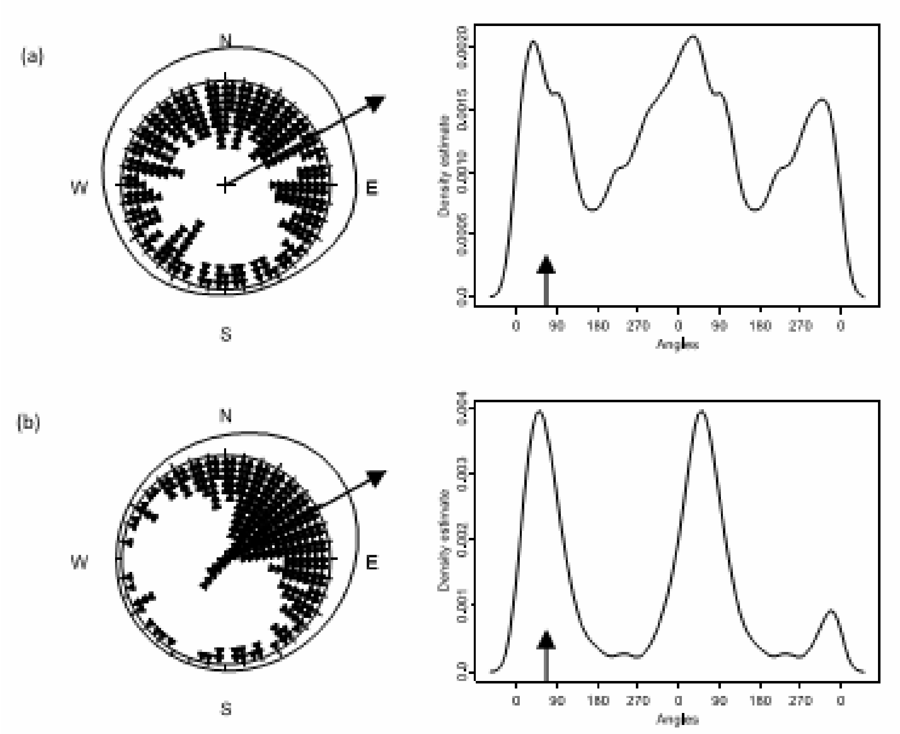

Fig. 4. Sun orientation at Oued Laou (a, left side of the river mouth) and Ka'asrass (b, right side of the river mouth) of *Talitrus saltator* (Montagu, 1808). The triangles show the directions of the samples; the line diagrams show the kernel density distributions, both in polar and Cartesian coordinates (in this case the diagrams are doubled to show circularity). Arrows show the direction seaward at each point: a, 178°; b, 201. Arrows show the direction seawards at both beaches (60°). Summary statistics of the distributions: a, mean angle=14 ±27°, r = 0.231, N = 31; b, mean angle=52±9°, r = 0.627,N=306.

suitable characteristics for a bioassay are: (1) availability of the biological material, (2) availability of the necessary equipment, (3) ease of execution by one/two persons after short training, (4) clear interpretation that follows from previous testing and comparison with existing baseline data, (5) reliability of the biological parameter measured, and finally (6) the possibility of repeated measurements. The rationale for proposing the behaviour of sandhoppers as early warning indicators of changes is that behaviour itself is an adaptation to changing environments, such as sandy beaches (Brown, 2006). Considering the whole set of case-studies analysed, it could be seen that the harshness of a sandy beach environment forces the inhabiting fauna to deal with physical constraints first, which influences not only species diversity, population abundance and structure, but also behaviour (see Defeo & McLachlan, 2005 for the Habitat Harshness Hypothesis and the Habitat Safety Hypothesis modelled on supralittoral amphipods; Fanini et al., 2009). The complex systems we considered in this study include different sources of impact, but only some of them are affecting the physical environment, and thus can be clearly detected by behavioural assays.

In the case of Collelungo, the beach under study was part of a natural park, with controlled numbers of visitors and activities on the beach; so the main physical constraint was the dynamics of erosion/accretion of the littoral. Orientation tests revealed consistency with the littoral dynamics shown by the significant interaction of the beach point factor in the SPLM analysis of the orientation distributions. The interpretation of this correlation also follows from previous studies that highlighted beach stability as major requirement for sun orientation adapted to the shoreline direction (Scapini et al., 1995). Being a behavioural feature, orientation represents one of the first responses to the environmental variations, and the time-scale in this case is small, consisting of a few months (within an individual's life span). On the Collelungo beach *Talitrus saltator* is the dominant species, so its behaviour must be well adapted to its life-environment, as documented in similar studies carried out in 2002-2003 on this beach (Scapini et al., 2005b; Ketmaier et al., 2010). The existence of previous data also offered the possibility of comparing results from before and after the intervention for the mitigation of the beach erosion process, and check the suitability of sandhoppers' orientation as an impact's bioindicator. An initial comparison with the orientation data from previous studies seems to indicate an overall worsening of the sandhoppers' performances at all sampled sites, probably because more time is needed for them to integrate the new artificial setting of the shoreline in their behaviour (Nourisson et al., unpublished data, 2011-2012). What is remarkable is that the results of this study in some way contradict those of 2002-2003, where the best orientation performances were those of the population of the intermediate beach segment under equilibrium between erosion and accretion (Scapini et al., 2005b), while in this study the best orientation was observed on the eroded beach sector. It seems likely that, after the stone bank construction on the beach near the river mouth, stabilization is occurring in the eroded site at 4 km from the river mouth, as shown by the increasing precision in orientation. Apparently an accretion process has started at 5 km of distance, increasing the variation of this segment of shoreline in terms of coastline morphodynamics. Here the sandhoppers showed a misadaptation of their behaviour, with a tendency to orientate to the opposite direction. The results of the beach point at 6 km distance confirmed those obtained in 2002-2003. Here, the effects of shoreline stabilization are probably not acting on the natural morphodynamics, and natural accretion dynamics are still occurring (as a decade ago) to which the sandhopper population responds with an increasing variability of orientation. The data presented here showed significant differences in the orientation performances among the three populations analysed, confirming that the behaviour expressed, which integrates all the information from the environment in the individual life-span besides the genetic bases (that may also differ among these populations, Ketmaier et al., 2010), can be used to assess even small habitat divergences before other effects on the beach community become evident. The variability observed in sun orientation may also reflect the observed variation in circadian rhythmicity. Activity rhythms highlighted a strong dependence on the habitat of the species, with less limiting habitats allowing for lower periodicity. *Orchestia gammarellus* expressed a more variable circadian rhythmicity with respect to *T. saltator*, as was expected for a population colonising a less limiting environment (roots and damp) compared with a sandy beach. In this case, impacts leading to habitat loss will have an effect in terms of retreating populations. Previous studies showed similar relationships between habitat preferences and the expression of circadian/circatidal rhythmicity (Rossano et al., 2008). Differences in the expression of circadian rhythmicity related to the presence/absence of stranded detritus and the possibility of finding protection against dehydration were observed also within the same species (Nardi et al., 2003; Rossano et al., 2009). Clearer conclusions can be drawn for the S. Rossore beach (Natural park of Migliarino-S. Rossore, Pisa, Italy; Rossano, unpublished data, 2001). That site had a beach reduced to a few meters as it was subjected to strong erosion, for the prevention of which the park managers had covered the beach with scattered branches and treetrunks that were used by talitrids as refuge against dehydration. Here slightly more precise and defined rhythms were observed for *T. saltator* (N=22, SNR=0.567) compared to the present data regarding the Maremma Park. However, a higher precision was observed for the same species on the beach of Castiglione della Pescaia (Grosseto, Italy; Rossano, unpublished data, 2001), a stable beach characterized by the absence of debris and strong anthropogenic impacts (N=26, SNR=0.746). A recent study, still in progress, on the rhythms of *T. saltator* from the same three beach points at Collelungo considered in the orientation experiments, seems to indicate that there are no significant differences in the mean periods of the three populations, implying that different morphodynamics are not enough to induce an adaptive divergence of this behavioural trait in the same species (Nourisson et al., unpublished data, 2011-2012). The observed trends regarding the precision of the rhythms suggest that also within the same species different sources of stress with synergic effects may cause behavioural and short-term specific responses. However, more case studies and comparisons are needed to draw a gradient of stressing factors that can be displayed in a hierarchy of strength in affecting rhythmic animal behaviour. Based on the data up to now, the use of rhythms as early warning indicators is not suggested, due to the excessive individual variability expressed by the activity rhythms in these populations.

The acceleration of the erosion dynamics described for the Maremma Park littoral (Pranzini, 2001) is likely to have produced both a short and long-term response of the beach animal community and different kinds of bioindicators would be required to follow the shoreline evolution in the medium- and longterm. The arthropods' biodiversity was analysed at the same site as a candidate indicator for the long-term effects, due to the primary role of these animals on sandy beaches and their strict link with their home range, which make them subject to symptomatic variations in case of significant alterations (Colombini et al., 2006). The time-scale for observing such shifts in the arthropods' community is of many months, or even years.

At Oued Laou and Ka'asrass the impacts on the beach were clearly different on the two sides of the river mouth. The buildings on the left side beach have probably affected the supralittoral dynamics as the aeolic transportation of substrate has been impeded by the houses built on the former dune, and the supply of sediments from the land is limited (Oueslati, 2006), while the higher number of people trampling the right side beach during the summer season, where the Municipality allows free camping on the beach, did not apparently affect orientation. The seasonality in beach use in this case occurs in July-August, i.e., outside the reproductive peaks of talitrids and in a period when individuals limit their activities to overcome the harshness of the habitat; thus, a minor effect of trampling on behaviour development is to be expected (Fanini et al., 2006). In this case, orientation tests detected an ongoing impact on the left side of the beach. All those impacts having direct or indirect effects on the physical environment an be monitored via behavioural assays. Impacts on the beach may derive from actions occurring outside the beach itself, like dam construction, river bed modification, hard structures (e.g., groynes, ports) or urbanization of the area. Such impacts are irreversible and are acting continuously, causing changes in substrate characteristics and limiting the availability of a habitat safe zone. The scale of the monitoring should be therefore extended, via tests on the supralittoral, to obtain information on the whole zone involved in energy and material exchange (Littoral Active Zone, according to Tinley, 1985). However, for a correct bioassay planning and interpretation of the data, a wider perspective, including behaviour, ecology, geomorphology and socio-economy (in terms of land use) is required. The behavioural response of resident sandhopper populations to human pulse impacts (like the case of trampling on the supralittoral) becomes relevant and can be distinguished from experimental noise only when the natural settings are stable. Morphometric features and fluctuating asymmetry were shown to be good indicators of stress, but only for those impacts occurring during a sensitive phase of the sandhoppers' life-cycle (Barca-Bravo et al., 2010; Ottaviano & Scapini, 2010). However, behavioural assays may provide a continuous updating of information, and are suitable for short- and medium-term monitoring of impacts (consistently with the periods of the sandhopper life-cycle, Marques et al., 2003). Comparing the indication capability of various features of the same species, morphometric characteristics were shown to be suitable for monitoring time- and space-limited impacts, while behaviour expresses integrated information related to the whole ecosystem throughout the life-span of the individuals. Contrary to human perception, which tends to identify impacts with an increased and visible human action (e.g., the increased number of tourists on a beach, buildings and infrastructures), animal behaviour integrates various actions and factors throughout the generations of a resident population.

To answer the main question of the paper, whether the behavioural adaptation of a key sandy beach species may indicate ongoing changes, we found that different behaviours may indicate different phenomena. Regarding the two behaviours analysed in this study, activity rhythms and sun orientation, the former appears to be more linked to the life style of the species, while the latter strongly depends on the physical characteristics of the beach. Orientation integrates motility, plasticity and rhythms; therefore orientation tests, analysed at population level, may provide information finely tuned to the environment. Other simpler behaviours such as substrate choice are more conservative, thus not suitable as bioindicators of ngoing trends (Fanini et al., 2012). Finally, orientation behaviour would appear to be a suitable bioindicator of environmental impacts.

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3.2 Case study 1: the Collelungo beach, Italy

The beach of the Italian study site is a well-studied environment, located in the Maremma Regional Park, a more than thirty years-old natural park, which is also the first protected area of Tuscany, extending on 89 km^2, with a littoral zone of about 20 km (Cassar et al. 2007). Here numerous researches have been carried out since a long-time, with the production of rich data-sets on the most disparate subjects, from botanical, through faunistical (both vertebrate and invertebrate) to archeological ones (Scapini and Nardi 2007). Such an availability of records, though sometimes difficult to obtain, represents a rare chance for scientists, which generally have to face a lack of long-term information, particularly concerning sandy beach ecosystems (Carpenter 2008; Schlacher et al. 2008; Defeo et al 2009). Here, in 2010 a hard engineering intervention was realized to the left of the Ombrone River mouth, to reduce the erosion acting on this beach sector, the causes of which are ascribable to several, long-term changes along the river course, reducing its sedimentary imputs to the coastal balance (Pranzini 2001; Ciampi 2007). A 1200 m seawall was realized parallel to the shoreline, from the river mouth to south-east, using cyclopean boulders. This offered an opportunity to evaluate the ecological effects of this kind of works in the best possible condition, a pristine environment with a reduced and controlled human disturbance. New experiments and samplings have been planned following the methods of the previous ones, to compare results from before and after the intervention. In particular, in 2002-2003 a field survey had been carried out along the beach, in which macrofaunal samplings had been made (Colombini et al. 2006) together with orientation experiments on *Talitrus saltator* (Scapini et al. 2005). Both samplings and experiments were repeated during this thesis work, to make a temporal comparison. We analyzed the sun orientation in parallel with findings on macrofaunal community, to evaluate the behaviour of *T. saltator* as an early warning bioindicator. Our working hypothesis was that, if the seawall reduced the shoreline erosion in the N-W beach sector, an increased accretion might be observed in the S-E ones; in this case, we expect that sun orientation of sandhoppers improves in the stabilized sctors and worsens in the changing ones, while the macrofaunal diversity should increase in the accreting zones, taking advantage of the major habitat availability.The main outputs from this study are described in the following three sections, while the discussion of the findings is at the end of the chapter, in section 3.4, after the description of the Portuguese case study.

3.2.1 Circadian rhythms in three *Talitrus saltator* (Crustacea, Amphipoda) sub-populations from an Italian beach with varying morphodynamics. A possible monitoring tool of shoreline stability.

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Abstract

The sandhoppers *Talitrus saltator* (Montagu) (Crustacea, Amphipoda), a widespread species inhabiting the sandy beaches of most Mediterranean and eastern Atlantic coasts, are known for their clear circadian rhythms of locomotor activity, entrained by periodic natural variables, permitting a temporal adaptation to the environment. In the Maremma Natural Park (Grosseto, Italy) three sub-populations from a sandy beach with different sedimentary dynamics along the littoral (from a severe erosion to a gradual accretion), were studied to analyze intra- and inter-population variation of the endogenous locomotor rhythms. The recordings were done using individual chambers for 21 days in constant darkness and controlled temperature and humidity conditions. The variation of the free-running period was studied at individual and population levels, and the dependence from intrinsic and extrinsic variables was analysed. Significant differences resulted in rhythm expression among the three sub-populations, in term of duration and definition of the circadian period, and a seasonal variation was also highlighted. The observed differences among the tested sub-populations may represent an adaptation to the coastline dynamics and may be used as a tool to evaluate the environment stability/variability on the short temporal scale, together with other behavioural traits, such as zonation and orientation.

Keywords: *Talitrus saltator*, Amphipoda, endogenous rhythms, bioindicators, sandy beaches.

Regional terms: Europe, Italy, Tuscany, Collelungo beach: 42° 40' 27" N; 11° 5' 59" E.

1. Introduction

The amphipods *Talitrus saltator* (Montagu, 1808) are well known in literature for their nightly activity and circadian rhythmicity (Bregazzi and Naylor, 1972; Williams, 1980) and for the variation of the patterns of activity depending on the environment colonized (Rossano et al., 2008). The adaptation to the light-dark cycle allows to avoid dehydration during the driest and hottest daily hours and can be observed in most of the mobile macrofauna species living on sandy beaches (Audisio, 2002). In talitrid amphipods, the variability in the expression of the locomotor endogenous rhythms may depend both on the latitude and local ecological conditions (Williams, 1980; Nasri-Ammar and Morgan, 2006; Rossano et al., 2008, 2009). The studies about locomotor activity rhythms highlighted a high variability between and within populations, during the different life-stage and throughout seasons (Williams, 1983; Edward and Naylor, 1987; Nardi et al., 2003). Among the main driving forces there are the meteorological factors, as demonstrated by comparing Italian and Tunisian populations from beaches with similar morphology: a better definition of the activity rhythms was observed in spring-summer and in winter, respectively, likely due to the extreme conditions of the North-African shores during summer, inducing a shift in the amphipods' activity to the mildest winter season (Scapini et al., 2005b; Nasri-Ammar and Morgan, 2006; Ugolini et al., 2007). Also the wrack presence and its distribution may influence the circadian rhythmicity, by offering a shelter during the daytime hours, so that on beaches with high detritus concentration a perfect synchronization of the activity to the night time is not required (Nardi et al., 2003). It was not yet investigated if also geomorphological dynamics of the coast may influence the locomotor activity rhythm expression, even if it is presumable that the coastal dynamics, by acting on the habitat suitability and safeness, can be responsible for deviant behaviours when the coastal stability is endangered. Bouslama et al. (2011) studied the behavioural response of *T. saltator* in two beaches differing in physical, geomorphological, and landscape characteristics and observed significant differences in the circadian period and rhythmicity rate between the two populations, with more defined circadian rhythms in the less impacted beach. Behaviour was already proposed as an early warning bioindicator (Gerhardt, 1996; Scapini, 2002) due to its variation as first adaptive response after habitat modification. For example, Sallam et al. (2006) proposed barnacles cirral activity pattern as a bioindicator for the presence of heavy metals in the water, being their rhythmicity directly and proportionally influenced by water copper concentrations at 24 and 96 hours, and Dissanayake et al. (2010) demonstrated on *Carcinus maenas* that ecologically-relevant behavioural observations may serve as valuable indicators of environmental quality, more than observations at cellular or physiological levels. Mass-mortality of animal populations or their disappearance may be a further effect of habitat modification, but at this point of the process the community may have been already strongly changed and the process itself may be irreversible. The coastal morphodynamics in the central and southern Tuscany is well documented (Maremma Natural Park, Pranzini, 2001; Ciampi, 2007) and a situation of dynamic equilibrium can be observed deriving from an eroded beach section at the left side of the Ombrone River mouth, evolving in an accreting section a few kilometres further south of the river mouth. This coastal area, thanks to the geomorphological studies performed at both sides of the river mouth, was the occasion to check the hypothesis that erosive phenomena or coastal changing can act as a perturbation for the rhythmic behaviour of beach animals. The specific origins of this perturbation can derive from habitat loss, changing in exposure or in debris deposition. Ketmaier et al. (2010) had identified a genetic variation along this extended beach through mtDNA polymorphism analysis distinguishing sub-populations of the sandhopper *Talitrus saltator* at distances of about 1 Km one from the other. In this study the activity rhythm of these sub-populations subjected to different morphodynamics were analysed.

2. Materials and methods

2.1. Study site

The beach of Collelungo, within the Maremma Natural Park (Grosseto, Italy, 42°39'33"N, 11°00'48"E) extends a few kilometres to the south of the Ombrone River mouth and is colonised by a rich fauna of vertebrates and invertebrates, among which an abundant population of the amphipod *Talitrus saltator*. Ongoing geomorphological and sedimentological studies document the presence of a strong erosive gradient from the river delta to about 3-4 Km to the south of the mouth, where an accretion area has started involving the next 2-3 Km of the beach. On this littoral, depending on the availability of sandhoppers (Ketmaier et al., 2010), three stations were selected from the river delta to collect the samples for the present study: at 4000 m (col4000, 42°38'26"N, 11°03'28"E), 5000 m (col5000, 42°38'10"N, 11°04'08"E) and 6000 m (col6000, 42°37'50"N, 11°04'45"E).

2.2. Experimental protocol

Samples of freshly collected adult amphipods (size >8 mm, supposed to be sexually mature according to Williams, 1978 and Marques et al., 2003, and big enough to be detected by the sensor) were collected in April, June, July and November 2011 at the above described three stations and, within a few hours, were transferred to the Department of Biology (University of Florence) in sealed containers. Each sandhopper was individually housed in a cylindrical recording chamber (12 cm in diameter, 16 cm high) of opaque PVC, in a temperature controlled room (Scapini et al., 2005b). In each chamber a 5 cm layer of humid sand freshly collected from the beach of origin was disposed, with “Tetramin” dry fish food provided *ad libitum* on a piece of untreated filter paper. In all the recording chambers an infrared beam, connected to a logger, spanned the radius across a small bridge, recording the interruptions due to the animal activity and downloading them on a personal computer every 20 minutes (apparatus and software had been provided by the workshop of the School of Biosciences of the University of Birmingham, UK). The recording sessions lasted 21 days under continuous darkness and at a constant temperature of 18±1°C, to produce free-running conditions for the assessment of the endogenous component of the locomotor activity rhythm (Aschoff, 1960). In each session a balanced number of sandhoppers from each station was placed in the 54 available recording chambers, with approximately 18 individuals from each sub-population. After 21 days of each recording session, the chambers were checked for the survival of the animals and for the potential presence of parasites or new-borns in the brood-pouches of the females (conditions that may affect the activity rhythm); the sandhoppers were then put in separate tubes with 75% alcohol, to establish the sex, the cephalic length (proportional to body length, Marques and Anastacio, 2002) and the number of articles of the second right antenna as proxy of age (Marques and Anastacio, 2002). A check of the sample structure was necessary for the interpretation of rhythm results; features linked to age were taken into account as it was observed in other amphipod species that the age can influence the activity pattern (Kennedy et al., 2000).

2.3. Data analyses

The data on the locomotor activity pattern of the single individuals were graphically represented for a first analysis in the form of actograms, using the software Chart 35

(provided by D.D. Green, Birmingham, UK). The activity was double plotted, showing two consecutive days (48 hours) in the two adjacent columns, to emphasize the continuity of the activity, and the 21 days of recording on the rows. The activity was expressed as a percentage of the maximum recorded each day. The dashed bars at the top of the actograms represent the light–dark period in nature on the first day of the experiment. Among the analysed animals, only those showing activity at least for 15 of the 21 days of each session were considered active and on these the following analyses of the period characteristics were performed.
The time series of the active animals were analysed with the periodogram technique, based on the Whittaker periodogram (Whittaker and Robinson, 1924), using a Time Series software package (Gerard Harris Computing, Rockwell Park, Bristol), and the circadian period (τ) and the definition of each individual were thus calculated. The circadian periods were calculated in a range from 10h 20' to 30h 20', and the confidence levels were obtained using the formula of Dorrscheidt & Beck (1975), modified by Harris and Morgan (1983). The definition of the period was reported as SNR (Signal to Noise Ratio), the ratio between the signal (correlation ratio) and the 95% probability line. All periods considered in the results resulted significant at the 95% level.

Table 1. Morphological features of the samples tested in the four months (April, June, July, November) and three stations (col4000, col5000, col6000)

April	N	survival %	Fm	Fi	M	sex ratio	ceph. mean	min max	ant. mean	min max
col4000	8	100	6	2	0		1.48±0.03	1.4-1.6	23±0	21-25
col5000	9	100	3	2	3		1.55±0.05	1.3-1.8	26±1	21-33
col6000	6	83	3	0	2		1.56±0.08	1.3-1.8	27±2	22-30
total	23	96	12 (57%)	4 (19%)	5 (24%)	0.31				
June										
col4000	12	92	1	1	6		1.35±0.07	0.9-1.6	25±1	19-28
col5000	15	80	1	2	5		1.49±0.07	1.2-1.8	23±1	19-27
col6000	12	83	1	0	5		1.37±0.10	1.2-1.8	23±2	18-30
total	39	85	3 (13%)	3 (13%)	16 (74%)	2.7				
July										
col4000	16	94	1	5	6		1.18±0.05	0.9-1.5	25±1	16-30
col5000	20	90	2	10	4		1.22±0.03***	1.0-1.6	23±1	19-28
col6000	19	100	0	8	4		1.29±0.04	1.1-1.5	24±1	20-29
total	55	95	3 (8%)	23 (58%)	14 (34%)	0.54				
November										
col4000	18	78	0	6	6		1.14±0.06**	0.9-1.5	21±2	15-31
col5000	18	83	0	7	5		1.25±0.07	0.8-1.6	24±1	19-32
col6000	19	89	0	8	6		1.32±0.05	1.0-1.6	25±1	19-30
Total	55	84	0 (0%)	21 (55%)	17 (45%)	0.81				

The specimens reported are those considered in the analysis.: Fm, mature females; Fi, immature females; M, males. Ceph. mean, mean of cephalic lengths in millimetres; ant. mean, mean of antennal articles. Standard errors are reported. * Significant differences within the same sub-population and among seasons. ° Significant differences within the same season and among sub-populations. */° = 0.05; **/°° = 0.01; ***/°°° = 0.001 Data sets were compared with Kruskall – Wallis rank sum test.

3. Results

The mean temperatures and humidities recorded during the months of the experiments (National Meteorological Archives, 2011), were similar to the temperature of 18 ± 1°C maintained within the recording chambers, while the humidities in the chambers were higher, between 87 and 90%. These values assured a good survival to the amphipods and confirmed a sufficiently good reproduction of the environmental conditions.A total number of 179 sandhoppers was tested and a high percentage of individuals survived throughout the recording sessions (Table 1) thus indicating a good fitness of the animals. However, some data were excluded from the analysis because of sand accumulation in front of the receiver (likely causing biased results) or the death of the animals without any significant activity expressed. Consequently the analysed percentage of specimens ranged from 58% to 100% of the samples (Table 2). An analysis was performed on intrinsic factors (e.g., sex and age) to highlight eventual differences in development within the sub-populations through the seasons or among the sub-populations during the same season. An inspection of the morphometric features of the tested samples was necessary to link the locomotor activity rhythm features to the sub-population features or/and to the environmental dynamics. All the tested individuals were adult *Talitrus saltator*, and the morphometric analysis showed that there was some seasonal and spatial variation among the stations (Table 1). Being the cephalic length proportional to the body length (Marques and Anastacio, 2002), the morphometric data showed that at col4000 and col5000 individuals were on the average significantly smaller at the end of the summer (November and July, respectively) compared to the other months at the same stations (Table 1).

Table 2. Summary of data for survival, number of circadian individuals, mean period (τ) and definition (SNR) for the four experiments performed in April, June, July and November 2011 at the three stations (col4000, col5000, col6000)

Subpop	N	Analyz.%	active	active%	circ	circ%	mean τ	mean SNR
April								
col4000	8	100	8	100	7	88	24h26'±7'	0.408±0.100
col5000	9	89	8	100	6	75	24h30'±18'	0.166±0.036
col6000	6	83	4	80	4	100	23h55'±31'	0.291±0.105
Total	23	91	20	95	17	85**	24h20'±11'	0.295±0.055
June								
col4000	12	67	4	50	4	100	23h55'±5'	0.372±0.041
col5000	15	60	7	78	4	57°	24h30'±13'	0.736±0.165
col6000	12	58	6	86	4	67	23h55'±26'	0.163±0.067
Total	39	62	17	71	12	71***	24h07'±10'	0.424±0.102
July								
col4000	16	75	7	58	7	100	24h57'±18'	0.436±0.155
col5000	20	80	11	69	11	100	24h25'±10'	0.622±0.138
col6000	19	63	10	83	10	100	24h06'±8'	0.735±0.151
Total	55	73	28	70*	28	100	24h26'±07'	0.616±0.085
November								
col4000	18	67	8	67	8	100	24h10'±50'	0.158±0.038
col5000	18	67	5	42°	5	100	24h12'±21'	0.182±0.036
col6000	19	79	8	53	8	100	24h20'±11'	0.334±0.059
Total	55	71	21	54**	21	100	24h14'±19'	0.230±0.032

* Significant differences among seasons. ° Significant differences among seasons within the same sub-population.*/° = 0.05; **/°° = 0.01; ***/°°° = 0.001, Fisher exact test.

The comparisons among the sub-populations within the same season indicated that the animals at col4000 were a little smaller than at the other stations, and the size difference was more relevant in April, when the number of articles of the second antenna in the col4000 sub-population was significantly lower than in the other two sub-populations (Table 1), thus revealing a population with younger individuals (less moulting events). In addition, the presence of a high percentage of mature females compared to immature females and males in April (Table 1) demonstrates that the April experiment was performed during the beginning of the reproductive season. The ratio between mature and immature females and that between females and males confirmed the end of the reproductive season before November (Table 1). An overall exploration of the activity data seasonally grouped showed a significantly lower activity in November (Fisher's exact test, $p = 0.007$), higher activity in June (Fisher's exact test, $p = 0.0443$) and July (Fisher's exact test not significant) and the highest activity in April (Fisher's exact test not significant,Table 2). Also the comparison of the number of periodic individuals resulted significant, with lower percentages in June (Fisher's exact test, $p = 0.001$), and April (Fisher's exact test, $p = 0.0218$) and the two highest figures in July and November (100%). By discriminating the data for sub-populations, a high percentage of specimens was active from April to July at stations col5000 and col6000, while the activity decreased in November, even if no seasonal comparison was significant except for the low proportion of active individuals at col5000 in November (Table 2, Fisher's exact test, $p = 0.0472$). At station col4000 the number of active animals was higher in April compared to the other stations, while it was lower and rather constant throughout the summer and autumn (Table 2). The percentage of individuals showing circadian periodicity was high during all the months, except for June at col5000 (Fisher's exact test, $p = 0.0486$) and col6000.

In Fig. 1 three actograms are shown, one for each season, selected to illustrate the range of variability exhibited by the individuals. In Fig. 1a the locomotor activity of an adult *T. saltator* (col4000 sub-population, tested in November) showed the most variable pattern with a quite dispersed activity characterised by some bursts of activity in the subjective day and a main nocturnal activity. Some interruptions occurred (days 5-7) and changes in the slope of the sequence, meaning changes in the period expression that indeed had a low definition (τ = 25h40’, SNR = 0.101). In Fig. 1b the activity of another specimen (col4000, tested in July) is an example of higher periodicity and period definition (τ = 25h, SNR = 1.009), but again the pattern was not constant throughout the recording session because of a prolonged activity during the first week and an interruption at the 14th day. In this case a period slightly longer than 24 hours was observed, producing a constant and gradual shift with respect to the actual light/dark regimen at the day of the sampling. In Fig. 1c, the pattern of an individual (col6000, tested in July) represents an example of a very well defined rhythm (τ = 24h20’, SNR = 1.659): this specimen was active almost every day, with a clear circadian rhythm and no bursts of activity separated from the main body of the activity. Here also a slight shift is visible, resulting in a shorter period compared to the other two examples. In the four months of the experiments, the mean circadian periods slightly varied around the 24 hours, but without a clear general trend: the shortest mean period was observed in June with 24h07'±10' and the longest in July with 24h 26'±07' and no significant difference was observed among the months. A more detailed analysis was performed considering the sub-populations in each month (according to what is known from the literature concerning seasonal variations of the amphipods rhythm, Nardi et al., 2003). The shortest period was observed at col6000 in April (23h 55’± 31’, N = 4, Table 2, Fig. 2), the longest at col4000 in July (24h 57’± 18’, N = 7, Table 2, Fig. 2). No clear gradient among the stations was observed except in July, when the period at col4000 was appreciably the longest, at col5000 intermediate and at col6000 the

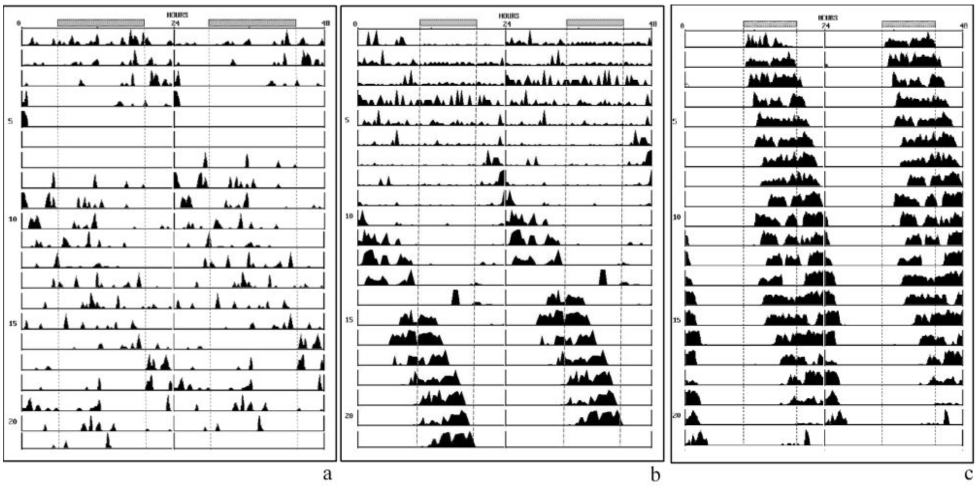

Fig. 1. Examples of three actograms to show the different patterns exhibited by the specimens tested. In (a) specimen from col4000 tested in November, τ = 25h40’, SNR = 0.101, (b) specimen from col4000 tested in July, τ = 25h, SNR = 1.009, (c) specimen from col6000 tested in July, τ = 24h20’, SNR = 1.659.

shortest. On the other hand, clear differences appeared among the months and stations regarding the mean definition of the period, which increased from April to July (April: 0.295±0.055; June: 0.424±0.102; July: 0.616±0.085, Fig. 3) and became lower again in November (0.230±0.032), with a significant difference among the months (Kruskal-Wallis rank sum test, chi-square = 11.6958, df = 3, *p* = 0.0085). Discriminating among the sub-populations, the minimum value of period definition was observed for col4000 in November (0.158 ± 0.038, N = 8, Table 2, Fig. 3) and the highest for col5000 in June (0.736 ± 0.165, N = 4, Table 2, Fig. 3). Two sub-populations reproduced the general trend of having a better defined rhythm in summer and a less defined rhythm in spring and autumn (see also Nardi et al., 2003), while the sub-population of col6000 showed the anomaly of having a very low definition in June (Fig. 3).

