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ECOLOGY OF SANDY BEACH MACROFAUNA AND ITS BEHAVIOURAL RESPONSE TO ENVIRONMENTAL CHANGE

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Structure of the thesis

This thesis is composed of three separate chapters, two of which are published, an introductory and a concluding chapter. The main question that led to the development of the studies presented in the thesis is the following: is it possible to compare behavioural and ecological studies at a large geographical scale in the context of the ecosystems of sandy beaches under environmental changes?

After the first introductory chapter, in the second chapter I present a review of studies in different (geographically distant) localities affected by similar distal and proximate drivers of climate change. In "Behavioural adaptations of sandy beach macrofauna in face of climate change impacts: A conceptual framework", we discussed if behavioural responses by sandy beach macrofauna were consistent with expectations under climate change. We formulated specific hypotheses for how behavioural adaptations in sandy beach macrofauna are predicted to respond to climate change impacts. We provided an overview of macrofauna behavioural adaptation features in different and varying environmental contexts, a summary of the effects of main climate change drivers on sandy beaches and a conceptual framework predicting behavioural adaptations of sandy beach macrofauna under climate change pressure.

In the third chapter, I present an experimental study linked to the main question for the ecological approach, to highlight which factors and variables in the physical-chemical environment influence the arthropodofauna distribution. In "Arthropodofauna richness and abundance across beach-dune systems with contrasting morphodynamics", I investigated the across-shore distribution of arthropods in two Uruguayan sandy beach-dune systems with contrasting morphodynamics. I performed a deconstructive analysis to describe faunal changes from the dunes to the shoreline, and assessed existing hypotheses on the species distribution in relation to beach morphodynamics.

In the fourth chapter, I present an experimental study focusing on talitrid ecology and behaviour in different localities, assuming that zonation and orientation behaviour of talitrids is an immediate response to environmental changes and considering behavioural adaptations as immediate responses to stressful environmental conditions, which may be dramatically increased with climate change. Starting from local comparisons, I analysed the relationships between the physical-chemical characteristics of six sandy beaches and the zonation and orientation behaviour of talitrid amphipods, ending with a comparison at a large geographical scale of two different species expressing similar behavioural adaptations in different localities.

In the Appendix I present a paper on ecological differentiation of plant species coexisting in a changing environment, written during a scholarship at the University of South Bohemia. “Ecological differentiation of *Carex* species coexisting in a wet meadow: Comparison of pot and field experiments” is a research work on the competition of phylogenetically similar species that share traits and resources. Differences were identified in phenotypic traits between seven coexisting *Carex* species and their responses to competition and fertilization in pot experiments; then, long-term field experiments were used to generate responses of *Carex* species to fertilization and mowing and to illustrate temporal variability between species. We assessed if the results of pot experiments were effective in predicting species responses in the field.
Another publication including the relevant results of the fourth chapter is in preparation for submission before the final defence of my thesis.

Chapter contributions
Chapter 2: FS and OD ideated the review. EID prepared the literature survey. FS, OD and EID discussed the scenarios and conceptual models and wrote the manuscript.
Chapter 3: EID, OD and FS designed the study. EID was responsible for ideating and preparing the protocols of the experiments, under the supervision of FS and OD. EID and collaborators collected the data. EID and OD analysed the data. EID wrote the manuscript. OD and FS edited the manuscript.
Chapter 4: EID, FS and OD designed the study. EID was responsible for ideating and preparing the protocols of the experiments, under the supervision of FS and OD. EID, FS, OD and collaborators collected data. EID analysed the data. EID wrote the manuscript. FS, OD and GS supervised the manuscript.

Name abbreviation
EID – Emilia Innocenti Degli (Ph.D. student)
FS – Prof. Felicita Scapini (Supervisor)
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Special thanks to my family and my friends, who have always believed in me, supporting me in joys and sorrows.

List of abbreviations

AIC - Akaike Information Criterion

GLM - Generalized Linear Model

HSH - Habitat Safety Hypothesis

LAZ - Littoral Active Zone

LRT - Likelihood Ratio Test

MDS - Multi-Dimensional Scaling

PCA - Principal Component Analyses

SAO - Southwestern Atlantic Ocean

SPLM - Spherically Projected Linear Models

TED - Theoretical Escape Direction

To my mother

Chapter 1 – General introduction: the challenge of change

1. Sandy beach ecosystems

The interest in sandy beaches has always been quite high both for commercial and tourism activities, being beaches environments in which it is pleasant to live, and which offer many useful services (Fanini et al., 2019). The ecosystems of sandy beaches have received increasing attention in recent years, as they are widely exploited and stressed from various drivers, and the animal species inhabiting sandy beaches have faced increasing challenges (Costa et al., 2020; Fanini et al., 2020; Defeo and Elliott, 2021). Populations of mammals, birds, reptiles and invertebrates nest, feed and reproduce on sandy beaches (McLachlan and Defeo, 2018). These environments are highly dynamic and harsh by nature, being at the edge between the land and the sea, and undergo continuous changes as a result of currents, tides and meteorological conditions: for this characteristic dynamism, the species which live on sandy beaches have evolved ethological and ecological adaptations, in order to survive under changing conditions (Scapini, 2014; McLachlan and Defeo, 2018). In addition to the stresses caused by natural changes, the ecosystems of sandy beaches must also cope with stressors caused directly or indirectly by human activities: recreation, mechanical cleaning, nourishment, sediment and water pollution, resource exploitation, biological invasions, coastal development and engineering, mining and, finally, climate change impacts (Defeo et al., 2009, 2021). Therefore, the quickest way for the macrofauna of sandy beaches to adapt to the changes underway (changes are not all slow and gradual, but they may be sudden and strongly impacting) is through behavioural plasticity, an immediate response to change (Watson, 2018). Behavioural adaptations of macrofauna to the sandy beach habitats have been studied in many localities worldwide (reviews are in Scapini, 2014 and McLachlan and Defeo, 2018).

Given that the above-mentioned impacts are common on sandy beaches and ongoing around the world, the macrofauna species that inhabit them are likely to adopt common strategies to deal with the same types of stress and may behave in a similar way. Behaviours such as orientation, zonation, foraging and those linked to reproduction, can change and adapt to new environments, or the adaptation may be not possible due to a sudden disruption. It was important to explore the behavioural adaptations adopted by sandy beach macrofauna in their specific environment, and the possible behavioural plasticity or fixity. To explore the possibility for sandy beach macrofauna to face global changes, it was important to analyse behavioural adaptations at a large geographical scale, following a common line of investigation. Considering different situations in the context of climate change, through the knowledge given by fragmented studies on behavioural adaptations of various species in the world, we hypothesized future scenarios for different species according to their current adaptability (chapter 2, Scapini et al., 2019).

To answer whether similar species in different localities can have similar adaptations to the (changing) environment, it was first of all essential to know the relationships between species and habitat characteristics. Enlarging the local scale without losing specific species-habitat relationships, allowed to conduct a comparative study between the across-shore distribution of the macrofauna of two beaches in Uruguay, with contrasting morphodynamics, reflective and dissipative, according to the definition by Short (1996) (chapter 3, Innocenti Degli et al., 2021). The Habitat Safety Hypothesis (HSH) states that supralittoral species of sandy beaches are more abundant in the more stable and protected backshore of reflective beaches than that of dissipative ones, which is more subjected to stressors due to wave actions (Defeo and Gómez, 2005; Defeo and McLachlan, 2011). Moreover, a further hypothesis predicts that the well-developed dunes of dissipative beaches may be a more favourable and safer environment for insects and arachnids than that of reflective beaches, and host more diverse and abundant species (Defeo and McLachlan, 2011; Barboza et al., 2012). The study of the relationships between different species and their environment, across the beach from the shoreline up the dunes, confirmed that it is important to carry out a deconstructive analysis of the biology (and behaviour) of the individual species, in order to understand which characteristics of the beach influence their across-shore distribution (chapter 3, Innocenti Degli et al.,2021).

Large-scale studies (geographical scale, possibly across continents) are still scarce, due to the difficulty of having comparable data, and sometimes they do not consider the specific characteristics of the studied beaches, or adopt different protocols to collect data. To focus the collection of data in the analysis of multiple beaches in different regions, it is important to select a bioindicator that should be simple to study and, at the same time, could give consistent answers to the main questions. Sandhoppers have been selected as bioindicators of beach ecosystem health: these crustaceans are usually abundant and unprotected species, widespread, easy to collect and analyse, with similar biology and behaviours expressed on different species (Cardoso, 2002; Scapini, 2006; Mezzetti et al., 2010; Nourisson and Scapini, 2015, and references therein). These crustaceans are semi-terrestrial, breathe through gills which must always be kept moist, and have a partially permeable cuticle; to avoid the risk of dehydration, they show behavioural adaptation to the beach of origin using solar orientation to reach the wet area of the beach, *i.e.* the shoreline (Scapini et al., 2005). A long-term stability of the shoreline allows the transmission of this adaptive behaviour to subsequent generations, while if the beach is not stable (under erosion, accretion or anthropogenic disturbance) the adaptation of the "winning" behaviour (orientation towards the shoreline) is not genetically transmitted, and mal-adaptation to the changing environment may be observed (Scapini et al., 1995; Ketmaier et al., 2010; Scapini et al., 2005; Scapini, 2006). I decided to analyse the zonation and orientation of two species of sandhoppers using the same protocols, selecting two geographical areas that differed in the physical characteristics of the beaches (Atlantic and Mediterranean), but which had a fairly similar climate (cold and wet winters and hot and dry summers). Two seasons, spring and autumn, were chosen in relation to the biology of sandhoppers, because in these seasons populations tend to be well structured, including the contemporary occurrence of juveniles and adults (Marques et al., 2003; Cardoso and Veloso, 1996). Moreover, in spring, the adults have likely overwintered, while in autumn, the populations are composed by adults born in spring and newborn juveniles. The two selected seasons allowed also the analysis of the periods before and after the greater summer touristic exploitation. I started from the local scale of the environment of each beach, comparing it with a different beach of the same locality or region (exploring the details of the relationships between species and environment), and then increased the scale of comparison to two different continents, to answer the question on the adaptations to common problems, such as those deriving from climate change (chapter 4).

Figure 1. Circular structure of the thesis: from fragmented studies on macrofauna behaviours on various sandy beaches in different countries, to local analysis on species-environment relationships, to large geographical scale studies, maintaining the local details.

In this way, the circle closes: I started from the general, looking for published information on behavioural adaptations of the macrofauna of sandy beaches in various countries, I went down into details, to define the relevant aspects of these ecosystems for macrofauna adaptations, and finally I increased the number of case studies on a wider geographical scale, without losing the details of the specific relationships between the species and their environment, in order to be able to draw general conclusions (Fig. 1).

2. *Aims of the thesis*

The Ph.D research herein presented investigated the behavioural adaptation and etho-ecology of macrofauna inhabiting sandy beaches at a large geographical scale. There are several local behavioural and ecological studies on the subject, but the link was missing between studies performed in different and geographically distant localities affected by similar climate change distal and proximate drivers. We have built hypotheses on possible strategies of sandy beach macrofauna to face climate change impacts (chapter 2); we focused on which factors and variables could influence species distribution and adaptations (chapter 3), and we highlighted differences and similarities between the behavioural adaptations of two species of sandhoppers that hypothetically face similar environmental conditions and could be influenced by climatic change impacts (chapter 4).

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Chapter 2 – Behavioural adaptations of sandy beach macrofauna in face of climate change impacts: a conceptual framework

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Abstract

Sandy beaches are severely under-represented in the literature on climate-change ecology, yet different lines of evidence suggest that the macrofauna inhabiting these narrow and dynamic environments located at the land-sea interface is being reorganized under the influence of this large scale and long-lasting stressor. This is reflected in macrofaunal sensitivity to increasing sea surface temperature, sea-level rise, extreme events and erosion of the narrow physical habitat. However, evidence of behavioural responses by sandy beach macrofauna that are consistent with expectations under climate change is scarce and fragmentary. In this paper, specific hypotheses are formulated for how behavioural adaptations in sandy beach macrofauna are predicted to respond to climate change impacts. Firstly, a conceptual framework and an overview of macrofauna behavioural adaptation features are provided. Secondly, the effects of main climate change drivers on sandy beaches are summarized. Thirdly, a conceptual framework is developed giving behavioural adaptations of sandy beach macrofauna under climate change pressure. The degree to which observations on behavioural adaptations of beach animals conform to expectations under specific climate change drivers (sea level rise, sea surface temperature, winds and storminess, rainfall, acidification and eutrophication) is explored. Taking into account the empirical evidence and the theoretical framework detailed in the paper, emergent hypotheses/predictions are proposed. Climate change drivers are expected to impact habitat features and consequently the behavioural expression of macrofauna as active responses to habitat changes. Behavioural adaptations are expected to be impaired, more variable or disrupted, thus decreasing fitness, causing local population extirpations and potentially triggering a range of cascading effects of ecological change in the beach ecosystem. Biodiversity loss will be

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the outcome of the negative pressures driven by climate change. The specificity of sandy beaches as narrow ecotones between sea and land may be lost under climate change pressure, adversely affecting fine-tuned macrofaunal adaptations and therefore ecosystem functioning. Strictly adapted endemic sandy beach fauna will be especially subjected to local extirpations, while species with a large reaction norm (*i.e.* phenotypic and behavioural plasticity) may face changes by dispersal and exploitation of new niches. Under climate change impacts, biodiversity loss is predicted, which would hamper beach ecosystem resilience. The limits to which sandy beach macrofauna responds and can behaviourally adapt to environmental change are worthy of exploration, in view of the increasing influence of the long-lasting climate driven stressors threatening these ecosystems at risk.

Key words
Climate change; sandy beaches; macrofauna; behavioural adaptation; individuals; populations

1. *Introduction*

Sandy beaches are threatened by a variety of stressors operating at different spatial and temporal scales (McLachlan and Defeo, 2018). These perturbations translate into ecological impacts that are manifested across several dimensions, affecting the physical and biological components of the beach system (Brown and McLachlan, 2002, Defeo et al., 2009). A worrying scenario is particularly given by the increasing occurrence of press perturbations, notably climate change. A conceptual framework was recently developed to construct explanatory hypotheses and predictions in sandy beach ecology under climate change expectations (Schoeman et al., 2014). Long-term information was used to test emergent hypotheses and related predictions on population abundance, structure, individual size, body condition, and extension of reproductive and recruitment periods (Ortega et al., 2012, 2016, Celentano and Defeo, 2016), as well as in contraction/expansion of distributional ranges (Carstensen et al., 2010, Hubbard et al., 2014; Schoeman et al., 2015; Donelson et al., 2019). Climate change has been linked to widespread and substantial changes in the distribution, abundance, reproduction and survival of beach populations, increasing the risk of local extirpations and functional extinctions (McLachlan and Defeo, 2018). Long-term changes in species richness, ecological diversity and ecosystem-level attributes were also detected as responses to climate-driven changes in local productivity associated with climatic shifts (Lercari et al., 2018). In a climate-change context, the survival of macrofauna is related to their adaptation potential, including genetic variation and phenotypic/behavioural plasticity (O’Connor et al., 2012). Behavioural adaptations of macrofauna to the sandy beach habitat have been subjected to a large amount of observational and experimental work (Scapini, 2014; McLachlan and Defeo, 2018), yet a general conceptual framework for explanatory and predictive hypotheses is lacking, which would go beyond fragmentary evidence (Watson, 2018).

Phenotypic plasticity in sandy beach macrofauna was recognized as an adaptation to these dynamic environments, emphasizing its importance as the “adaptation to adapt” (Brown, 1996). The concept of phenotypic plasticity was developed for plants that may reproduce asexually and give progenies sharing the same genotype, but adapted to different environmental conditions (Schlichting and Pigliucci, 1998). Phenotypic plasticity is part of the concept of reaction norm and may evolve under changing environments, including also responses to anthropogenic stressors such as climate change (Kelly, 2019). Behaviour is a particular case of phenotypic plasticity in animals, being expressed by individuals, which have unique genotypes and may respond to different environmental features that they encounter during development and free life (Scapini et al., 1988; Hazlett, 1995; West-Eberhard, 2003). Tolerant individuals more promptly avoid harmful stimuli or contexts through learning (negative conditioning), or they search again in the spot where they have previously found a resource (positive conditioning). In this respect, behaviour is contingent (*hic et nunc*, here and now), reflecting the past and actual conditions faced by individuals. However, individuals may show peculiar behaviours, acquired through imprinting-like learning processes, defined “behavioural syndrome” or “personality”, which may have evolutionary consequences (Scapini et al., 1988; Gherardi et al., 2012; Kralj-Fišer and Schuett, 2014).

Behaviour is expressed by animals in response to internal needs and external stimuli, genetics underlying behavioural expression through morphological, physiological and sensorial adaptations. Behaviour is an adaptation *per se,* important for survival and reproduction and, ultimately, for population and species survival (Campan and Scapini, 2002). Behavioural and physiological plasticity, which contributes to population survival, is a motor of evolution, but also a product thereof, giving rise to adaptations through processes of developmental canalization and/or genetic assimilation of gene expression (Badyaev, 2005). Inter-individual diversity is commonly observed in behavioural expression, which may provide fitness advantage to better adapted individuals, particularly in case of the colonization of new habitats, where natural selection may then establish new adaptations (O'Connor et al., 2012). Several behavioural adaptations were shown to be genetically determined, with differences within and between populations. These include rhythms, orientation, foraging behaviour, parental care, mate preference and antipredator behaviour (Pardi and Scapini, 1983; Scapini et al, 1985; Berthold et al., 1992; Sokolowski, 2001; Sinn et al., 2006; Kralj-Fišer and Schneider, 2012). Phenotypic plasticity and genetic variation may be considered as complementary attributes (Scapini et al., 1988; 1995). Genomic studies and molecular genetics evidence on gene expression and epigenetics offer a background for analyses of (changing) relationships between genes and environment during individual life and population evolution (Baker et al., 2001; Renn and Schumer, 2013; Clark et al., 2018).

Behavioural aspects have not been used yet in sandy beach ecology to construct hypotheses under climate change expectations. This could be due to the relatively poor understanding of how responses of individual organisms extrapolate to whole ecosystems (Schoeman et al., 2014). Hence this is an appropriate time to develop a conceptual framework of behavioural responses and flexibility in sandy beach macrofauna, in view of climate change impacts on beaches. In this paper, specific expectations are formulated for how behavioural adaptations in sandy beach macrofauna are predicted to respond to climate change related impacts. Firstly, a conceptual framework and an overview of behavioural adaptation features of sandy beach macrofauna are provided. Secondly, theory from climate change literature as applied to sandy beaches is summarized and the main drivers of change affecting the littoral active zone (LAZ) are detailed. Thirdly, a conceptual framework is developed for behavioural adaptations of sandy beach macrofauna under climate change pressure. Illustrative examples are presented for main climate change drivers that may affect behaviour in beach macrofauna, based on observational data series, field and laboratory experiments. The degree to which observations on behavioural adaptations of beach macrofauna conform to expectations under specific climate change drivers is explored. Taking into account the empirical evidence and the theoretical framework detailed in the paper, a suite of emergent hypotheses/predictions is also given.

2. *Behavioural adaptations in sandy beach macrofauna*

Behavioural adaptations characteristic of sandy beach macrofauna are dictated by the instability of the habitat, given by unpredictable and predictable environmental changes at different temporal scales, which may impact on individual survival and population persistence (McLachlan and Defeo, 2018). Animal behaviour may include various components of increasing complexity, from cue perception to behaviour expression (Figure 1). For example, tides are potentially harmful for supralittoral animals, for the risk of displacement from the suitable zone, and, at the same time, provide the "cues" that give the signal for and to orientate escape reactions, or act as *zeitgeber* (synchronizer) of tidal rhythms (Scapini, 2006; Naylor, 2010). "Responses" are reactions to single or multiple stimuli, being often innate (*i.e.* expressed at the beginning of life, without experience) and potentially shown by all individuals of the population, in many cases shared by different species inhabiting similar habitats or beach zones. Kinesis (movement with respect to environmental gradients, *e.g.* substrate temperature, humidity or salinity), taxis (movement with respect to a directional stimulus, *e.g.* shadow, light, gravity, magnetism, or more complex habitat-specific behavioural adaptations) are used to find the suitable habitat or avoid a hazardous situation. "Behavioural adaptations" are functional to the habitat where the population lives and may be genetically determined (evolved through natural selection) or acquired through individual experience. In the latter process, the adaptation to adapt or learning ability may be inherited.

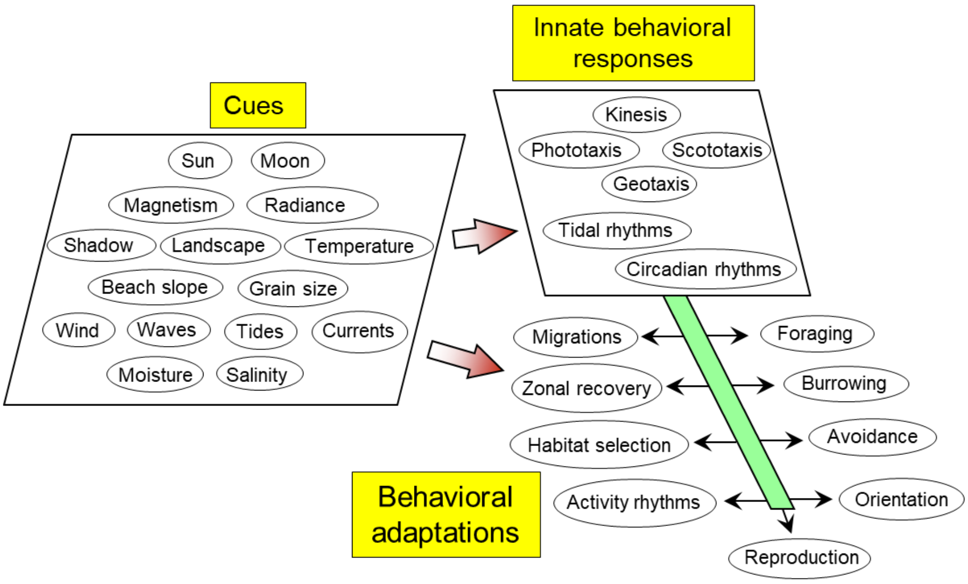

Figure 1. Behavioural adaptation features common in sandy beach macrofauna: **Cues** are significant environmental elements to which beach individuals react with **Innate behavioural responses**, according to species physiology; **Behavioural adaptations** are expressed on the beach for functions that contribute to individual survival and reproduction and may pass on to the following generations. Modified from Campan and Scapini (2002).

On sandy beaches, behavioural adaptations in mobile macrofauna are prevalently expressed to search and choose specific substrate conditions or avoid harmful ones, recover and maintain the suitable habitat in the LAZ, which lies between the outer limit of wave effects on bottom stability and the landward limit of sand transport by wind (Tinley, 1985) (Table 1S in Supplementary Material and references therein). Supralittoral species maintain or actively recover the beach zone through habitat selection, orientation and homing, tuning these behaviours to the conditions encountered (Vannini and Cannicci, 1995; Williams, 1995; Scapini, 2014). Biological rhythms contribute to express activities under optimal conditions, by synchronizing internal clocks to external cyclic variables, driven by night-day alternation, tides, lunar phases and seasons; activity rhythms are key adaptations of sandy-beach macrofauna, as they allow the anticipation of potentially stressful conditions (Naylor, 2010). Burrowing into the sediment is a common adaptation in sandy beach macrofauna to prevent hazardous conditions, such as dehydration, predation or dislocation by tides and storms, and is linked to sand granulometry and water content, which show gradients on beaches; this behaviour may be greatly impaired by direct human actions, such as beach nourishment, mechanical cleaning, car driving and trampling (Viola et al., 2014; Costa and Zalmon, 2019). In the intertidal zone, burrowing is expressed as vertical zonation change (Sassa et al., 2011; McLachlan and Defeo, 2018). Physical and chemical environmental driving forces prevail on sandy beaches, but biological ones are also relevant for behaviour expression (Table 1S). In the non-vegetated zone of the beach, animals are either predators or opportunistically forage on stranded carrion and wrack, which may be abundant, but unpredictably supplied and of varying nature (Pennings et al., 2000). Cannibalism was observed under particular conditions, such as high population density under oligotrophic conditions (Duarte et al., 2010). Supralittoral animals may also forage in the foredunes and dunes, avoiding the risk of being swept away by waves, dehydration risk and predation, and at the same time exploiting a rich food supply in the case of oligotrophic coastal waters (Colombini et al., 2013; Lagar et al., 2016). Gregarious behaviour may depend on the choice of the same zone (habitat selection) by several co-specifics; intraspecific competition may occur to defend resources (*e.g.* burrows, mates and food), population density being critical for the expression of competitive behaviour (Gherardi et al., 2012). Behavioural adaptations may differ between species with direct development and those with larval stages. The former may express behavioural adaptations specific to the beach of origin, which are further tuned to the home beach features during life (Gambineri and Scapini, 2008). Larvae may express innate habitat selection behaviour (settlement), such as taxis or kinesis to odors, sounds, salinity gradients and pressure (Stanley et al., 2012).

The expression of adaptive behaviours may be modified in response to changes of external cues and/or internal motivation and needs, as well as a consequence of individual experience. In non-homogeneous environments such as beaches, which present several land-sea physical and biological gradients, each zone of the beach may be differentially characterized; thus, animal displacement may itself cause a modification of the expressed behaviour. The needs of young and small individuals and their susceptibility to stressful factors may be different than those of adult and larger ones (Williams, 1995; Scapini and Dugan, 2008); females producing eggs or carrying broods use resources for the following generation, having therefore different needs than non-reproductive females, which results in differential behavioural expression (Borgioli et al., 1999).

Genetic variation within sandy beach populations is particularly important, as their habitats are subjected to frequent changes. In some sandy beach taxa, behaviours such as site fidelity, mate choice and contrasting random mating may produce genetically differentiated sub-populations, while dispersal and migration may establish meta-populations, maintaining genetic flow (Soares et al., 1999; Bezuidenhout et al., 2014). However, populations cannot tolerate the large-scale event of habitat disappearance, which may be a consequence of climate change impacts and encroaching development from expanding human populations on land (*e.g.* coastal squeeze, Defeo et al., 2009; Hubbard et al., 2014). In such case, dispersal and successful colonization of new beaches would allow species persistence and further evolution. The expression of innate behavioural responses to the beach environmental features and gradients would favor the success of colonization. The effects of environmental change on beach macrofauna will depend firstly on behavioural adaptability, which should be included in explanatory and predictive hypotheses, subjected to *ad hoc* experimental work.

There are specific limits or constraints to the behavioural expression, which should be considered when developing predictive hypotheses. Also, specific conditions are necessary to adjust behaviour to a changing environment, as the animals must actively track the environmental changes by adapting to modified and new contexts. Animal motility and the capacity of recovering a suitable beach habitat should be included in the conceptual framework. Learning is an adaptation strategy in most animals to adjust behaviour to new situations, but require time, memory and a nervous system (even a simple one). Constraints for behaviour adaptability depend also on the inherent characteristics of the species (life cycle, generation turnover, population abundance and genetic diversity). A certain population size and environmental temporal stability (with relation to population turnover) are necessary for adaptations to be stabilized. Prevailing climatic conditions on a sandy beach may interact with the tuning of behavioural expression, resulting in differential life-cycle and behavioural adaptations in different geographic areas (McLachlan and Defeo, 2018). The species potential for colonization of new habitats is a key factor under climate change, which implies the expression of adaptive behaviour in new contexts, whereas habitat connectivity would allow shifts of species ranges (O’Connor et al., 2012; Donelson et al., 2019).

3. Sandy beach ecosystems and climate change

Climate change has added a new global dimension to modifications of sandy beach ecosystems (Schoeman et al., 2014, McLachlan and Defeo, 2018). It has been postulated that sandy beach ecosystems are at risk because of concurrent and increasing impacts of different climate change drivers (Figure 2, Table 1). Indeed, in addition to the ecological consequences of temperature increase, sea level rise will increase erosion along sandy shores. Global warming may also be expected to cause increased storminess, as well as changes in rainfall patterns. Increased storminess will also result in erosion, retreat of beaches and dune scarping with vegetation loss. Predicted increase in rainfall patterns in some regions of the world may increase freshwater flow into marine environments, decreasing salinity. Salinity will also decrease due to ice cap melting, as has been observed in the Baltic Sea (Elliott et al., 2015). Excessive precipitation and flooding may cause a rise in groundwater levels, thereby increasing swash run-up distances and erosion rates. Warmer air and water have also been accompanied by an increase in the frequency and intensity of onshore winds (Bakun, 1990, Sydeman et al., 2014), which in turn increases wave energy, moving beaches towards more erosive states (Short, 1999). It has therefore become increasingly critical to understand how sandy beach ecosystems will respond to these long-lasting and pressing changes of the environment.

The most sensitive areas of the LAZ are the beach, backshore and foredunes (Figure 2), which together comprise a narrow stretch constrained by physical and anthropogenic limits, where impacts on sand budget and biodiversity are expected to occur. Human activities can accelerate the large-scale changes that are being generated by climate change drivers. For example, unplanned and intense urban development on land could affect the ecosystem service played by sand dunes in sand storage, increasing erosion rates. Acting together with rising sea levels, these drivers may affect the sand budget (storage and transport) across the LAZ, narrowing beaches up to the point that entire habitats could disappear, become severely restricted or move landwards, depending on the set of local conditions (Houston and Dean, 2012). Coastal squeeze is nowadays a major long-term threat that sandy beaches face worldwide (Defeo et al., 2009; McLachlan and Defeo, 2018). Particularly, climate change drivers (Table 1) are expected to affect the highly specialized sandy beach macrofauna restricted to inhabit the land-sea interface and thus lacking spatial refugia or compensatory habitats (Schoeman et al., 2014). Decreased salinity in coastal waters may affect fertility, reproduction, growth and survival rates in sandy beach macrofauna (Lima et al., 2000; Ortega et al., 2016). Predicted ocean acidification may further impact beach mollusks and crustaceans (particularly their dispersal stages), by reducing calcification rates and calcium metabolism (Jones et al., 2007). Nutrient enrichment that leads to eutrophication is another human-generated stressor in sandy beaches, driving to increasing occurrence of harmful algal blooms (Gianelli et al., 2019) and mass development of mats of drifting macro-algae (Quillien et al., 2015a), which, in turn, may affect beach macrofauna.

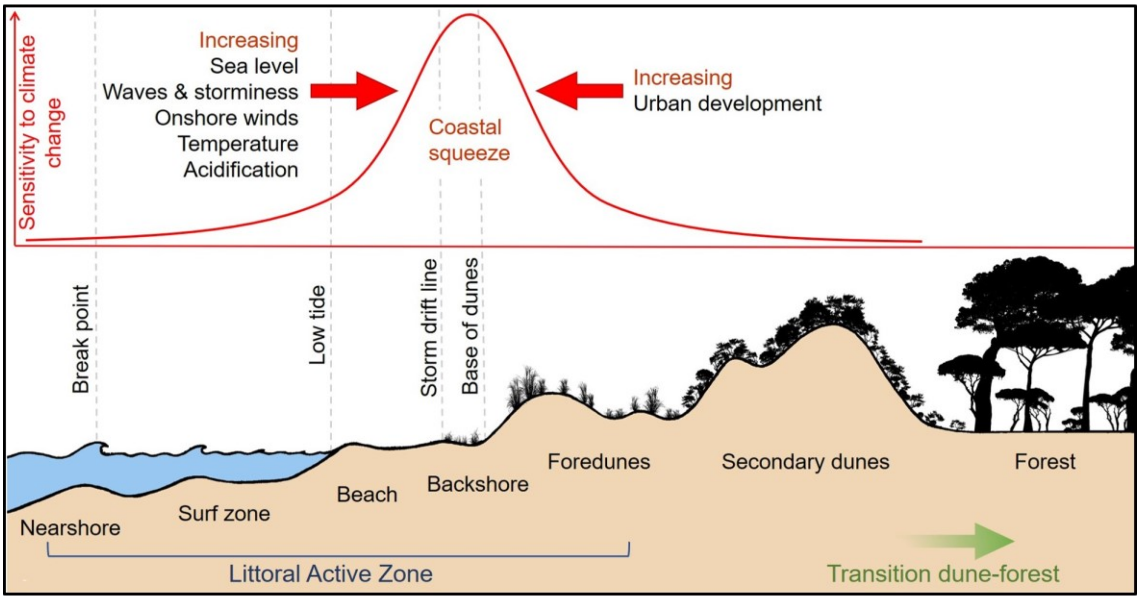

Figure 2. The Littoral Active Zone (LAZ) and its main components from the nearshore to the foredunes: the conceptual sensitivity curve suggests that the most sensitive areas to climate change drivers are the beach, the backshore and foredunes; expanding urban development on the dry (land) side in secondary dunes and the forest nearby interacts with climate change drivers mainly acting on the wet (sea) side to produce coastal squeeze. Modified from McLachlan and Defeo (2018).

4. Climate change impacts on behavioural adaptations

Given the above commentary, a conceptual framework regarding behavioural adaptation to sandy beaches under climate change should consider the following aspects: i) behavioural adaptation with relation to habitat availability/variability; ii) phenotypic plasticity, including behaviour, and its evolutionary role; and iii) species life-cycle traits (duration of life, dispersal, recruitment, developmental stages and intra-specific interactions).

It is expected that climate change will expose sandy beach macrofauna to significant alterations in their physical and biological environment, causing variations in behavioural expression. Under changing conditions, variability was observed in macrofaunal behaviour and increase in behavioural plasticity, *i.e.* rapid adaptive behaviour expressed in new contexts (Gherardi et al., 2012; Scapini, 2014). Comparative analyses of populations from different beaches showed an increase of behavioural variability and a decrease of genetic variation as a consequence of habitat loss (Scapini et al., 1995; Ketmaier et al., 2010). Behavioural plasticity may have different adaptive importance for dispersing or brooding species, with planktotrophic species showing less genetic diversity and higher phenotypic plasticity (Soares et al., 1999). The threat of global warming requires the ability to predict the differential effects of a changing environment on sandy beach species with dissimilar life cycles (Defeo and McLachlan, 2011).

Considering the importance of ambient energy variables (temperature and productivity) and local habitat conditions (beach slope and tides) as leading correlates of species richness and abundance (Defeo and McLachlan, 2013, Defeo et al., 2017) and behavioural responses as active reactions of individuals to environmental changes (Fanini and Scapini, 2008), a high priority should be given to the assessment of the effects of climate change on behavioural adaptations in beach macrofauna. Proximate drivers could impact the LAZ habitats and sandy beach macrofauna, including their behavioural adaptations and, consequently, fitness (Figures 2, 3).

Beach macrofauna interact with their microhabitat and respond to proximate drivers affecting the LAZ (Figure 3). The behaviour expressed by an individual may change during its life (T_0 to T_n), according to the external context (the microhabitat) and internal conditions. If individuals succeed in survival and reproduction, adaptations may eventually be passed on to the successive generations and upscaling to population level occurs. Referring to climate change impacts, caused by distal, anthropogenic drivers, the question then is to what extent macrofauna can (behaviourally, *i.e.* phenotypically) adapt to proximate drivers. Identifying constraints of behavioural plasticity is a critical issue to be explored. A direct impact of climate change drivers on behavioural adaptations has not yet been modeled. However, changes or disruptions of behavioural adaptations may represent indicators of impacts at the population level, which matters for evolution (Scapini et al., 2013; 2015; 2019). Here, some illustrative examples based on long-term observational data series, field and laboratory experiments (Table 1) are provided to explore the degree to which observations of the behaviour of beach macrofauna conform to expectations under climate change. The space-for-time substitution modeling approach is also used to infer temporal trends from spatial variation in population features and ecological processes across sites that vary in environmental conditions (Blois et al., 2013; Lester et al., 2014; Celentano and Defeo, 2016).

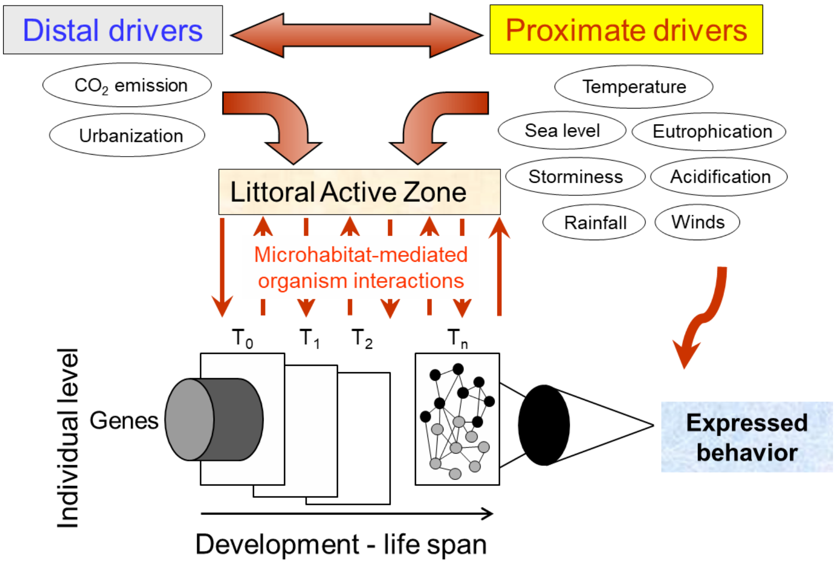

Figure 3. Climate change distal and proximate drivers affecting the Littoral Active Zone (LAZ) and microhabitat-mediated organism interactions influencing the expressed behaviour: distal drivers do not affect macrofaunal behaviour directly, but the LAZ where animals find their microhabitat; macrofauna are affected by proximate drivers, which in turn influence the LAZ. At any moment, in response to specific needs and cues, individuals express behaviours developed through interactions between genes and microhabitat features. Individual development follows the time horizon from T_0 to T_n, and interactions and expressed behaviours at time T_n are different from and depend on what happened in previous life times (T_1, T_2, ...), as visualized by the interaction network within the organism. Modified from Campan and Scapini (2002).

Table 1. Conceptual framework: emergent hypotheses for climate change impacts on behavioural adaptations in sandy beach macrofauna

Main proximate driver	**Potential effects on beaches**	**Implications for sandy beach macrofauna**	**Expected behavioural changes**	**Main predictions under global climate change**
Sea level	Sea level rise affects the narrow land-sea interface and increases erosion rates, modifying beach morphodynamics; sediment loss diminishes beach volumes and causes a retreat of the coastline.	Habitat loss and increase in temporal instability of the environment.	**Orientation** and **migration** become more variable; complex mechanisms (*e.g*. sun compass) are disrupted and substituted by simple taxes; **foraging** becomes opportunistic.	Increasing rates of local extirpations in supralittoral invertebrates with restricted distribution and dispersal.
Temperature	Augmenting temperature ranges between superficial and deep sediments changes sediment moisture properties in the upper littoral; sustained increase in sea surface temperature changes water and sand properties and modifies local hydrodynamic conditions.	Changes in macrofaunal thermal limits; increase in the energy cost of osmoregulation.	Disruption in **activity rhythms** expression; changes in the timing of **mating** and **recruitment**; changes in **burrowing** resulting from changes in oxygen availability and moisture; decrease in burrowing depth and increase in **competition** for burrows.	Reproduction and recruitment will be affected, lowering population fitness; range shifts and distributional changes; increased competition will decrease fitness.
Onshore winds and storminess	Warmer air and water increase storminess and frequency and intensity of onshore winds; more frequent and intense onshore winds together with rising sea levels augment swash width and strength, decrease beach width and accelerate beach erosion.	Larger wind waves cause stranding and mass mortalities of intertidal species, which are carried up the beach and are unable to return to the intertidal; habitat loss for supralittoral species with restricted distribution and dispersal.	**Escape** (avoidance behaviour) away from the stress source increases; changes in direction and timing of **orientation** and **migration** affect **zonation; competition behaviour** increases**.**	Increasing population extirpations due to habitat loss, particularly under coastal squeeze scenarios.

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Rainfall	Increasing freshwater flow into the marine environment decreases salinity; rise of groundwater levels increases swash run-up distances and beach erosion rates.	Decrease in salinity may alter osmoregulation and vital rates; rising groundwater and erosion may cause habitat loss for obligate beach species.	Tidal and seasonal **biorhythms** may change, due to unpredictability of synchronizing cues; changes in along- and across-shore **distribution and zonation**; disruption in **habitat recovery** and **orientation**; increase in **competition** between endemic species and invasive alien species from freshwater bodies.	Extirpations due to habitat loss for erosion/floods; recruitment failure and impaired reproduction activity; changes in community structure and function due to increasing representation of invasive species.
Acidification	Declining pH and carbonate saturation attributed to climate change are expected to alter water chemistry properties in the surf zone.	Reduced calcification rates and calcium metabolism in beach species with calcified structures in their anatomy.	Impaired **burrowing** in macrofauna with less robust shells; higher **substrate selectivity**; decreased **recruitment** for sensitive larval stages, whose motility and survival are impaired by acidification; affected **foraging** on wrack.	Increase in mortality rates and biodiversity loss; reproduction and recruitment affected in broadcast spawners with pelagic larvae; loss of fitness.
Eutrophication	Nutrient enrichment derived from climate change drivers combined with human activities increases eutrophication rates, driving to increasing frequency, intensity and periodicity of harmful algal blooms (red tides) and mass development of drifting macroalgae mats (green and golden tides), altering water and sediment properties.	Blooms of toxic algae may cause mass mortalities to suspension feeders, affecting the whole food web. Mass stranding macroalgae events decrease the diversity of intertidal benthic macrofauna, affecting some sub-surface deposit and suspension feeders.	Changes in **zonation** with species overlap; impaired specialized **foraging** and increasing representation of opportunistic species; reduced foraging rates in deposit and suspension feeders; disruption in **habitat selection** mechanisms.	Chronic changes in metabolism affecting growth and survival rates; extirpations and biodiversity loss over extended areas.

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4.1. Sea level

A projected rise in sea level will affect the narrow land-sea interface and increase erosion rates, thus modifying beach morphodynamics. Sediment loss will diminish beach volumes and cause a retreat of the coastline, which will greatly affect beach habitats, adding to the effects of coastal squeeze. Increasing human actions at the coast and shortcomings in management practices (*e.g.* nourishment, engineering structures such as groynes, revetments and breakwaters) also reduce sediment supply and therefore alter beach area, thus aggravating the scenario of concern given by sea level rise. Invertebrates with restricted distributions and dispersal inhabiting the upper zone of sandy beaches (*e.g.* crustaceans and insects) are extremely vulnerable to increasing habitat loss and fragmentation, as was documented in California and Italy on sandy beaches subjected to intense erosion and coastal squeeze (Hubbard et al., 2014; Nourisson et al., 2018; Scapini et al., 2018).

A comparison of beaches with different morphodynamic features along Mediterranean and eastern Atlantic sandy shores is used here to simulate future scenarios under climate change (Scapini et al., 2019). A strong dependence was shown of talitrid orientation on beach width and slope. On narrow and sloping beaches, higher behavioural variability was observed, indicating a disruption of the adaptation, which, under severe coastal squeeze and increasing occurrence of extreme events, will not prevent population extirpations and biodiversity loss, as was observed in pocket beaches and islands (De Matthaeis et al., 2000; Deidun, 2010). A reduced intertidal zone favors a shifting of foraging migrations of mobile macrofauna from the low shore to the vegetated sand dunes (Scapini, 1997; Colombini et al., 2013), a strategy that will not be possible with coastal squeeze. The destruction of the habitat or the reduction in organic supply from the sea will reduce adaptability of foraging behaviour (Laidre, 2013).

4.2. Temperature

A sustained increase in sea surface temperature (SST) may change water and sand properties and eventually modify local hydrodynamic conditions. Beach invertebrates are sensitive to changes in their thermal limits and, therefore, they are increasingly unable to acclimate to changing temperatures, particularly at the trailing range edge of their geographical distributions (Schoeman et al., 2014). Warming has a notable influence on life history traits and processes, including changes in individual growth, population structure and the extent of reproductive and recruitment periods (Marques et al., 2003). The intensity and directionality of these responses vary according to the phylogeographic origin of the species and the intrinsic characteristics of life history (McLachlan and Defeo, 2018).

Long-term trends were observed in demographic and biological traits of sandy beach species of cool-water origin (*e.g.* the yellow clam *Mesodesma mactroides* in the Southwestern Atlantic Ocean, SAO). These were correlated with the systematic increase in SST and consistent with expectations under climate change. These are: (1) occurrence of mass mortalities in concurrence with increasing SST, and (2) lower population abundance, rates of fecundity, recruitment and adult survival, clearly reducing fitness (Herrmann et al., 2011; Ortega et al., 2012, 2016). In contrast to the trends observed for this cool-water species, increasing abundance was found in species of SAO beaches that favor warmer conditions, such as the wedge clam *Donax hanleyanus* and the mole crab *Emerita brasiliensis* (Defeo, 2003; Herrmann et al., 2009). For the mole crab, a 20-year study on a Uruguayan beach showed that with increasing SST: (1) abundance and individual growth rates increased, and (2) reproductive and recruitment periods were more extended, recruitment increased and population structure was multi-modal (Celentano and Defeo, 2016). The advanced and extended breeding and recruitment seasons of *E. brasiliensis* denote a positive response of species with tropical affinities to increasing temperatures at the cold (leading) edge of its range, thus conforming to expected phenological responses to global warming (Schwartz, 2003; Parmesan, 2007; Poloczanska et al., 2013; Schoeman et al., 2014).

A main prediction of the adaptive behaviour to warming in sandy beach macrofauna may be an increase in the duration and phenology of reproductive and recruitment events in species with warm-water affinities at the leading edge of their distribution, whereas species with cold-water affinities will follow the reverse trend. These changes will be accompanied by others that also reflect a trend toward tropicalization, such as an increase in growth rates and decrease in individual size and life span. The narrow range endemic species would be at greatest risk, as their rates of range extension might be outpaced by changes in temperature, making these taxa particularly vulnerable to this proximate driver. A lack of thermal “safety net” at trailing edges leaves these species vulnerable to increasing temperatures, which could lead to mass mortalities and local extirpations and therefore contractions of their distribution ranges (McLachlan and Defeo, 2018).

Activity patterns of most species in temperate areas are related to habitat variability and seasonality, which may be impacted by temperature rise; as a consequence, beach populations may face a disruption of their annual rhythms and change the seasonality of rhythm expression (Nardi et al., 2003; Nasri-Ammar and Morgan, 2006; Rossano et al., 2008, 2018). Habitat selection, feeding, mating, recruitment and larval settlement are all linked to seasonality, synchronized by various *zeitgeber* (Naylor, 2010). In a scenario of tropicalization under global warming, some synchronizers may no longer be efficient, reducing fitness, with eventual biodiversity loss.

The predicted temperature rise may affect burrowing behaviour through changes in oxygen availability and water content within burrows. With increasing temperatures, the depth of burrows was observed to decrease and competition for burrows increase (Dugan et al., 2004; Sassa and Watabe, 2008: Gherardi et al., 2012). In intertidal mollusks, mean burrowing rates are expected to increase as a response to increasing temperatures (McLachlan and Defeo, 2018). In low-lying coastal areas, sea level rise could cause beach flooding, with consequent changes in groundwater level and suction-dynamics, affecting sediment compaction and, consequently, burrowing of supralittoral macrofauna (Sassa et al., 2014). The reduced fitness may cause species range shifts, distributional changes and biodiversity loss in sensitive species.

4.3. Onshore winds and storminess

Warmer air and water may increase storminess and frequency and intensity of onshore waves and winds, which in turn will impact the LAZ. More frequent and intense onshore winds, together with rising sea levels, will augment swash width and strength, decrease beach width and accelerate beach erosion rates, modifying beach morphodynamic features. The implications for the macrofauna may differ for intertidal species and supralittoral ones. Storm events in the surf can cause significant mortality in intertidal populations, which are stranded in the upper beach zones by larger waves, being unable to return to the intertidal (McLachlan et al., 1996). Supralittoral species with restricted distribution and dispersal are expected to experience habitat loss and mass mortalities with increasing storminess, particularly under coastal squeeze.

Supratidal macrofauna may anticipate extreme events by changing migration patterns, from the shoreline to the dune, where they may find a safe habitat (Scapini et al., 1992; Colombini et al., 2013). However, the loss of dune habitats may cause population mortality. Under abrupt decreases in atmospheric pressure, supratidal macrofauna was shown to anticipate storm events by changing orientation from seawards to landwards, towards a (predicted) safer zone (Scapini et al., 2002). Behavioural adaptations of marine animals appear fine-tuned to changes in pressure, yet these responses are still little studied in sandy beach macrofauna. In the intertidal zone, the motility of surfing gastropods for scavenging is driven by surf and surface currents and increasing storminess may disrupt this adaptation (Harris et al., 2017).

Habitat loss and population extirpations are the main predictions under a scenario of increasing storminess. Changes in zonation are also predicted, with increasing competition for resources, consequent decrease of fitness and population abundance and diversity.

4.4. Rainfall

Predicted rainfall increase may cause a decrease in salinity due to increased freshwater flow from land into the marine environment; at high latitudes, the melting of polar caps will add to this impact. Excessive precipitation and flooding will also raise groundwater levels, thereby increasing swash run-up distances and beach erosion rates. Sudden changes in salinity and salinity range may increase, particularly in transitional environments such as estuaries, therefore augmenting beach instability conditions (Lercari and Defeo, 1999, 2015; Colombini et al., 2006). The increase in rainfall and water transport by rivers interacts with sea level rise, impacting beach macrofauna communities. In estuarine beaches, salinity changes entrain tidal rhythms of activity, reproduction and recruitment (Naylor, 2010). Unpredictable salinity changes due to increased rainfall may disrupt the adaptation to tidal periodicity. While adult individuals will be able to counteract such changes through displacement or changes in the expression of activity rhythms, larvae settlement may fail under unsuitable salinity conditions, with negative consequences on recruitment. Habitat selection behaviour (substrate choice) in mobile beach species is part of the osmoregulation process, which can affect survival rates. Sandy beach crustaceans chose the most suitable salinity within their tolerance range, independently of the salinity of their home beach (Fanini et al., 2012; 2017). Avoidance reactions of abnormal salt concentration were observed in supratidal macrofauna after submersion in unsuitable salinity (freshwater or high salt concentration) (Scapini, 1979). Many mobile beach species do not adapt physiologically (osmoregulation) but behaviourally (substrate choice and orientation), which may be critical under a scenario of decreasing salinity.

A decrease in salinity through freshwater inflow and rising groundwater will cause habitat quality to deteriorate and may cause an increase in the relative representation of freshwater species, including invasive ones, eventually competing with resident species (Persson, 2001; Herkül et al., 2006; Fanini et al., 2017). Invasive intertidal beach-hoppers, clinging to wrack, have extended their distribution range in north-eastern Baltic beaches, where increased wrack mass has been transported by storms; the invasions by osmotically-tolerant alien species (*e.g. Platorchestia platensis*) or freshwater ones (*e.g. Cryptorchestia garbinii*) are aggravated by decreasing salinity caused by polar ice melting (Herkül et al., 2006). The Asian clam *Corbicula fluminea* has been increasingly documented in estuarine sandy beaches and a spread of this invasive species is predicted under climate change (Lercari and Defeo, 2015; Reyna et al., 2018). Under a scenario of increasing freshwater run-off and polar cap melting, it is predicted that invasive species will expand their distribution range from freshwater bodies to estuarine sandy beaches. Competition for resources with endemic species is expected, decreasing endemic population fitness and affecting community composition.

4.5. Acidification

Declining pH and carbonate saturation attributed to climate change are expected to alter water chemistry properties in the surf zone. Ocean acidification might affect beach species with calcified structures in their anatomy (especially mollusks and crustaceans), reducing calcification rates and calcium metabolism. However, very large quantities of biogenic carbonate already present in beach sediments constitute a buffer system flushing through the beach system, which may counteract ocean acidification effects on beach species (Schoeman et al., 2014).