Comparisons were done for period features (period and definition) among sub-populations within each month. In April and June no significant differences were highlighted. In July the period at col4000 resulted significantly longer than the period at col6000 (Kruskal-Wallis

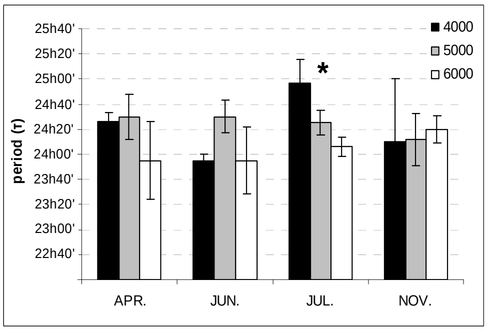

Fig. 2. Mean periods (in hours) and standard errors for the three stations col4000 (black), col5000 (gray) and col6000 (white) in the four months of April, June, July and November.

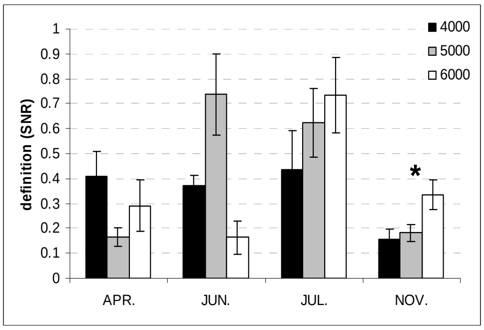

Fig. 3. Mean definition and standard errors for the three stations col4000 (black), col5000 (gray) and col6000 (white) in the four months of April, June, July and November.

rank sum test, chi-square = 6.5451, df = 2, *p* = 0.0379), with the period at col5000 positioned between these two (Fig. 2). In November the definition of the period at col4000 and col 5000 was significantly lower than col6000 (Kruskal-Wallis rank sum test, chi-square = 6.8648, df = 2, *p* = 0.0323), thus confirming an increase of definition from col4000 to col6000 (Fig. 3), a pattern that was observed also in July.

In Fig. 4 the SNR was plotted against the period and, despite the low number of individuals that were periodic in April and June, a typical seasonal pattern was recognizable, with the distributions showing a cloudy shape in April and November with the cloud of points dispersed along the y-axis, showing a flattened shape, and vice versa showing a ‘tower’ distribution in June and July. This result derived from the high definition of the period of few individuals around the mean in the hottest summer months, and a lower definition and more disperse distributions in the other two months corresponding to spring and autumn.

Comparisons were made for the frequencies of active and periodic animals between males, mature and immature females, without detecting differences except in July, when, by grouping all the three sub-populations, males appeared the most active (79%, N = 14), immature females a little less (74%, N = 23) and mature females not active at all (0%, N = 3) (Fisher's exact test, *p* = 0.0457).

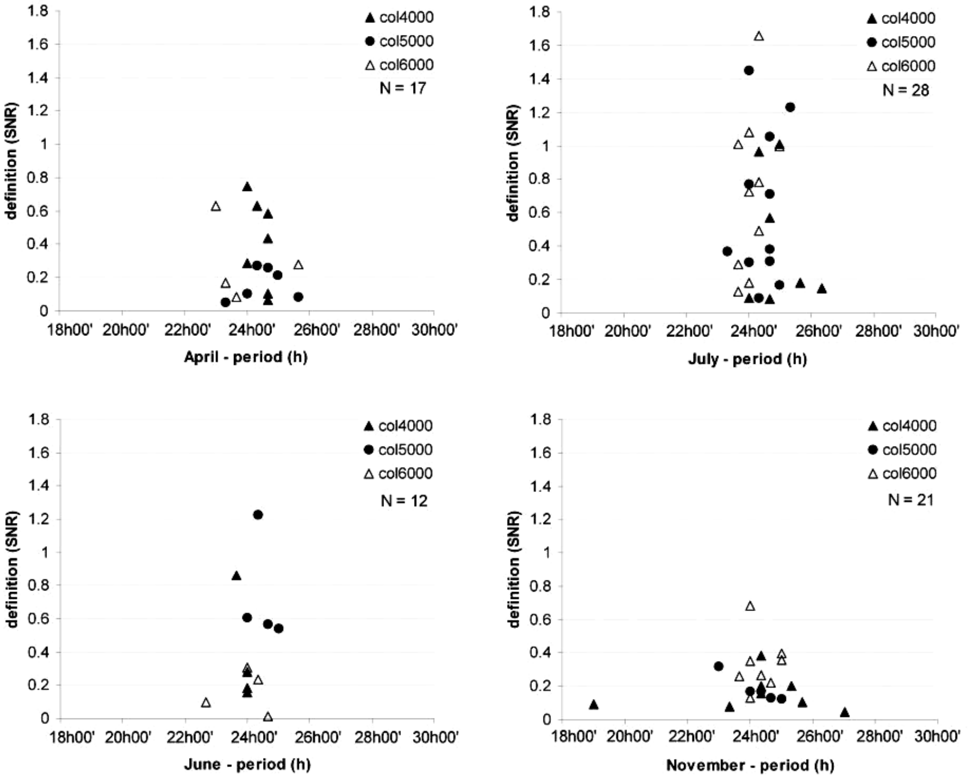

Fig. 4. X/Y plots of definition over period in the four months of experiment for the three stations, col4000 (black triangles), col5000 (black dots), col6000 (white triangles). The period is expressed in hours, the total number of the individuals tested is reported (N).

4. Discussion

The circadian endogenous rhythm of locomotor activity permitted a detailed comparison of populations from the three sites throughout the four months of experiments, in three different seasons. Consistently with the nocturnal pattern described by several authors (Bregazzi and Naylor, 1972; Williams, 1980, 1995), the first evidence was that the expression of the endogenous activity rhythm was very variable both between individuals and within the life-span. Activity rhythms changed in period and definition from April to November, in accordance with the previous observations by Nardi et al. (2003) on populations from the Thyrrenian coast. The activity pattern of individuals was clearly circadian for the whole registration session (Fig. 1c) or close to arrhythmicity (Fig. 1a), since the pattern changed many times during the registration, thought the test conditions were the same for all specimens and for all the 21 days. Some individuals, representing a small minority of the samples, were active but arrhythmic (not shown): again, a considerable behavioural plasticity emerged as one of the main features of beach animals, adaptability having a major role in the survival in extreme but changing environments like sandy beaches (Brown, 1996).

An interesting result was the significant difference observed in July among individuals of different reproductive phase, when the males and the great majority of immature females were active, while mature females did not show any activity at all. This may be a consequence of the different strategies for the egg-bearing gravid females with respect to the immature ones during the hottest season. The former may avoid dehydration and wave inundation by remaining buried in the wrack, so that for them a defined locomotor activity may not be strictly necessary, while the latter need to reach the foraging zone precisely during night hours to avoid extreme day conditions and recover before temperature and humidity reach dangerous levels. Anyway, the low numbers of individuals used in the present research do not guarantee solid results to discriminate activity rhythm based on sex. It would therefore be recommended to develop an ad hoc protocol to produce a more solid study in that direction. Even more interesting is the fact that in summer high percentages of active animals were observed and, among these, high percentages expressed circadian rhythmicity, while in spring and autumn several specimens were not active (November) and several active specimens were not circadian (April). As already discussed by Nardi et al. (2003) these differences in activity rhythms are clearly adaptive, as in summer the dehydration risk associated to the daily hours reaches its maximum and a high precision of the activity timing is required, while an increase of individual variability is allowed under mildest climatic conditions in the other seasons. On the other hand, no differences were observed in the numbers of active or rhythmic sandhoppers between the three stations, despite their differing sedimentary dynamics. The most informative features were instead the period and its definition that represented the most clear and interesting results of this work. About the circadian period, most of the free-running individuals had periods longer than 24 hours, even if a certain variability was always present. A significantly longer period was observed in July at col4000, decreasing as in a gradient, through col5000 toward col6000. This trend was finely joined to the increasing definition in the same direction (from col4000 to col6000) observed in July, becoming significant in November, thus indicating a less precise rhythmic behaviour at col4000 as compared to the other two stations. The more pronounced erosion at col4000 likely originated a more chaotic environment, with higher slope and frequent changing of the beach width that is generally shorter than in the other stations (personal observations on field; Colombini et al., 2012). The situation described here was comparable to the situation observed during winter months, when, responding to the changing environment, the locomotor activity rhythm is depressed, i.e. less defined. What is not clear is whether the observed rhythmic behavioural response is the direct consequence of the colonization of a variable environment or, according to the source-sink hypothesis (source-sink model, Rounge et al., 2006), it is the expression of the activity pattern of a sub-population coming from stations presenting more constant conditions along the same beach and colonizing a more disturbed environment. As a matter of fact the results of the morphometric measurements (intrinsic features of the individuals) seemed to separate the sub-population of col4000 from the other two, showing a different population structure: higher numbers of juvenile individuals occurred in April and November and elder individuals were present in June and July, not matching what was found in previous studies (Marques et al., 2003; Pavesi and De Matthaeis, 2009). For this beach an usual trend that presents bursts of juvenile individuals from March to September (spring and summer) and a prevalence of adults during the other months was shown in previous studies (Marques et al., 2003; Pavesi and De Matthaeis, 2009). On the other hand, Scapini et al. (2005a) stated that, due to beach erosion, one kilometer further from col4000 towards the river mouth direction the population was disappearing and col4000 was consequently the last point well colonized by *T. saltator*. Following that observation the sub-population col4000, with its smaller and younger individuals, may represent a sink and col5000 and col6000 a source. Even if our protocol was not produced for a study on population structure and abundances and an ad hoc protocol should be applied to confirm the trends observed here (Marques et al., 2003; Colombini et al., 2012) the observations may help to focus on the real dynamics in terms of colonization and behavioural changes occurring on the studied sandy beach.

Both previous data (Nardi et al., 2003) and these ones (see total monthly means of period and definition, Table 2, and plots of definition against period, Fig. 4) suggest that the rhythms and other behaviours of a so diffuse and sensitive taxon like Talitridae are in accordance with an on-going process of colonization of (or retirement from) an harsh habitat and can be used as an early-warning bioindicator of the beach ecosystem dynamics. On this coast, among all the seasons, the summer represents the most stressing one for the high temperatures and low humidity. The consequence is therefore the expression of a clearer circadian rhythm in summer as compared to the other seasons. Summer can so be chosen as the most suitable season to use locomotor activity rhythm and its deviation from the usual patterns of activity as a bioindicator of environmental perturbation, stress and/or instability of the ecosystem. The advantages in using activity rhythms as bioindicator derive from i) the reproducibility of the measurements that are done in laboratory, ii) the use of few individuals (recommended in endangered ecosystems and in natural parks). Moreover iii) behaviour is a synthetic bioindicator resuming the state of the ecosystem in responses that are the expression of the adaptation of living systems to the environment itself. We suggest that in the other seasons (here, the months April and November, with less severe environmental conditions) the intra-specific variability of the circadian activity rhythms of *T. saltator* is in itself so high that it has the effect of masking any other feature depending on the environment. In meteorologically variable conditions (winter, early spring or late autumn), the differences in sedimentary dynamics (if not enough pronounced) may be not able to produce adaptive divergences in the different sub-populations.

A similar decrease in the rhythm definition during the winter months was already described in a number of intertidal animals (Atkinson and Parsons, 1973; Holmström and Morgan, 1983; Bolt and Naylor, 1986; Cummings and Morgan, 2001) and was already described in a different Italian population of *T. saltator* by Scapini et al. (1992). In summer the short nights and the limiting daytime conditions induce the animals to synchronize their activity exactly to the night hours, while during the rest of the year such strict periodicity is not necessary.

Thus the locomotor activity rhythm matching to the environment in a way that reproduces its periodical changing (DeCoursey, 1983) has in addition a clear gradient of expression (less/more precise and defined) and should be considered in its variations. Even if activity rhythms are not directly linked to the direction of the waterline,their expression may depend on the modifications of the width of the beach (shortening/lengthening), the variation of slope (increasing/decreasing) and the presence of deposits of debris (deposition/removal), all factors that may produce increase/decrease of entropy and are linked to the erosion/deposition dynamics of the coastline.

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3.2.2 Seasonal variations in the orientation behaviour of *Talitrus saltatator* on a Mediterranean sandy beach: an ecological interpretation

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This manuscript was conceived when analyzing the data from the orientation experiments reported in section 3.2.3, as a noticeable dependence of subpopulations' performances was observed with respect to the seasons in which experiments were carried out. These findings throw light on the environmental effects acting on *T. saltator* behaviour and may furnish important information on the interpretation of orientation experiments when using them as impact bioindicator. As already observed in the section on the activity rhythm (section 3.2.1), also orientation was more precise during the summer, the hottest season, thus confirming this as the best season for the assessment of impacts via behavioural tests.

Abstract

The amphipod species *Talitrus saltator* (Montagu, 1808), widespread on the Mediterranean and Eastern Atlantic coasts, displays physiological and behavioural adaptations to life on sandy beaches. To avoid dehydration sandhoppers burrow in moist sand near water's edge during the daylight hours of the warmest seasons. They can navigate toward this safe zone (an adaptive behaviour named zonal recovery) using the sun compass and several additional mechanisms, both universal an local. In this study we analyzed the effects of several variables on the talitrids' orientation behaviour to deepen the relevance of the local cues and analyse the ecology of sandy beaches from an evolutionary perspective. For two years orientation experiments were made on a *T. saltator* population, at three sites of the same sandy beach, differing in shoreline sedimentary dynamics; the main environmental, climatic and individual variables known to influence talitrid orientation were registered during each experimental session. The statistics of circular distributions were calculated and a multiple regression analysis adapted to angular data was performed, to highlight the scaling triggered by environmental conditions. The results showed that seasonal climatic variables played a primary role, adaptively modifying the sandhoppers' behaviour. A significant interaction with the sites (differing sedimentary dynamics) was observed as well. An high behavioural plasticity seems to guarantee to *T. saltator* a good adaptation (both as abundance and geographical spreading) to life on sandy beaches, which are notably unstable environments.

KEYWORDS: *Talitrus saltator*, orientation, Mediterranean sandy beaches, seasonality, sedimentary dynamics.

1. INTRODUCTION

The sandhoppers *Talitrus saltator* Montagu, 1808 (Crustacea, Amphipoda, Talitridae) are abundant along the Mediterranean and the European and North-African Atlantic shores (BELLAN-SANTINI 1993), which makes the species suitable for studies on adaptive variations along geographical and climatic clines (e.g. MARQUES et al., 2003). These talitrids inhabit the supralittoral zone of sandy beaches, where the population features are determined mainly by physical constraints, with biotic interactions playing a minor role (MCLACHLAN & BROWN 2006). Despite being semi-terrestrial, sandhoppers are subject to dehydration risk, as they breathe through gills and have a partially permeable cuticle; this makes behavioural adaptations essential for their survival. As a rule, on Mediterranean sandy beaches talitrids move upshore toward the dune, on night, to forage on wracks and debris under conditions of higher air humidity and lower temperature, and they go downshore to the drift line at dawn, to burrow in moist sand at the water's edge (SCAPINI et al. 1992). The movement toward the shoreline to recover the optimal zone was defined zonal recovery (PARDI & ERCOLINI 1986). When displacements occur during the warm hours of the day, due to disturbance or unsuitable conditions (such as dry or moisture-saturated substrate), the dehydrating conditions motivate sandhoppers to escape seawards following the straightest path (TED, Theoretical Escape Direction). To succeed in this task, they rely on an innate sun compass, following a genetically determined direction that has been selected for most populations according to the local shoreline orientation (PARDI 1960; PARDI & SCAPINI 1983; SCAPINI et al. 1988). However, several other cues are used simultaneously, among which beach slope, differences in celestial radiance over land and sea, sky polarization, magnetic field and landscape view, all of which may enhance or interfer with solar orientation (ERCOLINI & SCAPINI 1974; HARTWICK 1976; ERCOLINI et al. 1983; UGOLINI et al. 2012). Such a redundancy of mechanisms was observed also for other crustaceans (ALTIZER & FORWARD 1993; CAMERON & FORWARD 1993; CHIUSSI & DÌAZ 2001), insects and spiders (COLLETT & COLLETT, 2000; MORSE 2002; BISCH-KNADEN & WEHNER 2003; WEHNER 2003, NØRGAARD et al. 2007). Also the ecological and morphological features of beaches are known to influence the behaviour of *T. saltator*, i.e. the rate of exposure to waves and tidal range (MEZZETTI et al. 1994; WILLIAMS 1995), the wrack abundance and distribution (BORGIOLI et al. 1999a; NARDI et al. 2003), the degree of stability of the shoreline (SCAPINI et al. 1995, BORGIOLI et al. 1999b; ELGTARI et al. 2000) and the seasonal climatic variations (WILLIAMS 1995; NARDI et al. 2003). According to these studies, talitrids express a high behavioural plasticity to survive on sandy beaches; natural selection may have determined the best direction for the solar orientation of each population, but it also may have maintained individual flexibility in the use of learned references and behaviour modulation (BROWN, 1996, MCLACHLAN & BROWN, 2006), permitting to sandhoppers to face both periodical, life-long lasting or sudden risks. About the periodical changes, many studies highlighted how macroinvertebrates' ecology and biological traits are substantially affected by seasonal variations. In temperate areas the main seasonal risk is represented by winter storms, which in their turn act on wave and tidal regimes (MCLACHLAN, 1988; MCLACHLAN & BROWN, 2006; MOREIRA et al., 2008), and hence on food availability. On Mediterranean shores, precipitation and temperature are the principal defining attributes showing seasonal patterns (ASCHMANN, 1973; GASITH & RESH, 1999), resulting in hot, dry summers and cool, wet winters. This pattern is the basis of several eco-ethological adaptations of sandhoppers, as the shift of circadian activity during the nocturnal hours, occurring particularly during the hottest, summer period (SCAPINI et al., 1992; NARDI et al., 2003, UGOLINI, 2003). Also the zonal recovery is generally more precise (concentrated towards the sea) under summer, dry conditions (SCAPINI et al., 2002; FANINI & SCAPINI, 2008), which is adaptive as in this season the dehydration risk become the highest. On the other side, also less predictable environmental changes may modify the talitrids' orientation, as a higher concentration seawards has been observed on beaches with a stable shoreline, compared to a major scattering on beaches where the shoreline is continuously changing direction (SCAPINI et al., 1995; BORGIOLI et al., 1999b; ELGTARI et al., 2000). In this study we investigated the orientation of *T. saltator* from an ecological perspective. We performed orientation experiments at three sites of the same extended beach, subject to different sedimentary dynamics (from an eroded to an accreting shoreline), and analyzed the orientation of the resident sub-populations (for the definition of sub-population in this context, see KETMAIER et al., 2010), aiming at: 1) assessing the effects of Mediterranean seasonality on the behaviour of a species that is widespread along a geographical cline encompassing a variety of sandy beaches; 2) understanding the role of local sedimentary dynamics on the observed behavioural differences among sub-populations.

2. METHODS

2.1 The study site

The Collelungo beach is a well-preserved sandy beach ecosystem, located in the Maremma Natural Park (Grosseto, Tuscany, Italy - 42°39'33''N, 11°0'48''E) and is an almost straight beach (Fig. 1a), extending about 6500 m on the left side of the Ombrone River mouth. The reduced sedimentary inputs from the river in the last century, ascribable to human actions along its course, have produced an erosive gradient along the beach (PRANZINI, 2001;

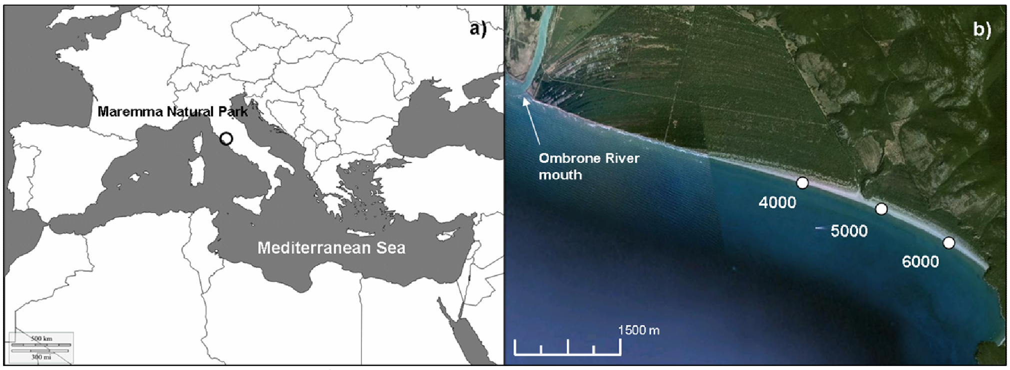

Fig. 1. The study site. a) Location of the Maremma Regional Natural Park (42°39′59.00″N, 11°05′36.30″E): Italy, Mediterranean Sea. b) The Collelungo beach, with the position and distance of the three experimental sites with respect to the Ombrone River mouth; photo credits for the right image: Google Earth, accessed May 2013.

Ciampi, 2010); a strong sand erosion acts close to the river mouth, decreasing with distance, until a condition of dynamic equilibrium is reached at about 5000 m from the river mouth. This sedimentary dynamics produces a gradual accretion in the last part of the beach. It is an exposed microtidal beach with a medium sand granulometry (Marques et al. 2003), and tide excursions never exceeding 0.40 m (Pranzini 2001). Three sites were chosen, differing in their erosion/accretion dynamics, at about 4000, 5000 and 6000 m from the river mouth (Fig. 1b). At point 4000 the dune was severely eroded and the beach was very narrow, while at point 5000 and 6000 the low fore-dune was stable, with a wide flat sandy beach. All three sites had vegetated dunes; at 5000 the beach was backed by a cliff, while at the 6000 site it was backed by marshes and hills. Orientation experiments were performed in spring, summer and autumn of 2011 and 2012. Experiments were not carried out in winter because in that season sandhoppers have a reduced activity and show very low densities (Scapini et al. 1992). At each session, the local environment was characterized determining the main physical features; beach width and slope were recorded at the three sites, along transects ranging from the first dune vegetation to the high tide mark and sand penetrability was measured (as a proxy for sand granulometry and moisture). In consideration of their possible effects on the orientation of *T. Saltator*, we measured air relative humidity and temperature (with an electronic thermohygrometer) and the global solar radiation (using a luxmeter), together with sun visibility, sky cover and shoreline direction, from which the TED (Theoretical Escape Direction seawards, the perpendicular to the seashore) was calculated.

2.2 Field experiments

In May (spring), July (summer) and September (autumn) 2011, and in May and October 2012 (spring and autumn, respectively), orientation experiments were performed at the three sites contemporaneously. Experiments were made on the field to test the effect of all, also unidentified, factors on the movements of animals, which may strongly influence the results obtained (Underwood & Chapman, 1985). Adult amphipods were caught in the morning before the tests and tested in experimental arenas having 72 pitfall traps of 5° each at their rim, as described by Scapini et al. (2005). A transparent screen covered the entire device and a white cardboard (10 cm height) was applied on it at alternate releases of groups of ten animals, to have one release with the landscape view allowed and the following one with the view screened off. For each experimental session at each site, we carried out four releases with the landscape view and four without it in the morning and we repeated the same protocol in the afternoon. After the tests, the angles of orientation to the North of trapped individuals were registered and the specimens were stored in alcohol 75% for later observations and measurements. In the laboratory, for each individual, the sex (male, female and juvenile, with no identifiable sexual characteristics) and the reproductive status (ovigerous, mature and immature females) were identified under the microscope. We also measured the cephalic lengths (as proxies of size) and counted the right antenna articles, as proxies of age (Williams 1983; Marques et al. 2003). All these intrinsic factors were considered as they may in some measure modify the individual behaviour, and thus resulting in a significant effect on the orientation performances.

2.3 Data analyses

The environmental variables of the three sites were compared using Kruskal-Wallis rank sum tests, while their mean values for 2011 and 2012 experiments were compared by two-sample Wilcoxon tests. Angular distributions of talitrids were displayed using the statistics for circular data (Fisher, 1993), applying an *ad hoc* library (Marchetti & Scapini, 2003) working in the S-plus Insightful software. The angles of orientation were plotted into circular distributions and probability density functions were estimated, smoothed with the kernel method, and double plotted on Cartesian graphs, to better show the peaks of the distributions. The mean vectors were calculated and the null hypothesis of uniform distribution was tested with Rayleigh's test at a P level <0.05. A multivariate analysis adapted to angular data was applied, by developing Spherically Projected Linear Models (SPLMs), to analyse the effects of all variables and factors on the orientation angles, the response variables (Scapini et al., 2002; Marchetti & Scapini, 2003). We considered the following potentially influencing variables: site (three sites), year (two years), season (three seasons), month (four months), time of the day (a.m./ p.m.), solar time (at each release), sun azimuth (at each release), air temperature, air relative humidity, landscape visibility (view allowed, not allowed), cloudiness (assessed on a conventional scale from 0/8 to 8/8), sun visibility (four levels, from visible to not visible), sex, cephalic length and number of antennal articles of the individuals. At the three sites, the shoreline orientation varied from one season to another less than 10° and, given that the intrinsic error of the orientation chamber is of 5°, this variable was considered constant over time and excluded from the analysis. In case of significant interactions between factors, different additive models differentiated by the interacting factor were developed. A best model was selected based on the smallest AIC (Akaike Information Criterion) and the significance of each variable was assessed by LRT (Likelihood Ratio Test), comparing the best model and the nested one without the variable being tested (Marchetti & Scapini, 2003).

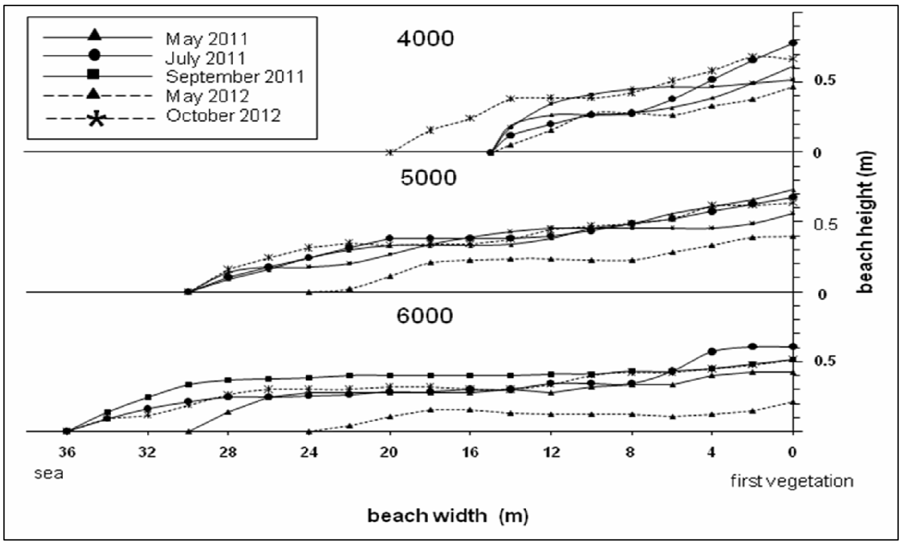

Figure 2. Beach profiles, as measured at the experimental sessions, from the sea level (on the left) to the first dune vegetation (on the right), for each one of the three sites.

3. RESULTS

3.1 Environmental features

The beach profiles (Fig. 2) clearly showed an erosion/accretion gradient along the shoreline, as the beach width measured 20 m at the 4000 site and 36 m at the 6000 one. The different erosive rate was also reflected in the beach slopes and widths at the three sites (Table 1), as the former were generally reducing from the 4000 to the 6000 site, while the latter increased. No significant differences were found concerning the environmental variables between the years (Wilcoxon test: $P > 0.05$), even between the sites (Kruskal-Wallis test: $P > 0.05$), but some observations can be made. The sand penetrability slightly decreased going further from the river mouth, with generally lower values in summer. Air temperatures were lower in spring in both the years, while the highest humidity was observed in July 2011. In both years the mean solar radiation was lowest in autumn (September and October), when it also showed the highest variability, linked to sky cloudiness. This one was very low in May in both years (sky cover close to 0/8), exceeded 3/8 in July 2011 and was around 2/8 in September 2011 and October 2012 (Fig.3a). In May 2011, September 2011 and May 2012,

Table 1. Main environmental variables at the three sites, for each experimental session. Wave and tidal regime were not considered due to very reduced variations.

	Session		***4000***	***5000***	***6000***
Beach width (m)	2011	May	15	30	30
		July	15	30	36
		Sept.	15	30	36
	2012	May	15	24	24
		Oct.	20	30	36
Beach mean slope (%)	2011	May	4.12	2.44	1.39
		July	5.22	2.27	1.67
		Sept.	3.48	1.86	1.43
	2012	May	3.14	1.62	0.87
		Oct.	3.35	2.12	1.43
Mean sand penetrability ± SE (cm)	2011	May	4.59 ± 0.33	4.04 ± 0.22	3.01 ± 0.18
		July	3.48 ± 0.35	2.41 ± 0.35	1.98 ± 0.19
		Sept.	3.89 ± 0.29	4.03 ± 0.27	4.09 ± 0.22
	2012	May	4.20 ± 0.35	3.45 ± 0.20	3.20 ± 0.28
		Oct.	2.64 ± 0.55	3.63 ± 0.27	2.85 ± 0.16
Mean air temperature ± SE (°C)	2011	May	28.50 ± 1.60	23.88 ± 0.54	26.92 ± 1.05
		July	34.42 ± 0.55	31.88 ± 0.38	32.31 ± 0.51
		Sept.	35.00 ± 0.75	29.75 ± 0.99	33.06 ± 1.49
	2012	May	25.10 ± 0.70	21.70 ± 0.50	21.10 ± 0.40
		Oct.	29.13 ± 0.86	27.91 ± 1.07	29.96 ± 0.91
Mean air relative humidity ± SE (%)	2011	May	41.19 ± 0.10	33.88 ± 2.88	30.06 ± 0.60
		July	51.64 ± 2.05	59.00 ± 1.99	62.25 ± 1.84
		Sept.	37.50 ± 3.52	32.25 ± 2.41	27.81 ± 2.57
	2012	May	41.30 ± 1.00	56.50 ± 2.10	56.30 ± 1.50
		Oct.	42.38 ± 1.96	47.53 ± 2.32	40.56 ± 2.33
Global solar radiation ± SE (Klx)	2011	May		108.74 ± 1.03	
		July		131.77 ± 0.85	
		Sept.		82.72 ± 1.65	
	2012	May		122.41 ± 1.04	
		Oct.		85.87 ± 1.81	

100% of animals were tested with visible sun, whereas in July 2011 and October 2012 a small percentage of them had limited sun visibility (Fig. 3b).

3.2 Orientation experiments

For the orientation experiments, a total of 2193 amphipods were tested, of which 454 in May 2011, 441 in July 2011, 456 in September 2011, 484 in May 2012 and 358 in October 2012. In general, a smaller number of individuals (642 sandhoppers) was tested at the 4000 site with respect to the other two (772 and 779 sandhoppers at the 5000 and 6000 site, respectively), despite the same sampling effort was made at the three sites, due to the minor density of the former sub-population. Also, amphipods from the 4000 site generally had the smallest mean size (Fig. 4a) and number of antennal articles (Fig. 4b), i.e. more juveniles were tested here, again due to a higher difficulty to find adults with the same sampling effort.