Observational and experimental evidence from other marine ecosystems (there are no examples for sandy beaches) showed that the reproductive behaviour, breeding success and survival of offspring will be less affected by acidic conditions in species with parental care (brooders and direct developers) than in broadcast spawners with pelagic larval development (Byrne, 2011, Lucey et al., 2015). Thus, it is predicted that behavioural adaptations related to reproduction will be differentially affected by ocean acidification in sandy beach macrofauna as follows (in increasing order): supralittoral peracarids < intertidal peracarids < intertidal mollusks and crustaceans with parental care (brooders) < broadcast spawners with external fertilization and planktonic larvae. Differential changes in behaviour related to avoidance of stressful conditions and habitat unsuitability are also expected, particularly during larval settlement, as this phase is strongly dependent on innate behavioural and sensory adaptations (Figure 1). Species with pelagic larval stages may also be directly affected by large scale, climate-driven changes in prevailing oceanographic systems, particularly affecting migration behaviour.

Many species showed a decrease in settlement under elevated $p\mathrm{CO}_2$ and reduced pH, which cause reductions in the larval sensory capacity and alter settlement substrates (Stanley et al., 2012; Espinel-Velasco et al., 2018). Reduced settlement and recruitment and increasing mortality rates are expected for species with a non-compensatory capacity (*i.e.* visual cues instead of chemical ones). Altered sensory capacity may also affect homing in sandy beach decapods, which would be more susceptible to predation and less efficient in competition for mate and space (Vannini and Cannicci, 1995; Gherardi et al., 2012).

Foraging may be altered by changes in pH. In the south-eastern Pacific littoral, *Orchestoidea tuberculata* forages on the brown algae *Durvillaea antarctica*, whose nutritive characteristics are modified by changes in CO_2 levels in seawater. Feeding preference was observed on seaweeds exposed to lower levels of CO_2, also suggesting a lower palatability of seaweed exposed to elevated CO_2 levels (Duarte et al., 2016). It is predicted that algal palatability would be affected by ocean acidification, forcing algal-consumers to display compensatory feeding (higher consumption of lower quality food), therefore increasing the feeding energetic cost. Changes in feeding behaviour and/or eventual colonization of new beaches are expected to occur under the prolonged absence of good nutritional items. Therefore, food preference, consumption and absorption efficiency may be affected by ocean acidification, causing a deterioration of animal body conditions, as an estimate of foraging success, ultimately affecting fitness.

4.6. Eutrophication

Red tides, also known as harmful algal blooms (HAB), have seriously affected the health of sandy beach ecosystems and regional economies, especially as their frequency, magnitude and duration are increasing. Nutrient enrichment derived from human activities, acting together with climate change drivers (including increasing SST and onshore winds), may be main factors of the worldwide increase in frequency, intensity and periodicity of red tides (Hoagland and Scatasta, 2006, Dyson and Huppert, 2010, Rodríguez et al., 2011, Anderson et al., 2012). Several suspension feeders, including clams and mole crabs, have been increasingly affected by red tides, which can cause mass mortalities or render these suspension feeders not fit for human consumption (McLachlan et al., 1996, Defeo, 2003, Gianelli et al., 2019), thereby affecting the whole food web (Lercari et al., 2018). Large-scale changes in the composition of phytoplankton community have been documented in the surf zone of sandy beaches together with HAB, including also an increasing representation of species with warm-water affinities (Martínez et al., 2017). Therefore, it is expected that the quality and quantity of food, and thus the foraging behaviour, will be affected in sandy beach suspension feeders.

In bivalves, valve activity is affected by food concentration and quality. With non-toxic food, clams maintain open valves to provide continuous ventilation and inflow through the gills and mantle cavity. However, valve closure was documented under the occurrence of toxic dinoflagellates, which may be a behavioural mechanism directed to control water inflow through the body cavity and avoid toxic cells (Basti et al., 2009). It is predicted that the increasing occurrence and magnitude of HAB will increase valve adduction activities, thus reducing the amount and quality of food and oxygen uptake, affecting body condition and fitness.

Mass occurrences of drifting macroalgae mats (green and golden tides) have been increasingly documented in sandy beaches, altering water and sediment properties (Smetacek and Zingone, 2013). Eutrophication is responsible for the increasing frequency and magnitude of green and golden tides worldwide (Charlier et al., 2008, Ye et al., 2011). Other proximate drivers related to global change (temperature, acidification), acting simultaneously, may accelerate this process (Xu et al., 2017). The production of toxic hydrogen sulphide (H_2S) from decomposition under anoxic conditions affects the physico-chemical features of the habitat and the biota. Therefore, these events may alter habitat quality and availability for the resident macrofauna and affect the beach food-web. Recent studies documented species-specific responses to green tides along the coastline of Brittany, France: herbivorous marine invertebrates and some suspension feeders benefited from the presence of *Ulva* mats, whereas large sub-surface deposit feeders and bivalve drifters, which surf up and down the shore with the tides were negatively affected (Quillien et al., 2015a). The overall diversity of intertidal benthic macrofauna decreased in the presence of green tides (Quillien et al., 2015b), but little effect was detected on subtidal communities or flatfish (Quillien et al., 2018).

It is predicted that intertidal forms will be more affected than subtidal and supralittoral ones by eutrophication because toxic compounds may accumulate in the sediment of the intertidal zone. Changes in community composition are expected under climate change driven eutrophication, with increasing occurrence of mobile supralittoral herbivorous species, which may increase their habitat and food quality and availability through adaptive changes of behaviour (habitat selection, migrations, food selection) and/or exploitation of the dune habitats (Colombini et al., 2013). By contrast, the linear and narrow habitat of intertidal species will be even more restricted and could cause local extirpations.

5. *Discussion and conclusions*

Population size or growth rate against a background of climate change is an integrated outcome of complex interactions among fundamental biological, ecological and evolutionary traits and processes, which together comprise adaptive capacity (Dawson et al., 2011). The relevance of behavioural adaptations to changing sandy beaches has been considered mainly as a response capacity in the framework of macrofauna adaptability to changing local conditions of the habitat. Our literature review has shown the widespread behavioural responses in sandy beach macrofauna to environmental features, which were valuable to postulate specific hypotheses on potential macrofaunal adaptations to a changing climate. Biota adaptations to climate change pressure include three interlinked processes: (1) plasticity, (2) dispersal and (3) evolution (O'Connor et al., 2012). Behavioural plasticity (1) assumes a major role for survival of sandy beach macrofauna in new environments during processes (2) and (3). The evolution of new adaptations requires time to occur, but behavioural responses will allow the survival of individuals in a new habitat. Behavioural adaptations contribute to fitness and, through evolutionary time, may become more and more adapted to specific microhabitat features. Under climate change, it is predicted that strictly adapted endemic sandy beach fauna will be especially subjected to local extirpations, while species with a large reaction norm (*i.e.* euryoecious species with phenotypic - physiological and behavioural - plasticity) may face changes by dispersal and exploitation of new niches. Nevertheless, there may be species that facilitate the survival and resilience of other (more sensitive) species, even under future climate conditions (Bulleri et al., 2018). Thus, biological interactions, including behavioural ones related to interspecific and intraspecific competition, prey-predator relationships, reproduction and recruitment, may play an important role in structuring sandy beach macrofauna communities under climate change. Beach environments are inter-connected (*e.g.* by coastal continuity, longshore currents, passive transportation of organisms by stranded material or boats), and therefore the colonization of novel habitats by sandy beach macrofauna is likely, particularly, but not exclusively, for those species with pelagic larval dispersal (Bishop et al., 2017).

Our literature review regarding behavioural adaptations of macrofauna to changing environments has revealed the loss of behavioural specificity and complexity under impacts on the microhabitat (Table 1S, Supplementary Material). Under climate change, the predicted outcome will be a decrease of fitness that could eventually lead to population extirpations and biodiversity loss (Table 1; Elliott et al., 2015). In this context, rigid adaptations to a specific microhabitat may result in a constraint and behavioural plasticity may be a better strategy for population survival under climate change. An increase in behavioural variation is predicted, which may represent an early warning signal. However, increasing pressure of climate change drivers interacting with human actions on sandy beaches could lead to impaired or disrupted behavioural adaptations, threatening fitness, population survival and eventually ecosystem functioning. Sandy beach ecosystems will always exist as boundaries between sea and land, but the specificity of these ecotones, characterized by fine-tuned macrofaunal adaptations, will likely be lost under climate change pressure. The limits to which behaviour can adapt to environmental changes are worthy of exploration, in view of modelling scenarios under climate change (Scapini et al., 2019).

Comparative analyses of behavioural adaptations in macrofauna populations with a wide geographic range (Table 1S, Supplementary Material) are suitable to highlight the adaptation potential under climate change impacts, also considering the large distribution range of most sandy-beach macrofauna species. On the other hand, common-garden experiments, while recommended to test specific hypotheses on the effects of microhabitat change, require time, depending on the life cycle of species (*e.g.* decades for plant populations, Germino et al., 2019). The same constraint exists for long-time series observations, which must reflect accelerating changes under climate change pressure and anthropogenic impacts (McLachlan and Defeo, 2018). Population substitution by invasive alien species, already notable and occurring in large areas in marine environments (Cardeccia et al., 2018), are also predicted for sandy beaches.

Climate change is a global process (Elliott et al., 2015), in which the sources of impact are multiple and interacting with many effects (Figure 2, Table 1), posing novel questions on the potential adaptation of sandy beach macrofauna and its narrow habitat. The predicted scenarios under climate change may thus be complex. Degrading environmental features will impact on ecological fitness and a loss of biodiversity will be the outcome of the negative pressures originated by climate change. If sandy beaches and the ecosystem services and societal goods and benefits they provide are to be conserved, detailed insights on macrofaunal adaptations (including behaviour) and the consequences of their predicted disruptions by climate driven stressors, are needed to provide mechanistic explanations of changes across geographic areas. The limits to which sandy beach macrofauna responds and can behaviourally adapt to environmental change require further exploration, in view of the increasing influence of the long-lasting climate driven stressors threatening these ecosystems at risk.

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7. Supplementary material

Table 1S. Overview of common behavioural adaptations studied in sand beach macrofauna, stressing their dependence on environmental and biological driving forces.

Behavioural adaptation	Physico-chemical driving forces	Biological driving forces	Geographical extension of observations	Relevant references
Activity rhythms	Tides; night/day; seasons; salinity; rainfall; air temperature & humidity; pressure	Food; mate; predation; cannibalism; competition	Chile, France, Great Britain, Italy, Spain, Tunisia	Mezzetti et al., 1994; Kennedy et al., 2000; Jaramillo et al., 2000; Nardi et al. 2003; Ugolini, 2003; Rossano et al., 2008, 2018; Lastra et al., 2010; Naylor, 2010; Colombini et al., 2013; Ayari et al., 2015; Nasri et al, 2017
Habitat selection	Substrate; temperature; moisture; salinity; granulometry; sediment organic content	Food; predation; competition	Australia, Great Britain, Italy, Poland, Tunisia, Uruguay	Williams, 1995; Colombini et al., 2002; Fanini et al., 2012; Fanini and Lowry, 2014; Fanini et al., 2017

Zonation & zonal recovery	Substrate; air temperature; moisture; salinity; granulometry; organic content; tides; day-time; season; storms	Food; mate; predation; competition	Brazil, Chile, France, Great Britain, Italy, Portugal, South Africa, Spain, Tunisia, Uruguay	Scapini et al., 1992; Williams, 1995; Brown and Odendaal, 1994; Colombini et al., 1994; Fallaci et al., 1996, 1999; Colombini et al., 2002, Jaramillo et al., 2003; Defeo and McLachlan, 2005; Rodil et al., 2006; Lastra et al., 2010; Colombini et al., 2013; Nourisson et al., 2014; Harris et al., 2017
Orientation	Air & substrate temperature & moisture; tides; storms; day-time; season	Food; predation	Brazil, Chile, France, Great Britain, Greece, Italy, Kenya, Morocco, Poland, Portugal, South Africa, Tunisia, Uruguay	Ugolini et al., 1995; Scapini et al., 2002; Ugolini et al., 2003; Meschini et al., 2008; Scapini and Dugan, 2008; Fanini et al., 2009; Colombini et al., 2013; Scapini, 2014; Nourisson and Scapini, 2015; Ugolini and Ciofini, 2016; Bessa et al., 2017; Ciofini and Ugolini, 2018; Scapini et al., 2019
Migration	Tides; storms; night/day; seasons	Mate; food; brood care	Ecuador, Ireland, Italy, Kenya, Tunisia	Davidson et al., 2004; Vanagt et al., 2008
Homing	Substrate; air temperature; moisture; day-time; tides; landscape	Food; predation; mate; brood care; competition	Italy, Japan, Kenya, Tunisia, USA,	Vannini and Cannicci, 1995

Burrowing	Substrate gradients; granulometry; moisture; tides; storms; night/day; seasons	Food; mate; predation; brood care; competition	Australia, Brazil, Canada, Great Britain, Indonesia, Japan, Kenya, Portugal, South Africa, USA	Wolfrath, 1992; McGaw, 2005; Lucrezi and Schlacher, 2010; Sassa and Watabe, 2008, 2011, 2014; Wardiatno et al., 2016; McLachlan and Defeo, 2018
Foraging	Salinity; pCO_2; moisture; organic content; tides; night/day; seasons	Food; competition; predation; cannibalism	Chile, Italy, South Africa, Spain, Sri Lanka, Tunisia, Uruguay, USA	Pennings et al., 2000; Duarte et al., 2010; Colombini et al., 2013; Lastra et al., 2015; Lagar et al., 2016; Cannicci et al., 2018; McLachlan and Defeo, 2018
Reproduction & recruitment	Habitat gradients; refuge; tides; night/day; seasons	Mate; brood; competition; predation	Brazil, Great Britain, Italy, New Zeeland, Portugal, Tunisia, Uruguay	Williams, 1978; Marques et al., 2003; Defeo and McLachlan, 2005; Stanley et al., 2012
Gregariousness & competition	Habitat extension	Population abundance and density, resource availability	Australia, Italy, Kenya, Sri Lanka, USA	Gherardi et al., 2012; Aquiloni and Tricarico 2015; Cannicci et al., 2018

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Chapter 3 – Arthropodofauna richness and abundance across beach-dune systems with contrasting morphodynamics

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Abstract

The across-shore distribution of arthropods in two Uruguayan sandy beach-dune systems with contrasting morphodynamics was investigated. A deconstructive analysis was performed to describe faunal changes from the dunes to the shoreline. The Habitat Safety Hypothesis (HSH), which states that sandy beach supralittoral species are more abundant in the backshore of reflective beaches than in dissipative ones, was assessed. A hypothesis that predicts more diverse and abundant supralittoral and dune insects and arachnids in dissipative beaches was also tested. Sampling was performed simultaneously in both beaches with pitfall traps that were kept active for 24 h at three transects that included 17 sampling levels. The reflective beach presented significantly higher elevation, sand temperature, grain size and sorting, and lower sediment compaction and moisture than the dissipative one. Total abundance of arthropods was significantly higher in the reflective beach, supporting the HSH. However, the deconstructive analysis revealed different patterns in the across-shore distribution, diversity and abundance among taxa. Crustaceans, coleopterans and dipterans exhibited higher abundance in the backshore of the reflective beach, whereas acarines were more abundant in the dunes of the dissipative beach. Ants were similarly abundant in the dunes of both beach types. Species diversity did not differ between beaches and was higher in the dunes than in the backshore. The higher abundance observed in the backshore of the reflective beach supports the HSH, reinforcing the idea that the backshore of reflective beaches can be a safer zone not only for beach crustaceans inhabiting this beach fringe, but also for some insects (coleopterans and dipterans). Other arthropods (acarines)

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inhabiting the dunes were more abundant in the dissipative beach. The similar abundance of ants found in both beaches suggests that dunes offer suitable microhabitats for them, overriding the effects of beach morphodynamic factors.

Key words
Species richness; Abundance; Arthropod diversity; Habitat safety hypothesis; Beach-dune system; Deconstructive analysis

1. *Introduction*

Sandy beaches dominate temperate and tropical coasts, representing about three-quarters of ice-free coastlines (Bascom,1980). The physical environment of these ecosystems is modulated by the interaction between tides, waves and sand, creating a range of beach morphodynamic types from narrow and steep microtidal reflective beaches to wide and flat macrotidal dissipative systems (Short, 1999; McLachlan et al., 2018). Reflective beaches are characterized by a smaller surf zone, coarser sand, steeper slope, shorter wave period and harsher swash climate than dissipative ones (Short, 1996; Short and Jackson, 2013).

Polychaetes, molluscs and crustaceans are representative taxa in the sublittoral and intertidal zones, whereas insects and crustaceans dominate the supralittoral backshore and dunes. are the most abundant terrestrial invertebrate group inhabiting sandy beaches (Dexter, 1983; McLachlan and Defeo, 2018). According to the Habitat Safety Hypothesis (HSH), supralittoral species have higher growth, reproduction and survival rates in microtidal reflective beaches than in dissipative ones (Defeo and Gómez, 2005; Defeo and McLachlan, 2011). It is postulated that reflective beaches provide a more stable and safer backshore environment than dissipative ones, by reflecting the wave energy from the beach face back to the sea, so decreasing the risk of submersion for supralittoral species (Defeo and McLachlan, 2011). In agreement with HSH predictions, supralittoral crustaceans showed higher abundance and species richness in the backshore of reflective beaches than in dissipative ones (Defeo and Gómez, 2005; Defeo and McLachlan, 2011; Barboza et al., 2012; Petracco et al., 2013).

Sandy beaches are closely coupled to adjacent surf zones and foredunes and, together, these systems form the core of the Littoral Active Zone (LAZ), which is characterized by wind and wave-driven sand transport (McLachlan and Defeo, 2018). In this context, most

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studies on sandy beach ecology focused on supralittoral and intertidal communities, whereas few studies were made on the terrestrial component of the LAZ (McLachlan and Defeo, 2018), namely the backshore and dunes (Giménez and Yannicelli, 2000; Carpaneto and Fattorini, 2001; Colombini et al., 2002a, 2003; Costa et al., 2006; Fattorini et al., 2012; Mourglia et al., 2015). Considering only sub-compartments of the LAZ separately could lead to a distorted perspective in across-shore biodiversity trends, with negative implications when delineating management programs (Fanini et al., 2020). Therefore, comparative studies between beaches with different environmental characteristics could add information on community and population abundance and distribution across dunes and beach zones, by taking into consideration species with different life histories and adaptations (Colombini et al., 2002b, 2003, 2009, 2013; Defeo and McLachlan, 2011).

The meta-analyses performed by Defeo and McLachlan (2011) and Barboza et al. (2012) revealed that beach insects showed a response opposite to that of crustaceans. These authors argued that dissipative beaches and their well-developed dunes could be more favourable and safer environments for insects and arachnids than reflective beach-dune systems. However, since insect distribution extends landwards rather than seawards, unlike other marine and semiterrestrial taxa inhabiting sandy beaches, the trend observed could be part of an incomplete picture, since the whole transects from shoreline up the dunes were not sampled. Insects, often overlooked in accounts of sandy beaches, occur on the backshore and dunes under all conditions and may sometimes even constitute the most abundant group present (McLachlan and Defeo, 2018). Thus, detailed across-shore sampling and further deconstructive analyses on crustaceans and insects are required, separating beach arthropods from migrant ones and flying insects from wingless arthropods.

This study analysed the across-shore variation in species richness and abundance of arthropod communities in beach-dune systems of the LAZ, in two microtidal beaches with contrasting morphodynamics (reflective and dissipative). The structure and distribution of arthropod communities were deconstructed to better understand taxon-specific, across-shore distributional patterns, depending on their ecological and morphodynamic peculiarities. It was predicted that: (1) the reflective beach hosted more abundant and more diverse crustaceans in the zone from waterline to the base of the dunes; (2) the dissipative beach hosted more diverse and abundant insects and arachnids, particularly in the dunes, from the base of the dunes towards the hinterland.

2. Materials and methods

2.1. Study sites

The study was carried out simultaneously in two microtidal (tidal range of about 0.5 m) exposed oceanic beaches of the Uruguayan coast, spaced 30 km from each other: Barra del Chuy (33° 49′ S, 53° 27′ W) and La Viuda (34° 03′ S, 53° 32′ W). Barra del Chuy beach has a gentle slope, fine to very fine sand, low sediment organic matter content, medium-high sediment compaction and high sediment water content; La Viuda beach has steep slope, medium sand, low sediment compaction and moisture (Celentano and Defeo, 2006). In addition, Barra del Chuy has a large swash zone and a well-developed surf zone with bars (longshore-bar-trough type; Short, 1996), whereas La Viuda has a narrower swash and surf zones (Celentano and Defeo, 2006). Both beaches have developed and vegetated dune systems and do not present major urban impacts, being also far beyond the area of influence of the estuarine discharge caused by Río de la Plata (Lercari and Defeo, 2006). Hereafter, the two beaches are referred to as “dissipative” (Barra del Chuy) and “reflective” (La Viuda). Both beaches were subdivided into two distinct zones: (1) the backshore, extending from the waterline to the base of the dunes; and (2) the dunes, from pioneer plants to established vegetation (Martínez and Psuty, 2004; Pranzini, 2004; Fanini et al., 2009a).

2.2. Sampling design

A 24-hour sampling of surface-active arthropods was carried out simultaneously on both beaches, by two trained teams on April 11th and 12th 2016 (starting at 2:00 p.m. on April 11th), to capture both night and day active species (Fanini et al., 2009b; Nourisson et al., 2018). Arthropods were collected with pitfall traps consisting of plastic cups (12 cm diameter and 12 cm depth), inserted into the sand up to their upper edge and filled with 150 ml of a 50% propylene glycol solution (Aristophanous, 2010), to prevent specimens from escaping. In each beach, three transects were set perpendicular to the shoreline, spaced 8 m apart. In each transect, one pitfall trap was placed every 4 m from the shoreline to 40 m inland, considering as point 0 the base of the dunes, positive values towards the sea and negative values landwards (see Colombini et al., 2003, for a similar protocol over a range of beach-dune systems). Traps were placed from station −40 m to +24 m, which was the upper swash level in both beaches at the sampling time, totalling 51 traps set in each beach, subdivided into 17 sampling levels (10 in the dunes and 7 in the backshore). After 24 h, the trap content was transferred into plastic bags for laboratory analyses.

Near each pitfall trap, the following environmental variables were measured: beach slope (Emery, 1961), which was transformed into elevation (cm); sediment temperature (°C, digital thermometer for soil) and compaction (kg · cm^{-2}; penetrometer HUMBOLDT). Surface measurements were collected simultaneously by the two teams in each beach. Sediment samples were taken (from surface to 10 cm depth, one sample for each sampling level in one transect) to estimate the granulometric parameters (Folk and Ward, 1957), sediment water content and organic matter content.

2.3. Laboratory analyses

In the laboratory, arthropods were sorted, counted and identified at the genus or species levels. When the identification at species level was not possible, recognizable taxonomic units were used, based on morphological differences (morphospecies), which were shown to be useful for the determination of diversity (Oliver and Beattie, 1993). For granulometric parameters, sediment samples of about 50 g were sieved with progressively smaller mesh size, according to the Wentworth scale (1922). Ten mesh sizes ranging from 1 mm to 0.063 mm were used, and the sediment retained in each sieve was weighed (precision = 0.01 g) and used to estimate the mean particle size, sorting and skewness (GRADISTAT v.6.0 software; Blott and Pye, 2001). A fraction of the sediment samples (about 10 g) was oven-dried at 80°C for 24h and weighed before and after drying to estimate sediment water content (% of the fresh weight). Samples were incinerated at 460°C for 4h and weighed again to determine organic matter content (% of the dry weight).

2.4. Data analyses

Linear or nonlinear approaches were applied to model variations in (1) physical characteristics, (2) species richness and (3) abundance as a function of the distance from the base of the dunes, for both beaches separately. Mean estimates for the three transects at each distance were used for temperature, compaction and elevation, while data from one transect only were used for modelling grain size, sediment organic matter content and moisture. Abundance estimates from the three transects were averaged by sampling level, whereas the total number of species was obtained by summing up the number of species observed in each of the three transects of each beach. The models that best explained the relationships were selected according to the coefficient of determination (R^2) and statistical significance. Between-beach differences in abiotic variables, species richness and abundance were analysed using one-way ANOVA, with beach as main factor, using R Software Package. Levene and Brown-Forsythe tests were performed to verify the assumption of variance homogeneity (Underwood, 1997).

Multivariate analyses were carried out with PRIMER v.6 software (Clarke and Gorley, 2006), using log-transformed and normalized environmental data and a fourth-root transformation for biological data, to reduce the influence of variables with high values. Cluster analyses and non-metric Multi-Dimensional Scaling (MDS) based on the Euclidean distance matrix for environmental variables and Bray-Curtis similarity matrix for abundance were used to create a two-dimension ordination of samples for both beaches.

A deconstructive analysis was carried out using two different grouping strategies, considering: (1) the six most abundant taxa (Crustacea, Insecta, Formicidae, Diptera, Coleoptera and Acarina); and (2) species/morphospecies identified as dominant species by SIMPER procedure (PRIMER v. 6 software; Clarke and Gorley, 2006). A fourth-root transformation was applied to abundance estimates for each beach level. The percentage contribution of each species to the similarity and dissimilarity between beaches was also estimated using SIMPER. The minimum percentage contribution was set to 10% for typifying species and 4.5% for discriminating species, to focus on those species that mainly contribute to similarity and dissimilarity, respectively.

Generalized linear models (GLMs, McCullagh and Nelder, 1989) were used to analyse across-shore variations in species richness and abundance in relation to physical variables, using non-transformed data and considering the three transects separately as replicates. Models were fitted using negative binomial (abundance) and Poisson (species richness) error distributions, both with a log-link function, which provided the best fit. First, two models with all environmental variables were fitted for both species richness and abundance: model 1, an additive model, and model 2, a model with the interaction with the fixed factor ‘‘beach’’. Both models were compared to test the significance of the interaction, *i.e.* the difference between beaches. The two models (additive and with interaction) were compared using a Chi-square test, obtaining the Likelihood Ratio test for Negative Binomial Models for abundance, and the Analysis of Deviance for species richness. Then, Akaike’s Information Criterion (AIC) was used to compare and select the best models with the interaction with the factor ‘‘beach’’, to verify the influence of each environmental variables on species richness and abundance in relation to beach type. To avoid the possibility of arbitrary decisions in selecting a single best model, multimodel inference approach and model averaging were applied (Burnham and Anderson, 2002). Corrected Akaike information criterion (AICc), log likelihood scores and AICc weights (AICcw) were estimated. Models were ranked by AICc values and those with a difference between AICc values (ΔAICc)>4 were excluded from the analyses. Lastly, additive models were fitted separately for each beach to verify which environmental variables could influence species richness and abundance. The analysis was conducted in R Software Package (R Core Team, 2017).

3. Results

3.1. Physical environment, species richness and abundance

Table 1 summarizes the physical characteristics of the two beaches recorded at sampling time. The dissipative beach had significantly lower temperature, grain size, sorting, organic matter content and elevation, and higher sand compaction and sand moisture (water content) than the reflective beach (ANOVA, $p<0.01$).

Temperature (Fig. 1a) decreased from the dunes to the shoreline in both beaches. Grain size (Fig. 1b) decreased linearly towards the sea in the dissipative beach, whereas no clear trends were found for the reflective beach. Across-shore variations in elevation (Fig. 1c) were explained by quadratic models for both beaches, tending to decrease seawards. Spatial variation in sediment compaction across the dissipative beach was explained by a quadratic model with increasing values towards the sea, while no significant trends were found for the reflective beach (Fig. 1d). Sediment water content increased towards the shoreline in both beaches, with a quadratic model explaining the variation across the dissipative beach (Fig. 1e).

Table 1. Characterization of physical variables (mean ± SE) of the dissipative (Barra Del Chuy) and reflective (La Viuda) beaches (Uruguay). ANOVA: ***$p<0.001$, **$p<0.01$

	Dissipative	**Reflective**	***p***
Sediment temperature (°C)	19.79 ± 0.04	20.73 ± 0.10	***
Sediment compaction (kg·cm^{-2})	4.21 ± 0.10	3.36 ± 0.06	***
Grain size (µm)	180.28 ± 1.32	294.84 ± 11.57	***
Sorting (µm)	1.28 ± 0.01	1.45 ± 0.02	***
Organic matter content (%)	0.22 ± 0.01	0.30 ± 0.03	**
Water content (%)	8.01 ± 1.44	3.13 ± 0.35	**
Elevation (cm)	102.97 ± 18.51	319.29 ± 57.10	**

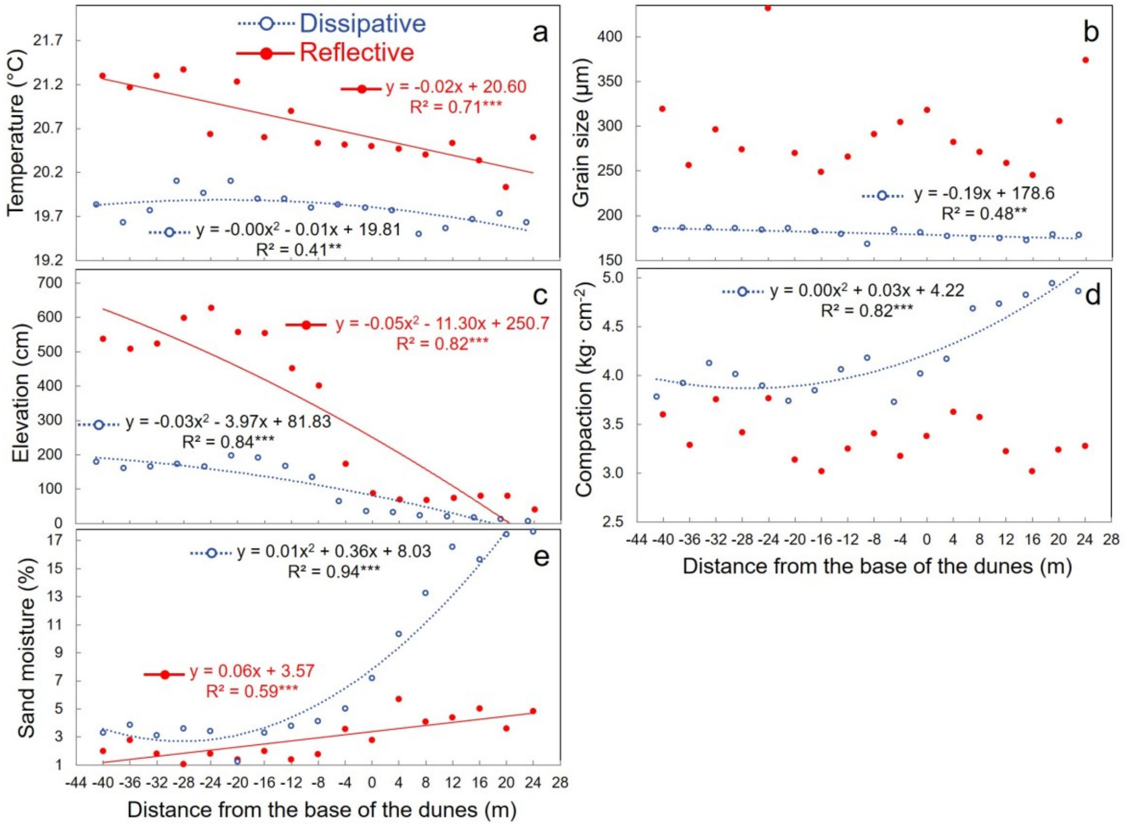

Figure 2. Across-shore variations in sediment variables in the dissipative Barra del Chuy (○) and reflective La Viuda (●) beaches: (a) temperature; (b) grain size; (c) beach elevation; (d) sand compaction; and (e) sand moisture (water content). The best models fitted between mean values per sampling level and the distance from the base of the dunes are shown: ****p*<0.001, ***p*<0.01 and **p*<0.05. The zero value in the X-axis indicates the base of the dunes, negative values indicate the landward direction and positive ones the downshore direction.

The MDS of environmental variables showed two distinct groups that clustered the samples of each beach (Fig. 2). The very low stress value (0.06) indicates a robust 2D representation of two groups within the Euclidean distance value of 4 (Figs. 2 and S1). Within both groups, it was possible to distinguish a further separation between the dunes and the backshore (Euclidean distance=2.5), except for the values at −4 m in the dunes of the reflective beach, which joined those of the backshore fringe (Figs. 2 and S1).

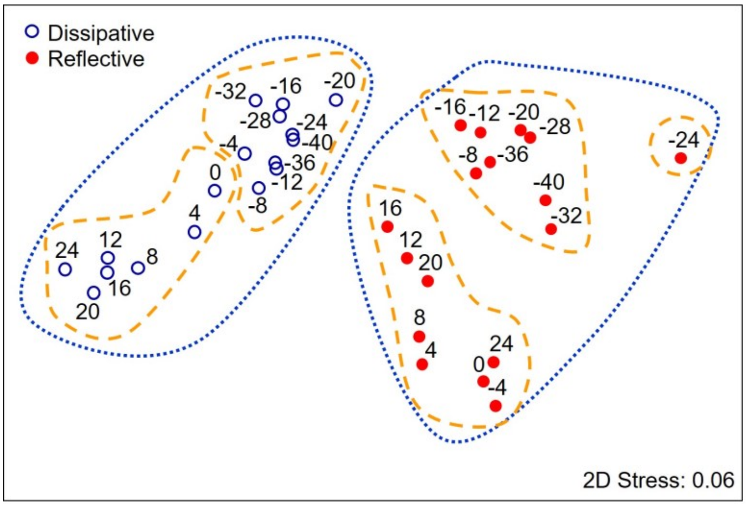

Figure 3. Non-metric Multi-Dimensional Scaling (MDS) of environmental data. The point numbers indicate the distance (m) from the base of the dunes to the shoreline (positive values) and landwards (negative values). The dotted and dashed lines show an Euclidean distance of 4 and 2.5, respectively. ○: dissipative beach (Barra del Chuy); ●: reflective beach (La Viuda).

A total of 1984 individuals (reflective beach: N=1368; dissipative beach: N=616) belonging to the three major taxa of arthropods (crustaceans, insects and arachnids) were collected, totalling 62 morphospecies (reflective beach: S=39; dissipative beach: S=44; Table S1). The most abundant taxa were crustaceans (62.1%), including amphipods, isopods and decapods, with a lower percentage in the dissipative beach (12.6%) than in the reflective one (49.6%). Other representative taxa (≥4.5%) were hymenopterans, dipterans, acarines and coleopterans, followed by other arthropods with relative abundance lower than 1% (Table S1). Regarding species richness, coleopterans had the highest percentages, followed by hymenopterans, dipterans and crustaceans (>8%). Acarines presented the lowest species richness, with only one morphospecies. MDS ordering did not show any clear grouping between beaches, but highlighted a strong demarcation between the dunes and the backshore at a 30% similarity level (Figs. S2a and S2b). Across-shore variations in species richness were significantly ($p<0.05$) explained by a quadratic model in both beaches (Fig. 3a). The highest values were found close to the dunes, decreasing towards the shoreline and, to a lesser extent, landwards. Species richness did not differ between beaches. Abundance (Fig. 3b) followed a quadratic model ($R^2=0.84$, $p<0.001$) in the reflective beach, with a strong increase in the backshore and lower values in the dunes. No significant pattern was found for the dissipative beach, characterized by low arthropod abundance across the whole beach-dune system (Fig. 3b). Abundance resulted significantly higher in the reflective beach than in the dissipative one (ANOVA, *p*<0.05; one outlier removed, see Fig. 3b).

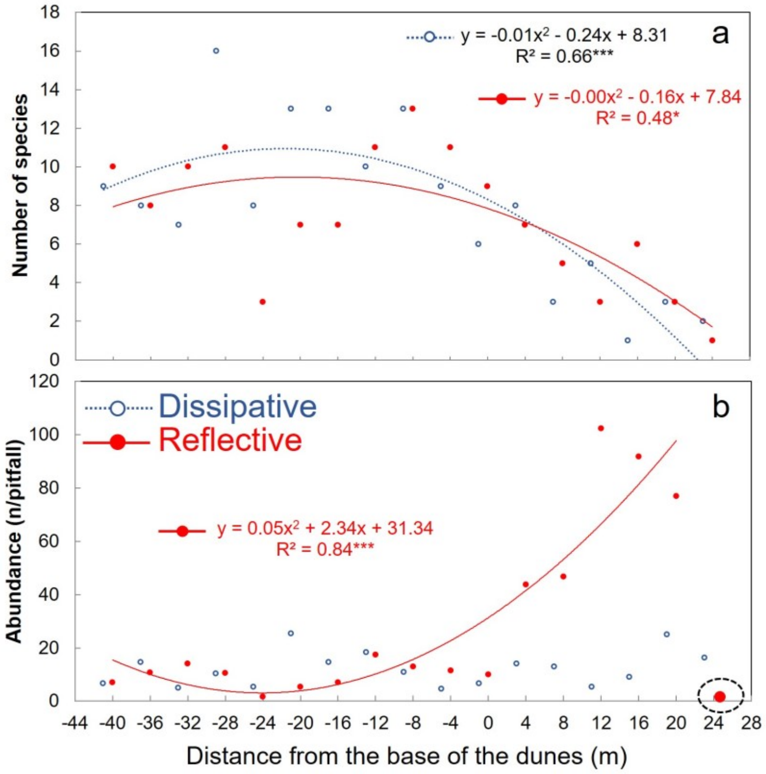

Figure 4. Across-shore variation in (a) species richness; and (b) abundance at the dissipative Barra del Chuy (○) and reflective La Viuda (●) beaches. The best models fitted between mean values of richness and abundance and the distance from the dunes are shown: ****p*<0.001, ***p*<0.01 and **p*<0.05. The zero value in the X-axis indicates the base of the dunes, negative values indicate the landward direction and positive ones the downshore direction. The outlier excluded from model fitting is encircled (dashed line).

3.2. Deconstructive analysis

The abundance of the six dominant taxa differed in their across-shore distribution (Fig. 4). Marine and semiterrestrial crustaceans (decapods, amphipods and cirolanid isopods, Fig. 4a) were collected in the backshore of both beaches, whereas the terrestrial balloniscid isopods only occurred in the dunes of the dissipative beach, increasing their presence landwards (Fig. 4a). Insect abundance increased landwards in the dissipative beach and presented three peaks between the backshore and the dunes of the reflective beach (Fig. 4b). Ants showed the highest abundance in the dunes, being absent in the backshore fringe of both beaches (Fig. 4c). Dipterans occurred in very low abundance, but tended to be more abundant in the reflective beach (Fig. 4d). Coleopterans showed a wider distribution with larger abundance across the shore in the dissipative beach (Fig. 4e). Acarines were found in the dunes of both beaches, with higher abundance in the dissipative beach (Fig. 4f).

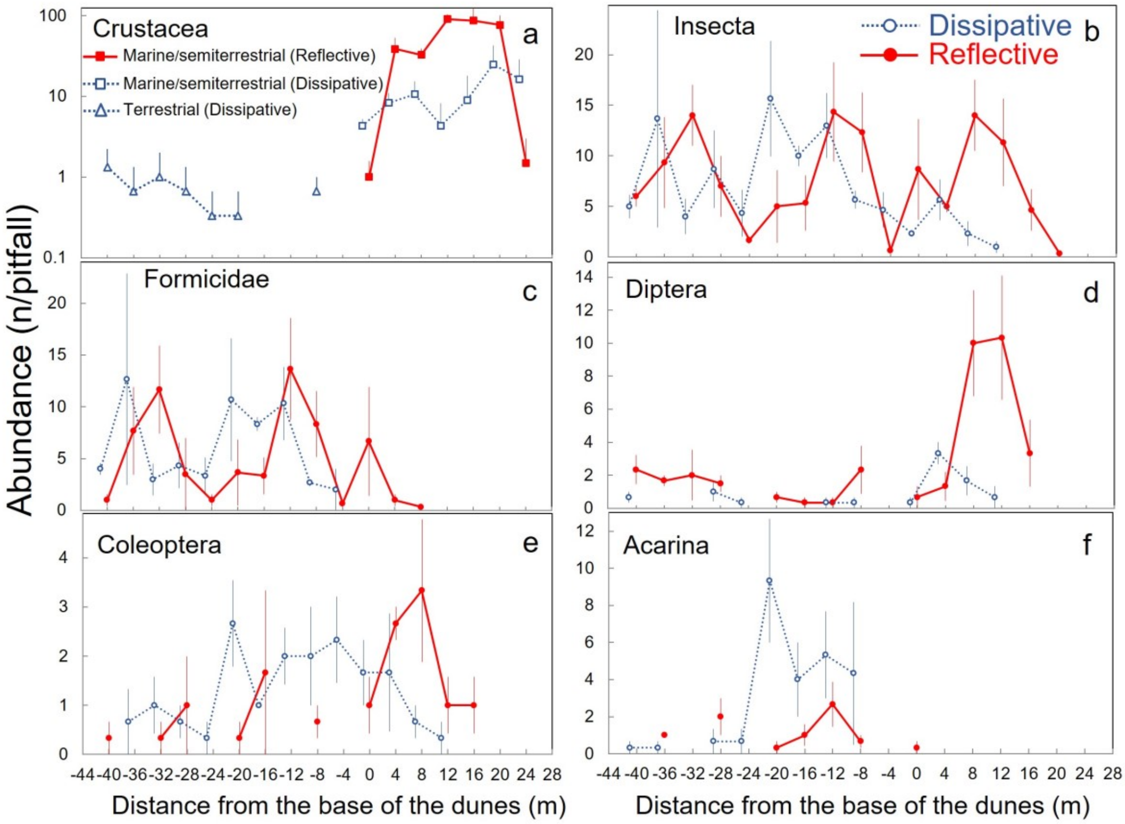

Figure 5. Across-shore variation in abundance (mean ± SE) for six selected taxa of the arthropod community in the dissipative, Barra del Chuy (○) and reflective, La Viuda (●) beach: (a) Crustacea subphylum (note the logarithmic scale); (b) Formicidae family; (c) Insecta class; (d) Diptera order; (e) Coleoptera order; (f) Acarina order. In (a) crustaceans were subdivided into marine/semiterrestrial species and terrestrial ones. The zero value in the X-axis indicates the base of the dunes, negative values indicate the landward direction and positive ones the downshore direction. Note the different scales in the Y-axis.

SIMPER analysis highlighted the following typifying species (Table S2): the ant *Dorymyrmex biconis* typified both beaches; the ant *Pheidole subarmata* typified the dissipative beach only; the talitrid *Atlantorchestoidea brasiliensis* and the ant *Brachymyrmex* sp. typified the reflective beach only. Discriminating arthropod species between beaches were: *A. brasiliensis*, *D. biconis*, *P. subarmata*, *Excirolana armata* (cirolanid isopod), *Tethina* sp. (canacid dipteran) and *Acarina* spp. (Table S2).

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A. brasiliensis showed higher abundance in the reflective beach than in the dissipative one (Fig. 5a). Other crustaceans showed an opposite trend: the cirolanid isopod *Excirolana armata* occurred only in the dissipative beach, while *Excirolana braziliensis* was found in the dissipative beach at 20 m only (Fig. 5b). The dipteran *Tethina* sp. (Fig. 5c) had higher abundance in the reflective beach, particularly in the backshore. Concerning ants, *Brachymyrmex* sp. showed a relatively higher abundance in the reflective beach (Fig. 5d); *D. biconis* was more abundant in the reflective beach (Fig. 5e), whereas *Pheidole subarmata* prevailed in the dissipative beach (Fig. 5f).

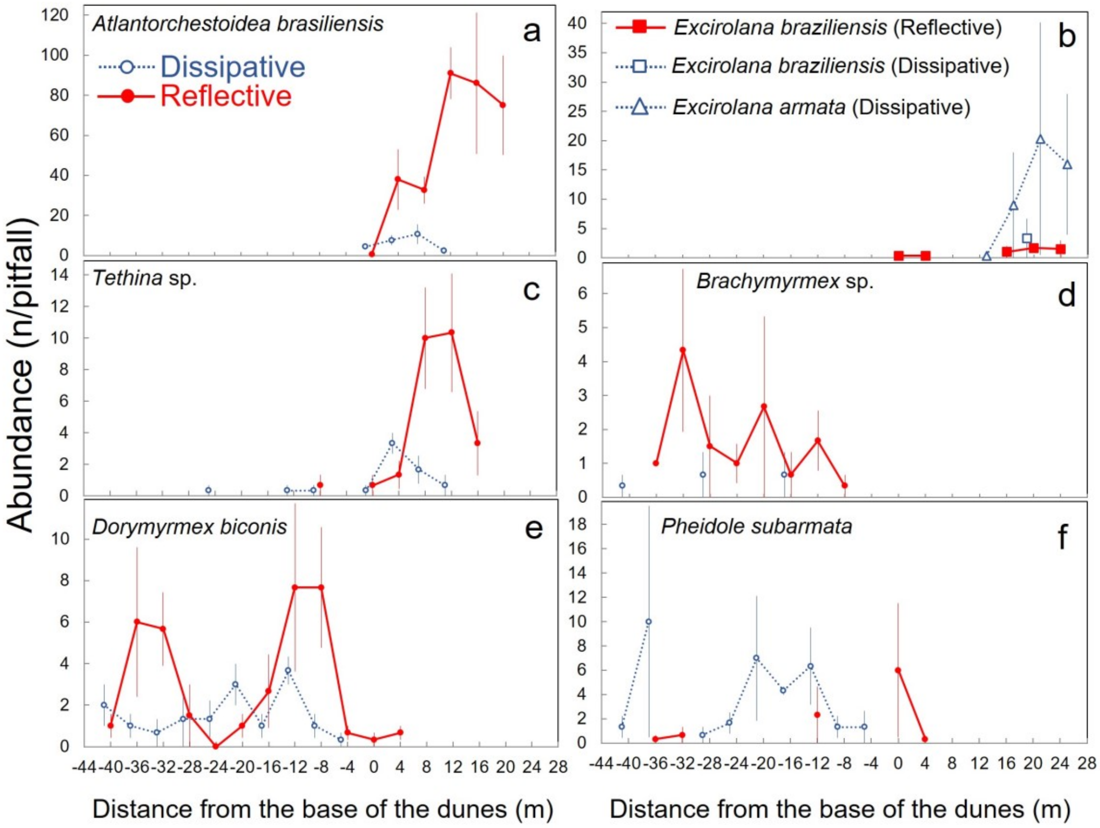

Figure 5. Across-shore variation in abundance (mean ± SE) typifying and discriminating species of the arthropodofauna community in the dissipative, Barra Del Chuy (○) and reflective, La Viuda (●) beach: (a) *Atlantorchestoidea brasiliensis*, Crustacea; (b) *Excirolana braziliensis* and *Excirolana armata*, Crustacea subphylum; (c) *Tethina* sp., Diptera; (d) *Brachymyrmex* sp., Formicidae; (e) *Dorymyrmex biconis*, Formicidae; (f) *Pheidole subarmata*, Formicidae. The zero value in the X-axis indicates the base of the dunes, the negative values indicate the landward direction and positive ones the downshore direction. Note the different scales in the Y-axis.

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3.2. Relationships between species richness, abundance and abiotic variables

GLMs showed that the interaction of the fixed factor "beach" with environmental variables was not significant for species richness, while it was significant for abundance ($p<0.001$, Likelihood Ratio Test; Table 2). For species richness, the effect of sand moisture resulted significant in both beaches, while the temperature effect differed between beaches; for abundance, organic matter content was significant in both beaches and its effect differed between beaches; the effect of beach elevation on abundance also differed between beaches (see the best models with the interactions in Table S3). The best models fitted for both beaches are shown in Table 3. In the dissipative beach, species richness significantly increased towards dryer and warmer beach levels, whereas organic matter and temperature were significant positive predictors for abundance. In the reflective beach, species richness significantly increased towards finer grains and dryer sands, whereas abundance significantly increased towards finer, less-sorted and more compacted sands and at gentle beach elevations with lower organic matter (Table 3).

Table 2. Generalized linear models. Comparisons between additive models and those with an interaction with the factor "beach".

Model 1 additive: Species richness ~ BEACH + Temperature + Compaction + Grain size + Sorting + Organic matter + Moisture + Elevation
AIC=416.81; DF=93; Residual Deviance=107.287

Model 2 with interaction: Species richness ~ BEACH * (Temperature + Compaction + Grain size + Sorting + Organic matter + Moisture + Elevation)
AIC=423.14; DF=86; Residual Deviance=99.613

Chi square test: *∆DF=7; ∆Deviance=7.6745; p=0.362*

Model 1 additive: Abundance ~ BEACH + Temperature + Compaction + Grain size + Sorting + Organic matter + Moisture + Elevation
AIC=779.15; DF=93; LogLik=-759.151

Model 2 with interaction: Abundance ~ BEACH * (Temperature + Compaction + Grain size + Sorting + Organic matter + Moisture + Elevation)
AIC=423.14; DF=86; LogLik=-718.948

Likelihood Ratio test: *∆DF=7; ∆LogLik=40.20383; p=0.000001*

Comparisons between the two models (additive and with interaction) was performed with a Chi-square test, obtaining the Analysis of Deviance for species richness, and the Likelihood Ratio test for Negative Binomial Models for abundance; DF, degrees of freedom; LogLik, log likelihood scores

Table 3. Generalized Linear Models (GLM) relating species richness and abundance with key environmental variables for the dissipative (Barra del Chuy) and reflective (La Viuda) beaches.

	Dissipative				**Reflective**			
	Estimate	SE	z-value	*p*	Estimate	SE	z-value	*p*
Species richness								
Best model	*DF= 4; LogLik= -99.06; AICc= 206.98; AICcw= 0.22*				*DF= 4; LogLik= -98.31; AICc= 204.49; AICcw= 0.22*			
Grain size	-0.031	0.015	1.901	#	-0.009	0.004	2.134	*
Moisture	-0.081	0.022	3.550	***	-0.170	0.078	2.140	*
Temperature	1.046	0.392	2.605	**				
Compaction					0.415	0.223	1.813	#
Abundance								
Best model	*DF= 4; LogLik= -174.83; AICc= 358.54; AICcw= 0.32*				*DF= 7; LogLik= -185.84; AICc= 388.29; AICcw= 0.25*			
Organic matter	6.976	2.908	2.340	*	-8.076	1.608	4.905	***
Temperature	1.443	0.610	2.305	*				
Grain size					-0.029	0.008	3.306	***
Sorting					10.067	4.502	2.186	*
Compaction					0.932	0.406	2.243	*
Elevation					-0.004	0.001	4.312	***

Averaged estimates were calculated using the best models selected through AICc values; SE, standard error; significant p values are highlighted with asterisks (*<0.05; **<0.01; ***<0.001; # almost significant); DF, degrees of freedom; LogLik, log likelihood scores; AICc, corrected Akaike Information Criterion; AICcw, AICc weights. For each beach, the starting models were Abundance/Richness ~ Temperature + Compaction + Grain size + Sorting + Organic matter + Moisture + Elevation

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4. Discussion

The study shows distinct patterns in the across-shore distribution, diversity and abundance among taxa in two beaches with contrasting morphodynamics and different environmental characteristics. The number and the location of pitfall traps (every four metres in three transects, with a total of 17 levels for each beach) provided a full coverage of the sampling sites across the main components of the LAZ (including the backshore and dunes) and gave confidence about the patterns described by the snapshot sampling. The non-random location of the pitfall traps across the beach-dune continuum allowed us to capture active organisms in different parts of the LAZ, *i.e.*, ‘‘linked to different niches’’ from the shoreline to the dune (Fanini and Lowry, 2016). It also allowed us to capture different types of organisms actively walking on the surface of the beach, thus reaching to unbiased estimates of biodiversity and abundance based on this sampling method (Fanini and Lowry, 2016, and references therein). Among these organisms, dipterans and other flying insects that use to shelter on stranded wrack or forage on the substrate, and also those that predate or parasitize beach organisms, were included in the analysis because of their importance as typical members of LAZ arthropodofauna (González-Vainer, personal communication, Universidad de la República of Uruguay, April 2016). Placing the sampling limit inland at 40 m was also useful for comparative purposes with previous studies that implemented the same or similar protocol (Colombini et al., 2003; Mourglia et al., 2015). The intensive one-day (24 h) sampling design was also suitable to avoid the effects of night-day and tides, which can substantially influence zonation in microtidal beaches even in the short term (McLachlan and Defeo, 2018). The synchronous comparison between beaches added robustness to the sampling design: it has been found that snapshot sampling can provide reliable information when the data must be taken as synchronous as possible to reflect actual species interactions (Fattorini et al., 2012). The distribution of the organisms highly depends on seasonality and other periodical or contingent events, and the two synchronous snapshot sampling provided a reliable picture of the interactions between the beach arthropodofauna and microenvironment (sediment) characteristics at the same time.