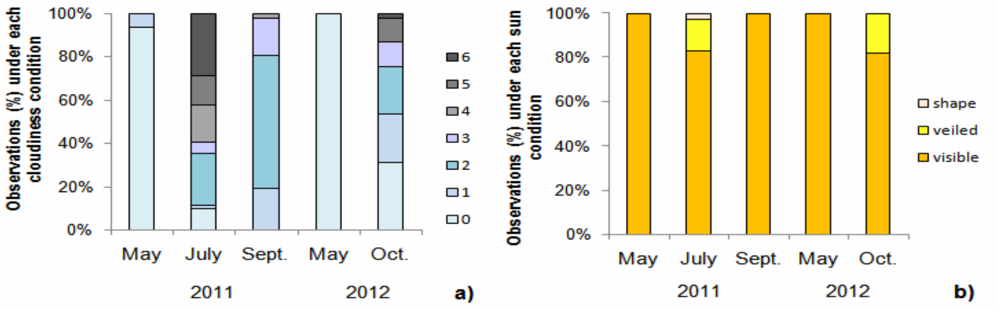

Figure 3. Sky and sun variables, as measured at each experimental session; a) Sky cover, scores on a conventional scale from 0/8 (no clouds) to 8/8 (sky totally cloudy); b) Sun visibility, from well visible sun to only sun shape.

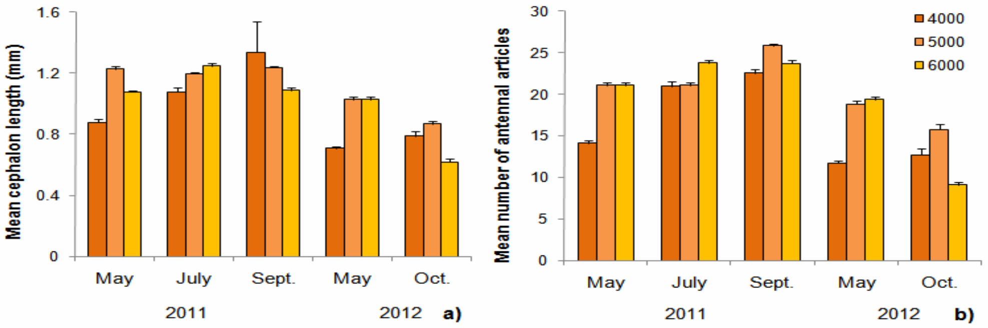

Figure 4. Individual variables, mean values ± SE for each experimental session at the three sites; a) Cephalon length, as a proxy of the animals' size; b) Number of antennal articles (right antenna), as a proxy of age (Williams 1983; Marques et al. 2003).

About the temporal trends of the age/size proxies, the both globally increased from May to September 2011, while a decrease was observed from May to October 2012. Sex ratio was generally male biased, confirming the findings obtained by Marques et al. (2003) in a population study at the 5000 site; the number of mature females substantially increased at September 2011, while more juveniles were observed in October 2012 (Table 2).

We performed a multiple regression analysis on the observed angular distributions; starting from a simple additive model, we also built alternative models including the interaction of the year, the season or the month with all the other parameters. Based on the AIC (maximum likelihood with the least number of parameters), the best model showed a significant interaction of the month with the other factors and variables, as follows:

Best model: Orientation ~ month***(site*** + landscape visibility*** + am/pm*** + air humidity*** + sky cover*** + cephalon length** + antennal articles**) + sun visibility** + solar radiation*

(Likelihood = 6681.1324, AIC = 6837.1324, degrees of freedom = 2115)

(*** $P < 0.001$; **$P < 0.01$; *$P < 0.05$; LRT)

Factors are displayed in a decreasing order of significance (LRT); the variables not included in the model did not improve its likelihood and factors outside the interaction acted on the talitrids' orientation independently from the month.

On the basis of this best model, we developed separate models for each month, obtaining:

May: Orientation ~ site***(landscape visibility*** + am/pm***+ air humidity*** + cephalon length*** + antennal articles*) + solar radiation

Table 2.Population variables for each experimental session, relative to the orientation experiments on *Talitrus saltator* at the three sites. Cephalon length and antennal articles: mean values ± SE.

		4000	**5000**	**6000**
ales/females	**May 2011**	60/57	73/65	52/109
ature/immature females		6/51	19/46	23/86
uveniles/adults		31/117	2/138	5/161
ales/females	**July 2011**	53/53	78/76	39/113
ature/immature females		16/37	8/68	17/96
uveniles/adults		23/106	4/154	2/152
ales/females	**Sept. 2011**	66/47	119/44	85/71
ature/immature females		25/22	23/21	21/50
uveniles/adults		17/113	0/163	7/156
ales/females	**May 2012**	50/23	48/82	55/87
ature/immature females		0/23	6/76	26/61
uveniles/adults		95/73	27/130	17/142
ales/females	**Oct. 2012**	16/25	49/46	8/17
ature/immature females		3/22	11/35	0/17
uveniles/adults		26/41	57/95	112/25

(Likelihood = 2993.6587, AIC = 3069.6587, degrees of freedom = 900)

July: Orientation ~ site***(solar time*** + solar radiation*** + sky cover*) + landscape visibility*** + air humidity** + air temperature** + sun visibility

(Likelihood = 1084.3151, AIC = 1152.3151, degrees of freedom = 407)

September: Orientation ~ site***(landscape visibility*** + solar time** + azimuth** + sky cover** + air humidity** + air temperature*) + am/pm

(Likelihood = 1303.391, AIC = 1391.391, degrees of freedom = 412)

October: Orientation ~ site*** + landscape visibility*** + solar time** + antennal articles*** + cephalon length**

(Likelihood = 1150.7013, AIC = 1178.7013, degrees of freedom = 344)

In these monthly models a significant interaction with the site was present, except for October 2012, where the site was still a highly significant factor. Also the landscape visibility was always highly significant, and the use of the sun compass was confirmed by the presence of factors like solar time (or time of the day: am/pm), sky cover, sun visibility, solar radiation and azimuth position. In no model the season was retained as significant. A higher variability was observed regarding climatic and intrinsic variables. In all the models, except October, the air humidity was retained, while air temperature was significant only in July and September. The sex of individuals was never included, while the size/age proxies (cephalon length and number of antennal articles) were significant in May and October.

All the circular distributions obtained at the three sites significantly differed from randomness (Rayleigh test: $P < 0.01$, Fig. 5; appendix A). The mean resultant length (r), indicating the concentration of the samples, was higher in summer (July 2011) and lower in autumn (September 2011 and October 2012) at the three sites, with intermediate values in spring. In all the experimental sessions the best mean direction was observed at the 5000 site, while the mean resultant length was not so steady. In May 2011 and October 2012 the 5000 samples were also the most concentrated, while in July 2011 the highest concentration was observed at the 4000 site, in September 2011 a tendency to bimodality was expressed at the three sites (with secondary peaks in the direction opposite to the expected one, see Fig. 5) and in May 2012 samples were more concentrated at the 4000 site. In October 2012 a strong dispersion was observed at the 4000 and 6000 sites (see Fig. 5).

A more detailed analysis of the distributions under each experimental condition showed that in September 2011 the amphipods were landwards oriented, when the landscape visibility was not allowed, and during the afternoon, when the sun azimuth was almost coinciding with the TED (Fig. 6a,b).

4. DISCUSSION

Animals have adapted their orientation to their particular environments through selection, thus we can roughly define the orientation fitness as the ability of an individual to be in the right place at the right time (JANDER, 1975). Regarding the movements perpendicular to shorelines, reviews are available concerning arthropods, amphibians and hatchling sea turtles (FERGUSON, 1971; MROSOVSKY, 1972; ALTIZER & FORWARD, 1993; CHIUSSI, 2003; SCAPINI, 2006). In all these groups, selected cross-shoreline orientation contains time compensated sun compass as an essential component, but a modulation occurs based on several environmental factors, contributing to increase the individual orientation fitness. In this study the best regression model explaining sandhoppers' orientation showed an interaction with the month of the experiments, indicating an effect of Mediterranean seasonal variations, that represent a periodical risk. On the other hand, the season was not retained in the best model, meaning that the month must also include finest climatic differences, particularly between September 2011 and October 2012, the only experimental sessions made in different months of the same season, i.e. autumn. Actually, differences were observed in air temperature and humidity (Table 1), sky cloudiness (Fig. 3a) and sun visibility (Fig. 3b); all of these climatic factors demonstrate a prompt response also to the sudden changes occurring on beach environments. Also biotic information (sample characteristics) may be included in the month, as a higher number of juveniles was observed in May (both 2011 and 2012) and October 2012 (Fig. 4). Together with the retention of the size/age proxies, this indicates that a different orientation was displayed by juveniles and adults, with the oldest animals relying more on learned information from the environment, as it is the rule for most invertebrates, which thus display also a good adaption to risks with a long-life exposure (UGOLINI et al., 1989, 1991; BOAL et al., 2000; CHIUSSI, 2003; KARSON et al., 2003). Besides the month, several other variables indicated a conspicuous weight of learned (linked to individual experience) references, particularly the site (differing mainly for the shoreline stability) and the landscape visibility (a local visual reference). The significance of air humidity is closely connected with dehydration risk; we consider this response a learned modulation of behaviour, as the animals must anticipate stressful conditions. Looking at the monthly models, the regression analysis kept together the May distributions of the two years, which is in accordance with a periodicity of the seasonal effects. Remarkably, the factors interacting with the site were only three in July 2011 and none in October 2012. Looking at retained factors, it seems likely that in July (high summer) the climatic and environmental parameters had a major weight, becoming independent from the erosive dynamics; on the other side, they did not reach prohibitive values (in terms of dehydration risk) in October 2012, when the intrinsic factors (independent from the site) were very significant, as this was the month when more juveniles were tested (Fig. 4).

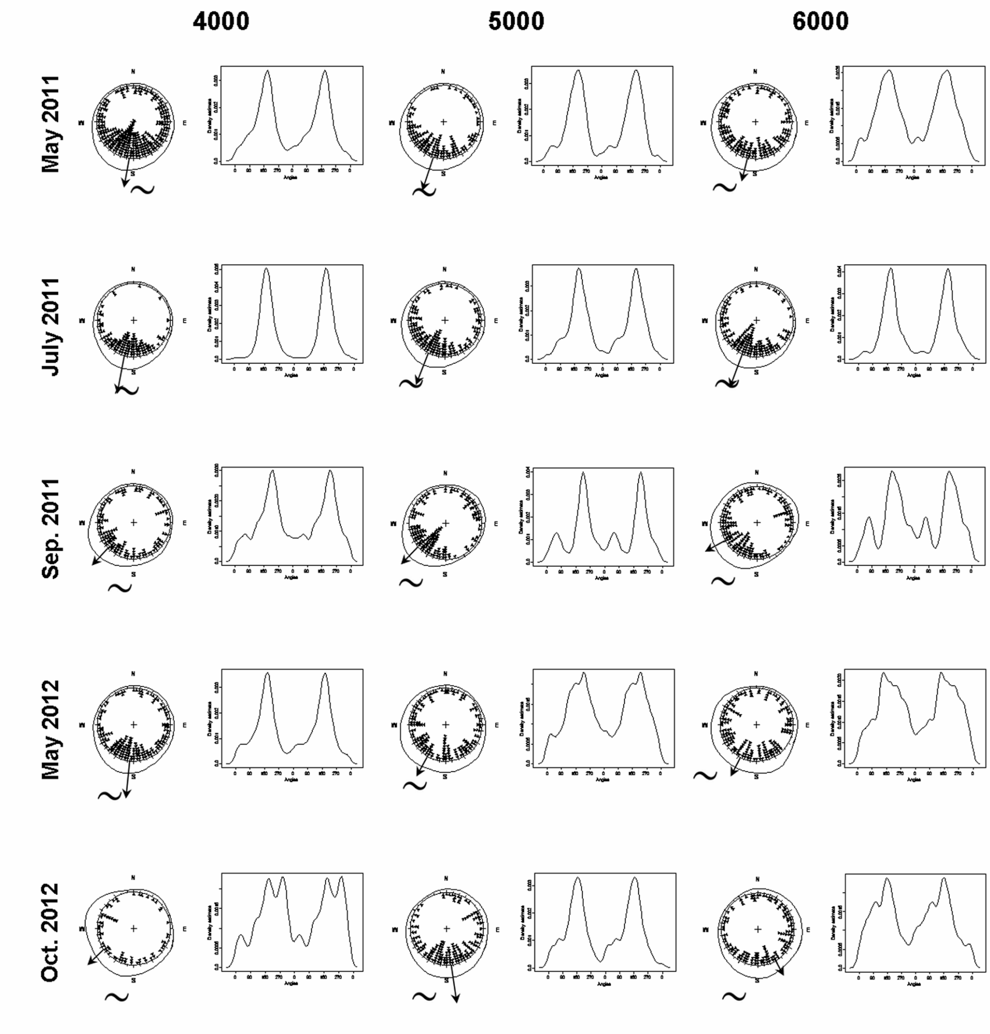

Figure 5. Angular distributions from the orientation tests on *Talitrus saltator* at the three sites for each experimental session. On the left: circular plots of the orientation angles; on the right, density estimates (kernel method), double plotted on Cartesian graphs. Black waves: sea direction, corresponding to the Theoretical Escape Direction (TED). Arrows: mean vectors, with lengths proportional to concentrations. Circular statistics and summaries reported in Appendix A.

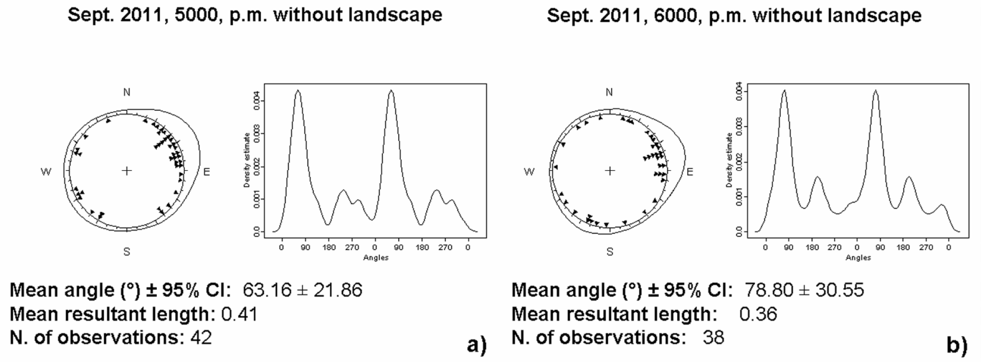

Figure 6. Angular distributions from the orientation experiments on *Talitrus saltator* selected from the global ones of Fig. 3 to highlight the conditions under which a peculiar orientation pattern was expressed. a) September 2011, 5000 site; b) September 2011, 6000 site. For each distribution the mean direction (with the 95% confidence interval), the mean vector length and the number of tested individual are reported.

Likely, the role of such individual factors is not influenced by the erosive dynamics of the sites, which might explain why in October the regression model is a simple additive one. Also, air temperature was discarded from the models of May and October, when they were the lowest (Table 1), indicating that climatic factors act on sandhoppers' orientation only when exceeding threshold warning values. The relation of circular distributions with seasonal variations is well visible in Fig. 5; the highest seaward concentrations observed in summer (July) and the lowest observed in autumn (October and September) are adaptive, as on Mediterranean coasts the high risk of dehydration in summer makes a correct zonal recovery essential for the sandhoppers' survival, while the mild climate common in autumn permits a certain degree of dispersion on the supralittoral zone also during the day hours. Such a dependence from seasonal climatic variations was already described for a variety of Mediterranean locations and behaviours of *T. saltator* (BORGIOLI et al. 1999a,b; NARDI et al. 2003; FANINI et al. 2007), while it was found that on beaches with more marked tidal excursions the sandhoppers are more influenced by tidal phases (linked to the submersion risk), as air temperature and humidity almost never reach prohibitive values (BORGIOLI et al., 1999b; GAMBINERI et al., 2008; ROSSANO et al., 2009; BESSA et al., 2013). This is true not only for this species, but for a variety of other invertebrates and was interpreted as phenotypic plasticity (HAZLETT, 1988; BROWN, 1996; SOARES et al., 1999; WEST-EBERHARD, 2003; GIURFA 2007). Also the physical features of each site significantly influenced the sandhoppers' orientation. The data on sand penetrability and beach profiles (Table 1, Fig. 2) permit to class the 4000 site as a more reflective beach type and the 6000 as a more dissipative one, with the 5000 site intermediate between the two (MCLACHLAN & BROWN 2006). Thus, the 4000 site was the poorest in terms of trophic resources and possible refuges, while the 6000 site had the highest habitat variety and abundance of stranded and vegetal debris, but also the longest distances (beach width). Considering the circular distributions at each site, generally the individuals from the 5000 site (intermediate sedimentary dynamics) showed the best orientation, while the worst performances (considering both direction and concentration) were observed at the 6000 site (Fig. 5). These results in some extent contradict those obtained on the sandhopper *Atlantorchestoidea brasiliensis* by FANINI et al. (2009), who observed a more precise seawards sun orientation on a reflective beach with respect to a dissipative one, in agreement with what expected according to the Habitat Safety Hypothesis (HSH, DEFEO & GOMEZ, 2005). According to this hypothesis, microtidal reflective beaches are the most stable beach type, with low backshore mobility and a substantially lower risk of immersion with respect to macrotidal dissipative beaches (DEFEO & MCLACHLAN, 2011); this more stable and safer environment would favour rather than negatively affect fitness features of supralittoral forms (that are not affected by the interaction between coarse sand and turbulent swash). For this reason, these species generally have higher growth, survival and reproduction rates on reflective beaches (DEFEO & GÓMEZ, 2005; DEFEO & MCLACHLAN 2011). According to the HSH also the average body size was expected to decrease from the more reflective (4000) to the more dissipative site (6000), as community density increases (MCLACHLAN & DORVLO, 2007). On the contrary, in this study the individuals from the 4000 site almost always had the smallest size and age (Fig. 4). Likely, the strong erosion at the 4000 site made it not stable nor safe for talitrids, as indicated by the reduced number of individuals that were found here. On the other side, both the behavioural tests and the individual features were generally in accordance with the HSH when considering only the 5000 and 6000 sites, where the beach was not subject to the erosive phenomenon. Coming back to the circular distributions, the best orientation observed at the 4000 site with respect to the 6000 one might to indicate that the latter was accreting more rapidly than the 4000 site was receding (see Fig. 2), i.e. the shoreline was more unstable at the 6000 than at the 4000 site. It is worthy to note that in September 2011 the sandhoppers of the 5000 and 6000 sub-populations that were prevented to observe the surrounding landscape moved also landward (Fig. 6). This bimodal orientation may mean that in these two sites the visual cues prevail on the sun compass, consistently with the findings of HARTWICK (1976), according to which populations with a well-developed landscape rely more on landscape features than populations from flat beaches. Moreover, both the 5000 and 6000 sites had vegetated dunes or marshes backing the beach and the possibility to find moisture and shadow in the opposite direction with respect to the seashore may also explain the observed bimodal distributions. As this phenomenon was observed in September 2011, when the animals were bigger (Fig. 4), this behaviour may be related to the different zonation patterns described for juveniles and adults in *T. saltator* on a near beach of the same coast (FALLACI et al., 2003) and in *Orchestoidea tuberculata* in south-central Chile (KENNEDY et al., 2000). In those populations, the oldest adults were found more landward than juveniles, likely exploiting their higher ability to burrow deeply in the sand to find moisture furthest from the sea (WILLIAMS, 1995; BOUSLAMA et al., 2009; AYARI & NASRI-AMMAR, 2011). Hence, the individuals orienting toward the dune may have been experienced, not so linked to the intertidal zone for their survival. BORGIOLI et al. (1999a) and NARDI et al. (2003) already highlighted that a highly precise sun compass seawards would be less adaptive than other mechanisms to find protection when abundant stranded materials may offer shelter or food sources. These observations stress how the local environmental conditions may contribute to the individual orientation performance. To sum up, we highlighted how both seasonality and local environmental features (particularly sedimentary dynamics) may act on the orientation of *Talitrus saltator* that may integrate external and internal information into a plastic behaviour, adaptive for survival in an unstable life-environment.

Appendix A. Summary circular statistics of the orientation experiments on *Talitrus saltator*. *P*-values: Rayleigh test for randomness.

		TED (° from N)	***Mean angle (°) ± 95% CI***	***Mean resultant length***	***Circular dispersion***	***Sample size (N)***	***P-value***
May 2011	*4000*	176	187.50 ± 12.88	0.47	1.91	148	< 0.001
	5000	202	200.90 ± 10.27	0.56	1.16	140	< 0.001
	6000	209	194.40 ± 15.85	0.38	3.22	166	< 0.001
July 2011	*4000*	185	195.50 ± 6.76	0.76	0.46	129	< 0.001
	5000	213	208.70 ± 10.29	0.54	1.31	158	< 0.001
	6000	210	201.00 ± 8.75	0.61	0.93	154	< 0.001
Sep. 2011	*4000*	194	222.70 ± 18.85	0.34	3.53	130	< 0.001
	5000	213	230.20 ± 11.99	0.39	1.83	163	< 0.001
	6000	215	240.20 ± 18.31	0.30	4.19	163	< 0.001
May 2012	*4000*	207	186.70 ± 11.49	0.46	1.74	168	< 0.001
	5000	212	209.10 ± 22.44	0.29	5.95	157	< 0.001
	6000	225	205.20 ± 25.57	0.25	7.71	159	< 0.001
Oct. 2012	*4000*	200	233.30 ± 33.73	0.30	5.38	67	< 0.01
	5000	205	172.70 ± 12.22	0.46	1.80	154	< 0.001
	6000	205	150.60 ± 25.53	0.26	6.62	137	< 0.001

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3.2.3 Orientation of *Talitrus saltator* and macrofaunal analyses on a dynamic sandy shore before and after the construction of an anti-erosive stone bank

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Abstract

The Collelungo sandy beach (42° 40' 27" N; 11° 5' 59" E, Maremma Natural Park, Italy) shows an erosion/accretion gradient, with strong erosion in the sector near to the Ombrone River mouth, gradually turning into accretion with increasing distance from there. To stop the sea water intrusion into the backing pasturage fields, in 2010 an anti-erosive stone bank was built 1200 m along the coast starting from the river mouth. Before the intervention (2002-2003), sun orientation experiments on the amphipod *Talitrus saltator* (Montagu, 1808) and macrofaunal samplings had been carried out along this beach. In 2011 the experiments and samplings were repeated, in the same stations and seasons, to assess the possibility of using sun orientation as a bioindicator of impact on sandy beaches stability, by using the macrofaunal analyses as a control. Circular statistics and multiple regression models for circular distributions were applied to the orientation data, while univariate and multivariate analyses were performed (Primer 6 & Permanova+ software) on macrofaunal data. In the present study the experiments performed in 2011 are reported and results compared with those obtained in 2002-2003: the worsening of sandhoppers' orientation at all the stations indicated that the variation in orientation may be used as a bioindicator of shoreline changeability, to monitor the effects of hard-engineering interventions on sandy beach ecosystems, which was confirmed by the information derived from macrofaunal community changes.

Keywords: *Talitrus saltator*, orientation behaviour, sandy beaches, shoreline stability, macrofauna community, impact bioindicator.

Regional terms: Europe, Italy, Tuscany, Collelungo beach: 42° 40' 27" N; 11° 5' 59" E.

1. INTRODUCTION

Talitrus saltator (Montagu, 1808) (Crustacea, Amphipoda) is common on the Mediterranean and eastern Atlantic sandy coasts (Bellan-Santini 1993). Sandhoppers take the oxygen from air by means of gills and have a cuticle without waxes, which makes them not adapted to the life in dry environments, as the upper zones of beaches often are. The whole life cycle of *T. saltator* occurs in sandy beaches, where physical constraints continuously shape the habitat, producing predictable and unpredictable conditions of risk or stress (McLachlan and Brown 2006). The physiological limitations are faced by individuals thanks to behavioural adaptations, common to most of the species of sandy beaches (Williams 1995; Defeo and Gómez 2005; Schlacher et al. 2008; Fanini et al. 2012). An important behavioural adaptation is the burrowing attitude, as the wet sediments near the water's edge protect beach invertebrates from dehydration thanks to the periodical wetting and oxygenation by sea water. Also motility and orientation toward the shoreline are fundamental behavioural adaptations for sandhoppers' survival. Their ability to recover the optimal zone is called 'zonal recovery' and is based on both universal (sun, moon, polarized light, Earth's magnetic field) and local cues (landscape, beach slope, wind), which are used in an opportunistic way, depending on the prevailing beach features (Scapini 2006). Orientation ability becomes even more crucial after accidental displacements (e.g, to escape from a predator) during daytime, when the dehydration risk is higher. In this case the sun compass, which allows maintaining a precise direction by compensating for sun movement throughout the day, represents the most reliable mechanism for a rapid recovery (Pardi and Grassi 1955; Pardi and Ercolini 1986; Walsh et al. 2010). Different populations may show innate directional tendencies, adjusted on the direction of the shoreline of origin, which may be fixed in the long term by a process of natural selection (Pardi 1960; Scapini and Pardi 1979; Pardi and Scapini 1983; Scapini et al. 1988a). However, sandhoppers may also rely on their individual experience to cope with changes acting faster than in evolutionary times, across generations (Scapini et al. 1988b; Ugolini and Scapini 1988; Fanini et al. 2009). This behavioural plasticity in orientation permits sandhoppers to face immediate variations determined by meteorological and ecological factors (Brown 1996; Soares et al. 1999; Price et al. 2003), as air temperature and humidity (Williams 1995), exposure to waves and tidal range (Williams 1995; Rossano et al. 2009; Bessa et al. 2013), wrack abundance and distribution (Dugan et al. 2003; Nardi et al. 2003). Also the shoreline stability may affect the orientation of *T. saltator*: sandhoppers orient consistently seawards, showing statistically significant distributions, on beaches with a stable shoreline and are more scattered when shoreline direction is continuously changing (Scapini et al. 1995). This may indicate a lack of adaptation of a population that may use simpler mechanisms than sun compass, as phototaxis or scototaxis (Gambineri and Scapini 2008). On these bases, Scapini et al. (2005) and Fanini et al. (2007) have proposed the sun orientation of *T. saltator* as a bioindicator of shoreline instability on beach ecosystems.

In autumn 2002 and spring 2003, along a Tyrrhenian beach in central Italy (Collelungo beach, Maremma Natural Park, Fig. 1a, b), Scapini et al. (2005) demonstrated that the natural erosion/accretion gradient (and consequent variations in shoreline stability) produced a corresponding gradient in the sun orientation performances of the resident sandhoppers' sub-populations (Ketmaier et al. 2010). On the other hand, on that beach coastal erosion produced a negative impact on the sea-linked tourism business, as the most eroded beach sector in the park was mainly devoted to recreational activities. In 2010 a seawall was constructed (Fig. 1c) in the most impacted sector near the river mouth, to avoid the reduction of the exploitable beach surface and prevent sea water intrusion in the area backing the beach. Such hard-engineering interventions are becoming more and more widespread all over the world (Charlier et al. 2005; Chapman and Underwood 2011, McLachlan et al. 2013), to contrast beach erosion and coastal squeeze phenomena, in their turn linked to the rising sea levels and increased storminess caused by global warming (IPCC 2007). While engineering and financial criteria are always considered in designing projects, the ecological consequences are rarely accounted, also due to an incomplete comprehension of the beach biotic interactions (Nordstrom 2000; Dean and Dalrymple 2002; Phillips and Jones 2006). At Collelungo beach a substantial slowdown of the erosion in the beach sector near the river mouth was expected, thus engendering changes of sedimentary dynamics along the whole shore. The current accretion of the furthest beach sectors, naturally nourished by the sediments transported by surface currents, might reverse or alternatively, the natural accretion might accelerate in other sectors of the beach, depending on changes in local hydrodynamics.

The availability of previous data sets on the same area represented an occasion to verify if the sun orientation of *T. saltator* may highlight some effects engendered by this kind of hard-engineering interventions on the beach ecosystem, particularly on shoreline stability, which would affect orientation (Scapini et al. 1995; Scapini et al. 2005). With respect to the previous experiments, an improvement of the orientation was expected in the sectors having the shoreline stabilized by the seawall, while a worsening of orientation was expected if strong changes had been engendered. Orientation tests were thus repeated in 2011, after the seawall construction, in the same stations of 2002-2003, with the same protocol, in the same seasons and under similar meteorological conditions. Furthermore, in the days of the orientation experiments of 2002-2003, samplings of the beach macrofaunal community and an assessment of its diversity were also made (Colombini et al. 2006). Macroinvertebrates communities are considered effective bioindicators of impacts because of their strict link with the life-environment; symptomatic variations may be observed in their composition and abundance in response to variations in the ecosystem (Cairns and Pratt 1993; Mattoni et al. 2000; Longcore 2003). Hence, in 2011 we performed macrofaunal samplings too, using a protocol quite similar to that used in 2002-2003. Though data were available for macrofaunal diversity before the impact, a rigorous before-after control-impact (BACI) study (Underwood 1994) was not feasible, as the seawall construction may have modified morphodynamics also in the furthest beach sectors, preventing the selection of impacted and control sites on that continuous extended beach. On the other hand if the changes ascribable to the intervention were substantial, a decrease in the macrofaunal diversity or a shift in the community composition were expected, likely more evident in the stations near the river mouth, which were already highly disturbed during the seawall construction phase.

It is worth noting that sandhoppers' orientation, as all behavioural adaptations, originates as an integration and feedback of the information from the environment to the animal throughout a learning process (Campan and Scapini 2002). Hence, behavioural changes may permit to assess environmental changes (in this case shoreline changes) over a short-time scale, that of the species' life-span, ranging from 6 to 9 months for *T. saltator* in the Mediterranean area (Marques et al. 2003). The time scale to observe changes in macrofaunal community is longer with respect to behavioural variations, and may range from few months (seasonal variations) to some years. Hence, these analyses may be considered as a medium-term control for the proposed behavioural bioindicator.

2. METHODS

2.1 Study site

The study site is an almost straight beach in the Maremma Natural Park (Grosseto, Italy, Fig. 1a), extending about 6 km eastwards from the mouth of the Ombrone River (42°39'33''N, 11°0'48''E), on its left side (Fig. 1b).

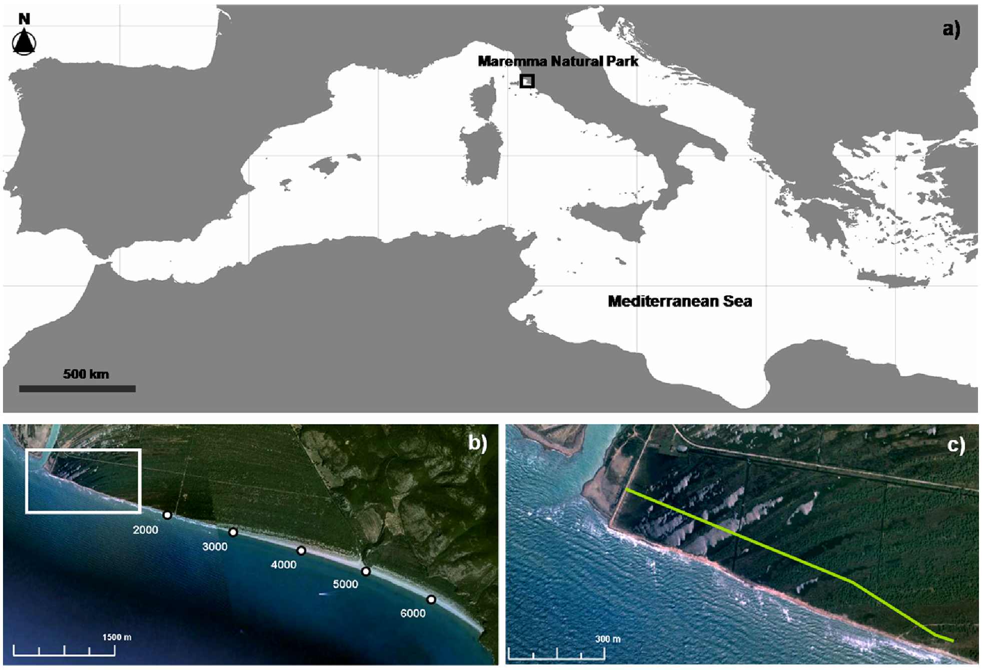

Figure 1. The study site; a) The location of the Maremma Natural Park, Tuscany , Italy (42°39′59.00″N, 11°05′36.30″E) in the context of the Mediterranean region; b) The Collelungo beach on the left side of the Ombrone River mouth; the circles represent the sampling stations, with their distance in meters from the Ombrone River mouth (in the upper left corner); c) A magnification of the white rectangle of fig. 1b to show the scale of the anti-erosive intervention realized in 2010. Photo credits for the images: Google Earth accessed in May 2013.