A major aim of this study was to test the HSH and related hypotheses across beach-dune systems with contrasting morphodynamics, and the two beaches chosen offered a good case study. Abundance was significantly higher in the reflective beach than in the dissipative one, thus supporting the HSH (Defeo and McLachlan, 2011). In the reflective beach, abundance increased considerably towards the shoreline, due to the high number of semiterrestrial crustaceans (mainly amphipods) collected. The distribution of beach crustaceans supported the HSH. However, for terrestrial crustaceans (balloniscid isopods), insects and acarines, trends were taxon-dependent. In this context, the deconstructive analysis revealed that species differing in taxonomic affiliation, body size, development mode or mobility responded differently to environmental features. In some cases, species exhibited contrasting patterns, which would be obscured if only aggregate richness was considered. Crustaceans and dipterans on the backshore supported the HSH, showing higher richness and abundance in the reflective beach, while dune species (mainly ants) showed dissimilar patterns between beaches and in the across-shore gradient, which could be explained by the markedly different physical environment. Across-shore variations in sediment compaction and moisture followed changes in elevation and demarcated the backshore and dunes as distinguishable zones, which were also markedly differentiated by the MDS. This was reflected in marked changes in the across-shore distribution of the species.

Arthropod communities of the two beaches showed a relatively large number of species compared with long-term studies on Uruguayan sandy beaches (Lercari and Defeo, 2006; Mourglia et al., 2015), with a slightly higher number in the dissipative beach than in the reflective one. The results of this study support well-established findings that showed an increase in species richness from reflective to dissipative beaches (Barboza et al., 2012; Defeo and McLachlan, 2013; Defeo et al., 2017). The number of individuals and species found in the dissipative beach in this study is consistent with the estimates provided by Mourglia et al. (2015) for the same beach. Species richness followed similar trends in both beaches, with higher values in the dunes than in the backshore, as was also found by Colombini et al. (2009) for macroinvertebrates studied throughout an annual cycle in a Mediterranean beach, although zonation patterns could change seasonally. The higher species richness found in the dunes was due to the greater presence of insects, which tended to increase in abundance landwards (Defeo and McLachlan, 2011; Barboza et al., 2012). These results were reinforced by the deconstructive analysis, which separated backshore species from dune-specific ones.

Crustacean distribution was concentrated in the backshore, from the waterline to the base of the dunes, except for the terrestrial balloniscid isopod *Balloniscus sellowii* (Brandt 1833), which was found in the dunes of the dissipative beach (Wood et al., 2012). Among insects, ants were mainly distributed in the dunes, as was found also in other studies (Colombini et al., 2002b; Mourglia et al., 2015). Dipterans presented a greater abundance in the backshore, due to the presence of the canacid species *Tethina* sp. Canacids can be found in the intertidal zone of marine coasts, associated with algae or plant debris, or in the dunes, associated with vegetation (Mathis, 1992). Coleopterans showed a higher abundance in the dissipative beach, except for the tenebrionid *Phaleria testacea* (Say 1824), which presented a higher abundance in the backshore of the reflective beach. This species can be usually found in American sandy shores (Watrous and Triplehorn, 1982), buried in the sand during the day and feeding on stranded wrack on the beach at night (Caldas and Almeida, 1985).

Typifying and discriminating species showed differences in distribution, according to their life history traits. The talitrid *A. brasiliensis* (Dana 1853), a beach omnivorous crustacean with direct development, was more abundant in the reflective beach, supporting the HSH (Defeo and Gómez, 2005; Defeo and McLachlan, 2011). Ants had a relatively high abundance in the dunes of both beaches, whereas *Brachymyrmex* sp. (Mayr 1868), Formicinae subfamily, was more abundant in the reflective beach. The species *Dorymyrmex biconis* (Forel 1912) was a typifying species for both beaches. These ants often nest in dry environments and soils without vegetation (Cuezzo and Guerrero, 2011). The ant *Pheidole subarmata* (Mayr 1884) was found with a relatively high abundance in the dissipative beach. This generalist forager was registered from Mexico to the south of Brazil in a wide variety of habitats, including coastal ecosystems (Wilson, 2003).

In summary, our results support the HSH for the abundance of crustaceans, dipterans and coleopterans (particularly *Phaleria testacea*) on the backshore, but not for species richness. The hypothesis proposed by Defeo and McLachlan (2011), which predicts that dissipative beaches and their well-developed dunes could be more favourable and safer environments for insects and arachnids than reflective beach-dune systems, was supported for acarines (arachnids) abundance, and coleopteran abundance and diversity. Regarding ants, which occurred only in the dunes, with no apparent differences in abundance between beaches, vegetation may offer suitable microhabitats independently of beach morphodynamic characteristics, overriding the effect of physical factors. These results reinforce the relevance of conducting deconstructive analyses by beach zone (backshore and dunes) in order to decipher across-shore distribution patterns in beach-dune systems. Future studies should also be directed to disentangle the effects of differences in physiology and behaviour on a longer temporal scale. The results provided here reinforce the need of conducting studies across the LAZ as a whole unit, whose integrity is jeopardized by the several threats acting simultaneously (Colombini et al., 2003; Fanini et al., 2020, 2021; Defeo and Elliott, 2021).

5. *Acknowledgements*

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7. *Supplementary Material*

Environmental data, arthropods sampled, and multivariate analyses

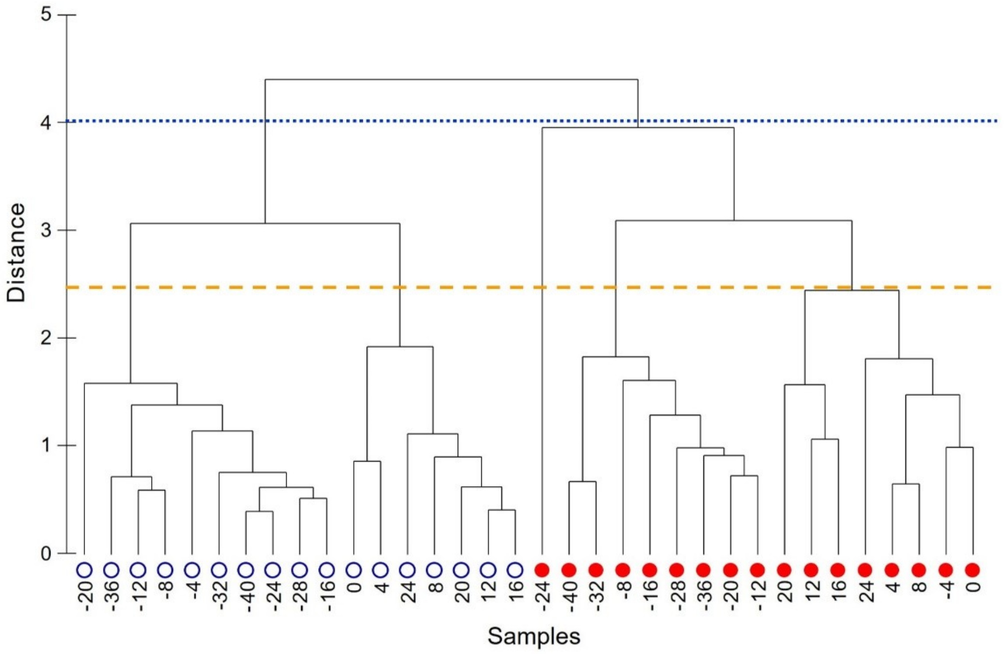

Figure S1. Cluster analysis of environmental data. The point numbers indicate the distance (m) from the base of the dunes to the shoreline (positive values) and landwards (negative values). The dotted and dashed lines show an Euclidean distance of 4 and 2.5, respectively. ○: dissipative beach (Barra del Chuy); ●: reflective beach (La Viuda).

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Table S1. Absolute (n° of individuals) and relative (%) abundance of the taxa captured in the two beaches, La Viuda and Barra Del Chuy, in April 2016, with pitfall traps across the beach-dune systems. Numbers of morphospecies indicate a recognition code

Class	Order	Family	Genus/Species	Absolute abundance (number of individuals)	Relative abundance (%)
Malacostraca					
	Amphipoda				
		Talitridae	*Atlantorchestoidea brasiliensis*	1045	52.7
	Isopoda				
		Cirolanidae	*Excirolana armata*	137	6.9
			Excirolana braziliensis	23	1.2
		Balloniscidae	*Balloniscus sellowii*	15	0.8
	Decapoda				
		Hippidae	*Emerita brasiliensis*	13	0.7
Insecta					
	Hymenoptera				
		Formicidae	*Dorymyrmex biconis*	149	7.5
			Pheidole subarmata	131	6.6
			Brachymyrmex sp.	43	2.2
			Mycetophylax sp.	19	1.0
			Formicidae morphosp. 28	11	0.6
			Formicidae morphosp. 17	5	0.3
			Solenopsis sp.	4	0.2
			Formicidae morphosp. 73	3	0.2
			Formicidae morphosp. 80	2	0.1
			Formicidae morphosp. 18	1	0.1
		Vespidae	Vespide morphosp. 2	8	0.4
			Anoplius sp.	2	0.1
			Vespidae morphosp. 26	1	0.1
	Coleoptera				
		Carabidae	*Peronoscelis pictus*	9	0.5
			Megacephala fulgida	7	0.4
			Cicindelinae morphosp. 62 larva	2	0.1
			Clivina sp.	2	0.1
			Bradycellus sp.	1	0.1

		Chrysomelidae	*Chrysomelidae* morphosp. 87	1	0.1
		Cleridae	Cleridae morphosp. 80	1	0.1
		Curculionidae	*Listronotus* sp.1	2	0.1
			Listronotus sp.2	2	0.1
			Curculionidae morphosp. 46	1	0.1
		Elateridae	Elateridae morphosp. 23	8	0.4
		Phalacridae	Phalacridae morphosp. 72	2	0.1
		Salpingidae	*Aegialitinae* morphosp. 70	2	0.1
		Scarabaeidae	*Aphodiinae* morphosp. 67	1	0.1
			Aphodiinae morphosp. 77	1	0.1
		Staphylinidae	Staphylinidae morphosp. 81	2	0.1
			Staphylinidae morphosp. 92	2	0.1
			Staphylinidae morphosp. 88	1	0.1
		Tenebrionidae	*Phaleria testacea*	39	2.0
			Pimeliinae morphosp. 38	1	0.1
			Pimeliinae morphosp. 45	1	0.1
			Alleculinae morphosp. 57	1	0.1
			Tenebrionidae morphosp. 59 larva	1	0.1
	Hemiptera				
		Cydnidae	Cydnidae morphosp. 76	3	0.2
		Delphacidae	Delphacidae morphosp. 50	11	0.6
		Gerridae	*Halobates micans*	4	0.2
	Diptera				
		Bombyliidae	*Asilidae* morphosp. 11	12	0.6
		Canacidae	*Tethina* sp.	100	5.0
			Canacidae morphosp. 25	13	0.7
		Empididae	Empididae morphosp. 54	3	0.2
		Milichiidae	Milichiidae morphosp. 24	4	0.2
		Sarcophagidae	*Microcerella* sp.	3	0.2
	Dermaptera				
		Labiduridae	*Labidura riparia*	14	0.7
	Neuroptera				
		Myrmeleontidae	Myrmeleontidae morphosp. 21	2	0.1
	Orthoptera				
		Tridactylidae	Tridactylidae morphosp. 39	1	0.1
	Archaeognatha				
			Archaeognatha morphosp. 47	1	0.1
Arachnida					

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Acarina				
		Acarina spp.	97	4.9
Araneae				
	Anyphaenidae	Anyphaenidae morphosp. 20 juvenile	1	0.1
	Lycosidae	*Allocosa brasiliensis*	6	0.3
		Allocosa sp. 1	2	0.1
	Miturgidae	Miturgidae morphosp. 79 juvenile	1	0.1
	Salticidae	Salticidae morphosp. 58 juenile	2	0.1
		Ailutticus nitens	1	0.1
	Zodariidae	*Cybaeodamus taim* juvenile	1	0.1

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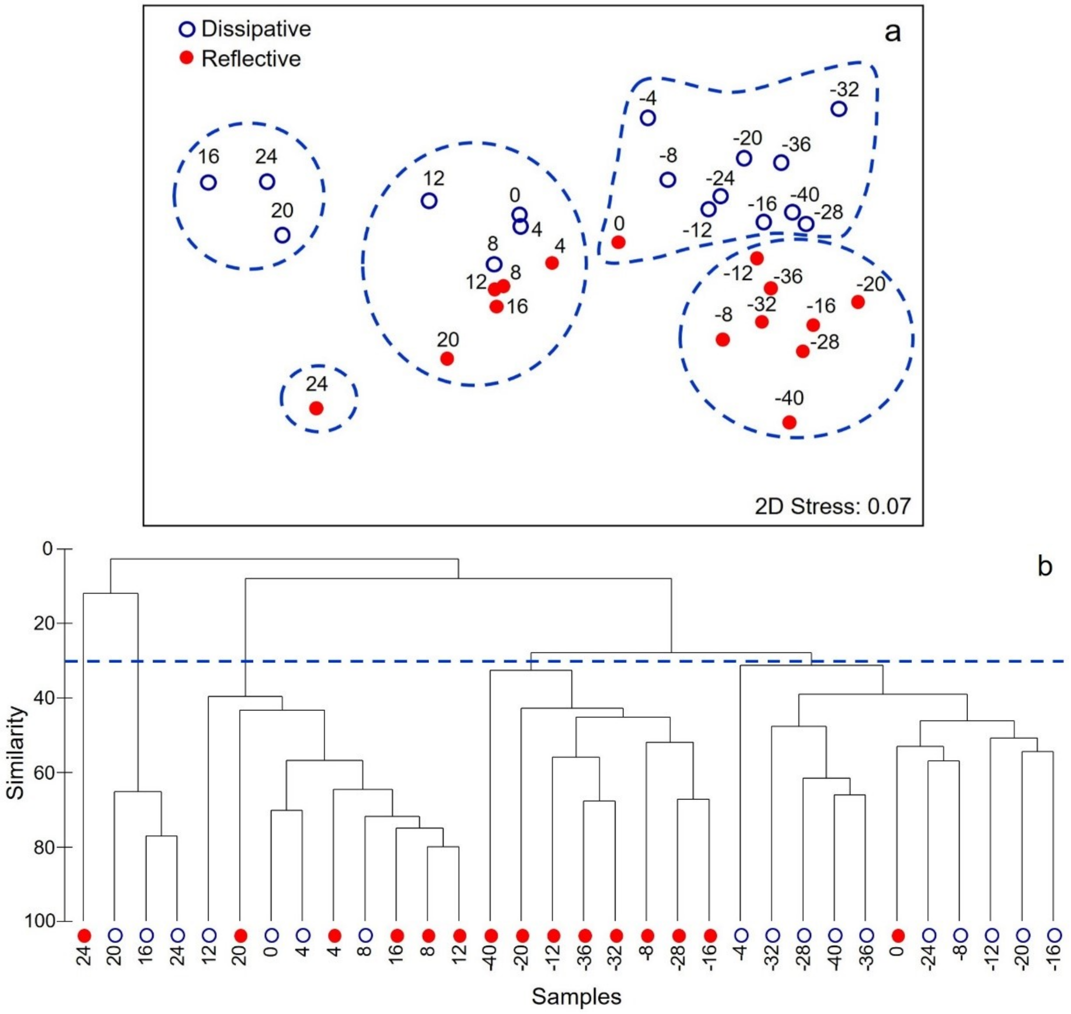

Figure S2. (a) Non-metric Multi-Dimensional Scaling (MDS) of arthropodofauna abundance; (b) Cluster analysis. The point numbers indicate the distance (m) from the base of the dunes to the shoreline (positive values) and landwards (negative values). The dashed lines denote a similarity of 30%. Values of samples -4 and -24 of the reflective beach were considered outliers; when these values were removed from the analysis, the model stress improved from 0.16 to 0.07. ○: dissipative beach (Barra del Chuy); ●: reflective beach (La Viuda).

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Table S2. SIMPER analysis: percentage contribution of species to the average similarity (typifying species>10%) and dissimilarity (discriminating species>4.5%) between beaches, dissipative: Barra del Chuy; reflective: La Viuda.

Typifying species	**Dissipative**	**Reflective**
Dorymyrmex biconis	15.29	25.23
Pheidole subarmata	13.75	
Atlantorchestoidea brasiliensis		15.67
Brachymyrmex sp.		12.05
Average similarity	21.88	21.29

Discriminating species	**Dissipative vs. Reflective**
Atlantorchestoidea brasiliensis	9.37
Dorymyrmex biconis	6.09
Pheidole subarmata	5.58
Excirolana armata	5.42
Tethina sp.	5.36
Acarina spp.	4.87
Average dissimilarity	84.63

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Table S3. Generalized Linear Model (GLM) relating species richness and abundance with key environmental variables for both beaches with the interaction with “beach” factor

	Estimate	SE	z-value	*p*
Species richness				
Best model	*DF= 5; LogLik= -200.69; AICc= 412; ∆AICc= 0.00; AICcw= 0.06*			
Intercept	-14.32	9.458	1.504	ns
Grain size	-0.015	0.011	1.362	ns
Moisture	-0.093	0.022	4.256	***
Sorting	3.548	2.213	1.585	ns
Temperature	0.791	0.438	1.796	#
Beach	13.94	9.876	1.400	ns
Compaction	0.265	0.184	1.422	ns
Beach:Temperature	-0.843	0.405	2.056	*
Beach:Grain size	0.020	0.016	1.239	ns
Beach:Moisture	-0.094	0.082	1.129	ns
Organic matter	-0.538	1.053	0.505	ns
Elevation	-0.000	0.000	0.034	ns
Beach:Compaction	0.141	0.340	0.411	ns
Beach:Sorting	-1.526	4.790	0.315	ns
Beach:Elevation	0.002	0.001	1.364	ns
Beach:Organic matter	-1.890	1.808	1.032	ns
Abundance				
Best model	*DF= 11; LogLik= -364.06; AICc= 753.06; ∆AICc= 0.00; AICcw= 0.05*			
Intercept	-13.171	22.951	0.572	ns
Beach	12.202	23.954	0.507	ns
Elevation	0.001	0.002	0.587	ns
Grain size	0.003	0.039	0.084	ns
Organic matter	6.473	3.222	1.985	*
Sorting	-1.100	7.223	0.151	ns
Temperature	1.111	0.832	1.327	ns
Beach:Elevation	-0.006	0.002	2.735	**
Beach:Organic matter	-14.206	3.727	3.769	***
Beach:Sorting	15.237	7.690	1.958	#
Compaction	0.231	0.405	0.565	ns
Beach:Compaction	0.980	0.567	1.707	#
Beach:Grain size	-0.062	0.037	1.669	#
Beach:Temperature	-1.432	0.823	1.720	#
Moisture	0.020	0.036	0.557	ns

Averaged estimates were calculated using the best models selected through AICc values; SE, standard error; significant p values are highlighted with asteriscs (*<0.05; **<0.01; ***<0.001; # almost significant); interaction estimates are referred to the reflective beach; DF, degrees of freedom; logLik, log likelihood scores; AICc, corrected Akaike Information Criterion; AICcw, AICc weights. For each beach, the starting models were Abundance/Richness ~ BEACH * (Temperature + Compaction + Grain size + Sorting + Organic matter + Moisture + Elevation)

Chapter 4 – Dealing with environmental changes on sandy beaches: behavioural adaptations of two sandhopper species

1. *Introduction*

Sandy beaches are highly dynamic ecosystems both in time and space, characterised by the interactions between abiotic and biotic factors in a narrow land-sea interface, and therefore subjected to impacts from land and sea (McLachlan and Defeo, 2018). It has been stressed the importance of studying the perturbations on sandy beach ecosystems and the responses developed by organisms to survive in these environments (Chapter 2; Scapini et al., 2019a, b). The analyses of abiotic and biotic factors, including sediment characteristics and behavioural adaptations of the macrofauna populations, may enhance the knowledge of sandy beach ecosystems and provide useful information for their protection and sustainable management.

Behavioural adaptations have been recognized as fundamental strategies to face the changing environment of sandy beaches and climate change impacts, which may increase instability of beaches (Chapter 2; Scapini et al., 2019b). In particular, orientation is an immediate response of sandhoppers to coastline changes, also being a bioindicator of beach perturbations (reviewed in Scapini, 2014; Scapini et al. 2019a). The zonation of sandhoppers has been considered an important behavioural adaptation to exploit the optimal zone on sandy beaches (Williams, 1995; Cardoso, 2002; Colombini et al., 2006, Bouslama et al., 2009; Colombini et al., 2013). Dehydration risk was shown as a main driver for sandhoppers to search the optimal zone between the shoreline and the dunes; other drivers depend on meteorological factors, seasons and local characteristics, such as the risk of being swept away by waves or dislocated by the currents in exposed beaches. During the Anthropocene, human direct or indirect impacts have dramatically increased, causing larger environmental changes in a shorter time frame (Nordstrom, 2000; McLachlan and Defeo, 2018). In a dynamic scenario, the study of behavioral responses is mandatory to assess how individuals and populations may respond, and predict future scenarios, possibly anticipating catastrophes regarding biodiversity, habitat and ecosystem loss (Chapter 2, Scapini et al., 2019b).

The sandhopper *Atlantorchestoidea brasiliensis* (Dana, 1853) inhabits south-western Atlantic coasts from Brazil to north Argentina (Cardoso and Veloso, 1996; Serejo, 2004; Defeo and Gómez, 2005) and *Talitrus saltator* (Montagu, 1808) is widespread both on Oceanic and Mediterranean sandy beaches, from the northern European coasts to the northern African ones (Mezzetti et al., 2010; Ketmaier et al., 2010). Recently, Lowry and Myers (2019) described three species of the genus *Talitrus* inhabiting the above-mentioned areas, identified as *T. saltator* until today: *T. saltator*, *T. platycheles* and *T.*

cloquetii. The Mediterranean species that we considered in this study was identified as *T. platycheles*, while *T. saltator* is spread on eastern Atlantic coasts (Lowry and Myers, 2019). For simplicity and continuity with previous studies, in this study we refer to *T. saltator*. On Mediterranean coasts, these talitrids burrow into the moist sand near the shoreline during daytime avoiding dehydration risk, and migrate inland during the night to forage on the beach and backshore up the dunes (Scapini et al., 1992; Colombini et al., 2013).

A diurnal activity was described of juveniles of both *T. saltator* and *A. brasiliensis* near the shoreline, which was explained assuming the lower predation risk and higher feeding needs of juveniles that may compensate the smaller foraged area during the night, and also avoid food competition with adults (Scapini et al., 1992; Cardoso, 2002). If disturbed during the daytime (by a predator, physical stress or researcher) sandhoppers orient towards the recovery zone near the shoreline thanks to the sun compass (they maintain a fixed direction to the sun azimuth by compensating its daily movement through the biological clock) and/or environmental cues, such as landscape vision, beach slope, radiation intensity and wave length differences between land and sea, or wind (reviewed by Scapini, 2006). The more stable the environment, the higher concentration of sandhopper orientation was observed (Scapini et al., 1995; Scapini et al., 2005).

Studies on the orientation of several sandhopper populations and species revealed different patterns according to the characteristics of the beach. Orientation experiments were conducted in the field both on Mediterranean beaches in Tunisia (Scapini et al, 2002; Fanini and Scapini, 2008), Italy (Fanini et al., 2005; Colombini et al., 2013, Nourisson and Scapini, 2015); and in other oceanic beaches in western France (Gambineri et al., 2008), Chile (Scapini and Dugan, 2008), Uruguay (Fanini et al., 2009), western Morocco (Fanini et al., 2012), Portugal (Bessa et al. 2014) and Brazil (Bessa et al., 2017). The study of sympatric species highlighted the evolution of similar/same behavioural adaptations in different species to achieve the same goal (Scapini et al., 2002; Bessa et al. 2014; Gambineri et al., 2008; Colombini et al., 2013).

Many researchers have stressed the importance of long-term analyses in discovering and monitoring both large and small climate change impacts in the same region, to evaluate possible actions to reduce ecological damages, and possibly prevent them in the future (Jorge-Romero et al., 2021). However, in many cases long term analyses cannot be carried out for the lack of previous data, aided by the fact that the focus on the ecosystems of sandy beaches and on long-term management were scarce to date (Defeo et al., 2021). Besides long-term analyses, it is also important to consider a large geographical scale to address global changes. It has been proposed to conduct large-scale geographical studies of areas affected by similar distal and proximate drivers of climate change, collecting (with the same protocol) and comparing data from different localities, regions and countries; such studies are scarce to date (McLachlan and Defeo, 2018). It has also been suggested to analyse the behavioural responses of different species facing similar problems, *i.e.* immediate responses to environmental changes, such as morphodynamic, meteorological and anthropogenic ones (Chapter 2; Scapini et al., 2019a, b). Collecting information regarding animal strategies facing the changes mentioned above can be useful to hypothesize or project future scenarios and understand how to manage sandy beaches in a conservation perspective in face of climate change impacts.

A scientific challenge is to extend the geographical scale of the comparisons from small (local or regional) to large (continental) scales, thus to give an overall picture without losing the characteristics that are relevant at the level of animal life and the habitat, where the behavioural adaptations are expressed (Colombini et al., 2005). The aim of this study was to verify the possibility of using the behavioural strategies of sandhoppers as case study and conduct comparisons at increasing geographical scales.

In this chapter, zonation and orientation of close and distant talitrid populations of the same or similar species were compared in relation with the (changing) physical-chemical and meteorological characteristics of sandy beaches. Two different species of Talitridae were considered: *Talitrus saltator* on four Mediterranean beaches, and *Atlantorchestoidea brasiliensis* on two Atlantic beaches. The six selected beaches, in two continents (southern Europe and south America) were microtidal, but differed in morphodynamics and sediment characteristics, which permitted to analyse a range of different aspects. The behaviours were analysed in spring and autumn along with meteorological variables and beach physical-chemical characteristics, before and after the summer touristic exploitation. It was decided to compare pairs of beaches within the same region, likely inhabited by very similar talitrid populations, and more distant beaches inhabited by different populations and species.

Considering previous hypotheses (*e.g.* the Habitat Safety Hypothesis, HSH, Defeo and Gómez, 2005; Defeo and McLachlan, 2011; Innocenti Degli et al., 2021: see Chapter 3), and experimental work on abundance and zonation (Williams, 1995; Cardoso, 2002; Defeo and Gómez, 2005; Bouslama et al. 2009; Colombini et al., 2013) and orientation (Scapini, 2006, 2014; Scapini and Dugan, 2008; Fanini et al., 2009; Bessa et al., 2017), predictions were to observe: regarding zonation 1) larger talitrid populations on beaches with coarser sand and higher elevation, where the safer supralittoral environment is more protected by inundations (HSH, which was verified on Uruguayan beaches, but was not yet analysed on Mediterranean ones); 2) a zonation closer to the shoreline on beaches with coarser and less compact sand (with lower water content in the interstices) and greater elevation (which is a limitation to landward zonation), regardless of the geographical area, considering the risk of dehydration greater than that of being swept away by waves; 3) juveniles zonated closer to the shoreline than adults because of their major risk of dehydration and less capability to burrow deeply into the sand; regarding orientation 4) a more concentrated orientation in beaches with coarser sand and higher elevation, thus with a more stable supralittoral zone; 5) the orientation of juveniles more seawards concentrated than adults, for the major risk of dehydration of the former, which has been rarely considered in the orientation tests; 6) the orientation of the Mediterranean populations more seawards concentrated with respect to that of the Atlantic populations because of the greater risk of dehydration on Mediterranean beaches (due to the higher air temperature and lower air relative humidity during the studied seasons).

To verify the last prediction (6), Scapini et al. (2019a) had carried out a meta-analysis of previous data from European Mediterranean and Atlantic beaches. However, an *ad hoc* planned comparative work was necessary at large (continental) geographical scale, characterising the beaches and populations using the same methods, also including sediment variables, which notably influence the recovery of the digging zone, but had not been considered in the studies of that meta-analysis.

Deviations from predictions were discussed by comparing different localities. A comparative approach was applied to evaluate possible similarities or differences among populations and the behavioural strategies in face of changing environmental conditions. This approach responds to the need to find common strategies to monitor, manage, protect and restore sandy beach systems. In the last International Sandy Beach Symposium in 2018 it was stressed that sandy beaches cannot be considered more or less stable areas to be exploited without any regard, but they must be managed as ecosystems (Fanini et al., 2020).

2. *Materials and methods*

2.1. Study sites

The study was carried out on six beaches, four in Italy and two in Uruguay, in two seasons (spring and autumn; Fig. 1). These six beaches are microtidal, mostly exposed, and with limited stranded material (seaweed and/or algae). Two of the Italian beaches are located in the south of Tuscany, bathed by the Tyrrhenian Sea: 1) Collelungo (42°37'54,3'' N, 11°04'40,0'' E), in the Maremma Regional Park (Grosseto), a sandy beach backed by a dune system, actually under erosion, and 2) Macchiatonda (42°24'02,2'' N, 11°21'10,4'' E; Capalbio, GR), near the Burano Lake, a Natural Reserve with a well-established and protected dune system. The other two Italian beaches are located in the north of the

Tuscan coast, bathed by the Ligurian Sea: 3) Bocca di Serchio (43°46'20.9" N, 10°16'10.7" E) and 4) Buca del Mare (43°42'43.1" N, 10°16'44.8" E), both in the Migliarino, San Rossore and Massaciuccoli Natural Park, backed by dune systems, actually under erosion. Italian beaches were chosen within or adjacent to protected areas, to have a minimal effect of tourism activities on macrofauna. The selected beaches had been previously studied, offering the possibility of comparisons in different times (Scapini et al., 1997; Fallaci et al., 2003; Fanini et al., 2007; Colombini et al., 2009; Nourisson and Scapini, 2015). In Uruguay, the two oceanic beaches are located in the south and south-eastern coasts: 5) La Viuda (34°03'00.6" S, 53°32'32.6" W), a large reflective beach, and 5) José Ignacio (34°50'09.9" S, 54°37'50.2" W), an intermediate beach, both backed by dune systems. Both beaches had been the subject of previous studies (Fanini et al., 2009; Barboza et al., 2012; Gómez and Defeo, 2012).

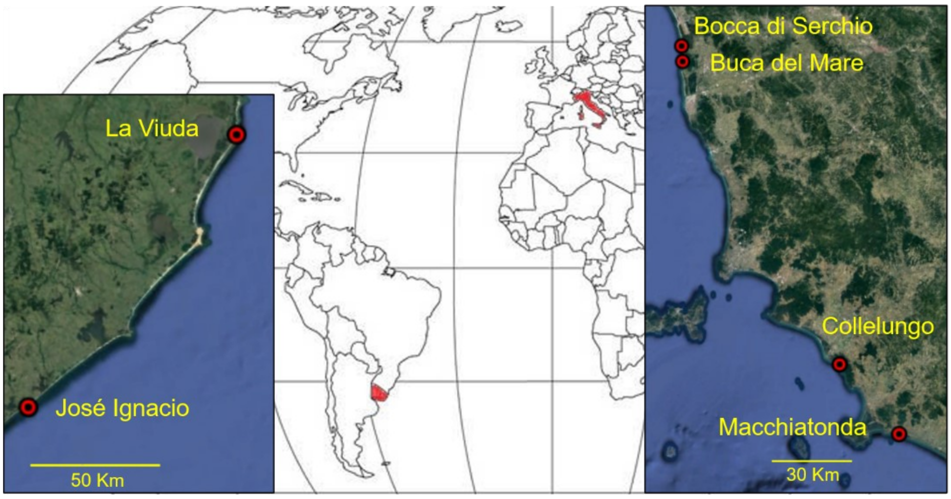


Figure 1. Location map of the 6 selected beaches (circles): 4 beaches in Italy (insert on the right) and two in Uruguay (insert on the left). Images from Google Earth 2021.

2.2. Sampling design

Spring and autumn were chosen for the zonation and orientation experiments, in consideration of the life cycle of sandhoppers: according to previous studies, in these seasons the populations are more abundant and better structured (Marques et al., 2003; Cardoso and Veloso, 1996; Table 1).

Field sampling and orientation experiments were carried out with clear weather, avoiding rain and overcast when possible. The meteorological conditions were recorded during the experiments.

Table 1. Sampling and experiment dates on the six beaches in the two seasons

	Macchiatonda	Collelungo	La Viuda	José Ignacio	Bocca di Serchio	Buca del Mare
Spring	22/04/2018	23/04/2018	08/12/2018	16-22/12/2018	06/06/2019	07/06/2019
Autumn	22/09/2018	23/09/2018	29/04/2019	25/04/2019	23/09/2019	24/09/2019

The two beaches on the same coast were analysed during the same time (in subsequent days, when possible): Collelungo and Macchiatonda, located at a distance of up to 30 km; La Viuda and José Ignacio, located at a distance of about 130 km; Bocca di Serchio and Buca del Mare are about 7 km away, but at the two sides of the stream Fiume Morto Nuovo, which keeps the populations of the two beaches separate. Simultaneously on the same beach, two trained teams conducted 1) the samplings of three transects, from the shoreline to the base of the dune, and 2) orientation experiments, to have a picture of sandhopper abundance, zonation and orientation behaviour during the same environmental conditions.

2.2.1. Environmental characteristics

Three transects spaced 8 meters apart were analysed on each beach from the shoreline to the base of the dune; sediment samples and physical-chemical measures were taken in correspondence of the stations where animals were found, adding the next three stations without specimens, to avoid the possibility of leaving out sandhoppers that might be in the highest part of the beach. Some beach characteristics were also known from previous studies, and eventually compared with the new ones. Beach profiles were obtained with standard topographic methods.

In the laboratory, sediment samples of about 100 g were sieved with ten mesh sizes ranging from 1 mm to 0.063 mm (Wentworth scale, 1922), and the sediment retained in each sieve was weighed (precision=0.01 g) and used to estimate mean particle size and sorting (μm; GRADISTAT v.6.0 software; Blott and Pye, 2001). Sediment samples of about 10 g were weighed before and after drying (oven-dried at 80°C for 24 hours) to estimate sediment water content (% of the fresh weight). The same samples were then incinerated at 460°C for 4 hours and weighed again to determine organic matter content (% of the dry weight).

2.2.2. Abundance, density and zonation

Sandhoppers were collected across-shore in the three transects on each beach, sampling standard areas every 2 m in Italy and every 4 m in Uruguay; sampling was performed collecting burrowed sandhoppers by hand within quadrats (Plexiglas square of 50x50 cm, in Italy) or sieving the sand collected with a corer (a cylinder of 16 cm in diameter, inserted into the sand three times, in Uruguay). The collected animals were preserved in 70%

alcohol and then analysed in the laboratory, where species and sex were identified, and individual morphological measurements were made. The length of the cephalon was measured (size indicator, Marques et al., 2003), the number of segments of the flagellum of the second right antenna was counted (age indicator, Williams, 1983); the presence of oostegytes and setae on oostegytes in females (*i.e.,* immature without setae, mature with setae) or of two penises in males were detected; small specimens without recognizable sex characteristics were classified as juveniles. Four classes for each species were detected: mature females, immature females, males, and juveniles. No intersexes were detected in the samples. The abundance (mean number of individuals collected; n/stations), density (ind/m^2) and zonation (across-shore distribution of each class and both size and age proxies) were obtained.

2.2.3. Orientation experiments

Sandhoppers were manually collected removing surface sand and eventual wrack from the zones with higher presence of animals (typically burrowed in wet and soft sand, approximately between 2 and 8 m above the waterline) by 3-5 operators for 30-60 minutes, on a surface of about 20 m^2; specimens were kept alive in containers with moist sand before every testing event. A circular arena made of Plexiglas was used for these experiments, having a diameter of 40 cm and provided of 72 pitfall traps along the rim, each one subtending an angle of 5° (Scapini et al., 2005); the 72nd trap was oriented to north. The arena was positioned horizontally on the beach, 1 m above the ground to prevent operators from being seen by the animals during the experiments. The sandhoppers were inserted in the arena through a transparent tube in Plexiglas and, after one minute of acclimatization, they were left free to move and fall into the traps, then were collected singularly and preserved in 70% alcohol for later measurements in the laboratory, as for the individuals of the zonation study. Sandhoppers were tested in groups of ten, every 15 minutes per 8 times, using approximately the same numbers of adults and juveniles (chosen by size, which was measured thereafter in the laboratory). The experimental sessions were conducted twice a day for about 90 minutes both in the morning and in the afternoon, around 9:00 a.m. and 2:00 p.m., using a total of about 160 individuals per beach. Orientation experiments were alternated with and without the view of the landscape, by shielding it with a white screen. At each release, the following environmental characteristics were registered: air temperature (°C) and relative humidity (%) with a thermo-hygrometer, cloudiness (from 0=clear sky to 8=sky completely covered by clouds, visual estimate), the view of the sun (visible, veiled, solar disk, not visible) and intensity of solar radiation (lux) with a luxmeter; sun azimuth and tide condition (rising or ebbing tide) were also registered. The pH and salinity of the sea/ocean water was measured with a pH-meter and a salinometer; the TED (Theoretical Escape Direction seawards, *e.g.* the shortest way from the backshore to the shoreline for a sandhopper subjected to dehydrating conditions) was measured with a magnetic compass, as the perpendicular of the shoreline direction.

2.3. Data analyses

Mean estimates per station were calculated for sand compaction, sediment organic matter content, sediment water content, sand temperature, grain size, sorting, elevation and sandhopper abundance and density. Differences between the two beaches located on the same coast and seasons (spring and autumn) were analysed using one-way ANOVA, with beach or season as the main factor respectively, using R Software Package (R Core Team, 2020); Levene and Brown-Forsythe tests were performed to verify the assumption of variance homogeneity; transformations were applied where necessary.

The variation of physical characteristics across the beach was modelled as a function of the distance from the shoreline, selecting the best models according to the coefficient of determination (adjusted R^2) and the statistical significance.

The variation of the density (ind/m^2) as a function of abiotic variables was modelled, using the square root of the total density (mean of the three transects) to reduce the influence of variables with high values, and selecting the best models according to the coefficient of variation (adjusted R^2) and statistical significance. This modelling approach was employed when at least four stations gave data on sandhopper density.

Population variables for each beach and season were calculated both for zonation and orientation experiments: the number of individuals of each class collected (juveniles, immature and mature females, and males), the proportion of each class to the total number of individuals (ind/tot), cephalic length, number of antennal segments, and the sex ratio (males/(males+females)). The cephalic length and antennal segments of each population were compared between beaches and seasons using one-way ANOVA, with beach or season as the main factor respectively.

The zonation of sandhoppers was calculated as the across-shore distribution of the following variables: percentage abundance of each class (square root of mean abundance), cephalic length (size proxy) and antennal segments (age proxy).

For each orientation experiments, environmental variables during the experimental sessions were recorded: cloudiness, sun visibility, air temperature, air relative humidity and solar radiation. Differences between beaches and seasons for air values and solar radiation were compared using the one-way ANOVA, with beach or season as the main factor respectively.

For the analysis of orientation circular distributions, the mean angle and vector length (r), 95% confidence intervals (CI) of the mean direction and sample circular dispersion were calculated for each distribution. The concentration of the individual directions around the mean was tested using the Rayleigh test for uniformity applied to the circular distributions, based on the length of the vectors (Batschelet, 1981). The density curves were smoothed with the kernel method and double plotted on Cartesian graphs, to better show the peaks of the distributions (Fisher, 1993).

To analyse the effects of environmental variables on orientation variation, multiple regression analyses adapted to circular data (SPLM, Spherically Projected Linear Models) were applied, using the angles of individual orientation as dependent variables, and meteorological (air temperature, air relative humidity, cloudiness), hourly (sun radiation, sun azimuth, day time, rising/ebbing tide), orienting (sun visibility, landscape vision) and intrinsic (general class: male, female, juvenile; cephalic length; number of antennal segments) variables, as independent variables or factors, together with factors beach and season; the models developed were both additive and with the interaction with factors "beach" or "season" (Scapini et al., 2002; Marchetti and Scapini, 2003). The best models were chosen using a backward selection and the Akaike Information Criterion (AIC); the significance of each influencing factor in the best models was tested with the Likelihood Ratio Test (LRT) (Scapini et al., 2002). Comparisons between populations from beaches with different orientation were made transforming the angles of the samples by subtracting the TED.

The observations made on the whole set of beaches were compared through multivariate analyses (PRIMER v.6 software, Clarke and Gorley, 2006), using log-transformed and normalized environmental data, to reduce the influence of variables with high values. Cluster analyses based on the Euclidean distance matrix for environmental variables were used to highlight eventual groups of samples among the six beaches in both seasons. Principal component analyses (PCA) were performed to investigate the relationships between environmental variables and the distance from the shoreline. Missing values were excluded from the analyses.

Mean zonations (m) of Uruguayan and Italian populations were calculated as the mean distance of densities (ind/m^2) from the shoreline in both seasons; the comparison of beaches was carried out with one-way ANOVA, with "sea/ocean" (Mediterranean or Atlantic) as the main factor; Tukey's Honestly Significant Difference (Tukey's HSD) post-hoc test for pairwise comparisons was performed to highlight differences among groups of beaches and seasons. The variations of density, mean zonation and concentration of orientation of the six beaches in both seasons as functions of environmental variables were modelled, selecting the best models according to the coefficient of determination (adjusted R^2) and the statistical significance.

3. Results

3.1. Collelungo and Macchiatonda: environment and zonation

In both seasons, Collelungo beach width was 20 m, while Macchiatonda beach had an extension of 30 m; the beach profile of Macchiatonda differed between the two seasons, presenting a higher elevation in spring (Fig. 2).

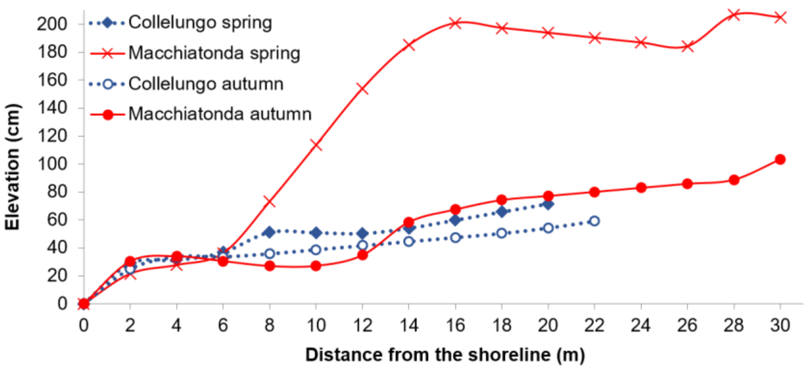


Figure 2. Beach profile (elevation) of Collelungo and Macchiatonda in spring and autumn, from the shoreline to the base of the dune.

Table 2. Characterization of physical-chemical and biological variables (mean ± SE) of Collelungo and Macchiatonda in spring and autumn. Comparisons between beaches and seasons. ANOVA: ***$p<0.001$, **$p<0.01$, *$p<0.05$, # $0.05<p<0.10$ and *ns* = non-significant.

	SPRING		AUTUMN		Beaches		Seasons	
	A: Ma	**B: Co**	**C: Ma**	**D: Co**	**AxB**	**CxD**	**AxC**	**BxD**
Compaction (kg·cm^{-2})	1.82±0.35	2.33±0.08	1.62±0.14	2.82±0.08	*	***	*ns*	***
Organic matter (%)	0.84±0.10	1.40±0.05	1.37±0.05	1.19±0.03	***	*	**	**
Sediment water content (%)	5.11±2.27	9.77±1.86	3.00±0.96	9.89±1.93	*ns*	*	*ns*	*ns*
Temperature (°C)	20.61±0.43	20.69±0.39	24.97±0.16	25.37±0.96	*ns*	*ns*	***	***
Grain size (μm)	502.08±14.99	222.81±8.57	485.53±3.43	224.45±4.61	***	***	*ns*	*ns*
Sorting (μm)	1.30±0.01	1.35±0.02	1.29±0.00	1.36±0.01	#	**	*ns*	*ns*
Elevation (cm)	136±19.30	45.54±6.08	56.39±7.50	38.69±4.49	*ns*	*ns*	***	*ns*
Abundance (n/station)	7.39±3.65	21.94±15.55	1.17±1.17	27.13±9.52	*ns*	#	*ns*	*ns*
Density (ind/m^2)	29.56±14.62	87.76±62.21	4.67±4.67	108.53±38.09	*ns*	#	*ns*	*ns*

Elevation data correspond to the whole beach, from the shoreline to the base of the dune. Ma, Macchiatonda; Co, Collelungo

In spring, Collelungo beach had significantly higher sediment compaction, organic matter content, almost significantly higher sediment sorting (ANOVA: *p*=0.05) and lower grain size than Macchiatonda; in autumn, Collelungo had significantly higher compaction, sediment water content, sorting, almost significantly higher sandhopper abundance and density (ANOVA: *p*=0.08) and lower organic matter and grain size than Macchiatonda (Table 2).

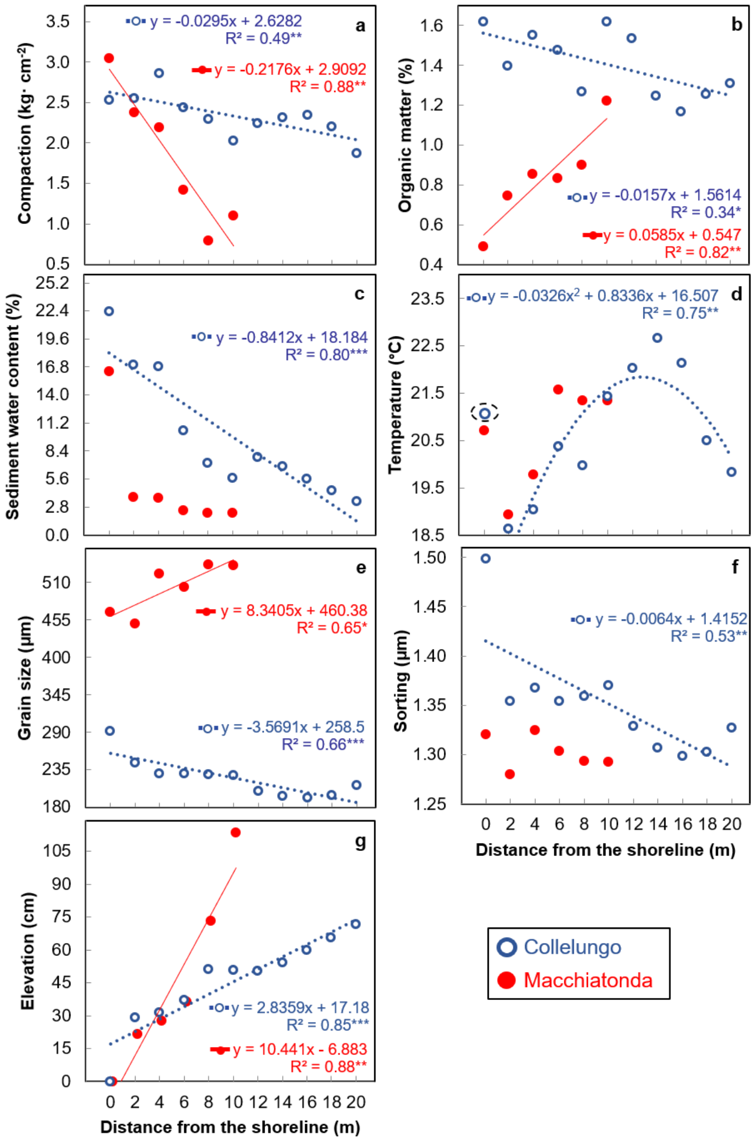


Figure 3. Spring across-shore variations in environmental characteristics on Collelungo (○) and Macchiatonda (●): a, compaction; b, organic matter; c, sediment water content; d, temperature; e, grain size; f, sorting; g, elevation. The significant best models fitted between mean values per sampling level and the distance from the shoreline are shown: ***p<0.001, **p<0.01 and *p<0.05. The outlier excluded from model fitting in (d) is encircled (dashed line).

Regarding differences between seasons, Macchiatonda in spring had lower organic matter and temperature and higher elevation with respect to autumn; Collelungo in spring had lower compaction and temperature and higher organic matter content with respect to autumn (Table 2).

In spring (Fig 3a-g), the variation of organic matter presented two opposite linear trends: increasing landwards on Macchiatonda and decreasing on Collelungo (Fig. 3b); a quadratic model explained the across-shore variation of temperature on Collelungo when station 0 was excluded from the model fitting, while no model was found for Macchiatonda (Fig. 3d); grain size linearly increased landwards on Macchiatonda and decreased on Collelungo (Fig. 3e).

In autumn (Fig. 4a-g), the across-shore variations in environmental characteristics were only explained for Collelungo beach, except for compaction, the variation of which was not explained by any model for either of the two beaches (Fig. 4a), and sorting, linearly modelled on both beaches, decreasing landwards (Fig. 4f).

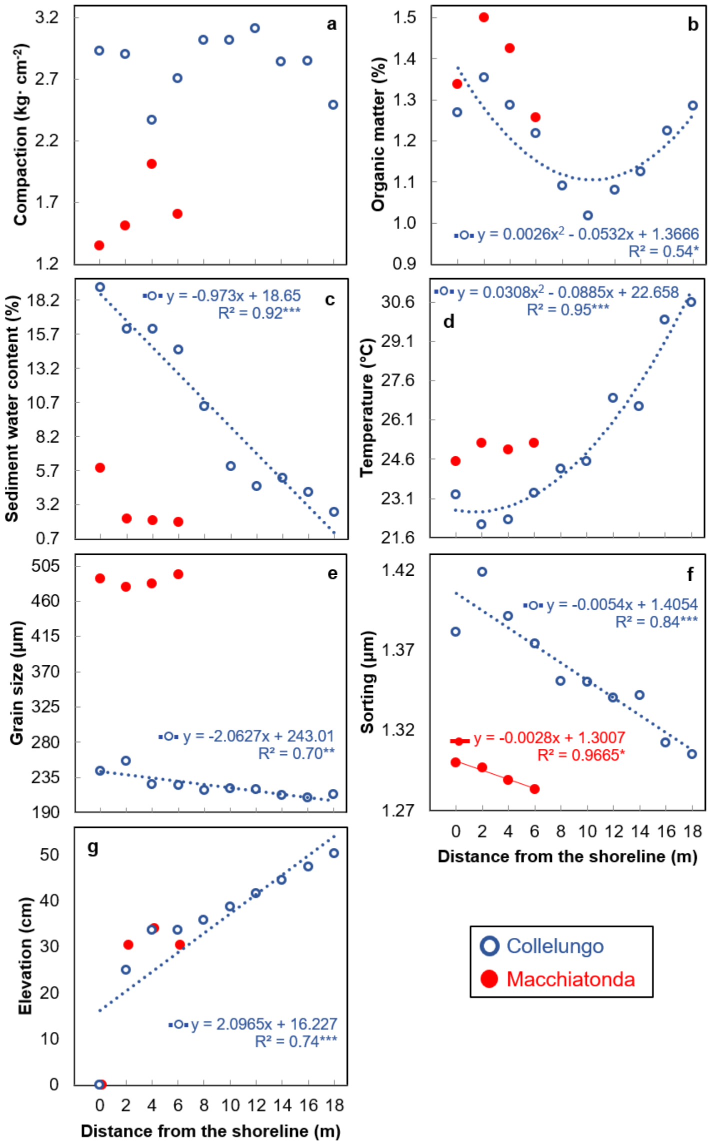


Figure 4. Autumn across-shore variations in environmental characteristics on Collelungo (○) and Macchiatonda (●): a, compaction; b, organic matter; c, sediment water content; d, temperature; e, grain size; f, sorting; g, elevation. The significant best models fitted between mean values per sampling level and the distance from the shoreline are shown: ***$p<0.001$, **$p<0.01$ and *$p<0.05$.