The sedimentary contribution by the river has decreased in the last century, due to natural and human-induced changes along its course (Pranzini 2001; Ciampi 2010). This generated an erosion gradient along the beach, with strong shore erosion close to the river mouth, gradually decreasing, reaching a condition of dynamic equilibrium at about 5 km of distance and turning into accretion in the last part of the littoral, at 6-7 km of distance (Scapini et al. 2005). In 2010 a protection seawall 1200 m long, parallel to the shore (Fig. 1c) was built with cyclopean boulders immediately at the left side of the river mouth. In 2011, following the protocol used in 2002-2003 (Scapini et al. 2005), five sampling stations were chosen, at 1000 m distance from each other, starting at 2000 m from the river mouth (station 2000) up to the S-E beach extremity, the 6000 station (Fig. 1b). The stations closest to the river mouth (0 and 1000) were not sampled as they did not present the typical features of a dune-beach ecosystem. In 2002-2003 the orientation experiments were not performed at the 2000 station, where not enough sandhoppers were found (Scapini et al. 2005).In 2011 the resident *T. saltator* sub-population had disappeared also from the 3000 station, due to a further loss of habitat; thus only the 4000, 5000 and 6000 stations were considered in this study. Station 4000 had an eroded dune and was very narrow, while at 5000 station the dune was apparently stable, backed by cliffs, and the beach was wide and flat. At 6000 station the beach was accreting and salt marshes were present behind the fore-dune. In each experimental season (spring and autumn), the beach profiles were drawn and the shoreline directions were recorded at all the stations; air relative humidity and temperature were also measured. No measures were taken of the tidal range, as this is a micro-tidal beach with tide variations never exceeding 0.40 m (Pranzini 2001).

2.2 Sun orientation experiments

The sun orientation experiments were performed in May (spring) and September (autumn) 2011 at the 4000, 5000 and 6000 stations at the same time by three researcher teams, taking all cares to have the same experimental conditions to avoid introducing uncontrolled variables (Borgioli et al. 1999a, b). Amphipods were collected on the beach before the experiments and tested in transparent circular arenas, following the protocol by Scapini et al. (2005). A white cardboard (10 cm height) was used to screen off the landscape view, in order to avoid the use of learned visual references and favour the expression of the innate sun compass; at each station about 40 individuals were tested in the morning and 40 in the afternoon. The angles of orientation to the North of the trapped individuals were registered and specimens were then preserved in 75% alcohol, for later observations by microscope in the laboratory. Here they were checked for species, sex (males, females and juveniles with sexual characters not yet visible) and reproduction state (ovigerous, mature and immature females). We also measured the cephalic lengths and counted the right antennal articles, as proxies of age (Williams 1983; Marques et al. 2003). Such individual features were considered to assess the role of intrinsic factors in the multiple regression analyses of the orientation distributions (Scapini et al. 2002; Scapini et al. 2005). The following environmental features were registered during the experiments, because of their possible effect on orientation: sun visibility (visible, veiled, shaped, not visible), sky cover (assessed on a conventional scale from 0/8 to 8/8), global solar radiation (measured at the 5000 station). From the shoreline direction we calculated the TED (Theoretical Escape Directions seawards), the perpendicular to the seashore at the release point. The data set of 2002-2003, relative to five experimental days, was reduced to have the same sample size of 2011, by choosing one day from the spring session and one from the autumn one, based on the most similar climatic conditions compared to 2011 experiments and samplings (Wilcoxon rank-sum test on air mean temperature and humidity: $P > 0.05$).

2.3 Macrofaunal sampling

In the same months of the orientation experiments, we sampled the macrofaunal community at stations 2000, 3000, 4000, 5000 and 6000 (Fig.1b). As it was made by Colombini et al. (2006), we placed two replicate transects perpendicular to the shoreline at each station, at 5 m from each other, ranging from the drift line to the base of the dune and composed by a set of pitfall traps positioned every 2 m. From 16 to 34 traps were placed across each station, depending on the beach width. The traps were constituted of plastic cups (20 cm high and 10 cm in diameter) pushed in the sand to their superior rim; they were kept active during one night and collected in the early morning, to avoid the desiccation of animals. The sampled specimens were preserved in 75% alcohol and carried to the laboratory, where they were sorted using a binocular microscope up to the species level, or at the genus when this was not possible. The procedure differed from that described by Colombini et al. (2006) for the sampling duration, as in that case it lasted 72 hours, while in the present study traps were removed after 12 hours. This decision was due to the Park managers’ request to impact as little as possible the existing macroinvertebrates’ community, especially in the S-E beach extremity, for the presence of rare or endangered animal and vegetal species.

2.4 Data analysis

2.4.1 Orientation experiments

For each station, the mean angular distributions were calculated using the total of spring and autumn orientation data. We applied the statistics of circular distributions (Fisher 1993) using an *ad hoc* library working in the S-plus Insightful software (Marchetti and Scapini 2003). The mean directions, mean vector lengths and sample circular dispersions were estimated; the density curves of the angular distributions were obtained with the kernel method and plotted on both polar and Cartesian graphs (in the latter case double plotting was used to better show any peaks). Rayleigh tests for uniformity (Fisher 1993) were carried out to highlight significant deviations from randomness. To assess the simultaneous effects of the variables and factors influencing orientation, multiple linear regression analyses adapted to angular data were performed (SPLM: Spherically Projected Linear Models, Scapini et al. 2002; Marchetti and Scapini 2003). The angles of orientation were considered as response variables, and the environmental (station, meteorological conditions, season) and intrinsic (biometrics and sex) variables as influencing factors. We started with an additive model including all variables and factors, and then we used AIC (Akaike Information Criterion; Akaike 1973) to compare models and select the best one, having the highest likelihood with the least number of parameters. The significance of each parameter was estimated with the LRT (Likelihood Ratio Test), based on the AIC difference between the best model and the nested one without the considered parameter.

2.4.2. Macrofaunal analyses

The capture frequencies from the two transects of each station were cumulated, as also data from spring and autumn samplings, to avoid seasonal fluctuations. The same treatment was applied to the data of Colombini et al. (2006). For both the data sets (2002-2003 and 2011) the capture frequencies in each station were standardized with respect to the total number of specimens, to avoid biases linked to the different number of traps. As the 2011 sampling was performed during the night, only nocturnal species, or with prevailing nocturnal activity, were considered in the analysis. The Brillouin (1962) index (H), suitable for non-random sampling designs (Colombini et al. 2002a; Khan 2006), and the Fisher index for α-diversity (Fisher et al. 1943), which is little influenced by the sample size (Magurran 2004) were calculated. A multivariate approach was also applied, more robust for disagreeing sample sizes (Clarke et al. 1996); a non-parametric (permutational) analysis of variance, through three-way PERMANOVA (Anderson 2001), was used to test hypotheses about macrofaunal assemblage differences before and after the seawall construction. The design included three factors: (1) Time (fixed; 2002-2003 vs. 2011), (2) Station (fixed; 2000, 3000, 4000, 5000 and 6000) and (3) Season (fixed; spring vs. autumn). The data were square-root transformed and converted to a Bray–Curtis similarity matrix before the tests, based on 9999 permutations. The assemblage responses were visualised by non-metric multidimensional scaling (nMDS) and the contribution by individual species to the dissimilarity between 2002-2003 and 2011 samplings was calculated using the SIMPER (Clarke 1993) procedure; taxa were considered important if their contribution to percentage dissimilarity was > 3%. All statistical analyses were performed using Primer v.6 with the PERMANOVA+ add on (PRIMER-E Ltd., Plymouth, UK).

3. RESULTS

3.1 Environmental features

Data on beach profiles are summarized in Table 1 and compared with those from Scapini et al. (2005) and Colombini et al. (2006), relative to autumn 2002 and spring 2003. In both the data sets there was an increase of the beach width going further from the river mouth, together with a slope reduction. The changes in slope and width from season to season were smaller in 2011, as only the 6000 station showed an accretion from spring to autumn. Moreover, in 2011 the shoreline direction resulted generally (7 out of 10 measurements) shifted to the south compared to 2002-2003, particularly at station 4000 (Table 1).
The climatic variables showed no significant differences between the test conditions during the experiments performed in 2002-2003 and those of 2011 (Wilcoxon rank-sum test on air mean temperature and humidity: P > 0.05, Figure 2).

Table 1. Summaries of the beach physical features at the five stations at the Collelungo beach (Maremma Regional Park, Italy, 42° 40' 27" N; 11° 5' 59" E). Data of autumn 2002 and spring 2003 are from Scapini et al. (2005) and Colombini et al. (2006).

	Season	*Beach mean slope (%)*		*Beach width (m)*		*Shoreline direction (° from North)*	
Distance from river mouth (m)		**2002-2003**	**2011**	**2002-2003**	**2011**	**2002-2003**	**2011**
2000	Spring	9.7	3.9	38	16	288	280
	Autumn	2.7	3.2	12	16	285	288
3000	Spring	9.8	3.9	13	18	295	295
	Autumn	10.2	4.3	14	18	290	285
4000	Spring	3.8	4.1	22	15	295	266
	Autumn	2.9	3.5	32	15	295	284
5000	Spring	1.8	2.4	34	30	300	292
	Autumn	2.2	1.9	30	30	303	303
6000	Spring	1.6	1.4	42	30	305	299
	Autumn	0.6	1.4	38	36	310	305

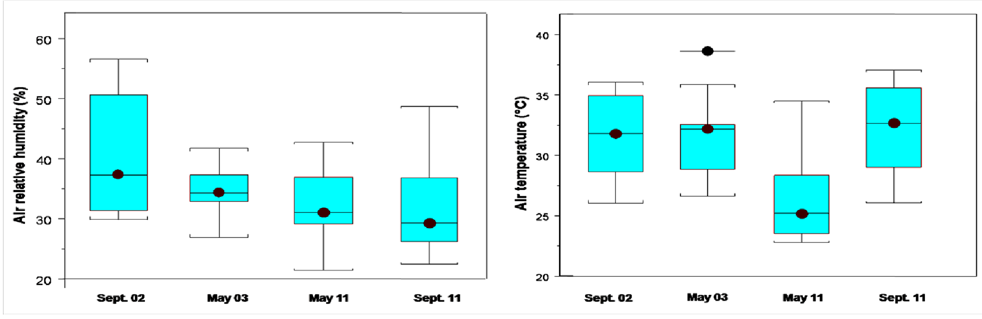

Figure 2. Box plots of the main climatic variables measured during the orientation experiments of 2002-2003 and 2011. On the left: air temperature (°C), on the right: air relative humidity (%). Sept. = autumn, May = spring.

3.2 Orientation experiments

For the orientation experiments, a total of 462 amphipods were tested in 2011: 143, 157 and 162 from the 4000, 5000 and 6000 station respectively (Tab. 2). The orientation distributions are graphically represented in Fig. 3, showing for each station the results of 2002-2003 (top) and of 2011 (bottom). A remarkable worsening was evident in the 2011 experiments at all the stations, making some circular distributions not significant (Rayleigh test *P*-values in Tab. 2).

Table 2. Summary circular statistics on the orientation experiments on *Talitrus saltator* in the three sites for 2002-2003 experiments (Scapini et al., 2005) and 2011 experiments. TED = Theoretical Escape Direction, perpendicular to the shoreline direction.

Site	*Summary circular statistics of the annual distributions*	*Year*	
		2002-2003	***2011***
4000	TED	205°	185°
	Observed direction ± ES (Confidence interval with α = 0.05)	208° ± 9°	179° ± 44°
	Mean vector length (r)	0.56	0.16
	Sample size (N)	159	143
	Rayleigh test for randomness	$P < 0.001$	$P < 0.05$
5000	TED	211.5°	207.5°
	Observed direction ± ES (Confidence interval with α = 0.05)	224° ± 8°	219° ± 43°
	Mean vector length (r)	0.68	0.14
	Sample size (N)	147	143
	Rayleigh test for randomness	$P < 0.001$	$P < 0.05$
6000	TED	217.5°	212°
	Observed direction ± ES (Confidence interval for α = 0.05)	231° ± 11°	194° ± 68°
	Mean vector length (r)	0.55	0.11
	Sample size (N)	159	162
	Rayleigh test for randomness	$P < 0.001$	$P > 0.05$

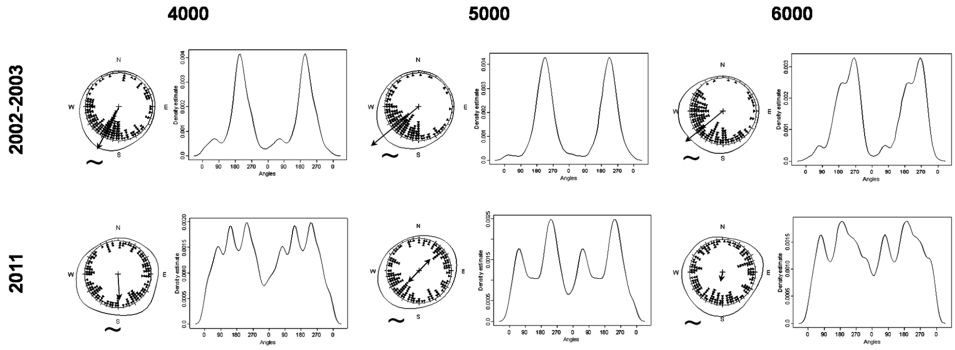

Figure 3. Circular distributions for the orientation tests on *Talitrus saltator* at the three stations, 4000, 5000 and 6000. On the top: 2002-2003; on the bottom: 2011. For each distribution, the circular plots of orientation angles are shown on the left and density estimates (Kernel method) on the right, double plotted on Cartesian graphs. Black waves: TED (Theoretical Escape Direction seawards). Arrows: mean directions of each distribution. Circular statistic and summaries are reported in Table 2.

The mean resultant lengths, indicating the concentration around the mean angle, became considerably smaller at all the stations, while the circular dispersions increased. In 2002-2003 the highest concentration around the TED was shown by the 5000 sub-population, and the worst performance was observed at the 6000 station. In 2011 a bimodality appeared at the 5000 station, reflecting a change of orientation from spring to autumn, while the best results were obtained at the 4000 station, both in terms of mean direction and concentration, and the most scattered distributions were observed again at the 6000 station.

The SPLM regression analysis indicated the following best models:

Table 3. Capture numbers of the most representative species at the five stations in 2002-2003 (data from Colombini et. Al, 2006), using a 72 hours sampling, and in 2011, using a 12 hours (night only) sampling. Species with prevailing nocturnal habits are indicated with *.

station	*2000*		*3000*		*4000*		*5000*		*6000*	
Year of sampling	*02-03*	*11*	*02-03*	*11*	*02-03*	*11*	*02-03*	*11*	*02-03*	*11*
*Tylos europaeus**	60	89	110	24	724	263	900	596	336	685
*Talitrus saltator**	18	0	14	0	637	3	2303	390	8498	1295
*Arctosa perita**	1	0	4	0	63	2	72	10	153	24
Cicindelidae	0	0	1	0	4	0	42	1	43	1
*Parallelomorphus laevigatus**	0	0	0	0	0	1	6	10	33	14
Histeridae*	1	0	12	0	17	0	19	7	67	4
Staphylinidae*	0	0	34	0	25	0	23	0	3	0
*Halammobia pellucida**	0	0	0	0	24	0	43	0	11	0
*Phaleria provincialis**	160	94	487	35	1080	124	709	211	433	170
Pimelia bipunctata	4	0	2	0	4	0	4	2	4	1
*Trachyscelis aphodioides**	0	0	8	0	50	0	28	0	37	0
Other Coleoptera	9	1	16	0	60	0	56	7	38	1
Neuroptera larvae*	1	0	4	0	25	0	12	3	12	2
Other Arthropoda	0	0	2	0	9	0	27	1	11	1
TOTAL	254	184	692	59	2713	393	4217	1238	9668	2198

<u>2002-2003</u>: Orientation ~ station***(am/pm*** + global radiation*** + sky cover*** + solar time*) + azimuth (Likelihood = 1041.5967; AIC = 1105.5967; df = 433);
<u>2011</u>: Orientation ~ station***(azimuth*** + air temperature*** + air humidity*** + sky cover***) + global radiation*** + sex (Likelihood = 1477.7161; AIC = 1553.7161; df = 424).
(***= P <0.001; ** = P <0.01; * = P <0.05; factors without * are not significant, but are maintained because reducing the AIC of models).
For both data sets a highly significant interaction was observable with the station, which includes substrate variables and local sedimentary dynamics. In both the models, some factors were not included in this interaction, meaning that they acted on the orientation in the same way at the three stations. The season, the cephalic length and the antennal articles were always discarded as non-significant factors, suggesting that orientation did not depend on season or size and age of animals. Comparing the two models, in the 2002-2003 model all the factors that were retained together with the site are involved in the sun compass use, indicating that the majority of the population used the solar orientation, being not significantly influenced by meteorological and intrinsic factors. On the contrary, in the 2011 model the immediate meteorological variables (air temperature and humidity) were highly significant and also the sex, although not significant, was retained as improving the likelihood of the model.

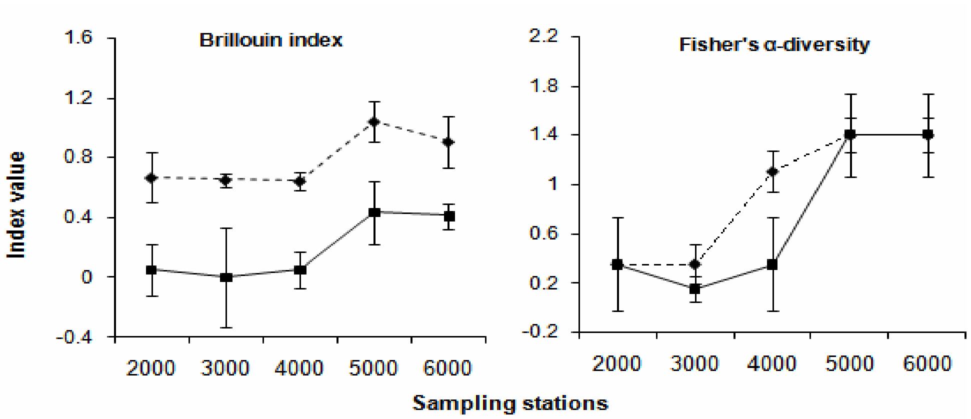

Figure 4. Diversity indices (calculated by considering only the species having nocturnal or mainly nocturnal activity) in the five sampling stations, 2000, 3000, 4000, 5000 and 6000. Solid lines : 2011; dashed lines : 2002-2003. a) Brillouin index; b) Fisher's α-diversity.

3.3 Macrofaunal analyses

During the samplings of 2011 a total of 4072 macroinvertebrates were captured, increasing from 184 at 2000 m from the river mouth up to 2198 at 6000 m (Table 3). The samples from 2002-2003 had a larger size, 1.38-2.37 times the capture frequencies of 2011 , due to the longer sampling period. In both 2002-2003 and 2011 the most represented species were the talitrid amphipod *Talitrus saltator*, the oniscid isopod *Tylos europaeus* and the tenebrionid beetle *Phaleria provincialis*, together reaching the 98% of the total community. About univariate analyses, only the spatial trends of the diversity indices were compared, while the numerical values were not considered due to the differing sample sizes. In both the samplings the Brillouin index (Fig. 4a) increased at the 5000 and 6000 stations. Fisher's α-diversity of 2011 (Fig. 4b, solid line) had low values in the 2000, 3000 and 4000 stations, with a sudden jump going toward the 5000 and 6000 ones; a similar trend was observed in 2002-2003 (Fig. 4b, dashed line), but in this case the difference between the 4000 and 5000 stations was less pronounced, while the difference increased between the 3000 and the 4000 station. The multivariate analysis of the assemblages highlighted other interesting aspects. The PERMANOVA test showed that the communities significantly differed before and after the seawall construction (Table 4), and the nMDS analysis showed a clear separation between the samples of 2002-2003 and those of 2011 (dashed line), and also between two

Table 4. Summary of one-way PERMANOVA comparing macrofaunal assemblages (species with mainly nocturnal habits) before and after the seawall construction in each of the five stations and for the two sampling seasons. Bold values = significant results.

Source	*df*	MS	Pseudo *F*	*P* (perm)	Unique permutations
A: Year (2002-2003 vs. 2011)	1	2343.8	**12.03**	0.020	9913
B: Site (2000, 3000, 4000, 5000, 6000)	4	2662	**13.66**	0.005	9959
C: Season (Spring vs. Autumn)	1	2933.5	**15.06**	0.016	9921
A X B	4	248.68	1.28	0.382	9962
A X C	1	909.4	4.67	0.091	9815
B X C	4	137.79	0.71	0.589	9970
Residual	4	194.84			
Total	19				

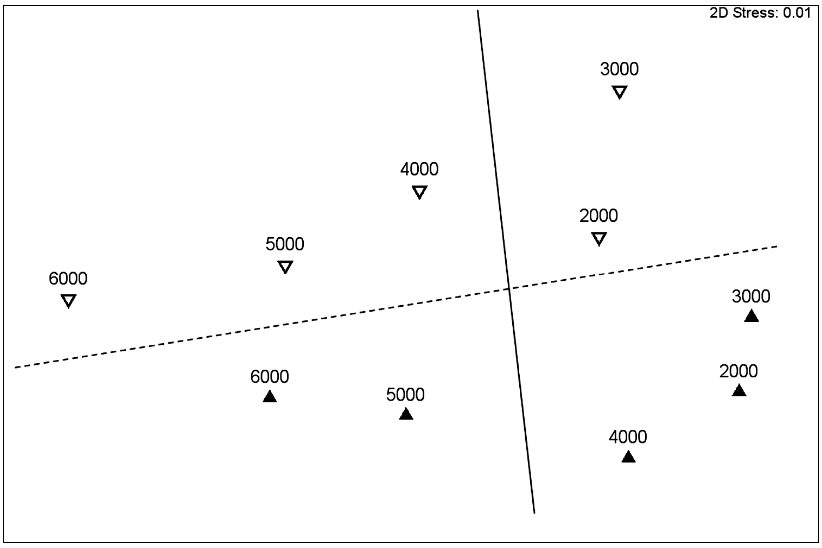

Figure 5. Non-metric multi-dimensional scaling ordination (nMDS) for variation in macrofaunal assemblages structure (nocturnal species) comparing 2002-2003 (white downward triangles) and 2011 (black topward triangles) samplings. A separation is visible between the two sampling sessions (dashed line) and between two groups of stations (continuous line): on one side the 2000 and 3000 stations, on the other the 5000 and 6000. The 4000 station was more similar to these latter in 2002-2003, and changed toward the first group in 2011.

groups of samples (continuous line), with the 2000 and 3000 stations at one extreme and the 5000 and 6000 at the other (Fig. 5). The 4000 assemblage was more similar to the latter group in 2002-2003, while in 2011 it resulted more comparable to the communities of the stations near the river mouth (Fig. 5). The SIMPER analysis (Tab. 5) confirmed that the species responsible for the dissimilarity between 2002-2003 and 2011 samples were *T. europaeus*, *T. saltator* and *P. provincialis*, together explaining the 69% of the differences between communities before and after the seawall construction; the other species giving a significant contribution were the spider *Arctosa perita*, the Tenebrionidae beetle *Trachyscelis aphodioides*, some Neuroptera larvae (genus *Myrmeleon*) and several rove beetles of the Staphilinidae family (mainly represented by *Phytosus nigriventris*). The dissimilarity between pairs of stations across all the samples adding data of the same stations from both data sets was also analysed (Tab. 6).

Table 5. Summary of SIMPER analysis showing the contribution of individual species to total dissimilarity between macrofaunal communities before and after the seawall construction. Only species with prevailing nocturnal habits were considered in this analysis.

Species	Order	**Average ab. (ind m^{-2})**		**Av. Diss.**	**Diss/SD**	**Contrib%**	**Cum%**
		02-03	***11***				
Tylos europaeus	Iso.	3.9	5.52	10.34	2.14	31.47	31.47
Talitrus saltator	Amp.	5.07	2.8	6.67	1.44	20.31	51.78
Phaleria provincialis	Col.	5.96	5.7	5.57	1.58	16.97	68.75
Arctosa perita	Ara.	1.13	0.52	2.45	1.52	7.45	76.21
Trachyscelis aphodioides	Col.	0.84	0	2.39	1.43	7.29	83.49
Neuroptera larvae	Neur.	0.67	0.11	1.85	1.34	5.64	89.13
Staphylinidae	Col.	0.59	0	1.71	0.62	5.2	94.33

The less different (i.e. the most similar) stations were the (proximate) ones 2000 and 3000 (~26% dissimilarity) on one extreme and the (distant) ones 5000 and 6000 (~24%) on the other, while the maximum dissimilarity was observed between stations 3000 and 6000 (~ 63%) and 2000 and 6000 (~ 60%) (Table 6).

4. DISCUSSION

Due to the worldwide increasing use of hard-engineering interventions for coastline protection, the need for effective tools to monitor the impacts on ecosystems has become more and more compelling. Several studies highlighted that seawalls, groynes and dikes often promote erosion by the development of rip currents (Martin et al. 2005; Phillips and Jones 2006; Hsu et al. 2007), altering the hydrodynamic regimes of the coastal zone. Substantial changes to the system ecology were generally observed after such interventions (James 2000; Brown and McLachlan 2002; Chapman and Bulleri 2003; Speybroeck et al. 2006); generally, a decrease in the beach width produces a reduced habitat disposability, which directly affects the macrofaunal diversity and abundance, with consequences for the whole biotic community (Sobocinski 2003; Martin et al. 2005; Bertasi et al. 2007; Dugan et al. 2008). Hence, macrofaunal community analyses represent an effective method to assess such medium/long-term impact; on the other side, a bioindicator permitting to evaluate the effects of beach sedimentary dynamics changes in short time-scales would permit to managers to quickly evaluate the ecological effects of these interventions and eventually counteract them before becoming irreversible. In this sense, the bioindicator based on behaviour that we propose here may help managers to focus on the short-term impacts of beach armouring procedures, especially those acting on the shoreline sedimentary dynamics. Despite behavioural adaptations are being more and more considered as a monitoring tool in environmental conservation, the studies on invertebrates from coastal areas deal almost only with the effects of chemical contaminants (Sharp and Stearns 1997; Lopes et al. 2004; Martins et al. 2007; Gutierrez et al. 2012).

The data on environmental variables highlighted the real possibility that in the short term the built of the cyclopean seawall has reduced the erosion at the stations near to the river mouth. By comparing the results of the sun orientation experiments on *T. saltator* of 2002-2003 and 2011, before and after the construction of a seawall, the worsening of the orientation of all the three sub-populations indicated that this behaviour has become unstable, likely reflecting the instability of the shoreline (Scapini et al. 1995; Borgioli et al. 1999a, El Gtari et al. 2000). In the 2011 experiments the best performance was displayed by the 4000 sub-population, which may confirm our working hypothesis that the hard engineering intervention at the Ombrone River mouth, by reducing the erosion in the proximate beach sector, produced an equilibrium condition already at 4000 m of distance from the river mouth, where the seawall was constructed, engendering an accretion process at the 5000 and 6000 stations. This seems to be confirmed by physical measures of the beach (Tab. 1), but an *ad hoc* study on sedimentary dynamics is required to exclude seasonal or occasional fluctuations (in course under the responsibility of the Park Direction). The bimodal distribution observed in 2011 at the 5000 station, with sandhoppers quite seawards concentrated in spring and more landward directed in autumn, was likely related to the mild climatic conditions registered in autumn, permitting animals to orientate to the dune for shelters and food (Charfi-Cheikhrouha 2000, 2001; Colombini et al. 2002b).

The two models obtained from the SPLM analysis on the orientation angular distributions highlighted that in 2011 the individual experience played a major role in the talitrids'

behaviour with respect to 2002-2003, as expected for sub-populations in changing environments, whereas under stable conditions the species tends to genetically fix a sun compass orientation mechanism (Scapini and Fasinella 1990; Scapini et al. 1995). A long term study, by using the same protocol, would be interesting to assess the impact of the intervention over talitrids' generations (evolutionary time) (planned by the authors).
About the macrofaunal univariate analyses, the Brillouin index spatial trend (Fig. 4a) did not show any significant differences between the data sets of 2002-2003 and 2011, which to some extent is a confirmation of reliability for the 12-hours nocturnal-only sampling of 2011. More interesting was the trend of Fisher's α- diversity (Fig. 4b), as the 5000 and 6000 stations showed the same values in 2002-2003 and 2011, despite the shorter duration of the last sampling. This seems consistent with the hypothesis of an accretion in these beach sectors after the seawall construction, which might have favoured macrofaunal diversity by furnishing a major habitat diversification (Colombini et al. 2006). At the assemblage level, the PERMANOVA results (Tab. 4) confirmed that a change occurred in macroinvertebrates' community from 2002-2003 to 2011, which was also confirmed by the nMDS analysis (Fig. 5). It is worth noting that here the samples from the different stations are located in the same relative positions in both data-sets, thus confirming that the 12-hours nocturnal-only sampling of 2011 was enough to give a satisfactory overview of the assemblage structure. The increased difference between the 4000 community and the 5000 and 6000 ones in 2011 compared to 2002-2003 is in agreement with the supposed changes (accretion and/or habitat enrichment) in the furthest beach sectors, which are likely becoming a diversity reservoir, with a macrofaunal community more various than all the other stations. In the last Park

Table 6. Summary of SIMPER analysis showing the contribution of individual species to dissimilarity between samples of each pair of stations, obtained grouping datasets of 2002-2003 and 2011. Only species with prevailing nocturnal habits.

Species	Order	**Average abundance (ind m^{-2})**		**Av. Diss.**	**Diss/SD**	**Contrib%**	**Cum%**
		2000	**3000**	**25.56**			
Tylos europaeus		5.06	3.78	10.56	0.83	41.31	41.31
Phaleria provincialis		7.61	8.63	6.24	0.73	24.43	65.74
Talitrus saltator		1.08	0.78	3.05	0.88	11.95	77.69
Staphylinidae		0	0.65	1.9	0.54	7.44	85.13
Trachyscelis aphodioides		0	0.62	1.78	0.87	6.98	92.11
		2000	**4000**	**29.72**			
Tylos europaeus		5.06	5.85	8.35	0.78	28.08	28.08
Phaleria provincialis		7.61	6.37	8.06	0.99	27.13	55.21
Talitrus saltator		1.08	2.85	5.33	0.91	17.95	73.16
Arctosa perita		0.25	1.11	2.56	1.15	8.6	81.76
Trachyscelis aphodioides		0	0.68	1.79	0.91	6.02	87.78
Halammobia pellucida		0	0.42	1.1	0.75	3.71	91.5
		2000	**5000**	**45.74**			
Talitrus saltator		1.08	6.46	16.35	2.82	35.74	35.74
Phaleria provincialis		7.61	4.17	11.3	1.52	24.71	60.45
Tylos europaeus		5.06	5.23	8.64	1.11	18.89	79.34
Arctosa perita		0.25	1.17	2.99	1.96	6.53	85.87
Parallelomorphus laevigatus		0	0.58	1.74	1.25	3.8	89.67
		2000	**6000**	**60.34**			
Talitrus saltator		1.08	8.49	24.04	3.62	39.84	39.84
Phaleria provincialis		7.61	2.37	17.01	1.98	28.19	68.03
Tylos europaeus		5.06	3.64	11.01	2.8	18.24	86.27
Arctosa perita		0.25	1.14	3.37	1.95	5.59	91.86
		3000	**4000**	**31.46**			

Tylos europaeus	3.78	5.85	10.45	0.81	33.22	33.22
Phaleria provincialis	8.63	6.37	8.81	1.07	28.01	61.23
Talitrus saltator	0.78	2.85	5.58	1.42	17.75	78.99
Arctosa perita	0.45	1.11	2.08	1.02	6.6	85.58
Staphylinidae	0.65	0.35	1.62	0.65	5.14	90.73
	3000	**5000**	**49.08**			
Talitrus saltator	0.78	6.46	16.57	5.83	33.76	33.76
Phaleria provincialis	8.63	4.17	13.61	1.93	27.73	61.49
Tylos europaeus	3.78	5.23	9.77	0.96	19.9	81.39
Arctosa perita	0.45	1.17	2.61	2.2	5.32	86.72
Parallelomorphus laevigatus	0	0.58	1.73	1.22	3.52	90.24
	3000	**6000**	**62.81**			
Talitrus saltator	0.78	8.49	24.01	8.01	38.22	38.22
Phaleria provincialis	8.63	2.37	19.77	3.09	31.48	69.71
Tylos europaeus	3.78	3.64	9.51	1.25	15.15	84.85
Arctosa perita	0.45	1.14	3.12	2.42	4.96	89.81
Parallelomorphus laevigatus	0	0.68	2.08	2.13	3.31	93.13
	4000	**5000**	**30.65**			
Talitrus saltator	2.85	6.46	10.08	1.86	32.9	32.9
Phaleria provincialis	6.37	4.17	7.01	1.21	22.89	55.79
Tylos europaeus	5.85	5.23	6.47	1.32	21.11	76.9
Arctosa perita	1.11	1.17	2.08	2.4	6.8	83.7
Parallelomorphus laevigatus	0.25	0.58	1.31	1.12	4.26	87.96
Halammobia pellucida	0.42	0.49	1.14	0.77	3.73	91.7
	4000	**6000**	**44.18**			
Talitrus saltator	2.85	8.49	16.39	2.73	37.11	37.11
Phaleria provincialis	6.37	2.37	11.19	1.7	25.33	62.44
Tylos europaeus	5.85	3.64	8.84	2.34	20.01	82.45
Arctosa perita	1.11	1.14	2.44	1.88	5.52	87.97
Parallelomorphus laevigatus	0.25	0.68	1.59	1.57	3.6	91.57
	5000	**6000**	**23.61**			
Tylos europaeus	5.23	3.64	5.71	1.26	24.17	24.17
Talitrus saltator	6.46	8.49	5.64	2.19	23.88	48.04
Phaleria provincialis	4.17	2.37	5.61	1.75	23.77	71.81
Arctosa perita	1.17	1.14	1.73	0.83	7.33	79.15
Halammobia pellucida	0.49	0.15	1.25	0.64	5.3	84.45
Parallelomorphus laevigatus	0.58	0.68	1.18	1.38	5	89.45
Neuroptera larvae	0.47	0.29	0.98	1.43	4.15	93.59

Management Plan this sector of the beach has been declared an integral reserve, where tourists cannot stay for leisure activities (Scapini 2010). The results on the macrofaunal community from the present study may support this conservation strategy.