In spring, the highest density value of *T. saltator* was found at station 2 and decreased seawards and landwards on both beaches (Fig. 5a). On Macchiatonda, density decreased linearly landwards ($y=-0.9925x+8.7563$, adjusted-$R^2=0.70$, $p<0.05$). In autumn, on Collelungo the highest density was found in station 2, decreasing landwards (with similar values in stations 4 and 8), while on Macchiatonda sandhoppers were found only in station 0 (Fig. 5b). On Collelungo, density linearly decreased landwards ($y=-1.0115x+17.171$, adjusted-$R^2=0.75$, $p<0.001$).

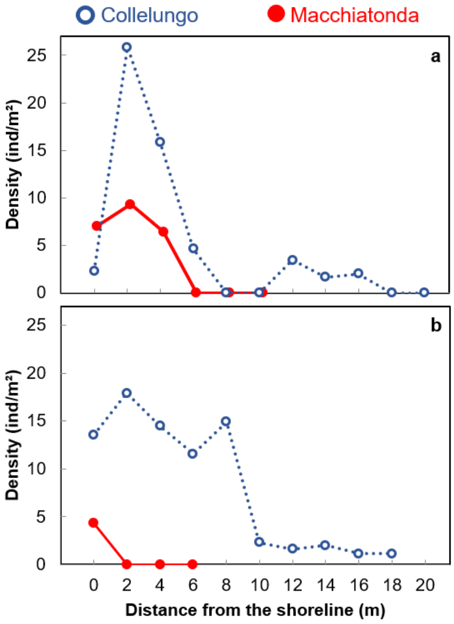


Figure 5. Across-shore density variations of *Talitrus saltator* of Collelungo (○) and Macchiatonda (●) beaches: a, spring; b, autumn.

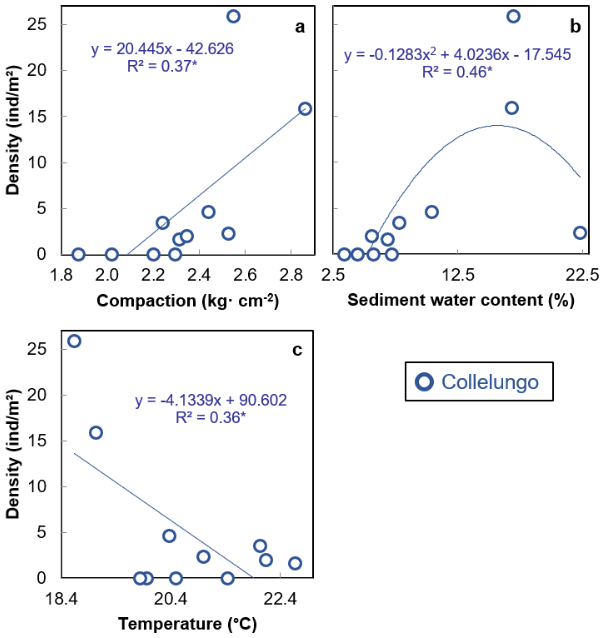


Figure 6. Spring density variations of *Talitrus saltator* on Collelungo (○) beach as a function of: a, compaction; b, sediment water content; c, temperature. The best models fitted between density values per sampling level and the environmental variables are shown: **p*<0.05.

In spring, on Collelungo beach, sandhopper density increased with compaction, and its highest value corresponded to a compaction equal to 2.6 kg/cm^2 (Fig. 6a); density was higher at intermediate percentages of water content (17%; Fig. 6b), and lower values of temperature (18.6°C; Fig. 6c).

In autumn, on Collelungo beach, density linearly increased towards higher percentages of water content (Fig. 7a), smaller values of temperature (Fig. 7b) and higher values of grain size and sorting (Figs. 7c and 7d); a quadratic model explained the density variation as a function of elevation (Fig. 7e). In Fig. 7b is also shown the linear model explaining the variation of sediment water content as a function of temperature, with higher percentages of water content at smaller values of temperature.

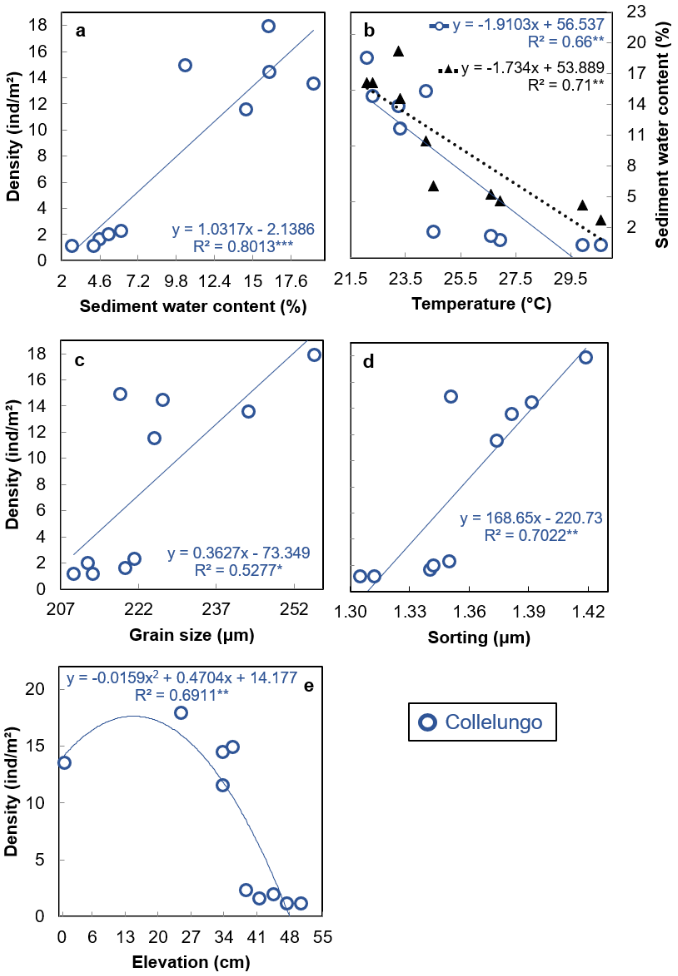


Figure 7. Autumn density variations of *Talitrus saltator* on Collelungo (○) beach as a function of: a, sediment water content; b, temperature; c, grain size; d, sorting; e, elevation. The sediment water content (▲) as a function of temperature is also shown in (b). The best models fitted between density values per sampling level and the environmental variables are shown: ***$p<0.001$, **$p<0.01$ and *$p<0.05$.

The population structure of *T. saltator* presented high relative percentages of abundance of juveniles at the shoreline in both seasons and beaches, decreasing from the shoreline going inland, while adult percentages increased landwards (Fig. 8). In spring, on Collelungo beach, adults were found starting from 2 m from the shoreline, while in autumn they were found also in the station 0, as on Macchiatonda beach in both seasons. On Collelungo beach, higher or similar percentages of females were found near the dune with respect to males, in both seasons (except for station 14 in autumn; Fig. 8). On

Macchiatonda beach, higher percentages of males with respect to females were found in spring near the shoreline, and only males (and juveniles) were found in station 0 in autumn (Fig. 8).

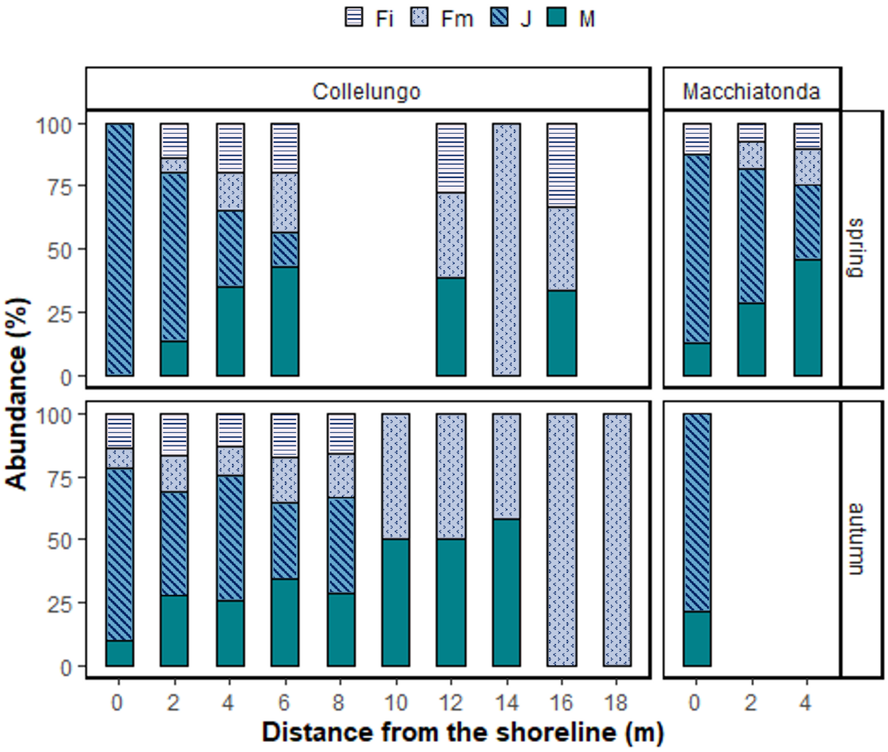


Figure 8. Across-shore population structure of *Talitrus saltator* in spring and autumn, on Collelungo and Macchiatonda beaches. Fi, immature females; Fm, mature females; J, juveniles; M, males.

Regarding size and age proxies of sandhoppers in the two seasons, on Collelungo, cephalic lengths and antennal segments were significantly lower in spring than in autumn (ANOVA: $p<0.001$), while the opposite was found on Macchiatonda (ANOVA: $p<0.01$). In spring, no differences were found for size and age proxies between beaches; in autumn, the two variables were significantly larger on Collelungo than on Macchiatonda (Table 3).

In both seasons and beaches, the proportion of juveniles out of the total number of collected individuals was the highest of the four classes considered, while females (mature and immature) always presented the lowest proportion; on Macchiatonda in autumn, no females and only one male were found (Table 3).

Table 3. Population variables of *Talitrus saltator* for Collelungo and Macchiatonda beaches, in spring and autumn. Comparisons between beaches, ANOVA: *$p<0.05$ and *ns* = non-significant.

	Collelungo	**J**	**Fi**	**Fm**	**M**
spring	Number of individuals	528	50	29	117
tot=724	Proportion: ind/tot	0.73	0.07	0.04	0.16
	Cephalic length (mm): mean ± se	0.52 ± 0.00	1.04 ± 0.01	1.29 ± 0.03	1.04 ± 0.01
	Number of antennal segments: mean ± se	7.33 ± 0.07	17.48 ± 0.27	23.24 ± 0.46	22.47 ± 0.39
	Sex ratio [M/(M+F)]	0.60			
autumn	Number of individuals	495	61	6	197
tot=814	Proportion: ind/tot	0.61	0.07	0.07	0.24
	Cephalic length (mm): mean ± se	0.49 ± 0.00	1.07 ± 0.02	1.27 ± 0.01	1.15 ± 0.01
	Number of antennal segments: mean ± se	6.69 ± 0.08	19.79 ± 0.41	23.52 ± 0.20	25.18 ± 0.31
	Sex ratio [M/(M+F)]	0.62			
	Macchiatonda	**J**	**Fi**	**Fm**	**M**
spring	Number of individuals	91	3	4	35
tot=133	Proportion: ind/tot	0.68	0.02	0.03	0.26
	Cephalic length (mm): mean ± se	0.61 ± 0.01	0.8 ± 0.00	1.34 ± 0.03	0.9 ± 0.05
	Number of antennal segments: mean ± se	8.86 ± 0.22	12.67 ± 0.88	22.75 ± 0.48	16.57 ± 1.25
	Sex ratio [M/(M+F)]	0.83			
autumn	Number of individuals	13	0	0	1
tot=14	Proportion: ind/tot	0.93	0	0	0.07
	Cephalic length (mm): mean ± se	0.50 ± 0.02	-	-	0.72
	Number of antennal segments: mean ± se	6.69 ± 0.54	-	-	14
	Sex ratio [M/(M+F)]	1			

		Collelungo	**Macchiatonda**	*p*
spring	Mean cephalic length ± se (min - max)	0.67 ± 0.01 mm (0.32 - 1.60)	0.71 ± 0.02 mm (0.4 - 1.6)	*ns*
	Mean: number of antennal segments ± se (min - max)	11.12 ± 0.25 (5 - 33)	11.39 ± 0.49 (5 - 32)	*ns*
autumn	Mean cephalic length ± se (min - max)	0.75 ± 0.01 mm (0.32 - 1.56)	0.52 ± 0.03 mm (0.40 - 0.72)	*
	Mean number of antennal segments ± se (min - max)	13.37 ± 0.31 (5 - 33)	7.21 ± 0.72 (5 - 14)	*

tot = total of captured individuals; se, standard error; M, males; F, females; J, Juveniles; Fi, immature females; Fm, mature females

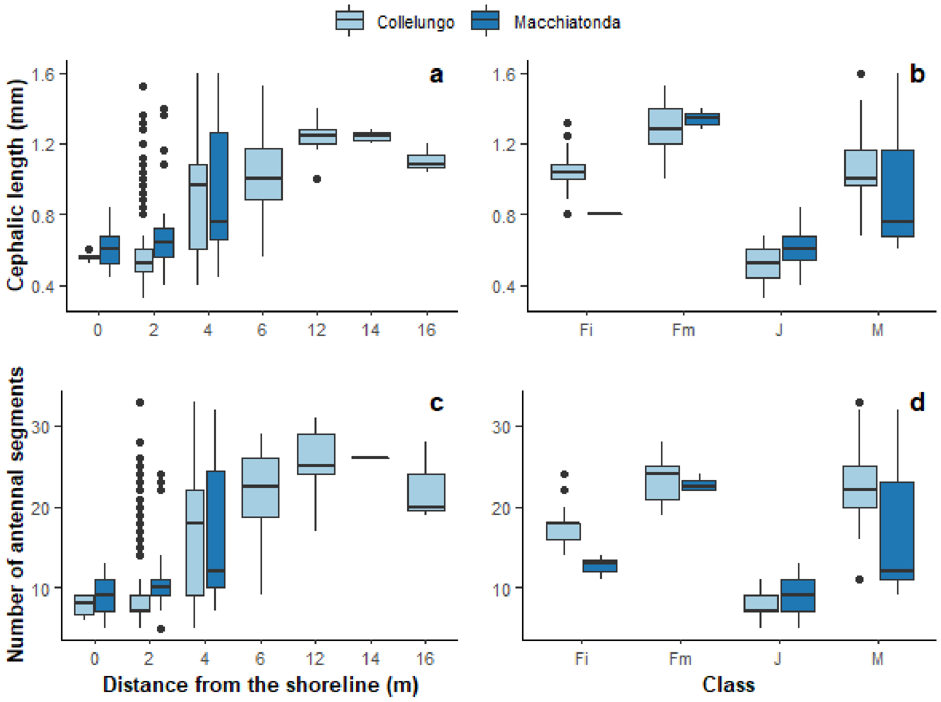


Figure 9. Across-shore (a and c) and among classes (b and d) distributions of population variables of *Talitrus saltator* on Collelungo and Macchiatonda beaches in spring. a and b, cephalic length; c and d, number of antennal segments. Fi, immature females; Fm, mature females; J, juveniles; M, males.

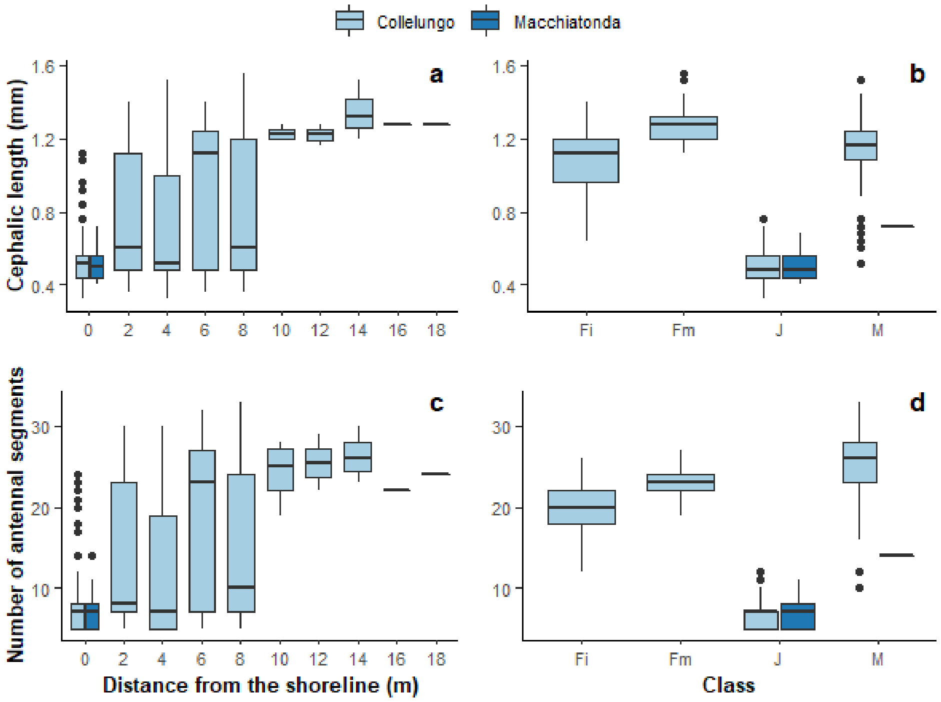


Figure 10. Across-shore (a and c) and among classes (b and d) distributions of population variables of *Talitrus saltator* on Collelungo and Macchiatonda beaches in autumn. a and b, cephalic length; c and d, number of antennal segments. Fi, immature females; Fm, mature females; J, juveniles; M, males.

Regarding the number of antennal segments, the highest values among classes were presented by mature females in spring and males in autumn; juveniles always presented the lowest values (Table 3; Figs. 9d, 10d). Sex ratio was always male biased (*n.b.* the absence of females on Macchiatonda in autumn; Table 3). The across-shore distributions of the cephalic lengths and number of antennal segments reflect the distribution of adults and juveniles, the latter being abundant near the shoreline (Figs. 9a, 9c, 10a, 10c; see also Fig. 8).

3.2. Bocca di Serchio and Buca del Mare: environment and zonation

Bocca di Serchio beach width was 24 m in spring and 14 m in autumn, while Buca del Mare beach had an extension of 20 m in spring and 18 m in autumn, with no apparent differences in elevation (Fig. 11).

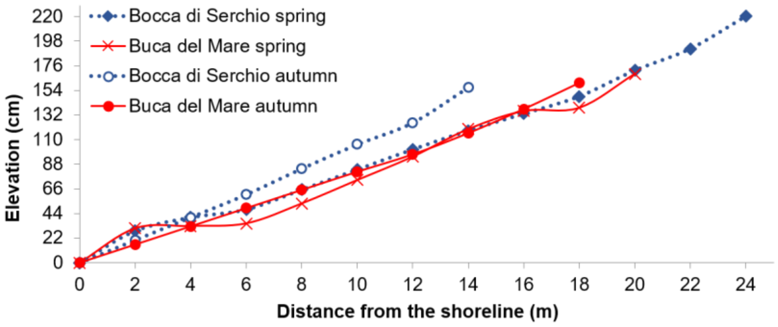


Figure 11. Beach profiles (elevation) of Bocca di Serchio and Buca del mare in spring and autumn, from the shoreline to the base of the dune.

In spring, Bocca di Serchio beach had significantly lower substrate compaction than Buca del Mare; in autumn, Bocca di Serchio had lower compaction and greater sediment organic matter content and grain size than Buca del Mare (Table 4). Regarding differences between seasons, Bocca di Serchio in spring had almost significantly higher temperature and grain size with respect to autumn (ANOVA: *p*=0.07); Buca del Mare in spring had significantly higher temperature and grain size with respect to autumn (Table 4).

Table 4. Characterization of physical-chemical and biological variables (mean ± SE) of Bocca di Serchio and Buca del Mare in spring and autumn. Comparisons between beaches and seasons, ANOVA: ***$p<0.001$, **$p<0.01$, *$p<0.05$, # $0.05<p<0.10$ and *ns* = non-significant.

	SPRING		AUTUMN		Beaches		Seasons	
	A: BS	**B: BM**	**C: BS**	**D: BM**	**AxB**	**CxD**	**AxC**	**BxD**
Compaction (kg·cm^{-2})	1.75±0.09	2.14±0.05	1.49±0.13	2.07±0.16	**	*	*ns*	*ns*
Organic matter (%)	0.88±0.08	0.81±0.04	0.94±0.03	0.77±0.02	*ns*	***	*ns*	*ns*
Sediment water content (%)	5.43±2.61	5.13±1.62	7.36±1.43	6.21±1.29	*ns*	*ns*	*ns*	*ns*
Temperature (°C)	24.73±0.53	25.19±0.73	23.75±0.21	23.09±0.42	*ns*	*ns*	#	*
Grain size (μm)	355.51±6.29	354.95±5.98	335.78±6.01	317.82±4.45	*ns*	*	#	**
Sorting (μm)	1.30±0.01	1.31±0.01	1.30±0.01	1.30±0.01	*ns*	*ns*	*ns*	*ns*
Elevation (cm)	103.84±18.67	80.20±16.50	74.21±18.99	75.52±16.71	*ns*	*ns*	*ns*	*ns*
Abundance (n/station)	20.75±12.47	34.27±17.96	5.04±1.69	5.10±2.27	*ns*	*ns*	*ns*	*ns*
Density (ind/m^2)	83±49.89	137.07±71.83	20.17±6.78	20.40±9.07	*ns*	*ns*	*ns*	*ns*

Elevation data correspond to the whole beach, from the shoreline to the base of the dune. BS, Bocca di Serchio; BM, Buca del Mare

In spring (Fig. 12a-g), on Bocca di Serchio, sediment temperature increased linearly going inland ($p=0.06$), whereas on Buca del Mare a quadratic model explained its across-shore variation (Fig. 12d). On Bocca di Serchio, sorting decreased linearly from the shoreline going inland, while on Buca del Mare a quadratic model explained its across-shore variation, which also decreased landwards (Fig. 12f).

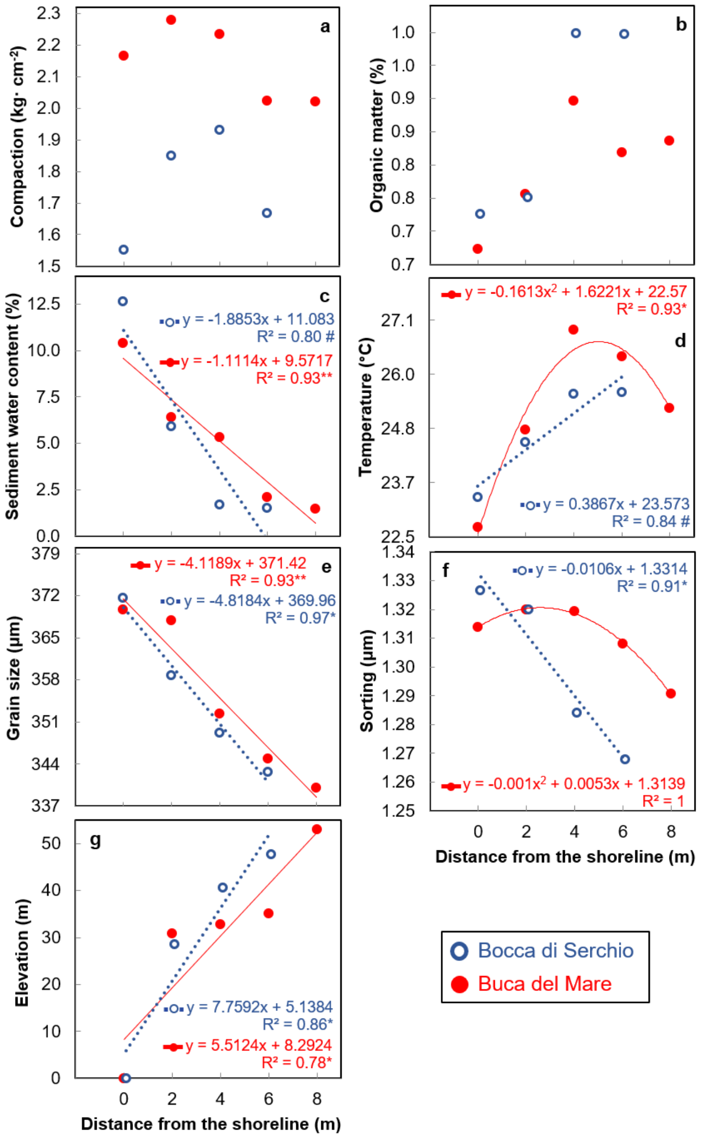


Figure 12. Spring across-shore variations in environmental characteristics on Bocca di Serchio (○) and Buca del Mare (●): a, compaction; b, organic matter; c, sediment water content; d, temperature; e, grain size; f, sorting; g, elevation. The best models fitted between the mean values per sampling level and the distance from the shoreline are shown: ***p*<0.01, **p*<0.05 and # 0.05<*p*<0.10.

In autumn (Fig. 13a-g), on Bocca di Serchio compaction increased linearly going inland, whereas on Buca del Mare a quadratic model explained its across-shore variation (Fig. 13a). Logarithmic models explained the variation of sediment water content, decreasing landwards on both beaches (Fig. 13c).