The detailed SIMPER analysis of dissimilarity among stations (Tab. 6) confirmed that the proximate (with relation to the impact) stations (2000 and 3000) and the distant ones (5000 and 6000) harbour quite different communities, with a dissimilarity of 63% from one extreme to the other. The 4000 station had intermediate dissimilarity values with respect to both kinds of stations, which confirms it as a transition point from eroded to accreting stations.

To conclude, the behavioural indicator and the macrofaunal analyses obtained in this study validated reciprocally, as both showed a change (in orientation and macrofaunal community) at about 4000 m of distance from the river mouth. Apparently, the seawall modified coastal sedimentary dynamics immediately after his construction, as the effects on the macrofaunal community were already evident less than one year after the end of the works.

About the scope of this study, i.e. the validation of sun orientation of *T. saltator* as a bioindicator of impact, the sun orientation behaviour has short time scales, which is desirable for an early bioindicator and makes this kind of studies a promising path to follow, particularly when studying short-medium term impacts, such as those of engineering interventions. It would be interesting to repeat this kind of experiments on shorter time-scales, possibly few weeks before the beginning and after the end of the analysed impact, to evaluate how much it is actually effective on the very short period. However, it is rarely possible to perform field experiments during hard engineering works. The deep existing knowledge regarding talitrids' orientation and ecology (Williamson 1951; Pardi and Papi 1952, 1953; Scapini et al. 2002; Scapini 2006), coupled with their short life-span, the rapid generation turn-over and their abundance and diffusion on temperate sandy shores, may make the sun orientation of *T. saltator* a reliable bioindicator of impact on sandy beach stability, which is not expensive, not time-consuming, does not require to capture rare or endangered species and furnishes results that are easy and immediate to interpret.

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3.3 Case study 2: the Leirosa beach, Portugal

The Leirosa beach is located to the south of Figueira da Foz, midway along the Portuguese Atlantic coast. Here a long, well developed dune system protects the village of Leirosa and two paper factories.In 1995 a submarine pipeline was built to discharge the industrial and civil effluents, and the dune was rebuilt using sand and rocks as inner core for protection.However, the continuity of the dune system was seriously damaged; moreover, the use of heavy machinery aggravated the coastal erosion already caused by a groyne located less than 1 km north, which caused the retention of large amounts of sand and increased the erosion problems southwards (Antunes do Carmo et al. 2010). Due to these erosive dynamics, by the end of 1999 the protection dune was almost broken and flat; to protect the aeration and load chamber of the outfall pipe, a rehabilitation of the dune started in March 2000 (Schreck Reis et al. 2008). Sand (very similar to that of the dunes in terms of texture and nutrient content) was brought from a nearby inland site, and the foredune was rebuilt along an extension of about 200 m. After the intervention, the sand dune system was replanted with transplants of *Ammophila arenaria* (L.) Link, the most common grass used in these situations, easy to collect and transplant and rapidly propagating (Van der Putten 1990), which is considered to be the most appropriate, durable, and effective way to rehabilitate dunes (Van der Putten 1990;Van der Laan et al. 1997), but unluckily in winter 2000–01 strong winds and unusually heavy precipitations caused great damage on the coast, and the ocean front of the dune system was destroyed. After evaluating several methods for a new rehabilitation of the dune, in February 2005 and geotextile containers where placed, filled with sand (geocontainers) in a pyramidal formation parallel to the coastline, to a height of 8 metres. Once the sand containers were in place, a 1.0 m layer of sand was deposited and a dune revegetation was performed with *Ammophila arenaria* (L.) transplants. However, the hydrodynamic forces that affected the dune led to the opening of the geotextile layers in some places, and some containers were also shifted from their initial position. Hence, geotextile tubes were chosen to reinforce the bottom layers of the rehabilitated stretch of the dune. In July 2008 the tubes (approximately 20 m long and 1.60 m in diameter) were prefabricated to avoid failure of the joints, placed at the bottom of the existing structure and then filled with a suspension of sand and water, to give additional stability to the dune structure (Antunes do Carmo 2010). However, already in mid-December 2008 strong erosion effects were felt in Leirosa, with a considerable amount of sand removed from the beach and the front of the dune, which left the geotextile tubes completely exposed. Anyway, the structure as a whole was not affected, so it was planned to cover the sea face of the dune with sand again, and a revegetation was performed in April 2009, to ensure better fixation of the sand and stabilization of the front of the Leirosa dune system. The dune stretch under intervention has remained stable for the last 7 years, whereas the sides to the left and right of it have suffered strong erosion along hundreds of meters, showing a regression of about 4/5 m each year (Antunes do Carmo 2010). As no data were available on talitrids' behaviour and macrofaunal community on this beach before the intervention, we decided to evaluate behavioural adaptations of sandhoppers and macrofaunal features in two points, the first one close to the rehabilitated dune and the second one in front of the natural, eroded dune, to make a spatial comparison, instead of the temporal one that was made in the Italian case study. In this case, what was assessed is the suitability of the behavioural indicators to monitor the long-term effects (more than 4 years ago) of a soft-engineering intervention, which are still poorly understood. The following two published papers describe the outputs from this study, and a comprehensive discussion, on both the Italian and Portuguese case study, can be found in the 3.4 section.

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

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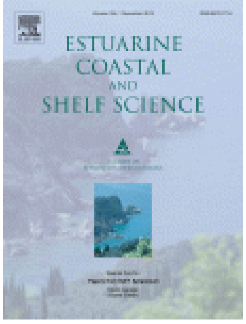

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Regional terms: Europe, Portugal, Eastern Atlantic coast, Leirosa beach

Geographic bounding coordinates: 40°02’57.33’’ N; 08°53’35.01’’W

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 (stabilization 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 stabilization 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.

1. 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, 2008;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, urbanization, 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. Socalled "soft engineering" interventions (nourishment processes) seek to mitigate beach erosion, but may cause ecological damage to sandy beach habitats (Nordstrom, 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 stabilization 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 beach 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 and Scapini, 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,b).

The sandhopper *Talitrus saltator* (Montagu, 1808) is a 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 have 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 *Talitrus 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 a 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 (Bregazzi and Naylor, 1972; Scapini et al., 1992, 1996, 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 stabilization of the dune system trough 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 beachedune system and provide baseline information about the pattern used by this species to cope with the physical constrains 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 *Talitrus saltator* behaviour, through a preand 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 stabilized environment (foredune) may lead to a stable behavioural performance.

Fig. 1. Location of the study area, the Leirosa beach (40° 02’ 57.33’’ N, 08° 53’ 35.01’’ O) on the eastern Atlantic coast of Portugal (Photo credits for the right image: Google Earth, Version 6, accessed January 2012).

2. Materials and methods

2.1. The study site

The Leirosa sand dune system (40°02’57.33’’N, 08°53’35.01’’W) is located south of Figueira da Foz, on the European Atlantic Coast (Portugal) and has an extension of about 10 km (Fig. 1). The beach is characterised by a well-developed sand dune ecosystem with low pressure from tourism. The beach is mesotidal with semidiurnal tides, with waves that frequently reach amplitudes of about 3 m. 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 beachedune system (Reis et al., 2008;Carmo et al., 2010). This coastal region is currently characterized 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 geotextilesfilled 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 .

2.2. Orientation experiments

Orientation experiments were performed on 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 40 cm 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-min in a Plexiglas tube. Tests were carried out in the morning, starting at 9:00 a.m. solar time, and in the afternoon, starting at 3:00 p.m. 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 inMcLachlan 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).

2.3. Endogenous activity rhythms

In May and September 2011, live samples of freshly collected adult *Talitrus 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 (12 cm in diameter, 16 cm 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 offiltering paper. Each recording chamber was connected to a logger, downloading every 20 min 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°C±1°C for 21 days.

2.4. Data analysis

2.4.1. Orientation

Individual data of orientation expressed as angles to the North (°) 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.

2.4.2. 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 48 h 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 lightedark 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 10 h 20’ to 30 h 20’. 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).

3. Results

According to theMcLachlan’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 on the beach at the ebbing tides, and a supralittoral zone of about 30 m 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). 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 1 mm 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).

3.1. Orientation tests

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

Characteristic	Leirosa
Beach width (m)	46 ± 8
Supralittoral area extension (m)	29 ± 6
Average slope (%)	6 ± 0.4
Foredune height (m)	8 ± 1
Sediment granulometry (mm)	Medium (0.250 - .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

The circular distributions of the orientation angles of *T. 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 in the north-western quadrant with a unimodal distribution of orientation angles, and a mean direction of 312° ± 10° that included the TED (310°) in its confidence interval. The Rayleigh test for uniformity was significant for the whole distribution (*p*

< 0.001). The variables considered in the regression SPLM analysis were: month (May/September), sun azimuth, landscape vision (landscape/non 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. Morning/afternoon (am/pm) and rising/ebbing tide gave the same information (ebbing tide in the morning, rising tide in the afternoon), so we did exclude morning/afternoon from the analysis). We start with the additive model including all the variables and factors (10) that could affect orientation:

Orientation ~ month + sun azimuth + landscape vision + tide + sun visibility + sky cover + air temperature + air humidity + antenna articles + cephalic length; Likelihood = 844.0636; AIC = 896.0636; Degrees of freedom = 251.

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 =487.0301; AIC =511.0301; 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 (Fig. 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 (Fig. 3). In September, sandhoppers were less dispersed in comparison 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 (Fig. 3). In fact, without landscape vision significant deviations from the TED were confirmed by the confidence intervals of the mean direction (70° ± 22°, not

Table 2. Environmental and population variables observed during the orientation experiments in Leirosa beach (May and September). Mean values ± 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 ± SE	Median (Range)		Mean ± 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 ± 0.1	0.8 (0.2 - 1.3)		1.0 ± 0.1	1 (0.4 - 1.9)
Air temperature (°C)		28.1 ± 0.4	26 (22 - 35)		24.4 ± 0.2	24 (22 - 28)
Air humidity (%)		54.3 ± 1.3	66 (26- 75)		75.3 ± 0.9	77 (60 - 92)
Sky Cover			1 (0-8)			1 (0- 8)
Sun visible (n)	134			99		

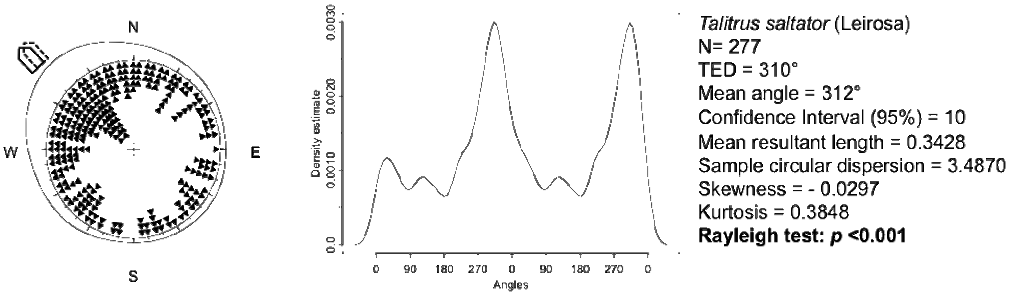

Fig. 2. Circular plot and summary statistics of orientation angles of *Talitrus saltator* (Montagu, 1808) tested on Leirosa beach. Density estimates (kernel method) are double plotted on the Cartesian graphs (on the right). Arrow with continuous line: Theoretical Escape Direction seawards (TED); Arrow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

including the TED, Table 3). The best model for May also described 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 (seeFig. 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, Fig. 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).

3.2. Endogenous activity rhythms

More than 90% of the individual 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 (Fig. 4a and b) 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 24 h 15’. A second activity peak (bimodal pattern) was significant at 12 h 8’for the 26% of the active animals, but with a minor definition (SNR = 0.06 ± 0.04) in comparison with the circadian peak (SNR = 0.22 ± 0.04). During both months, the activity of several adults peaked also just before the high tide hours during the spring tide (arrows, Fig. 4a). The same pattern of activity was found with the periodogram analysis (Fig. 4c and d), which represents the free-running periods of the individuals, estimated over the entire time

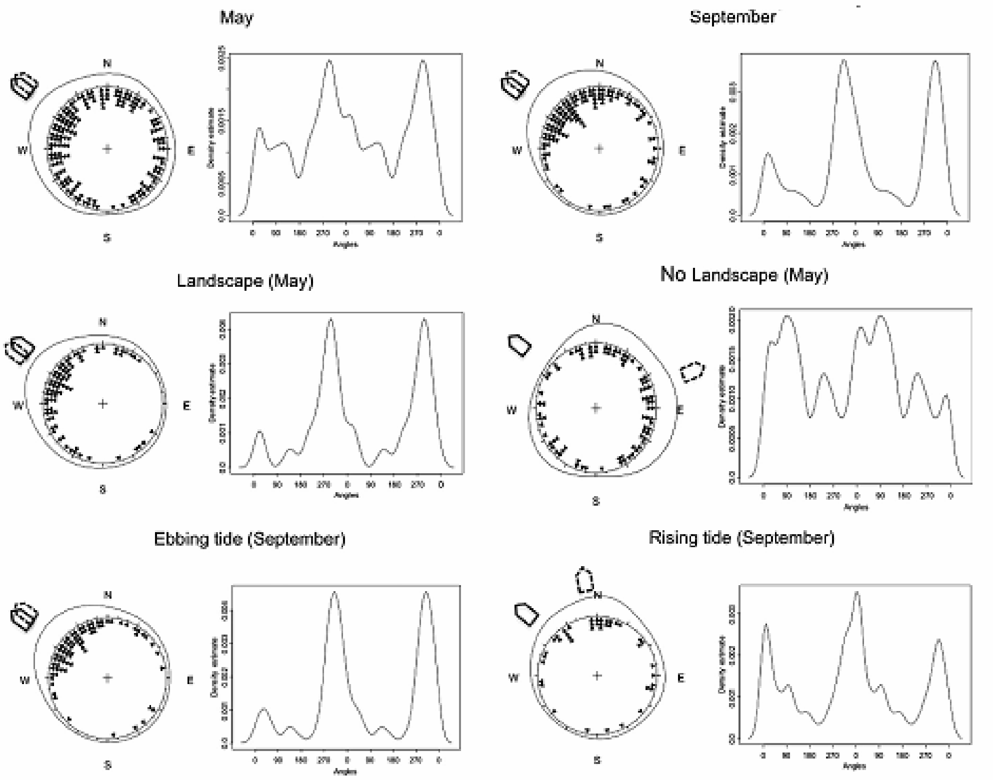

Fig. 3. Circular plots for angular distributions considering some significant factors resulted from the best orientation model for both months. Arrow with continuous line: Theoretical Escape Direction seawards (TED); Arrow with punctuated line: Mean Angle. The respective summary statistics are reported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

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

series (21 days), showing a circadian period and a bimodal period peak recorded in some individuals (e.g. Fig. 4c). In order to check the bimodality, periodogram and MESA analyses were performed on 5 individuals that showed clear bimodality (Fig. 5). These times series confirmed a clear circadian periodicity around 24 h 20' (periodogram) and 24 h 50' (MESA) for the five individuals with a second (bimodal) period of 12 h 20' (periodogram) and 12 h 50' (MESA). The difference of about 30 min between the periodogram and MESA analyses is known and was explained byPalmer (1995). For the bimodal actograms (Fig. 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 (Fig. 5a, see arrows).

4. 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 *Talitrus 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 the water's edge. This capacity is in agreement with the literature available for *Talitrus 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 response to landscape vision suggests that this feature represents a mechanism other than sun compass used by sandhoppers to orient

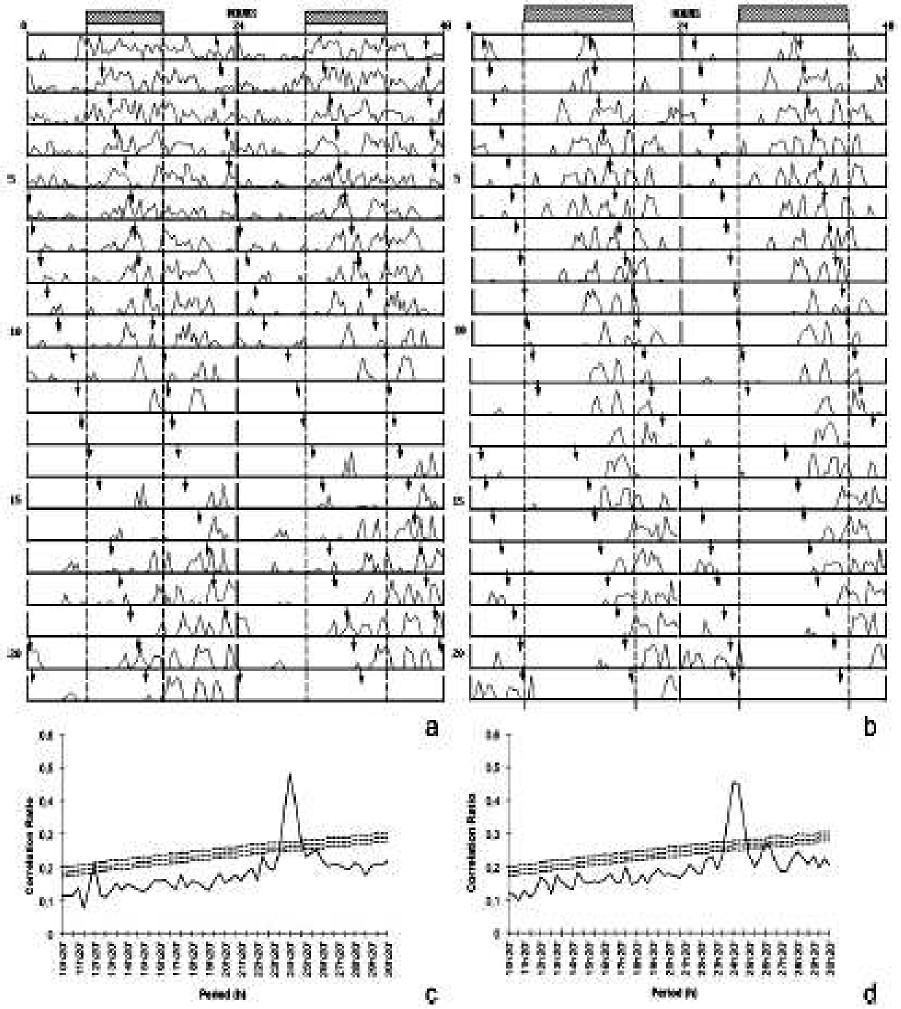

Fig. 4. Actograms and periodograms of two individuals of *Talitrus saltator* (Montagu, 1808) recorded singly in May and September. Dashed bars at the top of actograms indicate times of natural darkness at the day of sample collection; arrows represent high tide peaks during the recording period. Activity 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) *τc* (circadian period) = 24 h 20’ , SNR = 0.912, *τb* (bimodal period) = 12 h 20’, SNR = 0.013. (b) Experiment of September, animal 7 with respective periodogram (d) *τc* = 24 h 20’, SNR = 0.816.

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, 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 onlandscape 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

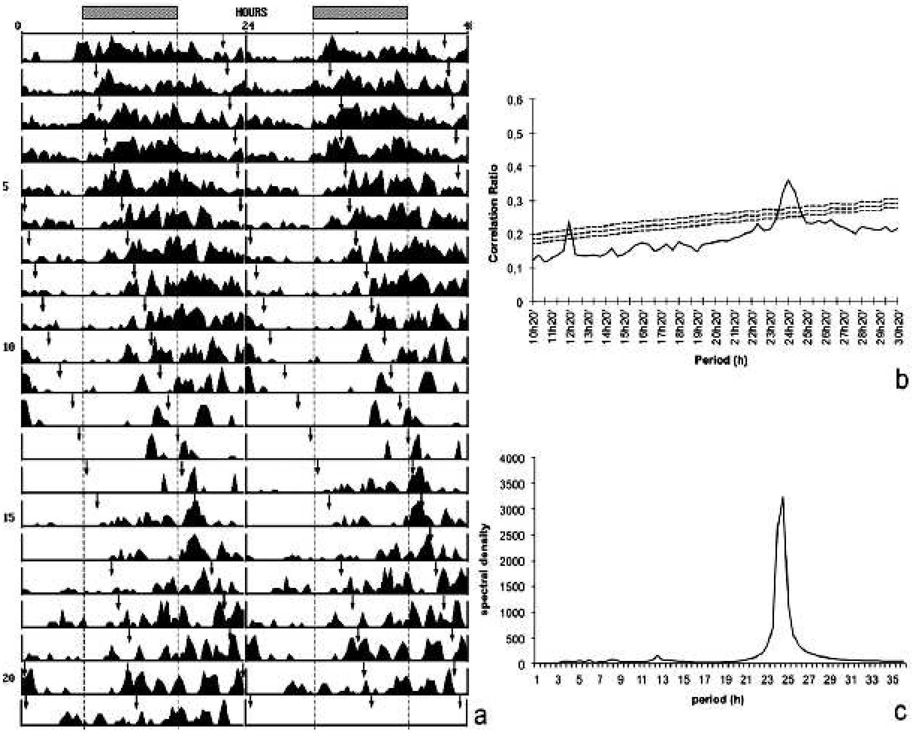

Fig. 5. Mean of activity of 5 bimodal individuals (a) and respective periodogram and MESA analysis. Dashed bars at the top of actograms indicate times of natural darkness on the collection day and arrows represent high tide peaks during the recording period. Probability lines in the periodogram are, from the top to bottom, the 99.9%, 99% and 95% probabilities for peak significance. (b) Periodogram: *τc* (circadian period) = 24 h 20’, SNR = 0.431, *τb* (bimodal period) = 12 h 20’, SNR = 0.103; (c) MESA analysis: *τc* = 24 h 50’,spectral density ¼ 3238, *τb* ¼ 12 h 50’, spectral density = 136.

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 agree 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, 2006). From these results we infer that dunes stabilization, despite the changes in the landscape, favours 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, 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 byTessmar-Raible and Raible, 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 *Talitrus saltator* from a mesotidal beach in the eastern Atlantic coast with an artificial foredune. The periodogram analysis showed a clear endogenous rhythm in *Talitrus saltator* close to 24 h (24 h 15’ ± 6’) throughout the registration period of 21 days and a second bimodal activity peak near 12h (12h 8’ ± 7’), which appeared just before the high tide hours. The high tide and night-time patterns of activity clearly suggest that *Talitrus saltator* population from Leirosa possesses both 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 *Talitrus 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, *Talitrus 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 12.4 h, 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) hypothesized 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 stabilization of the beach environment under study. The actual stabilization 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 *Talitrus saltator*. A continuous stabilization 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 *Talitrus 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 programmes and policy strategies.

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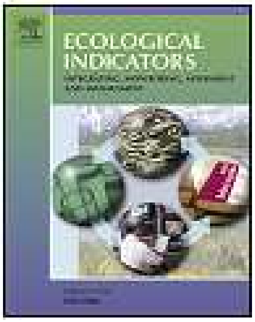

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ABSTRACT

In the last decades the increasing urban development on coastal areas have produced extensive modifications on shores all over the world, making critical the active management of pressures on sandy beaches. The use of engineering solutions to counteract beach erosion has been significantly increasing; the ecological indicators used to monitor these interventions generally focus on short- and medium-term effects, while little is known on their effectiveness on long-term temporal scales. The following ecological indicators have been tested in the present study: (a) macrofaunal community abundance and diversity and (b) orientation behaviour of *Talitrus saltator*, a talitrid amphipod widespread on Mediterranean and European Atlantic sandy beaches. Two sites were considered on a sandy beach of the Portuguese Atlantic coast, one located in front of a natural dune and the other at about 500 m of distance, where the dune had been rebuilt between 2000 and 2008 using geotextile tubes. In 2011 and 2012, macrofauna sampling and orientation experiments on *T. saltator* were performed at both sites in spring and autumn; contemporaneously the main environmental variables were registered. Macrofaunal data were analysed through multivariate statistical tests, and for the orientation distributions the circular statistics were calculated and multivariate analyses for angular data were performed. Geotextiles appeared to be successful in stabilising the recovered dunes; accordingly, the diversity of the macrofaunal communities and the orientation performances of *T. saltator* showed no differences between the altered and control sites. Significant reductions were nevertheless observed in the artificial-dune site regarding the abundance of *T. saltator* and, to a lesser extent, macrofaunal densities, likely ascribable to the presence of geotextiles instead of a vegetated natural dune, preventing invertebrates to burrow into the sand. These results, complementing a more comprehensive study on these two sites, indicate the abundance of *T. saltator* as the best indicator to follow long-term effects of this kind of soft-engineering intervention. The use of this bioindicator may be recommended for the late phases of monitoring procedures in dune-recovery processes.

1. Introduction

Sandy beaches are among the most important environments for human activities (Schlacher et al., 2008), being intensively exploited especially in the last two centuries (Nordstrom, 2000). In the coming decades about three quarter of the world’s population will live within 60 km of the shoreline (Povh, 2000) intensifying human pressures both as direct and indirect impacts. Sandy beaches will be squeezed between rising sea level and coastal erosion on the marine side and expanding human populations and development on the landward side (Schlacher et al., 2008; van der Weide et al., 2001). To slow down this trend, an active management has become necessary for most coastal zones. Currently, managers involved in coastal defence tend to focus on physical and geomorphological features (James, 2000; Micallef and Williams, 2002), despite more and more evidences have been accumulated of ecological change in beach ecosystems due to human interventions (Brown and McLachlan, 2002; Defeo et al., 2009; Dugan et al., 2010; Jones et al., 2007; Schlacher et al., 2007, 2008). Sandy beaches are dynamic habitats, with specialised living communities structured mainly by physical forces (Defeo and McLachlan, 2005; McLachlan and Brown, 2006). A deep knowledge of biotic responses to modifications of the physical environment is therefore a critical step, and the effects of interventions on local communities should be always considered when planning management, conservation, or restoration strategies. The use of geosynthetic containers was initiated more than 50 years ago in the USA, The Netherlands and Germany (Saathoff et al., 2007), and has become increasingly popular. Although this intervention impedes the natural morphodynamics of the coast y preventing erosion, so that a static shoreline may develop, geosynthetic containers are considered a soft solution because, if an unforeseen environmental impact ensues, they can easily be sliced open and removed, spilling sand back onto the beach (Corbella and Stretch, 2012). Moreover, the use of geotextile tubes to create artificial dunes reduces costs, time and environmental damage, with the advantage of being adaptable to the morphology of the dune system and using locally available sand (Oumeraci et al., 2003; Stokes et al., 2012). Recent examples of successful application of geotextile tubes come from the coastal zones of USA (Harris and Sample, 2009), Australia (Jackson et al., 2004) and the Republic of Korea (Shin and Oh, 2007), where positive outcomes were obtained in terms of coastal protection. However, very little is known on the effects of dune recovery with geotextiles on biotic communities, if compared to the several studies on the ecological impacts of both beach nourishments (reviewed in Goldberg, 1988; Peterson et al., 2006; Speybroeck et al., 2006) and hard-engineering interventions (Bertasi et al., 2007; Dugan et al., 2008; Martin et al., 2005; Sobocinski, 2003). Negative nourishing effects were observed at the population, community and ecosystem levels on all the biotic components (Bishop et al., 2006; Fanini et al., 2009; Speybroeck et al., 2006). Generally, if the interventions were carefully planned, they may represent short-term, pulse disturbance (Peterson et al., 2006) and a rapid ecological recovery may occur after few months, as sandy-beach species are adapted to severe physical disturbances (Hall, 1994). On the other side, hard-engineering interventions often promote erosion by the development of rip currents (Hsu et al., 2007; Martin et al., 2005; Phillips and Jones, 2005), altering the hydrodynamic regimes of the coastal zone, which in turn drive the composition of benthic macrofaunal assemblages (Defeo and McLachlan, 2005; McLachlan and Brown, 2006). Substantial changes to the system ecology were generally observed after these interventions (Brown and McLachlan, 2002; Chapman and Bulleri, 2003; James, 2000; Speybroeck et al., 2006). Frequently, as eroding beaches tend to become narrower, the reduced habitat disposability directly impacts diversity and abundance of biota in the upper intertidal zone, with consequences at all the trophic levels, thus engendering long-term ecological effects on the whole community (Bertasi et al., 2007; Dugan et al., 2008; Martin et al., 2005; Sobocinski, 2003). To monitor the effects of dune-recovery using geotextiles, managers need effective ecological indicators. Furthermore, being the ecological effects of such interventions relatively unknown, various indicators should be considered to obtain indications fitted to different ecological compartments or functional roles, depending on the impacts to be assessed (Dale and Beyeler, 2001; Niemerijer and de Groot, 2008; Pinto et al., 2009; Salas et al., 2006). The diversity and the structure of the macrofaunal community are considered representative bioindicators of impacts on sandy beaches (Fanini et al., 2009; Lercari and Defeo, 2003; Peterson et al., 2000; Schoeman et al., 2000). Intertidal invertebrates are at the basis of the food chain (Audisio, 2002; McLachlan and Brown, 2006), thus representing a key-component of the beach ecosystem. Arthropods in particular, well adapted to harsh climatic conditions and involved in the processing of nutrients from both sea and land, have been recognised as reliable bioindicators of ecosystem stability and recommended for use in conservation planning of sandy beaches (Colombini et al., 2003; Finnamore, 1996; Mattoni et al., 2000). However, a relatively little attention was given to the longterm effects of human-induced disturbances on the macrofaunal community structure of beaches (Bessa et al., 2013a; Lercari and Defeo, 2003; Schoeman et al., 2000). Another potential indicator of shoreline changes is the behaviour of the invertebrates dwelling sandy beaches, which may represent a first response to changes in their life-environment. Almost all beach arthropods display common adaptive behaviours, such as digging attitudes, rhythmic activity patterns, and orientation abilities to quickly recover the safest zone on the littoral (Audisio, 2002; Schlacher et al., 2008). Several behavioural studies were carried out on the sandhopper *Talitrus saltator*, a widespread talitrid amphipod that often dominates (in term of abundance) sandy beach communities at temperate northern latitudes on Mediterranean and Atlantic coasts (Scapini, 2006). One of the most interesting behavioural aspects shown by this and related talitrid species is zonal recovery, namely the ability to come back to the intertidal beach zone after spontaneous or accidental displacements, searching for the right moisture conditions to burrow into avoiding dehydration risk

(Pardi and Ercolini, 1986). The correct orientation is ensured by a redundancy of mechanisms, both heritable (sun-compass, beach slope) and learned (landscape features, reviewed by Scapini, 2006); orientation can also be modified according to the immediate climatic and ecological beach characteristics, adapting to an increasing risk of dehydration or submersion. Populations of *T. saltator* exhibit a higher seaward concentration on beaches that are stable in time and result more scattered if changes frequently occur (Borgioli et al., 1999a; ElGtari et al., 2000; Scapini et al., 1995). For this reason talitrid sun orientation was proposed as an indicator of human-impact on shoreline stability (Fanini et al., 2007; Scapini et al., 2005). Since the species' life-span in the Mediterranean area ranges from 6 to 9 months (Marques et al., 2003), orientation is expected to reflect the response to quite recent changes, while little is known about the effects on the populations' orientation over longer periods.

In this paper, the diversity and abundance of the macrofaunal community and the orientation behaviour of *T. saltator* were analysed on a sandy beach, where a dune-recovery intervention was carried out three years before. Two sites were defined, having the same physical conditions, to highlight eventual impacts ascribable to the intervention: (a) a control site, where no relevant alterations have been observed over the last two decades and (b) a site in front of the artificial dune. We aimed to assess which long-term impacts are susceptible of being detected by the selected indicators, assuming that three years represent a reasonably long temporal range in very dynamic and changeable environments such as sandy beaches (Dugan et al., 2008; Martin et al., 2005; McLachlan and Brown, 2006; Sobocinski, 2003). This study represented an extension of two previous studies in the same sites; the first one analysed the macrofaunal community features to detect eventual effects of the dune-recovery intervention (Bessa et al., 2013a), while the second represented a first assessment of behavioural adaptations in *T. saltator* sub-populations on the same rehabilitated sandy shore (Bessa et al., 2013b). Here the same features were studied in parallel and the two sites were compared, to assess whether and which bioindicator may be proposed for long-term monitoring of dune recovery with geotextiles.

2. Materials and methods

2.1. The study-site

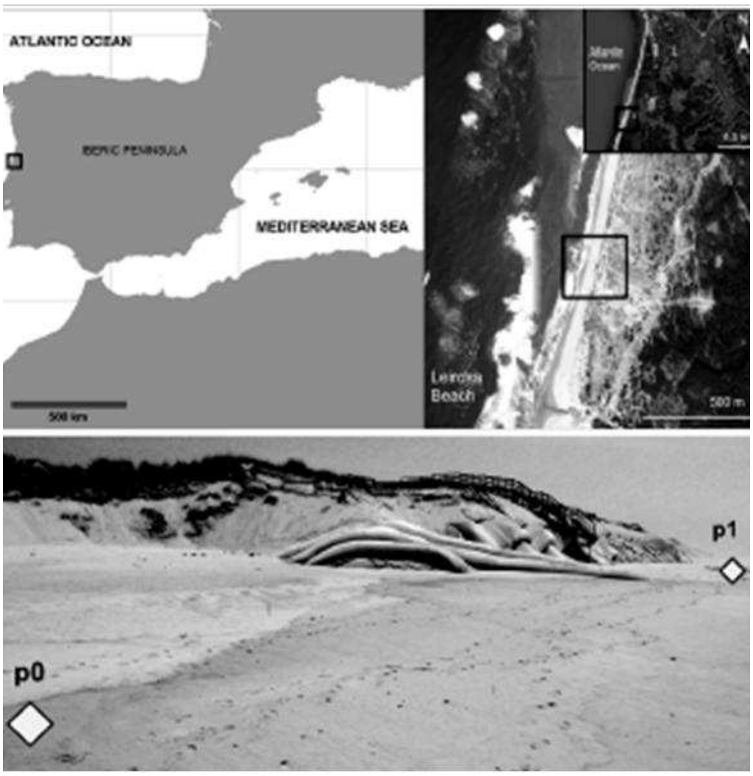

Fig. 1. (a) Study site: location of the Leirosa beach on the Portuguese coast (40°02’57.33’’N, 08°53’35.01’’W); photo credits for the image on the right: Google Earth, Version 6, accessed January 2012, adapted from Bessa et al. (2013b). (b) The experimental sites: p0 = shore in front of the natural dune, p1 = shore in front of the reconstructed dune, where the geotextile tubes are well visible (photo by D.H. Nourisson).

The Leirosa beach (40°02’57.33’’N, 08°53’35.01’’W) is located to the south of Figueira da Foz, midway along the Portuguese Atlantic coast (Fig. 1a). The beach is mesotidal with semidiurnal tides, and waves frequently reach amplitudes of about 3 m. The well-developed sand dune system is about 10 km long, and protects two cellulose pulp and paper factories located behind it. In 1995 a submarine pipeline was built to discharge the industrial effluents, damaging the continuity of the dune system due to the use of heavy machinery. After this intervention the dune was rebuilt (March 2000), but in the following years a considerable erosion affected the whole coastline south of Figueira da Foz, due to important changes engendered by the various human interventions in the surroundings on the shore sedimentary dynamics (Antunes do Carmo et al., 2010; Duarte and Reis, 1992; Reis et al., 2008). Additional works were thus carried out to rehabilitate the sand dune system, the last of which in 2008, with the installation of geotextile prefabricated tubes filled with locally available sand and water. Although the structure as a whole has endured, strong erosion effects were still visible in Leirosa (Antunes do Carmo et al., 2010) and the tubes today are completely exposed, showing signs of geotextile disruption (Fig. 1b). The two beach points chosen to assess the effects of the reconstruction were called p0 (control site, in front of the natural dune) and p1 (the impacted site, in front of the artificial dune), at about 500 m to the south (Fig. 1c). In 2011 and 2012, sampling of macrofauna and orientation experiments on *T. saltator* was performed at the two sites, in spring and autumn. To characterise the environmental features we registered, during the ebbing tides, beach slope and width, the extension of the intertidal zone, sand temperature, water salinity, wave height and period. During the orientation experiments we also recorded air temperature, air humidity, sky cover (assessed on a conventional scale from 0/8 to 8/8) and solar radiation because of their possible effects on the orientation of *T. saltator*. The sky cover directly influences the solar visibility, thus allowing or preventing the use of the sun compass, while the solar radiation includes also information about climatic conditions, in particular increasing the dehydration risk of amphipods during the tests. The sun azimuth was estimated from the geographical coordinates of the site and the time of the day of each orientation test. The shoreline direction was also registered, rom which the TED (Theoretical Escape Directions seawards) for sandhopper orientation was calculated, i.e. the perpendicular to the seashore.

2.2. Macrofauna sampling

Macrofaunal communities were sampled during low neap tides, simultaneously at p0 and p1, in May and October 2011 and in June and October 2012. Samples were taken at regular intervals along two shore-normal transects extending from the swash zone around the low tidewater mark to the base of the foredune; ten equidistant sampling levels were considered across each transect. The mean high water neap tide mark was used as a reference point to define the supralittoral and the intertidal zones. Five sampling levels were considered in the supralittoral zone of the beach and five in the intertidal one, adjusting the intervals between levels as necessary through the year. This sampling procedure was able to account for differences in the spatial distribution of macrofauna throughout the year (Gonçalves and Marques, 2011). For each level and each transect, three cores (inner diameter 25 cm, 30 cm deep) were taken. The sand was sieved through 1 mm mesh bags in the swash and the samples were fixed in situ with 5% formalin, to separate and identify the collected animals in the laboratory. To determine sediment characteristics, triplicate sediment cores (2.5 cm diameter, 30 cm deep) were also taken at each sampling level and kept in airtight plastic bags to analyse them in the laboratory. There sand granulometry was determined using the Wentworth scale (Brown and McLachlan, 1990), together with organic matter in the sediment and moisture content, according to the procedures described in Marques et al. (2003) and Gonc¸ alves et al. (2009), using the GRADISTAT 8 software (Blott and Pye, 2001).

2.3. Orientation experiments on T. saltator

Orientation experiments were performed simultaneously at p0 and p1, in May and October 2011 and in June and October 2012. Adult amphipods were removed from the sand in the morning of the experiments and were tested in two experimental arenas (40 cm of diameter) having 72 pitfall traps of 5° each at their rim, placed horizontally at one metre height (Scapini et al., 2005). A transparent cylindrical screen covered each arena device and a white cardboard (10 cm height) was applied around it at alternate releases of 10 animals, to have one release with the landscape vision allowed and the following one with only the sky visible. About 80 individuals (4 releases of 10 animals with the landscape view and 4 with screened landscape) were tested in the morning (from 9:00 to 11:00, solar time) and 80 in the afternoon (from 15:00 to 17:00, solar time). After having registered the angles of orientation to the North of the trapped individuals, these were individually stored in alcohol 75% for later observations and measurements. In the laboratory, the sex (males, females and juveniles of a length < 5 mm, having no visible external sex characteristics) and the reproductive status (ovigerous, mature and immature females) were determined for each individual. The cephalic length and counted the number of right antenna articles of each individual as proxies of age were also measured (Marques et al., 2003; Williams, 1983).

2.4. Data analysis

To assess the effects of the artificial dune on the beach features a Principal Component Analysis (PCA) was performed using an Euclidean distance resemblance matrix from normalised data of the following variables: water salinity, beach width and slope, wave height and period, sediment organic matter contents, sediment moisture and temperature. Univariate biotic metrics (total faunal density, species richness, Shannon–Wiener diversity index) and the abundance of most abundant species were compared for p0 and p1 through a series of Permutational Analyses of Variance, PERMANOVA (Anderson et al., 2008), after converting them in Euclidean distance similarity matrices. The design included three fixed factors: (A) site (natural vs. artificial dune), (B) beach zone (supralittoral vs. intertidal) and (C) time (May 2011, October 2011, June 2012, October 2012). Multivariate differences in assemblage structure between p0 and p1 were tested through PERMANOVA tests, with the same three factors design used for the univariate metrics. When interactions between factors resulted significant, pairwise comparisons (Anderson et al., 2008) were also made. The abundance data were fourth-root transformed and converted on a Bray–Curtis resemblance function. Both the global analysis and the pairwise comparisons were based on 9999 permutations. All the statistics on macrofaunal assemblages were made using the software Primer 6 with the Permanova+ add on (http://www.primer-e.com). Regarding the orientation data of *T. saltator*, the analysis of circular distributions was carried out and the following statistics were calculated: the mean angle, the mean resultant length (r) and the 95% confidence intervals of the mean direction (Fisher, 1993). The Rayleigh test for uniformity was applied to the circular distributions, based on the length of the mean vectors to test the concentration of the individual directions around the mean (Batschelet, 1981). The density curves, smoothed with the kernel method, were estimated and double plotted on Cartesian graphs, to better show the peaks of the distributions. Multiple regression analyses adapted to circular data (SPLM, Spherically Projected Linear Models) were applied, with the angles of orientation as dependent variables and environmental and intrinsic variables as independent ones (Marchetti and Scapini, 2003; Scapini et al., 2002). The best models, having the maximum likelihood with the least number of parameters, were chosen using the AIC (Akaike Information Criterion). The significance of each influencing factor was tested through the Likelihood Ratio Test (LRT), by comparing the best model with the nested ones without the tested variable.

3. Results

3.1. Environmental features

The mean values of the environmental variables during the macrofauna sampling are given in Table 1. The beach slopes and widths were measured separately at p0 and p1, as the erosion effects were different at the two sites, producing higher slopes and smaller widths in the control site with respect to the reconstructed one. Leirosa beach had medium sand grains, according to the Wentworth scale, and organic contents of about 36% in the intertidal zone and 17% in the supralittoral one. Moisture about ten times higher in the intertidal zone’s sediment than in the supralittoral one, while the sand temperature was

Table 1. The main beach environmental variables, from measures obtained during the macrofaunal communities samplings (2011–2012). Values are indicated as mean ± SE and, when adequate, range (min–max).

Variables	**Leirosa beach**	
	p0	p1
Beach slope	12.9 ± 1.8 (12.0 – 13.7)	9.8 ± 0.8 (7.2 – 12.0)
Beach width (m)	69.8 ± 3.7 (60.0 – 76.0)	43.8 ± 1.3 (40.0 – 46.0)
Sediment granulometry (mm)	Medium sand (0.25 – 0.50)	
Shoreline direction (°)	220 ± 0	
Seawards direction (°)	310 ± 0	
Organic matter - intertidal (%)	35.8 ± 8.5 (29.6 – 42.1)	
Organic matter - supralittoral (%)	16.8 ± 1.6 (16.7 - 17.0)	
Sediment moisture - intertidal (%)	12.3 ± 3.6 (8.7 – 15.9)	
Sediment moisture – supralittoral (%)	1.3 ± 0.6 (0.6 – 1.9)	
Sediment temperature - intertidal (°C)	14.5 ± 1.3 (12.0 – 18.0)	
Sediment temperature - supralittoral (°C)	23.8 ± 2.7 (21.0 – 28.0)	
Water salinity (PSU)	35.5 ± 0.2 (35.0 – 36.0)	
Wave height (m)	2.2 ± 0.3 (1.0 – 3.1)	
Wave period (s)	9.3 ± 0.5 (8.0 – 10.0)	

almost ten degrees lower in the intertidal zone with respect to the supralittoral one (Table 1). The data on sand granulometry, wave period and height and intertidal width allow to classify Leirosa as an exposed beach (exposure rate: 15), according to the McLachlan's (1980) rating scheme. The PCA analysis indicated that five factors were responsible for the 95.2% of the total variance observed between p0 and p1, with the first principal component PC1 (accounting for the 39.8% of the variance) mainly influenced by the sediment features (moisture, temperature and organic matter contents) and the second principal component PC2 (18.3%) linked to wave height and water salinity. During the orientation experiments on *T. saltator* (Table 2), the air temperature ranged from 18.2°C in October 2012 to 35.5°C in May 2011, the relative humidity ranged, conversely, from 26.7% in May 2011 to 100% in October 2012, when a storm interrupted the orientation experiments before the end of the experimental session. In October 2012 the solar radiation assumed the minimum value observed, of 10.1 lx, while it reached 133.4 lx in June 2012. In half of the observations, there were not clouds in the sky, but a minor percentage of sandhoppers was also tested with a sky cloudiness reaching 6/8 (in the conventional scale from 0 to 8/8) in October 2012.

Table 2. Environmental variables registered during the orientation experiments on *Talitrus saltator* in the experimental period (2011-2012). Sky cloudiness percentages are referred to the ratio number of observations under each condition/ total of observations. Values are indicated as mean ± SE - range (min – max).

Variables	**Leirosa beach**
Air temperature (°C)	23.9 ± 0.1 (18.2 – 35.5)
Air relative humidity (%)	70.6 ± 0.6 (26.7 – 100.0)
Solar radiation (lux)	74.6 ± 1.2 (10.1 – 133.4)
Sky cloudiness (% of observations)	0/8=48; 1/8=23; 2/8=2; 4/8=10; 5/8=6; 6/8=11

3.2. Macrofaunal communities

A total of 739 individuals of 8 species were counted in the samples collected for this study. Six species were crustaceans, one was an insect and one was a polychaete. Numerically, crustaceans represented the 99% of all individuals, and among these 5% were amphipods, 30% were isopods and 5% were mysids. The most abundant species was *T. saltator* (42%), followed by *Tylos europaeus* (20%), *Talorchestia brito* (12%), *Haustorius arenarius* (11%) and *Eurydice pulchra* (10%). PERMANOVA tests on the univariate descriptors (Table 2) showed significant differences between the beach zones and the sampling time (month) concerning several univariate metrics, but between the natural and the artificial dune sites only the abundance of *T. saltator* resulted to be significantly different when considering the whole beach (Pseudo F = 7.63, P (perm) = 0.007, Table 3, Fig. 2a). Due to the significance of the interaction Site X Zone for both the abundance of *T. saltator* and faunal density (Table 2), pairwise PERMANOVA tests were carried out a posteriori, highlighting that both dependent variables were significantly lower in the supralittoral zone of p0 and p1, which is consistent with the strongest impact of the dune-recovery intervention in the latter sector of the beach (Fig. 2b and c). Other significant differences were found concerning the interaction Site X Time (Table 3), but the pairwise comparisons showed that no regular trends can be inferred, as some descriptors significantly differed between p0 and p1 in only one of the four sampling times, and the pattern was not the same for the whole experimental period. The PERMANOVA analysis

Table 3. Summary of one-way PERMANOVA comparing univariate descriptors at the natural (p0) and reconstructed site (p1) at each of the two shore zones (supralittoral vs. intertidal) and during four different times (M11 = May 2011, O11 = October 2011, J12 = June 2012 and O12 = October 2012). *df* residual = 64, total = 79. Bold values: significant results.

Source		**A: Site (p0 vs.p1)**	**B: Zone (sup. vs. intert.)**	**C: Time (M11, O11, J12, O12)**	**A X B**	**A X C**	**B X C**
df		1	1	3	1	3	3
Total density	MS	208.83	948.82	779.04	1564.2	723.17	387.62
(ind. m^{-2})	Pseudo *F*	0.78	3.56	**2.93**	**5.87**	**2.72**	1.46
	P (perm)	0.389	0.063	0.040	0.016	0.049	0.241
Species richness	MS	0.01	5.51	13.08	1.01	3.45	2.41
(S per sample)	Pseudo *F*	0.01	**4.30**	**10.21**	0.79	2.69	1.88
	P (perm)	0.918	0.043	<0.001	0.372	0.053	0.138
Species diversity	MS	0.004	1.50	5.94	0.03	0.88	1.60
(Shannon-Wiener	Pseudo *F*	0.01	2.01	**7.94**	0.05	1.17	2.13
index)	*P* (perm)	0.94	0.161	<0.001	0.830	0.329	0.100
Abundance	MS	5.49	17.60	1.19	2.98	0.55	0.51
Talitrus saltator	Pseudo *F*	**7.63**	**24.46**	1.65	**4.14**	0.76	0.70
(ind. per sample)	*P* (perm)	0.007	<0.001	0.184	0.044	0.526	0.570
Abundance	MS	6.05	14.45	14.9	5	1.88	5.02
Talorchestia brito	Pseudo *F*	1.53	3.65	**3.77**	1.26	0.48	1.27
(ind. per sample)	*P* (perm)	0.224	0.052	0.013	0.264	0.712	0.30
Abundance	MS	14.45	217.8	9.48	16.2	53.68	9.43
Tylos europaeus	Pseudo *F*	1.24	**18.74**	0.82	1.39	**4.62**	0.81
(ind. per sample)	*P* (perm)	0.284	<0.001	0.520	0.261	0.003	0.517

Table 4. Summary of three-way PERMANOVA comparing macrofaunal assemblages at the natural (p0) and reconstructed site (p1) in each beach zone and at four times (M11 = May 2011, O11 = October 2011, J12 = June 2012 and O12 = October 2012). Data fourth-root transformed. Bold values = significant results.

Source	***df***	**MS**	**Pseudo *F***	***P* (perm)**	**Unique permutations**
A: Site (p0 vs. p1)	1	2157.1	2.62	0.06	9957
B: Zone (supralittoral vs. intertidal)	1	21403	**26.03**	<0.001	9961
C: Time (M11, O11, J12, O12)	3	5310.1	**6.46**	<0.001	9935
A X B	1	1008.0	1.23	0.322	9969
A X C	3	1606.8	**1.95**	0.050	9932
B X C	3	1621.6	**1.97**	0.047	9946
Residual	64	822.3			
Total	79				

analysis (Table 4) confirmed the differences the times of the sampling, while between the two sites no significant differences were found. A pairwise test carried out a posteriori on the interaction Site X Time showed that a significant difference in the community of the two sites existed in May 2011 (t = 1.98, P (perm) = 0.017, unique perms. = 9968) and October 2011 (t = 2.94, P (perm) = 0.002, unique perms. = 9964), but not in the samples of 2012, when the two sites showed more similar communities.

3.3. Orientation experiments on T. saltator

For the orientation experiments a total of 1090 individuals of *T. saltator* were tested, of which 291 in May 2011, 272 in October 2011, 284 in June 2012 and 243 in October 2012. The main population characteristics' summaries for p0 and p1 are reported in Table 5. At both sites the individuals had highest numbers of antennal articles in October 2012, while the cephalon lengths were shortest in June 2012 both at p0 and p1. The sex ratios were generally in favour of males, the immature females were always much more abundant than the mature ones, and the same was observed for the adults with respect to the juveniles. No significant differences appeared between p0 and p1 (antennal articles and cephalon lengths = Wilcoxon rank sum test; frequencies of males, females, mature, between the beach zones and immature females, juveniles, adults = Chi-square test P-values > 0.05). In the whole experimental period the seawards direction, representing the theoretical escape direction for the sun orientation of *T. saltator*, was at 310° to the North, in both sites and the observed directions were generally quite adherent to the TED (Table 6). The highest deviations were observed when the individuals were tested with the landscape screened off, when they also showed the lowest concentrations around the mean. The Rayleigh tests indicated that all distributions obtained with visible landscape were highly significant, while the distribution observed in October 2012 at p0 without the landscape cues resulted scattered (Rayleigh test: P-values > 0.05). The circular distributions obtained at p0 and p1 with visible landscape (Fig. 3) showed the main differences between the two sites in October, both 2011 and 2012, while in May 2011 and June 2012 the experimental results at the two sites were very similar. On the other side, when tested without landscape (Fig. 4) sandhoppers were orientated in a similar direction in October 2011 and June 2012, when they were quite well seawards directed, while in May 2011 and October 2012 differences were observed between the two sites, with mean vectors directed longshore or dune-wards. The SPLM analyses highlighted the effects

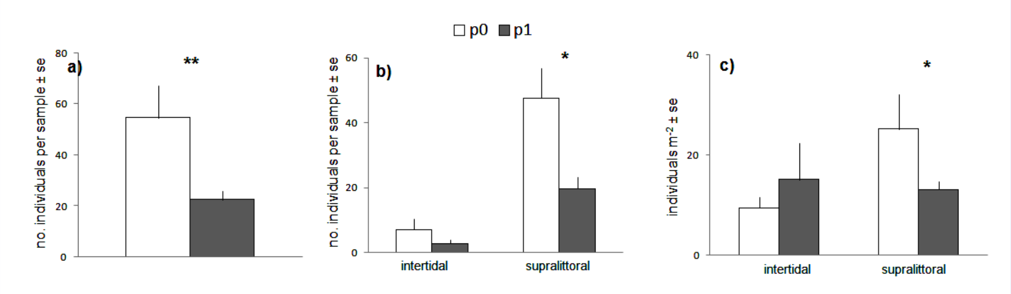

Fig. 2. Differences between sites: (a) total abundance of *Talitrus saltator*; (b) abundance of *T. saltator* in each beach zone; (c) faunal density in each beach zone. Mean values (±SE). Significance values: PERMANOVA tests described in Table 2 and in the text: ***P < 0.001; **P < 0.01; *P < 0.05.

on the orientation distributions of the following variables and factors: site (p0, p1), year (2011, 2012), month (May, June, October), season (spring, autumn), tide (ebbing, rising), landscape visibility (visible, not visible), time of the day, sun azimuth, cloudiness (from 0/8 to 8/8), global insulation, air temperature, air humidity, sex and reproductive status, number of antennal articles and cephalon length of the individuals. Starting from a simple additive model, we also developed alternative models including interactions of the most significant factors with all the other parameters. The best model chosen according to the AIC, showed a significant interaction of the landscape with the other factors, as follows:

Orientation ~ landscape visibility*** (month*** + sun azimuth*** + solar time*** + cloudiness***) + air humidity** + air temperature* + tide

(Likelihood = 3465.6524, AIC = 3525.6524, degrees of freedom = 1060; *p < 0.05; **p < 0.01; ***p < 0.001). Factors without *(p > 0.05), although not significant, were retained as they improved the AIC of the model; the factors not included in the interaction acted in the same way on the orientation behaviour independently from the landscape visibility.

4. Discussion

In our study, the geomorphological measurements seem to confirm that the intervention lowered the effects of the sea erosion, as in the reconstructed site p1 the beach was flatter than in the control site, p0 (Table 1). Nevertheless, a specific study should be required (Mendonça et al., 2012), to assess the erosive rates at both the sites, in order to exactly evaluate whether the observed differences are ascribable to the dune-recovery intervention. In other words, p1 became a more dissipative beach with respect to p0 that showed a more reflective typology (McLachlan and Brown, 2006). This change is likely to have engendered variations also in the biotic assemblage structure that is driven mainly by physical forces. However, the macrofaunal community showed a quite undiversified species composition, as it is the rule for exposed mesotidal beaches (Brazeiro, 2001; Defeo and McLachlan, 2005); crustaceans were the most abundant, and *T. saltator* represented the dominant species during the whole experimental period. Looking at the univariate macrofaunal descriptors, only the abundance of *T. saltator* showed a significant reduction from the natural- dune site to the reconstructed one considering the whole shore-normal transects(Table 3, Fig. 2a). This result is in accordance with the findings obtained by Bessa et al. (2013a) on this same beach in 2010–2011,

Table 4. Summary of three-way PERMANOVA comparing macrofaunal assemblages at the natural (p0) and reconstructed site (p1) in each one of the two beach zones (supralittoral vs. intertidal) and at four different times (M11 = May 2011, O11 = October 2011, J12 = June 2012 and O12 = October 2012). Data fourth-root transformed. Bold values = significant results.

Source	*df*	MS	Pseudo *F*	*P* (perm)	Unique permutations
A: Site (p0 vs. p1)	1	2157.1	2.62	0.06	9957
B: Zone (supralittoral vs. intertidal)	1	21403	**26.03**	<0.001	9961
C: Time (M11, O11, J12, O12)	3	5310.1	**6.46**	<0.001	9935
A X B	1	1008.0	1.23	0.322	9969
A X C	3	1606.8	**1.95**	0.050	9932
B X C	3	1621.6	**1.97**	0.047	9946
Residual	64	822.3			
Total	79				

Table 5. *Talitrus saltator* population relative to the samples used in the orientation experiments at p0 and p1.

		p0	***p1***
May 2011	**Antennal articles**		
	mean ± SE	22.7 ± 0.5	20.5 ± 0.5
	range (min – max)	(10 – 38)	(6 – 35)
	Ceph. length (mm)		
	mean ± SE	1.13 ± 0.03	1.24 ± 0.04
	range (min – max)	(0.37 – 2.05)	(0.44 – 2.11)
	Sex ratio m/f	49/94 = 0.20	12/109 = 0.11
	Fem mat/immat	22/76	3/106
	Juveniles/adults	0/143	27/121
Oct. 2011	**Antennal articles**		
	mean ± SE	22.26 ± 0.37	20.60 ± 4.33
	range (min – max)	(11 – 38)	(11 – 38)
	Ceph. length (mm)		
	mean ± SE	1.28 ± 0.02	1.18 ± 0.03
	range (min – max)	(0.56 – 2.11)	(0.67 – 2.44)
	Sex ratio m/f	46/107 = 0.43	41/76 = 0.54
	Fem mat/immat	2/105	3/73
	Juveniles/adults	2/153	0/117
June 2012	**Antennal articles**		
	mean ± SE	21.01 ± 0.42	21.81 ± 0.49
	range (min – max)	(12 – 33)	(14 – 35)
	Ceph. length (mm)		
	mean ± SE	0.93 ± 0.02	0.95 ± 0.03
	range (min – max)	(0.52 – 1.48)	(0.44 – 1.68)
	Sex ratio m/f	55/87 = 0.63	69/66 = 1.05
	Fem mat/immat	9/78	4/62
	Juveniles/adults	4/142	2/135
Oct. 2012	**Antennal articles**		
	mean ± SE	25.98 ± 0.39	25.62 ± 0.44
	range (min – max)	(15 – 36)	(16 – 40)
	Ceph. length (mm)		
	mean ± SE	1.26 ± 0.02	1.21 ± 0.02
	range (min – max)	(0.61 – 2.00)	(0.72 – 1.89)
	Sex ratio m/f	65/62 = 1.05	54/63 = 0.86
	Fem mat/immat	0/61	0/63
	Juveniles/adults	0/126	0/117

difference between the control and the artificial-dune sites resulted to be concentrated in the supralittoral sector (Fig. 2b), where also the faunal density was smaller at p1 with respect to p0 (Fig. 2c). This is likely ascribable to the geotextiles emergence, as most of the coverage sand was removed by the sea and wind that left a sandy layer too thin for animals burrowing into. It is noteworthy that, on the contrary, faunal density was higher, though not significantly, in the intertidal zone of the artificial dune site compared to the control one (Fig. 2c). The conditions at p1 after the soft-engineering intervention seem to favour in some way the intertidal species, which is an opposite finding with respect to what was observed for the hard-engineering solutions (Bertasi et al., 2007; Dugan et al., 2008; Martin et al., 2005; Sobocinski, 2003). Such differences between the two main beach zones are in agreement with the results of the PCA, indicating that the most influencing environmental variables were those linked to the sediment features that distinguish the intertidal beach zone from the supralittoral one (Table 1). At the assemblage level no separation was produced between the samples from p0 and p1. In fact, the pairwise PERMANOVA tests on the interaction Site X Time highlighted that the composition at the two sites was different in May 2011 and October 2011, but not in the following year (2012). It is possible that the effects of the dune reconstruction were still visible in2011 and had totally vanished in 2012, but this explanation is speculative, as seasonal/random variations may also explain these differences.

Table 6. Summary circular statistics for the orientation experiments on *T. saltator* at the two sites (p0 = natural dune, p1 =artificial dune) for the period of the study (2011–2012). TED = theoretical escape direction, perpendicular to the shoreline direction. TED and observed directions: degrees (°) to the North. SE: confidence interval for the mean direction, α = 0.05.

	Landscape		**Site**	
			p0	**p1**
May 2011	visible	TED (°)	310	310
		Observed direction ± SE (°)	289.80 ± 16.01	296.40 ± 13.34
		Mean vector length (r)	0.53	0.60
		Sample size (N)	71	71
		Rayleigh test for randomness	*p* <0.001	*p* <0.001
	not visible	TED (°)	310	310
		Observed direction ± SE (°)	57.85 ± 46.06	80.52 ± 43.73
		Mean vector length (r)	0.21	0.22
		Sample size (N)	72	77
		Rayleigh test for randomness	*p* <0.001	*p* <0.001
Oct. 2011	visible	TED (°)	310	310
		Observed direction ± SE (°)	297.00 ± 18.48	328.80 ± 15.68
		Mean vector length (r)	0.47	0.57
		Sample size (N)	73	60
		Rayleigh test for randomness	*p* <0.001	*p* <0.001
	not visible	TED (°)	310	310
		Observed direction ± SE (°)	334.20 ± 32.01	334.80 ± 17.01
		Mean vector length (r)	0.28	0.51
		Sample size (N)	82	57
		Rayleigh test for randomness	*p* <0.001	*p* <0.001
June 2012	visible	TED (°)	310	310
		Observed direction ± SE (°)	282.60 ± 12.43	277.20 ± 15.77
		Mean vector length (r)	0.62	0.57
		Sample size (N)	73	70
		Rayleigh test for randomness	*p* <0.001	*p* <0.001
	not visible	TED (°)	310	310
		Observed direction ± SE (°)	309.60 ± 29.71	301.30 ± 21.81
		Mean vector length (r)	0.31	0.37
		Sample size (N)	73	68
		Rayleigh test for randomness	*p* <0.01	*p* <0.001
Oct. 2012	visible	TED (°)	310	310
		Observed direction ± SE (°)	275.70 ± 21.86	243.40 ± 17.30
		Mean vector length (r)	0.43	0.58
		Sample size (N)	66	58
		Rayleigh test for randomness	*p* <0.001	*p* <0.001
	not visible	TED (°)	310	310
		Observed direction ± SE (°)	124.10 ± undet.	177.50 ± 23.97
		Mean vector length (r)	0.07	0.41
		Sample size (N)	60	59
		Rayleigh test for randomness	n.s.	*p* <0.001

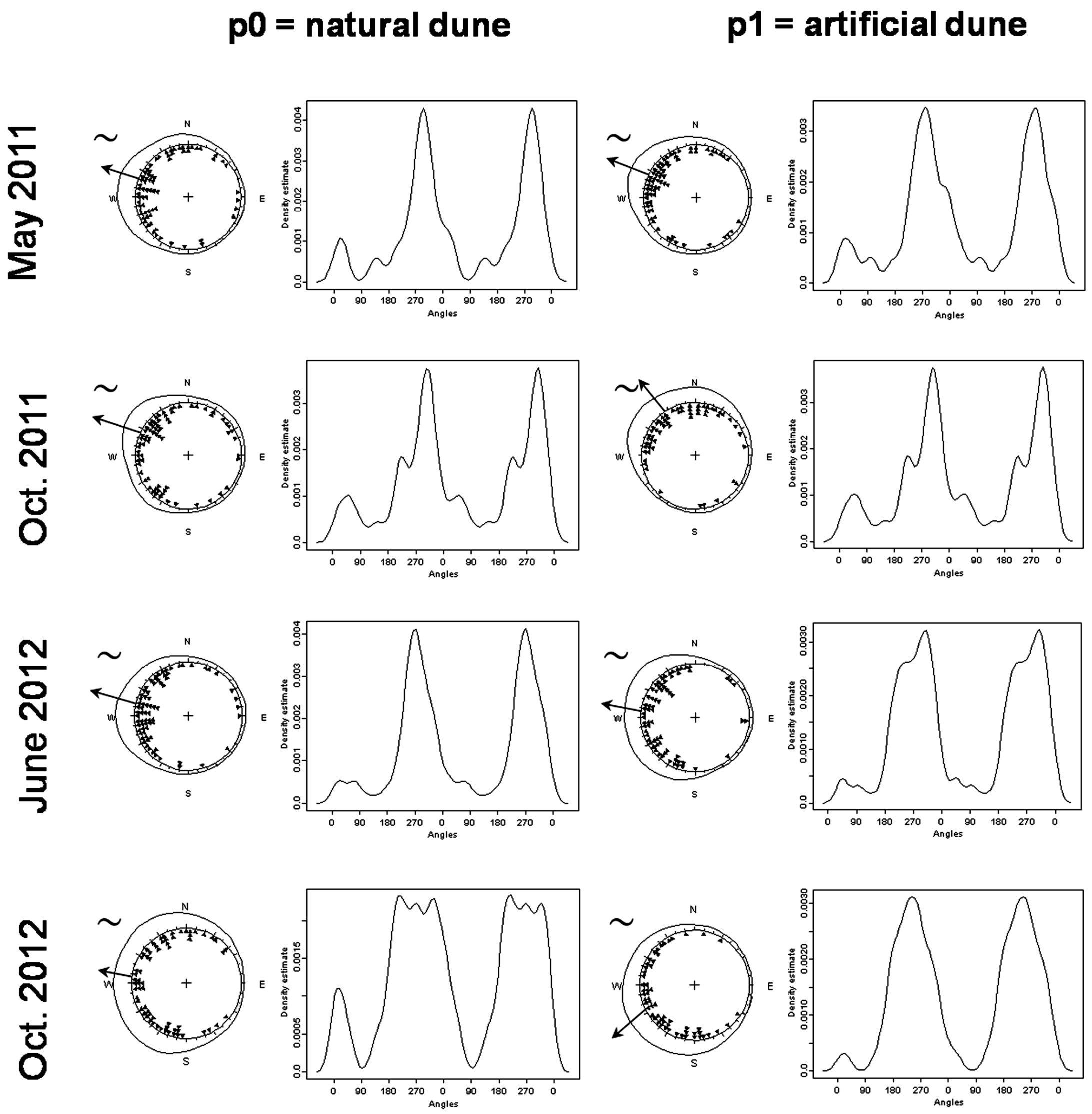

Fig. 3. Angular distributions obtained in the sun orientation experiments on *T. saltator* carried out with visible landscape. On the left: circular plots of the orientation angles. On the right: density estimates (kernel method) double plotted on Cartesian graphs. Black waves: sea direction, corresponding to the Theoretical Escape Direction (TED) for seawards orientation. Arrows: mean vectors for each distribution, with the lengths proportional to the concentration (r, mean vector length that may vary from 0 to 1). Summaries of circular statistics for each distribution are reported in Table 6.

Concerning the orientation performances of *T. saltator*, the amphipods were tested under various environmental conditions (Table 2) and in different life-stages, including juveniles, males mature and immature females according to the expected population frequencies for these latitudes (Marques et al., 2003). The main factor affecting the orientation performances resulted to be the landscape visibility (Figs. 3 and 4, Table 6), which interacted with the other variables. This result was expected on this beach that had a well-developed dune system and is in agreement with previous findings on Mediterranean coasts (Borgioli et al., 1999b; Scapini, 2006) and on the same Leirosa beach (Bessa et al., 2013b), confirming that populations from beaches with a prominent dune rely mainly on landscape visual cues, in contrast to populations from flat beaches, which use preferentially the sun compass (Hartwick, 1976; Scapini et al., 1992). Other highly significant factors on orientation were those

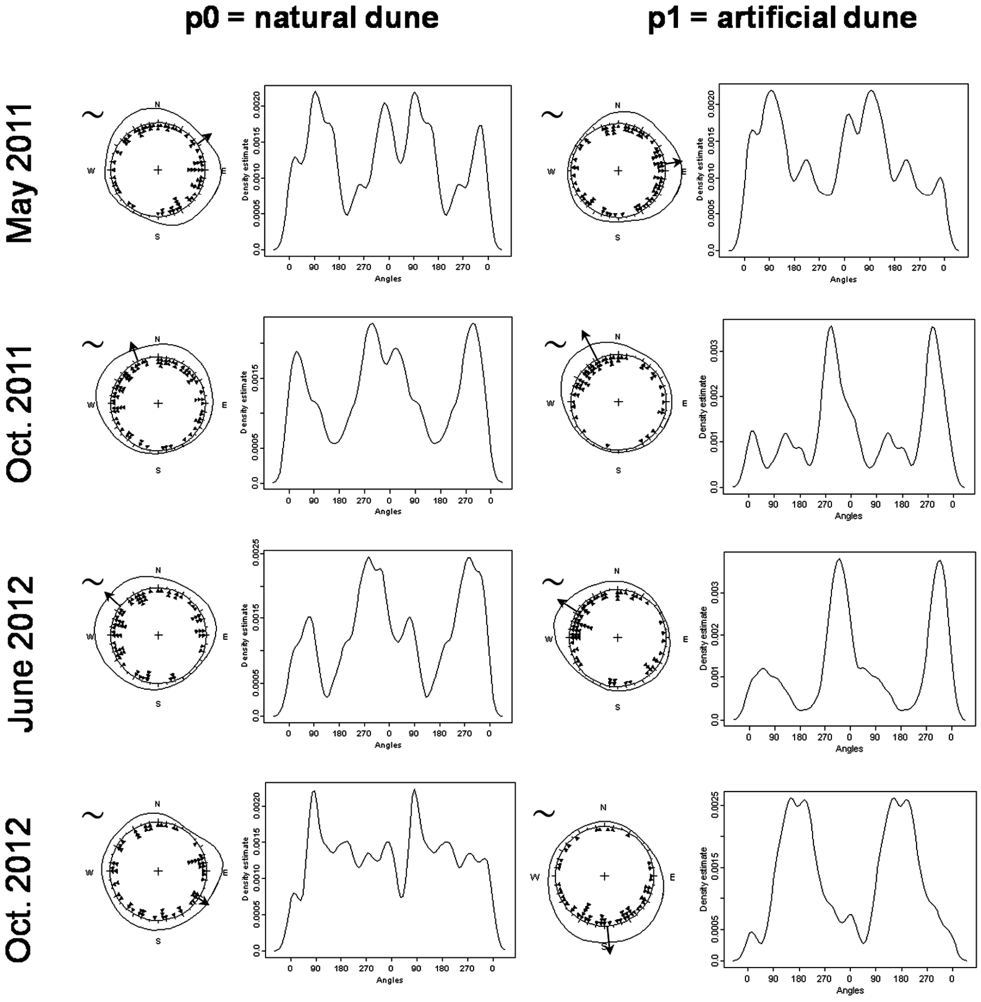

Fig. 4. Angular distributions obtained in the sun orientation experiments on *T. saltator* carried out with screened landscape. The meaning of graphs and symbols is the same as in Fig. 4; summaries of circular statistics for each distribution are reported in Table 6.

involved in the use of the sun compass (sun azimuth, solar time, sky cloudiness) that played its role mainly when the landscape was not visible, and the time (month) of the experiments. This result highlights a close relation between the amphipods' behaviour and the immediate climatic conditions (Nardi et al., 2003; Scapini et al., 2005), and is confirmed by the significance of air humidity and temperature and the tidal phase. The site was not included in the model, which actually is not surprising, as behaviour represents the immediate response of animals to changes in their life-environment. Thus, orientation may be an excellent early warning indicator, but it is not suitable to highlight long-term effects. On the other hand, if the dune recovery at p1 had not stabilised the beach morphodynamics, some effects on the behaviour of *T. saltator* should be still visible; hence the lack of differences can be considered as a proof of the effectiveness of the recovery in terms of shoreline stability.

5. Conclusions

This study showed that the use of geosynthetic containers in coastal defence, even though less impacting than building seawalls or groynes, has some long-term effects making these interventions not fully comparable to soft-engineering procedures, generating short-term pulse disturbance (Leewis et al., 2012; Peterson et al., 2006). In this case, the main issue seems to have been the partial removal of the sandy coverage after the dune-recovery, preventing the beach animals to burrow into the sand. This phenomenon is not uncommon, and mathematical models used in planning interventions may fail in forecasting the evolution of erosive processes, particularly when these are produced by several factors acting at the same time (Jetten et al., 2003; Thieler et al., 2000). Two main aspects were highlighted by this study; firstly, the importance of designing long-term monitoring studies when planning interventions on beaches, especially regarding the impacts of relatively recently introduced methodologies (as it is the case for the geotextile containers), which can modify the ecosystem features in unforeseen directions. Environmental restoration has to be planned with a long-term vision, but current practices tend to concentrate on immediate socio-economic benefits and rarely consider the future developments of the impacted ecosystems (McLachlan et al., 2013; Phillips and Jones, 2005). Secondly, there is a need to combine different ecological indicators in order to include the maximum number of possible effects on the biotic ecosystem components, choosing specific sets of indicators for the different phases of the monitoring procedure (Osenberg and Schmitt, 1996). The macrofaunal community analysis and the orientation behaviour of *T. saltator*, proven effective bioindicators to monitor the immediate, short and medium-term impacts of human interventions on sandy beaches, have proven to be unsuitable on longer temporal scales. However *T. saltator*, a key-species of beach ecosystems, showed a long-term effect in a reduced population abundance that therefore may be considered for the later phases of monitoring plans. The total faunal density resulted a less powerful bioindicator in detecting the impacts at the whole beach scale; however, a significant difference was shown for the supralittoral zone, the most impacted beach sector. To conclude, there is a need of a major integration between ecologists and engineers (Chapman and Underwood, 2011); both the categories have to engage to fulfil gaps in what is known on coastal defence intervention effects, aiming at reducing impacts as much as possible and reach equilibrium between the immediate socio-economical needs and environmental sustainability.

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3.4 Discussion and conclusions

In this chapter we focussed on the monitoring of human impacts on sandy beaches. These ecosystems experience numerous anthropogenic pressures, which can be hierarchically arranged into a causal chain, starting with ultimate pressures (root causes) and ending with proximate pressures (the immediate factors eliciting a biological response). The lack of public appreciation of beaches as habitat supporting unique assemblages is an ultimate factor leading to many other pressures, such as inadequate beach management and land use planning (Schlacher et al. *in press*). Actually, the main threat to sandy beach ecosystems comes from the interaction of human coastal development and climate change, both of which are, ultimately, a product of economic and human population growth. Climate change may generate an accelerated shoreline erosion (via sea level rise and increased storminess), which can produce extreme effects, as habitat heavily eroded or lost. Paradoxically, measures designed to manage the shoreline often create other problems and become pressures themselves; armouring (e.g. building of seawalls and similar structures) protects adjacent urban assets from erosion, but has several unwanted ecological effects. For example, seawalls reduce the size and number of intertidal zones and cause a loss of upper beach and dry sand habitat (Dugan and Hubbard 2006; Dugan et al. 2008), which can drown a beach over time because natural shoreline retreat is halted by the structure (Pilkey and Wright 1989). Ideally, shorelines should be allowed to freely landward migrate under conditions of higher sea levels. When retreat or quasi-natural shoreline movement is not a viable option, the alternative to the use of fixed seawalls is to restore or maintain eroding beaches through beach nourishment or other soft-engineering interventions. This strategy has gained popularity because it maintains both sandy habitat for biota and beach amenities for humans. Actually, nourishment often causes a range of negative ecological impacts (Speybroeck et al. 2006); however, if the process employs carefully matched practices, it may represent a less ecologically damaging option with respect to hard-engineering works (Finkl and Walker 2004). Although the initial ecological effects are usually severe, a recovery of some taxa may occur within few months, if the properties of the added sediment are closely matched to the original beach sand (Jones et al. 2008). On the other hand, where fill sediments are poorly matched, ecological impacts may persist for years (Peterson et al. 2000, 2006). Moreover, repeated nourishment episodes (e.g. annual, biannual) can degrade beach biota and inhibit recovery and ecological functions (Dolan et al. 2006). When planning an intervention, managers with limited funds need to know which impacts matter (Jones, 2003): although several criteria for the ecological significance of impacts (e.g. local extinctions, loss or reduction of critical breeding habitat, ecosystem stability, primary productivity, etc.) have been suggested, no consensus definition of an ecologically significant impact has been reached (Schlacher et al. *in press*). Actually, a universally accepted criterion for assessing the degree, duration or severity of environmental impacts is unlikely to be found; judging the limits of tolerable change and valuing biodiversity will vary according to a country’s or society’s historical, cultural and philosophical roots, and will also depend on its economic development status and financial resources (Scapini 2010). In this context, ignorance of beach biology and the consequent lack of appreciation of beaches as ecosystems can be a major threat for biodiversity (Fanini et al. 2007b).

Trying to answer to some of these issues, particularly the monitoring of impacts from hard and soft-engineering works on beach ecosystems, we decided to focus on a widespread, key species of European and North African shores, the sandhopper *Talitrus saltator* (Montagu, 1808), which was already known to readily answer to physical changes in its life environment, mainly by modifying its behaviour (Scapini 2006). Such a prompt response might be suitable as an early warning bioindicator of impacts on sandy beaches, able to highlight ongoing ecological variations more rapidly than other bioindicators having longer response times.Two sandhoppers’ behavioural traits, the circadian rhythm of locomotor activity and the sun orientation, were hence analyzed in different case studies, subject to various human impacts.

The activity rhythm showed to be dependent mainly on the habitat of the species, with less limiting habitats allowing for lower periodicity, but a notable individual variability was also highlighted (Scapini et al. 2013, section 3.1.1). In the case of the recent impact on shoreline dynamics produced at Collelungo beach by the construction of a seawall, significant differences in the sub-populations’ period definition were found only during high summer. The animals from the more eroded beach sector displayed a less precise rhythmicity compared to those from the accreting one, likely due to a more chaotic environment, with frequent changing of the beach width (generally shorter than in the other stations). This difference between sub-populations, though probably related to the differing shoreline dynamics, was not observed in the other seasons. It was probably hidden by the high individual variability that increases under mildest climatic conditions (in terms of dehydration risk), which allow a less defined rhythmicity (Nardi et al. 2003; Rossano et al. in preparation, section 3.2.1). hence, circadian activity patterns resulted hence unsuitable to monitor short-term changes of shoreline stability engendered by anti-erosive works, due to their too high variabilty. In the Leirosa beach, where we considered the long-term impact caused by a soft-engineering intervention, a well-defined rhythmic activity was observed in the site that had been subject to the intervention, indicating that environmental conditions were relatively stable there (Rossano et al 2008; Rossano and Scapini 2011, Bessa et al. 2013, section 3.3.1). Also in this case, a high individual variability was highlighted, confirming that this behavioural feature cannot be used as a monitoring tool, particularly in the case of long-term impacts. On the other side, the rhythmicity observed here may be considered a proof of the effectiveness of the dune rehabilitation, at least in terms of environmental stability.

The other behaviour of *T. saltator* that was analyzed, sun orientation, resulted to be more closely dependent on the physical characteristics of the beach, which makes this behaviour appear as a suitable bioindicator of environmental impacts. In the Moroccan case study (Scapini et al. 2013, section 3.1.1), orientation tests clearly detected an ongoing impact on the beach at the left side of the river mouth, where a higher urbanization (compared to the right side, with traditional settlements) probably affected the supralittoral dynamics; here in fact the supply of sediments from the land was limited and the wind transportation of substrate has been impeded by the houses built on the former dune (Oueslati 2006). When considering the specific purpose of this thesis, i.e. the assessment of the sun orientation as a bioindicator of impacts due to anti-erosive interventions, the Italian case study gave some interesting results, thanks to the comparison that could be made with the findings obtained on this same beach before the seawall construction. First of all, sun orientation worsened along the whole littoral, indicating that important changes had occurred in the beach environment. Furthermore, the best orientation, which in 2002-2003 was shown by the sub-population at 5000 m from the Ombrone River mouth, in 2011 was observed closer to the river mouth, at 4000 m. This may indicate that the seawall had modified the sedimentary dynamics along the littoral, likely reducing the erosion in the first beach sector, stabilizing the shoreline 1000 m further upstream and engendering a beach accretion in the last sectors. This hypothesis seems confirmed by the macrofaunal analyses, as a change was detected in the arthropods community from 2002-2003 to 2011. By using a Multi-Dimensional Scaling (MDS), we found that while in the previous samplings the stations at 2000, 3000 and 4000 m clustered together and the 4000, 5000 and 6000 stations formed another group, in the 2011 the 4000 community resulted to be more similar to the first group, confirming that a change happened in that sector of the beach. Also, the univariate diversity indices, which in 2011were expected to have lower values in all the stations, due to a considerably shorter sampling duration with respect to 2002-2003, showed almost the same values at stations 5000 and 6000, which seems to indicate that diversity increased in the furthest beach sectors. This is in agreement with our working hypothesis, according to which an accretion engendered by the seawall construction in the last littoral stretch may have favoured a rise of diversity by furnishing a major habitat availability (Brazeiro 1999; Lastra et al. 2004; Rodil et al. 2006; Deidun and Schembri 2008). Hence, sun orientation results were validated by macrofaunal analyses, and this opens the way for its use as an early warning bioindicator of shoreline stability, as it fulfils the main requirements for a suitable ecological indicator (Salas et al. 2006). In fact, it has an agreed scientifically sound meaning (behavioural change is the first response of individuals to environmental variations), it is representative of an important environmental aspect for the society (the shoreline stability), it provides valuable information with a readily understandable meaning (the orientation of subpopulations is directly related to the shoreline dynamics), it is meaningful for external audiences (with few simple explanations), it helps in focusing information necessary for answering important questions (the global issue of coastal erosion) and it assists decision-making by being efficient and cost-effective in terms of use, as it was the case in both the Italian and Portuguese study-cases (where the orientation experiments resulted less time-consuming and required a smaller effort, in terms of man power and costs, with respect to macrofauna samplings. On the other hand, in the Portuguese case study we assessed the effectiveness of sun orientation also as a bioindicator for long-term impacts of soft-engineering works, but we did not find any difference between the orientation of individuals living in front of the rehabilitated dune and those staying in front of the natural one. This confirms that behaviour, as an immediate response of animals to changes in their life-environment, can not be considered to assess long-term impacts, neither due to hard-engineering or soft-procedures. Nevertheless, here *T. saltator* was able to detect an ecological effect not by its behaviour, but by a reduction of its abundance in the sector interested by the dune-recovery, confirming to be a key species on temperate sandy beaches. A difference, although less significant, was also observed for the total macrofauna density, thus confirming that also soft interventions may produce long-lasting effects on beach ecosystems (Nourisson et al. 2014). This finding is of considerable interest, as until today no studies were made on this subject, being geotubes a quite recent methodology in coastal protection. Hence, though this topic needs to be deepened with other studies, our results seem to indicate the abundance of *T. saltator* as a suitable bioindicator for the monitoring of long-term effects ascribable to geotextiles and geotubes in the dune-rehabilitation processes along the Mediterranean and Eastern Atlantic shores.

4. GENERAL DISCUSSION AND CONCLUSIONS

Since the Earth Summit of 1992 in Rio de Janeiro, more and more attention was given worldwide to concepts as holistic environmental management, integrated pollution control and ecosystem approach, which combines natural and social sciences in dealing with environmental problems (Apitz et al. 2006). In Europe the process led to the approval of the Water Framework Directive (2000/60/EC), the implementation of which has been a major challenge. The EU Member States are spending time and resources to develop tools to gain the required data, towards an harmonisation of classification and water monitoring methods across Europe (Hering et al 2010). Many methods have been developed to sample and investigate aquatic ecosystems and large amounts of data are being generated, focussing on biological communities rather than on the more limited aspects of chemical quality (Moss 2007). This was recognised as being a more effective integrative way to measure ecological quality, but also requires complex and dynamic communities to be quantified into a single numeric score, rather than qualitative species lists, to establish reference conditions from which to estimate the change (Hering et al. 2010). Efforts to develop methods fulfilling the requirements of the WFD were huge and many national and international projects contributed (for coastal and transitional waters see Borja 2005; Borja et al., 2004, 2007). Likely, when writing the WFD the authors had in mind a simple assessment system, with just a few metrics to be applied across Europe, but this proved to be not realistic nor achievable, due to several important differences among stressors affecting ecosystems, water body types and sizse, bioindicator taxa and knowledge on these taxa (Schmidt-Kloiber et al. 2006). This becomes even more challenging when considering also non-EU Mediterranean countries. Hence, various monitoring procedures were proposed, aimed to detect ecological status changes following restoration/rehabilitation processes, thus providing a general guidance to the measures required.The challenge is to predict how the biota will respond to restoration and what management actions are best suitable. These questions are easier to answer for lakes and marine ecosystems, predominantly affected by eutrophication, where the main restoration measure is the reduction of nutrient load. It is more difficult for transitional waters (e.g. coastal lagoons), where a high turbidity and naturally poor light penetrating into the water complicates the response of the ecosystem to anthropogenic changes, or for coastal waters, which are also affected by hydromorphological degradation on different spatial scales (Elliott et al. 2007; Hering et al. 2010). Ideally, all studies of disturbance and recovery should be based on the assessment of a deviation from an undisturbed condition. When previous data are not disposable, a robust statistical design would include three types of sites: (i) restored sites, (ii) control (reference) sites, and (iii) sites similarly impaired as those restored but not restored (e.g. Downes et al. 2002). According a review by Jones and Schmitz (2009) on 240 recovery studies across terrestrial and aquatic ecosystems made under the WFD guidelines, pre-perturbation data were available for only 20% of them, a circumstance that made the assessment of recovery in 80% of the studies rather subjective, particularly when the three types of sites cited above were not included.

In this thesis, different situations were faced. In the case of the Ghar El Melh lagoon complex (section 2), suffering of increasing pollution and eutrophication, no recovery or restoration were planned, but the monitoring that we carried out may represent a reference condition if interventions will be undertaken. The sampling stations were chosen with the aim to cover most of the spatial variability, including stations with various levels of degradation and a control station (presumably not, or less impacted) close to the channel opening toward the sea From the study emerged the importance of water optical properties in the assessment of the ecological status of shallow coastal lagoons, as the optical classification showed a good overlapping with the ecological one, based on the macrophytes-community composition. The sectors with clear/moderate waters showed a high coverage of late-successional species, while those with the most turbid waters showed a major abundance of opportunistic ones. The use of macrophytes as a bioindicator of eutrophication for shallow coastal systems is accepted and recommended (Duarte 1995; Valiela et al. 1997; Orfanidis et al. 2001; Tilman and Lehman 2001; McGlathery et al. 2007). In this thesis we obtained a reliable water classification with just a visual survey of macrophytes coverage, a method that can easily be repeated also in particularly sensitive environments. In our study it is noteworthy the primary role of the optical water properties, in some measures confirming what stated by Peeters et al. (2009), i.e. that water transparency (or its counterpart, turbidity) can be a sufficient criterion to assess the status of small water bodies like lakes and lagoons. We took optical measures by means of a Portable Underwater Mini-Spectroradiometer (PUMS), which allows to assess optical properties also in very shallow water bodies and furnishes the spectra of reflectance and vertical attenuation coefficient (R and K_d) in the whole visible band. These two important optical features make the use of PUMS preferable with respect to the Secchi disk. The latter can not be used in too shallow waters and furnishes just a numeric score, less informative than spectral optical properties, which instead are able to discriminate which substances are more active in light attenuation by means of their spectral analysis. Of course, biotic indicators are generally preferable in environmental quality assessment, due to their ability of integrating the various environmental changes over time, but the link between water optical features and the ecological conditions should not be overlooked, as it might represent a suitable secondary indicator for the monitoring of shallow coastal lagoons. The method needs a finer calibration for Mediterranean coastal waters specificities, which is actually being performed in several Mediterranean port areas in the context of the MAPMED project (CBC-MED programme, 2011-2014). The outcomes of our survey at Ghar El Melh suggest that optical water properties are a reliable indicator for the monitoring of Mediterranean coastal water bodies.

At the Collelungo beach (section 3), the best situation was disposable for a study of disturbance and recovery, as data were available collected before the impact to make comparisons; moreover the site is located in a protected area, which permits to isolate the considered disturbance from many other stressors acting on beach ecosystems (a review in Schlacher et al. 2008). Here the main issue was the reduced sand delivery to the littoral zone by the Ombrone River (Pranzini 2001; Ciampi 2007). This is an important large-scale process that has become crucial worldwide for beaches conservation (Willis and Griggs 2003; Masters 2006), as it interacts with sea-level rise in determining an increased beach erosion (Defeo et al. 2009; Doney et al. 2012). Sand-shed management will be more and more important for maintaining beach health in some regions (Revell et al. 2007), and a number of strategies emerged from examining the theory and practice of beach and dune conservation, leading to a range of practical intervention options that have been proposed to achieve positive conservation outcomes (Schlacher et al. *in press*). In the Collelungo case study, a seawall was recently built parallel to the shoreline, a hard-engineering intervention that was already known for having substantial effects on the ecosystem features (Dugan and Hubbard 2006; Dugan et al. 2008; Defeo et al. 2009). The comparison of the macrofaunal community features highlighted that changes affected the whole littoral, and a clear separation was observed of the two furthest stations from the seawall, at 5000 and 6000 m from the river mouth, from the closest ones, with a substantial increased arthropods' diversity likely ascribable to an accretion of the beach in the furthest sector. This change in coastal morphodynamics and its ecological effects were clearly detected also by comparing results from the sun orientation experiments on *Talitrus saltator* performed before (previous studies) and after (this study) the construction of the seawall, as a worsening was visible at the 5000 and 6000 stations with respect to the previous experiments, while the best performance was now obtained at the 4000 station, which was likely stabilized by the seawall. In other words, the information given by an accepted and established bioindicator of ecosystem stability, such as the macrofaunal diversity and community structure, largely overlapped with that from behavioural tests, when considering the sandhoppers' solar orientation. For sure, macrofauna community has to be considered a high-profile indicator when analysing the whole ecosystem health, as it integrates all the possible impact sources affecting beach habitats, but the behaviour of *T. saltator* may represent a reliable alternative particularly when considering particularly short-time effects of the impact source on shoreline dynamics. The method is less time-consuming and less expensive than macrofauna samplings and provides a more rapid response to ongoing changes, as behavioural adaptations are expressed as prompt responses to environmental variations (Brown 1996; McLachlan and Brown 2006). Actually, behaviours are contingencies by definition, occurring in a particular place and at a particular time, modifiable by internal and external factors and individual experience (Campan and Scapini 2002). This may make challenging a generalization and repetition of the procedure in different environments and for different populations, but appropriate statistical methods were developed to test hypotheses on behavioural responses under (changing) natural conditions (Underwood and Chapman 1985; Marchetti and Scapini 2003). Hence, the differences observed among the experimental results of different workers on different beaches, which led authors to disagree on the possibility of using monitoring procedures based on behavioural changes (etho-assays), may be the result of a variety of adaptations that represent the basis of the evolutionary processes driven by natural selection (Scapini 2013). Given the changes (possibly irreversible) that sandy beaches are facing, a better understanding of behavioural adaptation of beach animals should become a research priority, especially if some of them, as it is the case for the sun orientation, appear to be suitable as early-warning indicators for the monitoring of the ecological impacts of hard-engineering interventions on sandy beaches. The findings decribed in this thesis should represent a step forward in this direction, which has to be deepened with further studies on other temperate sandy beaches.

Finally, we considered the Leirosa beach in Portugal (section 3), from which no previous data were available on the behavioural adaptations of sandhoppers. Here a dune-recovery was carried out ten years ago with goetextile tubes, an intervention of which the long-term effects had never been previously assessed. To have a quite robust experimental design, we considered a site that had been impacted and an other, on the same beach, not interested by the works. Here the results clearly showed that the behaviour of *T. saltator* did not vary between the two sites, as it was expected considering the long time elapsed from the intervention, but an interesting difference was observed in sandhoppers' abundance, making this population feature a possible long-term indicator for this kind of interventions, till now considered ecologically not impacting (Phillips and Jones 2006; Cisneros Linares 2013). Also, the data obtained in this study may become a reference point when other works will be undertaken against beach erosion, which is still acting on this sector of the Portuguese littoral representing a threat for human settlements. This might offer the occasion to assess sun orientation of *T. saltator* as an early warning indicator also for soft-engineering procedures, on Atlantic beaches where the main driving forces are not represented by seasonal climatic variations, but by tidal and wave regimes (Antunes do Carmo et al. 2010; Bessa et al. 2013, section 3.3.1).

In conclusion, the aim of this thesis was to suggest specific tools for the ecological monitoring of coastal environments, which are more and more threatened by a variety of human impacts. The need exists for coastal ecosystems to be represented specifically in policy and environmental legislation, starting from the assessment of their ecological status. Management at local, provincial/state and/or national/federal levels must take international scales and processes into account, without forgetting the problem of multi-jurisdictional issues, as it is the case for the Mediterranean coasts shared between EU and non-EU contries. Legislation should simplify jurisdiction regarding coastlines to foster consistent and efficient management for good conservation outcomes (Schlacher et al. *in press*). The WFD, though representing an important improvement in this direction, must now be implemented by the harmonization, at the appropriate spatial scales, of the numerous outcomes produced since its first application, considering all the countries that in some way are involved in coastal ecosystem management across the Mediterranean. Above all, an effective conservation of the unique biodiversity of coastal environments is a primary duty of society placing a greater value on maintaining the ecological properties of these systems and committing the willingess and resources to conserve them for the future.

5. APPENDIX

The subject of this manuscript has nothing to do with the theme of the thesis, but it was inserted here as the knowledge of circular statistics, learned for analysing the results of the orientation experiments on *Talitrus saltator*, revealed to be suitable also to analyze the distribution of activity fixes in relation to the lunar phase, wich is a circular variable. This work was conceived after a meeting od PhD students (promoted by the Italian Society of Ecology, SItE), where Dr. Emiliano Mori was presenting his work on crested porcupines and declared the intention to deepen the lunar influence on the porcupine behaviour. From a first discussion on the topic, the possibility of a collaboration arose, and the following manuscript represents the first version of the paper that is currently in preparation. This is a good example of how the knowledge gained during three years of PhD can be ‘recycled’ in other, different fields, if the occasion is given to PhD students to share their results and discuss on how to improve their researches. To be multitasking is for sure desirable for a future researcher, and it should be encouraged during the PhD formative experience. Basic animal behaviours can be investigated with methods that are independent from the considered taxonomic rank, which makes possible to use them for invertebrates as for vertebrates, though some arrangements are necessary.
Moreover, the four study sites, located in Tuscany, include the Maremma Regional Park, where the porcupines also frequent the beach-dune ecosystem; hence this research represents a further step towards the conservation of biodiversity in this environment, which actually is one of the main topics discussed in the previous sections of this thesis.

“Once in a blue moon” in the full moon. Nocturnal behaviour of the crested porcupine.

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INTRODUCTION

Predation pressure elicits specific behavioural traits in mammal species (e.g. Lima and Dill 1990; Ferrari et al. 2009); main tactics to reduce predation risk involve a modulation of spatial behaviour (e.g. Price et al. 1984; Daly et al. 1992; Lima 1998). In conditions of reduced light, the hunting abilities of many nocturnal predators diminish (e.g Lima Sàbato et al. 2006; Cozzi et al. 2012, but see Gursky 2003; Packer et al. 2011). Potential preys, advantaged by the decreased predator effectiveness, usually tend to concentrate their movements in dark (e.g. Kolb 1992; Clarke 1983; Penteriani et al. 2013) and/or sheltered (e.g. dense vegetation cover and shrub canopies: Stokes et al. 2001; Mandelik et al. 2003) environments to minimize predation risk (Lizcano and Cavelier 2000; Börk 2006). So far, the effect of moonlight intensity on ranging behaviour of rodents has been tested mainly in desert environments (e.g. Alkon and Saltz 1988; Bowers 1988; Eilam 2004), and North American prairies (Clarke 1983; Brillhart and Kaufman 1991; Topping et al. 1999), while data from European temperate areas are poor (Corsini et al. 1995). Old world porcupines (*Hystrix spp*., *Atherurus spp*. and *Trichys spp*., hereafter OWP) are large rodents with nocturnal habits. They spend most of the daylight hours in dens (e.g. Alkon and Saltz 1988; Corsini et al. 1995), which are located in natural caves or underground galleries, possibly in steep and compact soils covered with dense vegetation (e.g. Kayani et al. 1990; Monetti et al. 2005). Although cryptically coloured and well-defended by a quill-armed body (Ellerman 1940), OWPs are sometimes attacked by large predators (Mori et al. in press) as felids and hyenas (e.g. Alkon and Saltz 1988; Mills and Shenk 1992; Breuer 2005). Their large size and short legs respectively increase their visibility and limit their escape success (Mohr 1965). Since a direct defence against possible predators is energetically costly (Mori et al. in press), it has been suggested that moonlight avoidance have been developed by OWPs as a strategy to reduce encounters with possible predators (Brown and Alkon 1990). A clear tendency of the Indian crested porcupine *Hystrix indica* Kerr, 1972 to avoid moonlight and daylight has been observed both in the Negev desert (Israel: Alkon and Saltz 1988) and in the subtropical grasslands and forests of the Suklaphanta Wildlife Reserve (Western Nepal: Fattorini and Pokheral 2012). Both these areas are characterized by the presence of common leopard *Panthera pardus*, which potentially prey on porcupines (Lovari et al. in press). Alkon and Saltz (1988) recorded a different behaviour depending on the season: during the winter, porcupines avoided moonli ght exposure, while during the summer, when the night gets shorter, the radiotagged individuals remained active throughout the night regardless of moon’s phase. This evidence seems to be confirmed by Fattorini and Pokheral (2012), who collected date only in winter.

In Italy, predation on crested porcupines *Hystrix cristata* L., 1758 has been rarely reported only by red fox (mainly on cubs: Lucherini et al. 1995) and by wolf (Scandura et al. 2000). Poaching of this species still occurs in Italy with a special regard to the historical range (Mori et al. 2013). Ranging behaviour of crested porcupine is significantly affected by habitat richness (Lovari et al. 2013), but information on the activity of this species with regard to moon’s phase is limited to two studies with a very small number of radiotagged individuals (1 individual: Pigozzi and Patterson 1990; 4 individuals: Corsini et al. 1995). The mean duration of the nocturnal activity of *H. cristata* in a year was reported to be 8 h 18’±30’ per night (Corsini et al. 1995). Crested porcupines seem to reduce their activity during the cold season, while the major movements occur in the warmer nights (Corsini et al. 1995); overall, diurnal activity is scarce and irregular. Preliminary data suggested that influence of moon’s phase on *H. cristata* activity is slight and recorded just in extreme moonlight summer nights (Pigozzi and Patterson 1990; Corsini et al. 1995). Corsini et al. (1995) supposed that concentration of ranging movements in thick vegetation areas may represent the strategy evolved by *H. cristata* to avoid illuminated areas, hence reducing their visibility, as also reported for H. indica in the Israeli coastal plains (Sever and Mendelssohn 1991). This is not confirmed for *H. indica* in Suklaphanta Wildlife Reserve (Fattorini and Pokheral 2012) and for Atherurus africanus (Emmons 1983), which exhibit moonlight avoidance also in forests and shrubwoods.

In this work we aimed to test if lunar cycle influences the activity pattern of 27 radiotagged crested porcupines, each one followed for at least six months (one hot/cold season), from four sites in Southern Tuscany (Central Italy) with different levels of habitat heterogeneity. Following the hypothesis that the activity of the crested porcupine could be affected by the moonlight, then moon’s phase is expected to provide a regular temporal variation in its ranging behaviour.

MATERIAL AND METHODS

Study sites

We carried out this study at four sites in Southern Tuscany (Central Italy: Fig. 1), characterized by different environmental features. The two main potential predators, red fox and wolf (or hybrids dog × wolf: Caniglia et al. 2013) were present in each study site. Three sites (San Miniato, Le Malandrine and Maremma Regional Park) has been described in detail in a previous work dealing with spatial behaviour of the crested porcupine (Lovari et al. 2013). The fourth study area is included in the SCI “Poggi di Prata”, in the Metalliferous Hills of the Province of Grosseto (Cantini et al. 2013). Tab. 1 summarizes the main habitat types of each study site.

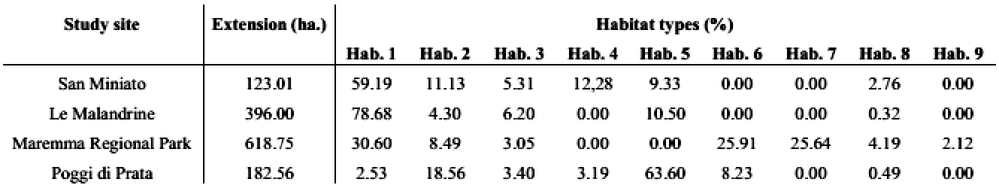
Figure 1. Location of the study sites.

Study methods and data analysis

Adult crested porcupines (N = 27) were trapped with metal box traps (Lovari et al. 2013), sedated (Massolo et al. 2003) and equipped with VHF radio -collars (AVM, USA; Biotrack, UK). Individuals were radio-tracked continuously for a minimum of 6 months and a maximum of 12 months (cold season: October-March; hot season: April-

September). Crested porcupines' activity was determined by means of an activity sensor, which detected the variation of signal intensity during 60 seconds (Garshelis and Pelton 1980). The same radio-telemetry protocol was used throughout all the study sites, i.e. a combination of distance (median, 1 fix/37.5 min, Q1– Q3 = 30–63.75; median location error = 58 m, Q1– Q3 = 52–64) and homing in locations (1 fix/15 min; median location error = 17.85 m, Q1– Q3 = 6–31.58), for a median value of 53.36 fixes/month/individual, Q1 –Q3 = 43.72–56.97. Fixes were categorized into four moon phases as follows: phase 1) from new moon to ¼ (3305 fixes collected in 211 nights); phase 2) from ¼ to ½ (2930 fixes collected in 202 nights); phase 3) from ½ to ¾ (3272 fixes collected in 200 nights); phase 4) over ¾ (3075 fixes collected in 196 nights). Moonlight variation has been reported also over the 29.5 days of lunar cycle (day 1 = new moon: Fig. 2). Radiotracking was performed under any weather condition, confirming that precipitation and wind do not affect the behaviour of *H. cristata*, as previously stated by Corsini et al. (1995). We reported three levels of moon visibility (visible, veiled or completely hidden by clouds) to limit potential biases due to different levels of ambient light at night. We assumed that the predation risk (including poaching) did not vary over a year. Habitat types were grouped in two categories: open habitats (cultivations, uncultivated fields, human settlements, wetland and coastal dune), and close habitats (shrubwood, Mediterranean "macchia", deciduous woodland and coniferous woodland). A Pearson's chi-square test without Yates' continuity correction was applied to compare frequencies of active/inactive fixes under all the conditions previously described, i.e. in open/close habitat types, with visible/veiled/hidden moon, during cold/hot season, for each lunar phase, in each of the four study sites. To test whether active/inactive fixes were randomly or uniformly distributed along a lunar cycle, the analysis of circular distributions was carried out and the following statistics were calculated: the mean angle, the mean resultant length (r) and the 95% confidence intervals of the mean direction (Fisher 1993).

Table 1. Extensions and habitat types composition of the four study sites. Hab. 1, cultivations; Hab. 2, uncultivated fields; Hab. 3, shrubwood; Hab. 4, human settlements; Hab. 5, deciduous woodland; Hab. 6, coniferous woodland; Hab. 7, Mediterranean "macchia"; Hab. 8, wetland; Hab. 9, coastal dune.



Study site	**Extension (ha.)**	**Habitat types (%)**								
		Hab. 1	**Hab. 2**	**Hab. 3**	**Hab. 4**	**Hab. 5**	**Hab. 6**	**Hab. 7**	**Hab. 8**	**Hab. 9**
San Miniato	123.01	59.19	11.13	5.31	12,28	9.33	0.00	0.00	2.76	0.00
Le Malandrine	396.00	78.68	4.30	6.20	0.00	10.50	0.00	0.00	0.32	0.00
Maremma Regional Park	618.75	30.60	8.49	3.05	0.00	0.00	25.91	25.64	4.19	2.12
Poggi di Prata	182.56	2.53	18.56	3.40	3.19	63.60	8.23	0.00	0.49	0.00

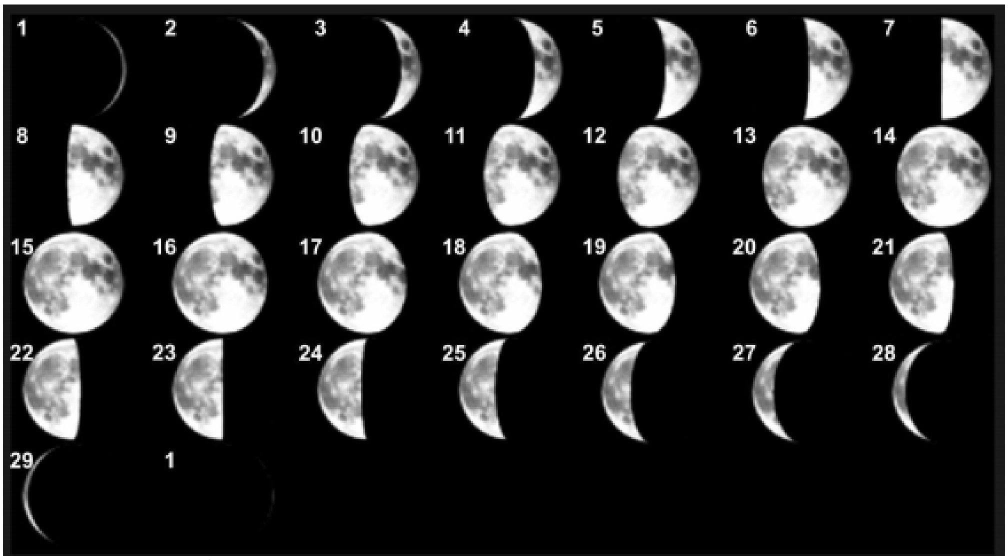
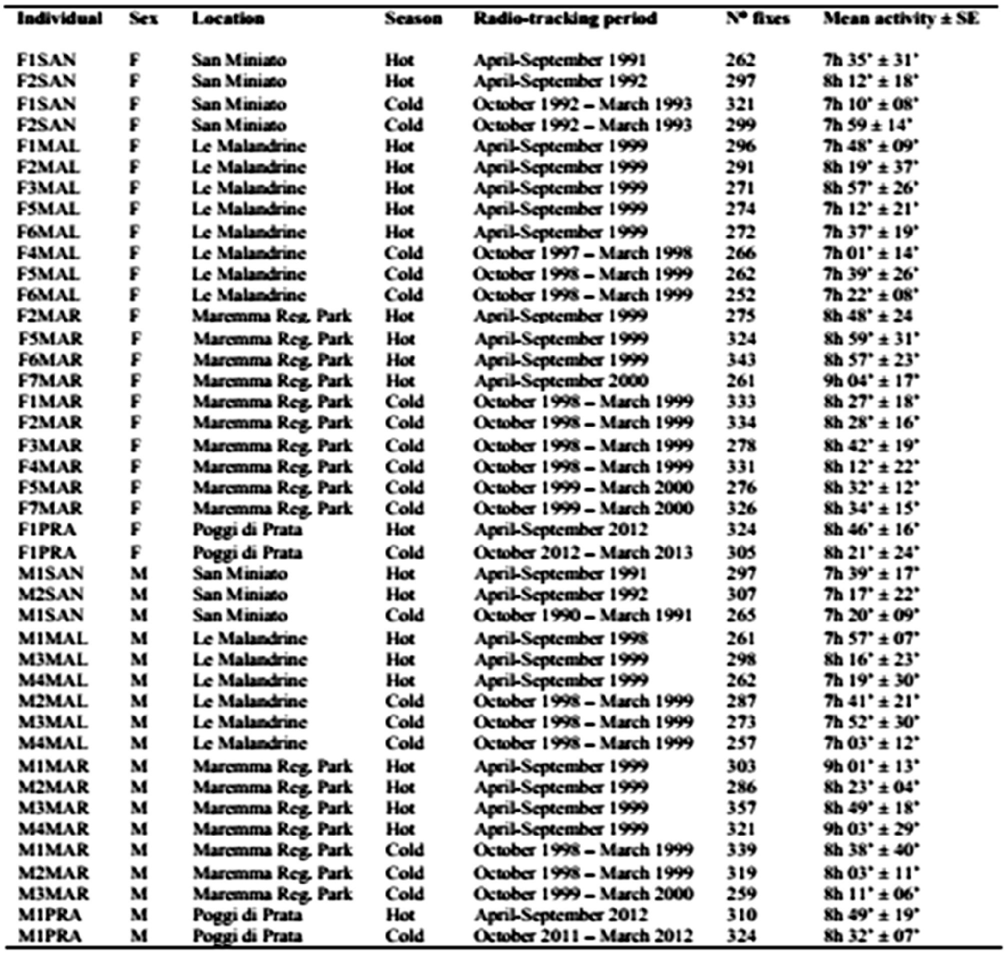


Figure 2. Numbering of days according to a lunar month.

The Rayleigh test for uniformity was applied to the circular distributions, based on the length of the mean vectors, to test the concentration of the individual directions around the mean (Batschelet 1981; Di Bitetti et al. 2006). The density curves, smoothed with the kernel method, were estimated and double plotted on Cartesian graphs, to better show the distribution peaks. Doubled line diagrams were used to show circularity (Fisher 1993; Marchetti and Scapini 2003).

Table 2. Mean (± SD) duration of nocturnal activity of each radio-tagged crested porcupine. Individual ID, sex, study site, season, radiotracking period and number of fixes per individual are also reported.

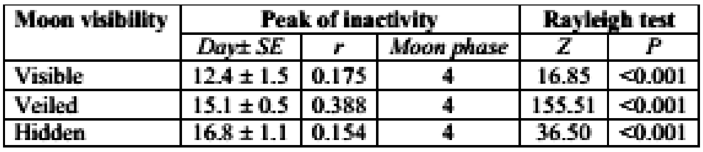


Individual	Sex	Location	Season	Radio-tracking period	N° fixes	Mean activity ± SE
F1SAN	F	San Miniato	Hot	April-September 1991	262	7h 35' ± 31'
F2SAN	F	San Miniato	Hot	April-September 1992	297	8h 12' ± 18'
F1SAN	F	San Miniato	Cold	October 1992 – March 1993	321	7h 10' ± 08'
F2SAN	F	San Miniato	Cold	October 1992 – March 1993	299	7h 59 ± 14'
F1MAL	F	Le Malandrine	Hot	April-September 1999	296	7h 48' ± 09'
F2MAL	F	Le Malandrine	Hot	April-September 1999	291	8h 19' ± 37'
F3MAL	F	Le Malandrine	Hot	April-September 1999	271	8h 57' ± 26'
F5MAL	F	Le Malandrine	Hot	April-September 1999	274	7h 12' ± 21'
F6MAL	F	Le Malandrine	Hot	April-September 1999	272	7h 37' ± 19'
F4MAL	F	Le Malandrine	Cold	October 1997 – March 1998	266	7h 01' ± 14'
F5MAL	F	Le Malandrine	Cold	October 1998 – March 1999	262	7h 39' ± 26'
F6MAL	F	Le Malandrine	Cold	October 1998 – March 1999	252	7h 22' ± 08'
F2MAR	F	Maremma Reg. Park	Hot	April-September 1999	275	8h 48' ± 24
F5MAR	F	Maremma Reg. Park	Hot	April-September 1999	324	8h 59' ± 31'
F6MAR	F	Maremma Reg. Park	Hot	April-September 1999	343	8h 57' ± 23'
F7MAR	F	Maremma Reg. Park	Hot	April-September 2000	261	9h 04' ± 17'
F1MAR	F	Maremma Reg. Park	Cold	October 1998 – March 1999	333	8h 27' ± 18'
F2MAR	F	Maremma Reg. Park	Cold	October 1998 – March 1999	334	8h 28' ± 16'
F3MAR	F	Maremma Reg. Park	Cold	October 1998 – March 1999	278	8h 42' ± 19'
F4MAR	F	Maremma Reg. Park	Cold	October 1998 – March 1999	331	8h 12' ± 22'
F5MAR	F	Maremma Reg. Park	Cold	October 1999 – March 2000	276	8h 32' ± 12'
F7MAR	F	Maremma Reg. Park	Cold	October 1999 – March 2000	326	8h 34' ± 15'
F1PRA	F	Poggi di Prata	Hot	April-September 2012	324	8h 46' ± 16'
F1PRA	F	Poggi di Prata	Cold	October 2012 – March 2013	305	8h 21' ± 24'
M1SAN	M	San Miniato	Hot	April-September 1991	297	7h 39' ± 17'
M2SAN	M	San Miniato	Hot	April-September 1992	307	7h 17' ± 22'
M1SAN	M	San Miniato	Cold	October 1990 – March 1991	265	7h 20' ± 09'
M1MAL	M	Le Malandrine	Hot	April-September 1998	261	7h 57' ± 07'
M3MAL	M	Le Malandrine	Hot	April-September 1999	298	8h 16' ± 23'
M4MAL	M	Le Malandrine	Hot	April-September 1999	262	7h 19' ± 30'
M2MAL	M	Le Malandrine	Cold	October 1998 – March 1999	287	7h 41' ± 21'
M3MAL	M	Le Malandrine	Cold	October 1998 – March 1999	273	7h 52' ± 30'
M4MAL	M	Le Malandrine	Cold	October 1998 – March 1999	257	7h 03' ± 12'
M1MAR	M	Maremma Reg. Park	Hot	April-September 1999	303	9h 01' ± 13'
M2MAR	M	Maremma Reg. Park	Hot	April-September 1999	286	8h 23' ± 04'
M3MAR	M	Maremma Reg. Park	Hot	April-September 1999	357	8h 49' ± 18'
M4MAR	M	Maremma Reg. Park	Hot	April-September 1999	321	9h 03' ± 29'
M1MAR	M	Maremma Reg. Park	Cold	October 1998 – March 1999	339	8h 38' ± 40'
M2MAR	M	Maremma Reg. Park	Cold	October 1998 – March 1999	319	8h 03' ± 11'
M3MAR	M	Maremma Reg. Park	Cold	October 1999 – March 2000	259	8h 11' ± 06'
M1PRA	M	Poggi di Prata	Hot	April-September 2012	310	8h 49' ± 19'
M1PRA	M	Poggi di Prata	Cold	October 2011 – March 2012	324	8h 32' ± 07'

RESULTS

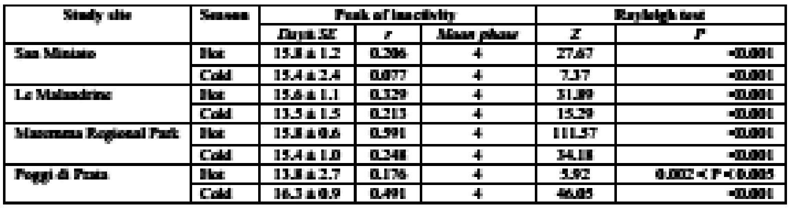
All porcupines were monitored (median number of fixes/individual, 296: Q1 - Q3, 271.5 - 321) for 16-23 hours/week/individual. In general, temporal activity (median value per night, 7h 38’: Q1 - Q3, 8h 16’- 8h 44’) did not vary significantly among seasons (Wilcoxon sum range test: W = 277; P = 0.20) and among sexes (Wilcoxon sum range test: W = 243; P = 0.67; N = 25 females and 18 males). Mean temporal activity for each individual is reported in Tab. 2. Animals were mainly active in the hot season ($\chi^2 = 83.38$, P << 0.01). Crested porcupine ranged in open habitats mainly in nights characterized by low moon visibility (veiled or hidden: $\chi^2 = 8.43$, P = 0.0147). The inactive fixes were significantly more concentrated in close habitats independently from the season $\chi^2 = 882.74$, P << 0.01). During the full moon (phase 4) a higher number of inactive fixes was recorded ($\chi^2 = 94.97$, P << 0.01); active fixes were mainly recorded in open habitats ($\chi^2 = 285.93$, P << 0.01). Circular statistics analyses provided further confirmation to these last results. The highest peak of inactivity for all the animals was recorded at day 15.3 ± 1.54 (day 15.3 ± 0.5; Rayleigh test: Z = 158.10; P < 0.001; r = 0.225), during the full moon, in close habitats (day 15.3 ± 0.5; Rayleigh test: Z = 164.40; P < 0.001; r = 0.242), in both cold (day 15.0 ± 1.0; Rayleigh test: Z = 40.22; P < 0.001; r = 0.155) and hot (day 15.3 ± 0.5; Rayleigh test: Z = 136.57; P < 0.001; r = 0.307) season (Fig. 3 a-d), at each level of moon visibility (Tab. 3; Fig. 3 e-g) and in all the study sites (Tab. 4; Fig. 4).

Table 3. Peaks of inactivity for each level of moon visibility, main circular statistics (Rayleigh test). SE: confidence interval for the day of highest inactivity, α = 0.05; r: mean resultant length of the circular distribution.



Moon visibility	Peak of inactivity			Rayleigh test	
	Day± SE	*r*	*Moon phase*	*Z*	*P*
Visible	12.4 ± 1.5	0.175	4	16.85	<0.001
Veiled	15.1 ± 0.5	0.388	4	155.51	<0.001
Hidden	16.8 ± 1.1	0.154	4	36.50	<0.001

Table 4. Peaks of inactivity for each level of moon visibility, main circular statistics (Rayleigh test). SE: confidence interval for the day of highest inactivity, α = 0.05; r: mean resultant length of the circular distribution.



Study site	Season	Peak of inactivity			Rayleigh test	
		Day± SE	*r*	*Moon phase*	*Z*	*P*
San Miniato	Hot	15.8 ± 1.2	0.206	4	27.67	<0.001
	Cold	15.4 ± 2.4	0.077	4	7.37	<0.001
La Malandrina	Hot	15.6 ± 1.1	0.329	4	31.89	<0.001
	Cold	13.5 ± 1.5	0.213	4	15.29	<0.001
Maremma Regional Park	Hot	15.8 ± 0.6	0.591	4	111.57	<0.001
	Cold	15.4 ± 1.0	0.248	4	34.18	<0.001
Poggi di Prata	Hot	13.8 ± 2.7	0.176	4	5.92	0.002 < P < 0.005
	Cold	16.3 ± 0.9	0.491	4	46.05	<0.001

DISCUSSION

Many nocturnal mammals correlate their ranging behaviour to moonlight intensity (Erkert 1974; Börk 2006; Lima 1998). For instance, most predators are more effective when some source of light is provide as they increase visual detection of potential preys (Cozzi et al. 2012; Rockhill et al. 2013). As a response, potential preys may use light as an indirect cue of predation susceptibility, and tend to shift their habitat utilization from open/enlightened to dark/sheltered sites (Clarke 1983; Brown et al. 1988; Upham and Hafner 2013). For instance, hunting effectiveness seems to be higher during bright moonlight nights for many canids (e.g. Lima Sàbato et al. 2006; Kotler et al. 2010), including red foxes (Molsher et al. 2000) and wolves (Theuerkauf et al. 2003). Red foxes, in particular, are able to shift prey selection when moon’s phase change (Molsher et al. 2000). Only where larger predators are present, red foxes shift their activity and select slightly darker nights to minimize encounters with them and maximize predation success (Mukherjee et al. 2009; Penteriani et al. 2013). Crested porcupines are large nocturnal rodents distributed in North Africa and, south to the Sahara desert, from Senegal to Northern Tanzania.

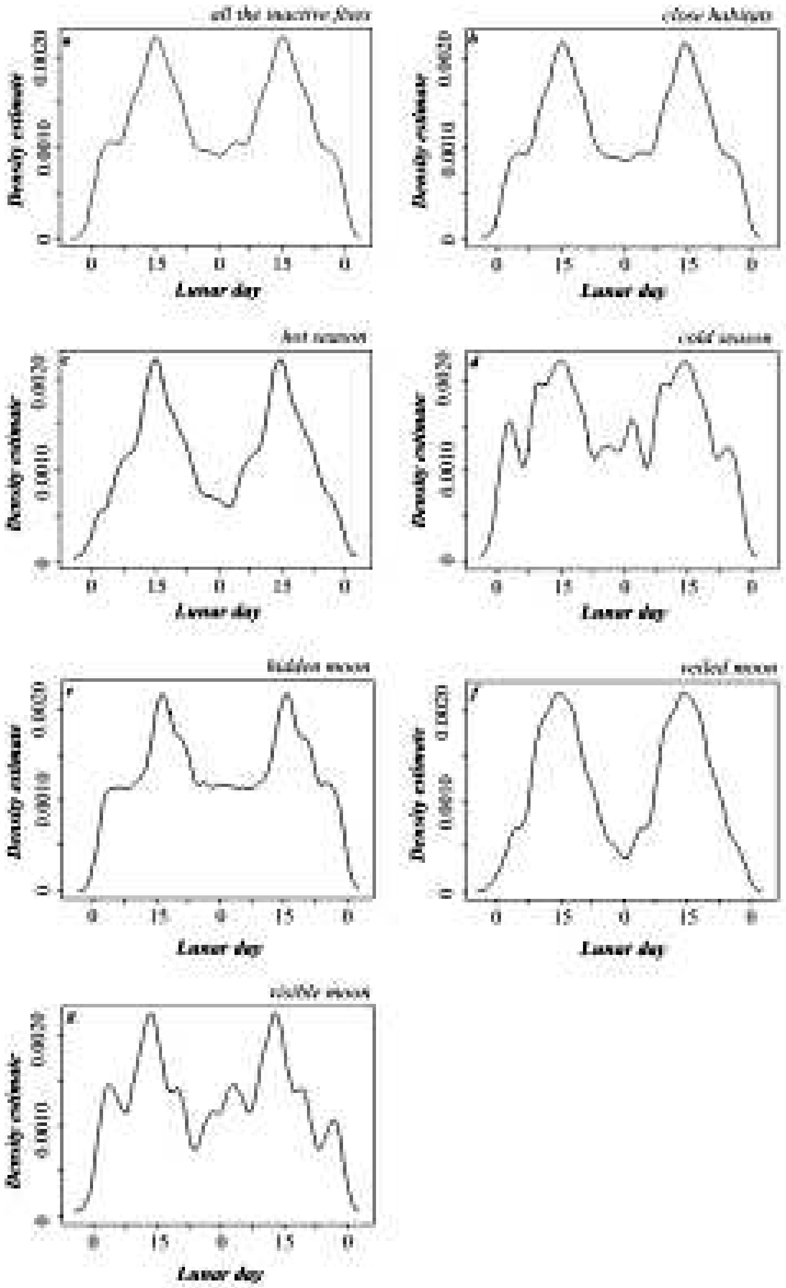


Figure 3. Frequency of inactive fixes variation along the lunar cycle; a) with all the inactive fixes; b) in close habitats; c) during the hot season; d) during the cold season; e) when the moon was hidden by clouds; f) when the moon was veiled; e) when the moon was completely visible.

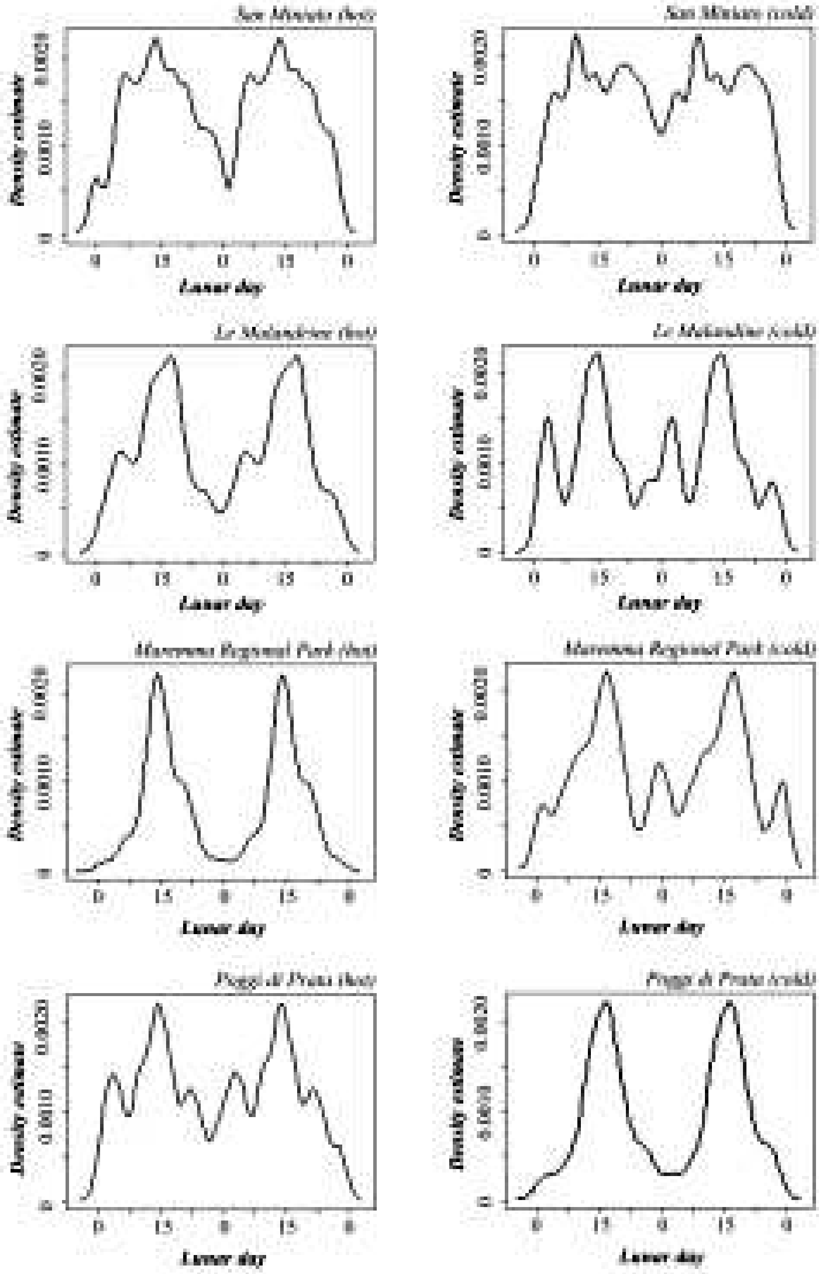


Figure 4. Frequency of inactive fixes variation along the lunar cycle for all the study areas, during hot (left) and cold (right) season.

Results of this study are based on a high number of radio-tagged individuals from four sites in Southern Tuscany; median activity was 7h 38' (Q1 - Q3: 8h 16'- 8h 44'). Variation in the daily period of activity was not significant neither among seasons, nor among sexes. Anyway, *H. cristata* seems to be more active (in terms of number of nights) during the hot season , consistently with Corsini et al. (1995).

Predation on *H. cristata* by lions *Panthera leo*, African wild dogs *Lycaon pictus* and spotted hyenas *Crocuta crocuta* has been reported in Africa (Mills and Shenk 1992; Breuer 2005). Despite data about *H. cristata* in Africa are not available, other porcupine species living in areas characterized by the presence of large predators, usually avoid exposure to bright moonlight (Emmons 1983; Alkon and Saltz 1988; Fattorini and Pokheral 2012). As for Italy , this quill-covered rodent represents just a negligible part of the diet of red foxes (Lucherini et al. 1995). Scandura et al. (2000) reported crested porcupine in the diet of the wolf, although other, geographically more extended studies on the feeding habits of this wild Canid do not support this finding with further information (for a review, see Meriggi et al. 2011). According to these considerations, predation on *H. cristata* is thought to be almost rare in Italy so that a non-significant effect of the moon's phase in the activity of this rodent is expected. However, our results clearly claimed that crested porcupines exhibit a lunar phobic nocturnal behaviour. This behaviour is not limited to summer nights, as reported by Corsini et al. (1995), but it is extended all over the year, independently from the visibility of the moon. According to Griffin et al. (2005), a definite reproductive season may increase attacks by possible predators or modify the ranging behaviour of the potential preys regardless to the moon's phase. This seems not to be the case of *H. cristata*, that does not show a clear seasonality of births (Santini 1980, A. Sforzi and E. Mori, personal observations). The origin of the Italian population of *H. cristata* has been debated for long. The presence of Pliocene and Pleistocene fossils of the genus *Hystrix* in Europe (Van Weers 1994) and the analyses of morphometric variation of the skull (Angelici et al. 2003) suggest the hypothesis of autochthony. On the other hand, the absence of late-Holocenic reports and genetic analyses based on mitochondrial DNA confirm that the crested porcupine was introduced from North Africa (maybe from Tunisia), probably since 600 AD (Trucchi and Sbordoni 2009; Masseti et al. 2010). Despite being protected from the Italian (National Law 968/1977) and the EU law (Berne Convention, all. II; Habitat Directive; all. IV), the species is still poached for meat in Italy (Mori et al. 2013). Moreover, *H. cristata* can cause serious crop damages (Bruno and Riccardi 1995), especially where locally abundant; damage to river banks are also complained (Convito and Paci 2003). Despite impact on crops has so far proved to be less than is often assumed (Bruno and Riccardi 1995), an effective solution of the conflict with human activities has to be found yet. Captures and removals represent only a temporary measure, as the empty space is rapidly re-colonized by other individuals, and the use of visual deterrents and electric fencing seems to be ineffective (E. Mori, personal observation). We may suggest that moonlight avoidance for the Italian population of *H. cristata* may represent a behaviour evolved by this rodent in Africa, where the species would be arisen (Trucchi and Sbordoni 2009), and maintained after the introduction (or the colonization) of the Italian peninsula, to avoid the limited predation risk and possibly to limit its visibility, thus vulnerability to the strong poaching pressure.

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