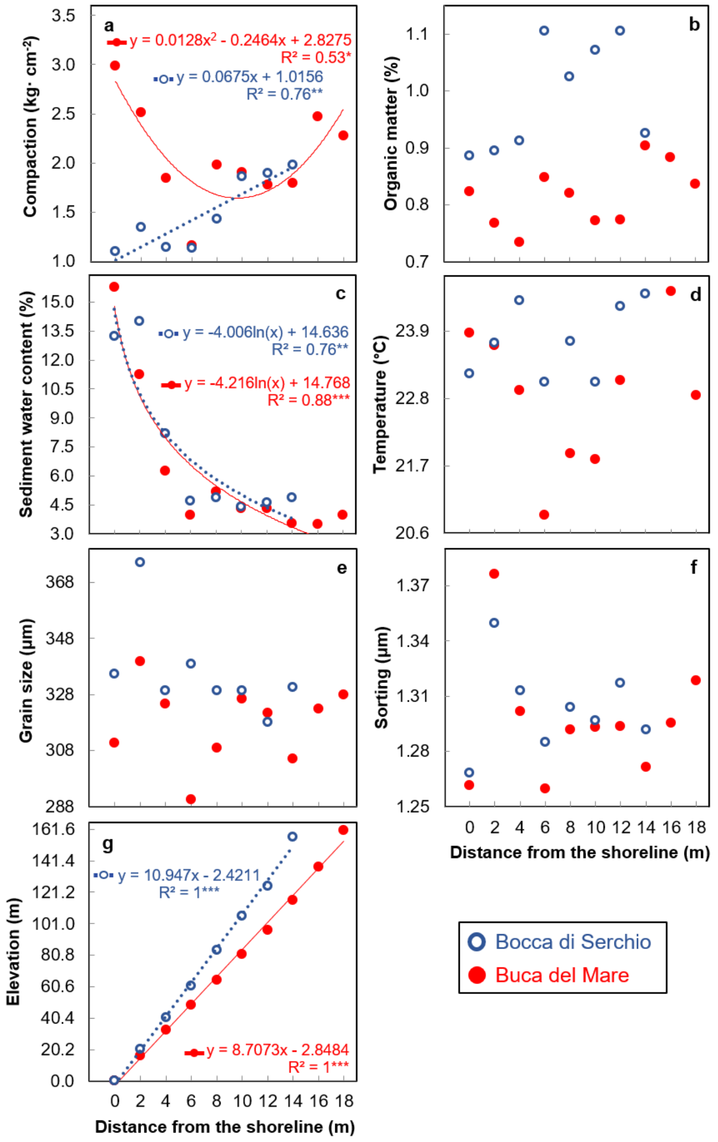


Figure 13. Autumn across-shore variations in environmental characteristics on Bocca di Serchio (○) and Buca del Mare (●): a, compaction; b, organic matter; c, sediment water content; d, temperature; e, grain size; f, sorting; g, elevation. The best models fitted between mean values per sampling level and the distance from the shoreline are shown: ****p*<0.001, ***p*<0.01 and **p*<0.05.

In spring, higher density values of *T. saltator* were found at stations 0 and 2 and decreased landwards on both beaches (Fig. 14a). In autumn, the highest density was found at station 4 in both beaches, decreasing seawards and landwards, with a more extended across-shore distribution with respect to spring (Fig. 14b). On Buca del Mare, in both seasons, density decreased linearly landwards (spring: $y=-2.6457x+19.233$, adjusted-$R^2=0.8663$; autumn: $y=-0.3644x+6.8168$, adjusted-$R^2=0.4999$; $p<0.05$).

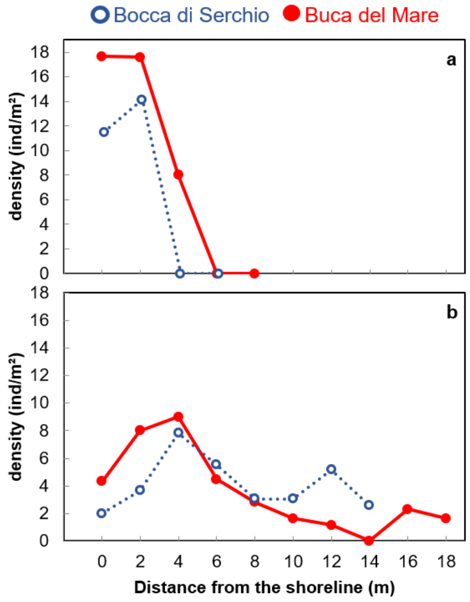


Figure 14. Across-shore density variations of *Talitrus saltator* of Bocca di Serchio (○) and Buca del mare (●) beaches: a, spring; b, autumn.

On Buca del Mare, in autumn, the variation of sandhoppers density as a function of organic matter was linearly modelled (Fig. 15a): although the correlation was almost significant (p=0.06) and the coefficient of variation quite low (adjusted-R^2=0.3), it can be noticed that the highest density values (square-root ind/m^2 equal to 8 and 9.02) corresponded to the lowest values of organic matter content (0.72% and 0.68%), in stations 2 and 4 (Figs. 14 and 15a); then the variation of density decreased with higher values of organic matter content (Fig. 15a). Density decreased landwards with the increase of elevation (Fig. 15b).

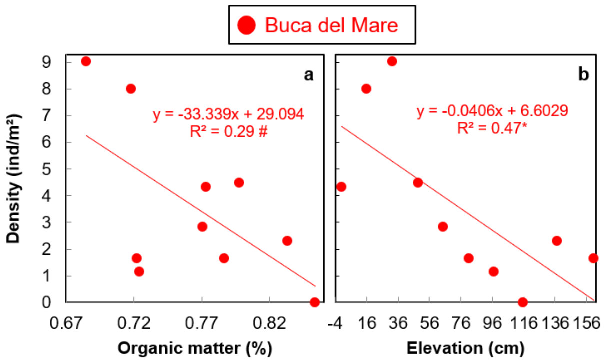


Figure 15. Autumn density variations of *Talitrus saltator* on Buca del Mare (●) beach as a function of: a, sediment organic matter content; b, elevation. The best models fitted between density values per sampling level and the environmental variables are shown: **p<0.01 and # 0.05<p<0.10.

The population structure in spring presented higher relative percentages of abundance of adults than juveniles on Bocca di Serchio beach, while on Buca del Mare the relative percentages of females (immature and mature), males and juveniles were similar, the latter slightly decreasing from shoreline to inland, while adult relative percentages increased landwards; the relative percentages of mature females were lower than immature females on both beaches (Fig. 16). In autumn, only juveniles were found on Bocca di Serchio at stations 0 and 2, their relative percentages decreasing landwards, while on Buca del Mare juveniles were similarly present from station 0 to 8; the relative percentages of males were greater or similar than those of females on both beaches; on Buca del Mare only males were found in stations 10 and 12; the relative percentages of mature females were lower on Bocca di Serchio (station 12), or equal (station 18) to the ones of immature females on Buca del Mare (Fig. 16).

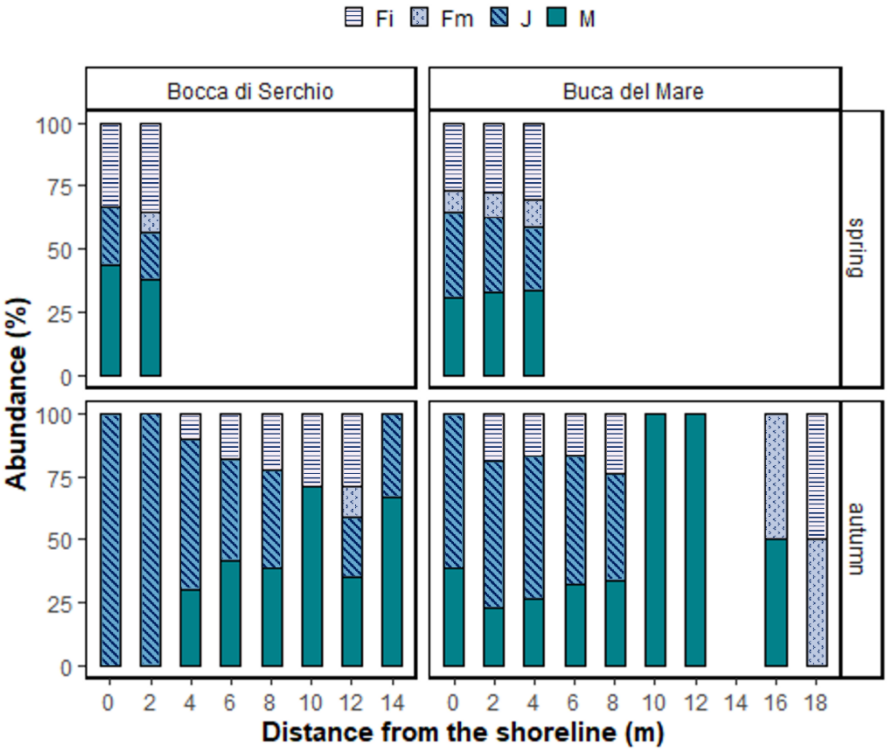


Figure 16. Across-shore population structure of *Talitrus saltator* in spring and autumn, on Bocca di Serchio and Buca del Mare beaches. Fi, immature females; Fm, mature females; J, juveniles; M, males.

Regarding size and age proxies, on both beaches cephalic length and antennal segment differed between seasons (ANOVA: $p<0.001$), the values in spring being larger than in autumn. In both seasons, the two variables were significantly greater on Bocca di Serchio than on Buca del Mare (Table 5).

Table 5. Population variables of *Talitrus saltator* for Bocca di Serchio and Buca del Mare beaches, in spring and autumn. Comparisons between the two beaches, ANOVA: ***$p<0.001$.

	Bocca di Serchio	**J**	**Fi**	**Fm**	**M**
spring	Number of individuals	32	91	3	123
tot=249	Proportion: ind/tot	0.13	0.37	0.01	0.49
	Cephalic length (mm): mean ± se	0.61 ± 0.01	0.94 ± 0.01	1.35 ± 0.13	0.92 ± 0.01
	Number of antennal segments: mean ± se	9.47 ± 0.25	16.34 ± 0.20	23.67 ± 1.33	17.93 ± 0.37
	Sex ratio [M/(M+F)]	0.57			
autumn	Number of individuals	67	11	1	42
tot=121	Proportion: ind/tot	0.55	0.09	0.01	0.35
	Cephalic length (mm): mean ± se	0.54 ± 0.01	0.89 ± 0.07	1.2	0.93 ± 0.03
	Number of antennal segments: mean ± se	8.30 ± 0.21	18.1 ± 1.6	23	20.07 ± 0.95
	Sex ratio [M/(M+F)]	0.78			
	Buca del Mare	**J**	**Fi**	**Fm**	**M**
spring	Number of individuals	177	138	16	183
tot=514	Proportion: ind/tot	0.34	0.27	0.03	0.36
	Cephalic length (mm): mean ± se	0.59 ± 0.01	0.92 ± 0.01	1.19 ± 0.03	0.87 ± 0.01
	Number of antennal segments: mean ± se	9.03 ± 0.15	16.38 ± 0.19	22 ± 0.26	17.03 ± 0.32
	Sex ratio [M/(M+F)]	0.54			
autumn	Number of individuals	108	11	3	31
tot=153	Proportion: ind/tot	0.71	0.07	0.02	0.20
	Cephalic length (mm): mean ± se	0.53 ± 0.01	0.80 ± 0.03	1.23 ± 0.05	0.75 ± 0.04
	Number of antennal segments: mean ± se	8.01 ± 0.20	15 ± 0.38	23.33 ± 0.33	14.48 ± 0.99
	Sex ratio [M/(M+F)]	0.69			

		Bocca di Serchio	**Buca del Mare**	*p*
spring	Mean cephalic length ± se (min - max)	0.89 ± 0.01 mm (0.44 - 1.60)	0.80 ± 0.01 mm (0.4 - 1.48)	***
	Mean number of antennal segments ± se (min - max)	16.33 ± 0.27 (7 - 30)	14.25 ± 0.22 (5 - 28)	***
autumn	Mean cephalic length ± se (min - max)	0.71 ± 0.02 mm (0.36 - 1.28)	0.61 ± 0.01 mm (0.36 - 1.28)	***
	Mean number of antennal segments ± se (min - max)	13.40 ± 0.64 (5 - 31)	10.12 ± 0.38 (5 - 29)	***

tot = total of captured individuals; se, standard error; M, males; F, females; J, juveniles; Fi, immature females; Fm, mature females

The proportion of the four classes differed between beaches and seasons (Table 5). Mature females always presented the lowest proportion; in spring, on both beaches, the highest proportion among classes was presented by males; on Bocca di Serchio, males were followed by immature females and juveniles, while on Buca del Mare, juveniles presented a similar proportion with respect to males. In autumn, on both beaches, juveniles presented the highest proportion, followed by males and females. The greatest cephalic length among classes was always presented by mature females, while juveniles had the smallest values, and immature females had higher values with respect to males, except for Bocca di Serchio in autumn (Table 5; Figs 17b, 18b). Regarding the number of antennal segments, mature females had the highest values, followed by males and immature females (except for Buca del Mare in autumn, where immature females presented higher values than males); juveniles presented the lowest values (Table 5; Figs 17d, 18d). In both beaches and seasons, sex ratios were male biased. The across-shore distributions of the cephalic length and antennal segments reflect the distribution of adults and juveniles, presenting in spring similar across-shore values for the presence of almost all classes in each station (Figs. 16, 17a, 17c), and in autumn showing higher frequencies of juveniles near the shoreline, which decreased landwards, while adults presented an opposite trend (Figs. 16, 18a, 18c).

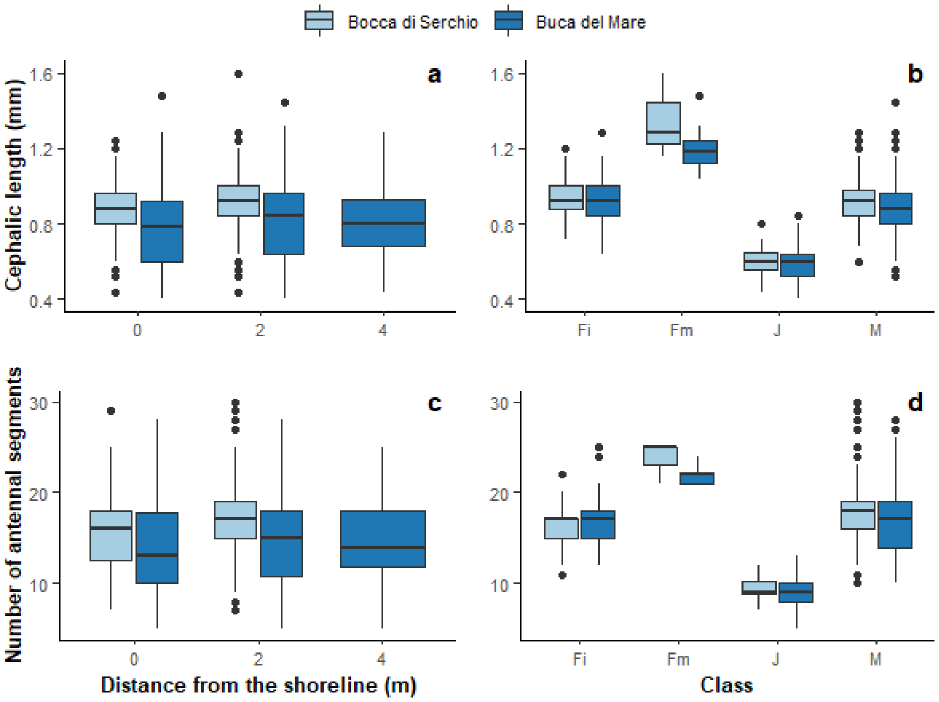


Figure 17. Across-shore (a and c) and among classes (b and d) distributions of population variables of *Talitrus saltator* on Bocca di Serchio and Buca del Mare beaches in spring. a and b, cephalic length; c and d, number of antennal segments. Fi, immature females; Fm, mature females; J, juveniles; M, males.

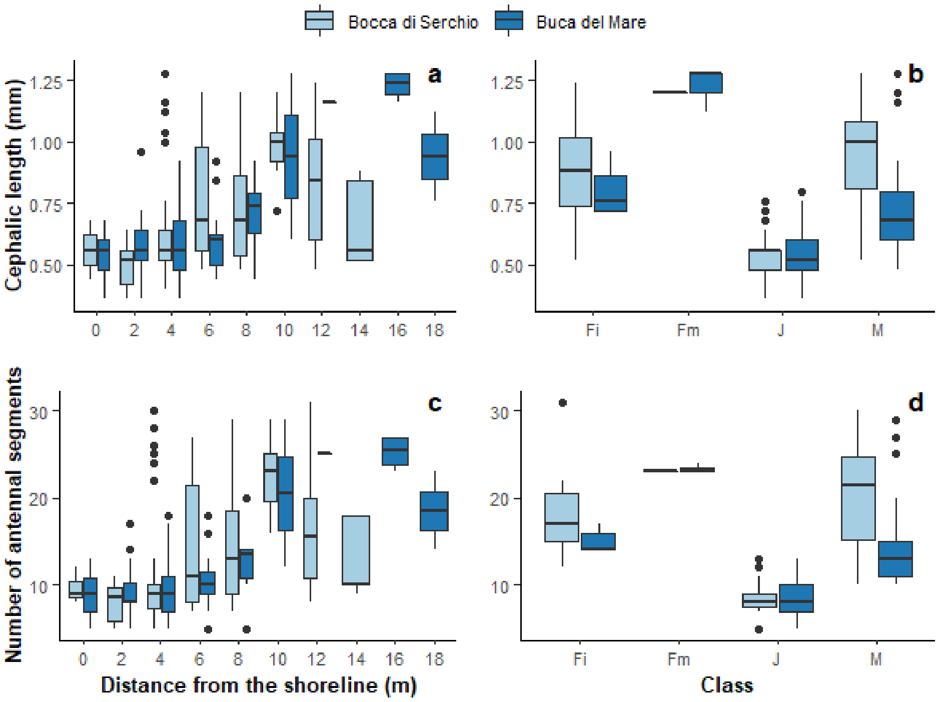


Figure 18. Across-shore (a and c) and among classes (b and d) distributions of population variables of *Talitrus saltator* on Bocca di Serchio and Buca del Mare beaches in autumn. a and b, cephalic length; c and d, number of antennal segments. Fi, immature females; Fm, mature females; J, juveniles; M, males.

3.3. José Ignacio and La Viuda: environment and zonation

José Ignacio beach width was 16 m in spring and 20 m in autumn, while La Viuda beach had an extension of 44 m in spring and 48 m in autumn (Fig. 19).

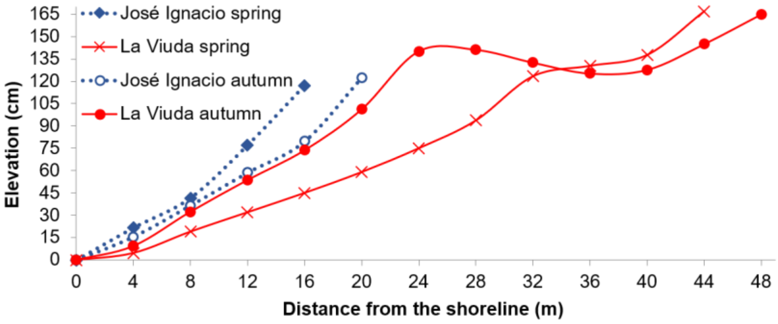


Figure 19. Beach profile (elevation) of José Ignacio and La Viuda in spring and autumn, from the shoreline to the base of the dune.

In spring, La Viuda beach had significantly greater organic matter content and lower sorting than José Ignacio; in autumn, La Viuda had greater organic matter and grain size than José Ignacio (Table 6). Regarding differences between seasons, La Viuda in spring had significantly higher temperature with respect to autumn; José Ignacio in spring had significantly higher temperature, grain size and sorting with respect to autumn (Table 6).

Table 6. Characterization of physical-chemical and biological variables (mean ± SE) of La Viuda and José Ignacio in spring and autumn. Comparisons between the beaches and seasons, ANOVA: ***p<0.001, **p<0.01, *p<0.05 and *ns* = non-significant.

	SPRING		AUTUMN		Beaches		Seasons	
	A: Vi	**B: JI**	**C: Vi**	**D: JI**	**AxB**	**CxD**	**AxC**	**BxD**
Compaction ($kg \cdot cm^{-2}$)	3.41±0.25	3.78±0.10	3.77±0.17	3.60±0.17	*ns*	*ns*	*ns*	*ns*
Organic matter (%)	0.33±0.02	0.22±0.02	0.35±0.03	0.22±0.01	*	**	*ns*	*ns*
Sediment water content (%)	8.40±2.09	5.71±0.83	7.35±2.04	3.85±0.77	*ns*	*ns*	*ns*	*ns*
Temperature (°C)	24.72±0.73	25.91±0.39	20.54±0.17	20.17±0.36	*ns*	*ns*	***	***
Grain size (µm)	341.08±21.32	374.72±31.89	331.09±13.10	235.58±15.86	*ns*	**	*ns*	**
Sorting (µm)	1.50±0.04	1.90±0.09	1.46±0.02	1.39±0.07	**	*ns*	*ns*	**
Elevation (cm)	73.89±16.25	51.47±20.71	95.91±15.49	52.17±18.27	*ns*	*ns*	*ns*	*ns*
Abundance (n/station)	0.4±0.19	1.08±1.08	0.52±0.26	0.28±0.16	*ns*	*ns*	*ns*	*ns*
Density (ind/m^2)	6.67±3.14	18.06±18.06	8.73±4.35	4.63±2.65	*ns*	*ns*	*ns*	*ns*

Elevation data correspond to the whole beach, from the shoreline to the base of the dune. Vi, La Viuda; JI, José Ignacio

In spring (Fig. 20a-g), sediment water content decreased linearly on La Viuda beach, showing two separate groups of values: higher values between stations 4 and 20 (12.1-16.5%), and lower values between stations 24 and 40 (1.6-4.4%; Fig. 20c); no trend was found for José Ignacio. On La Viuda beach, the values of sorting were separated in two groups, as for sediment water content: analysing the two groups separately, from station 4 to 20, sorting decreased landwards ($y=-0.014x+1.5564$, adjusted-$R^2=0.98$, $p=0.001$), while no significant trend was found from station 24 to 40 (Figs. 20f). Two groups of La Viuda samples were also evident for organic matter content and grain size (Figs. 20b and 20e).

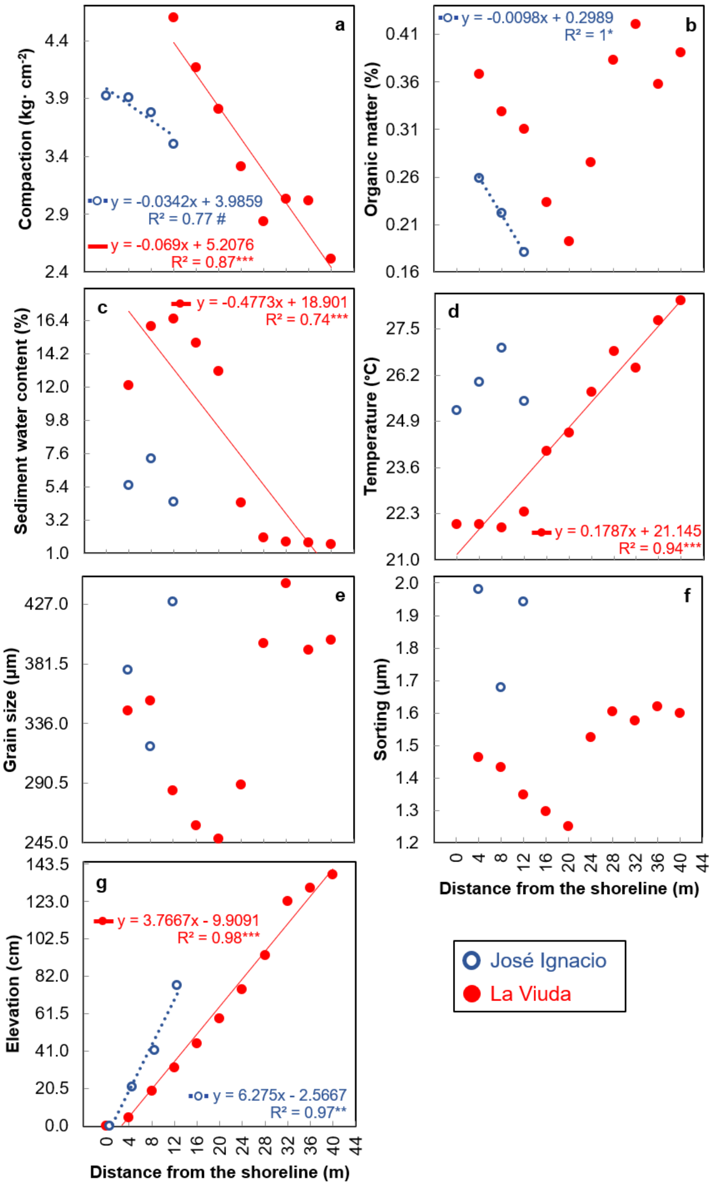


Figure 20. Spring across-shore variations in environmental characteristics on José Ignacio (○) and La Viuda (●): a, compaction; b, organic matter; c, sediment water content; d, temperature; e, grain size; f, sorting; g, elevation. The best models fitted between the mean values per sampling level and the distance from the shoreline are shown: ****p*<0.001, ***p*<0.01, **p*<0.05 and # 0.05<*p*<0.10.

In autumn, on José Ignacio beach, a quadratic model explained the across-shore variation of organic matter, while on La Viuda beach the organic matter decreased linearly (Fig. 21b). On José Ignacio beach, a quadratic model explained the across-shore variation of temperature, while on La Viuda beach sediment temperature linearly increased landwards (Fig. 21d). Two quadratic models explained the variation of grain size on both beaches: the highest value on José Ignacio was at station 20 (295.97 µm), while the highest two values on La Viuda beach were at stations 4 and 24 (362.3 µm and 369.84 µm respectively; Fig. 21e). On José Ignacio beach, a quadratic model explained the variation of sorting, although almost significant (*p*=0.06), while no trend was found for La Viuda beach (Fig. 21f).

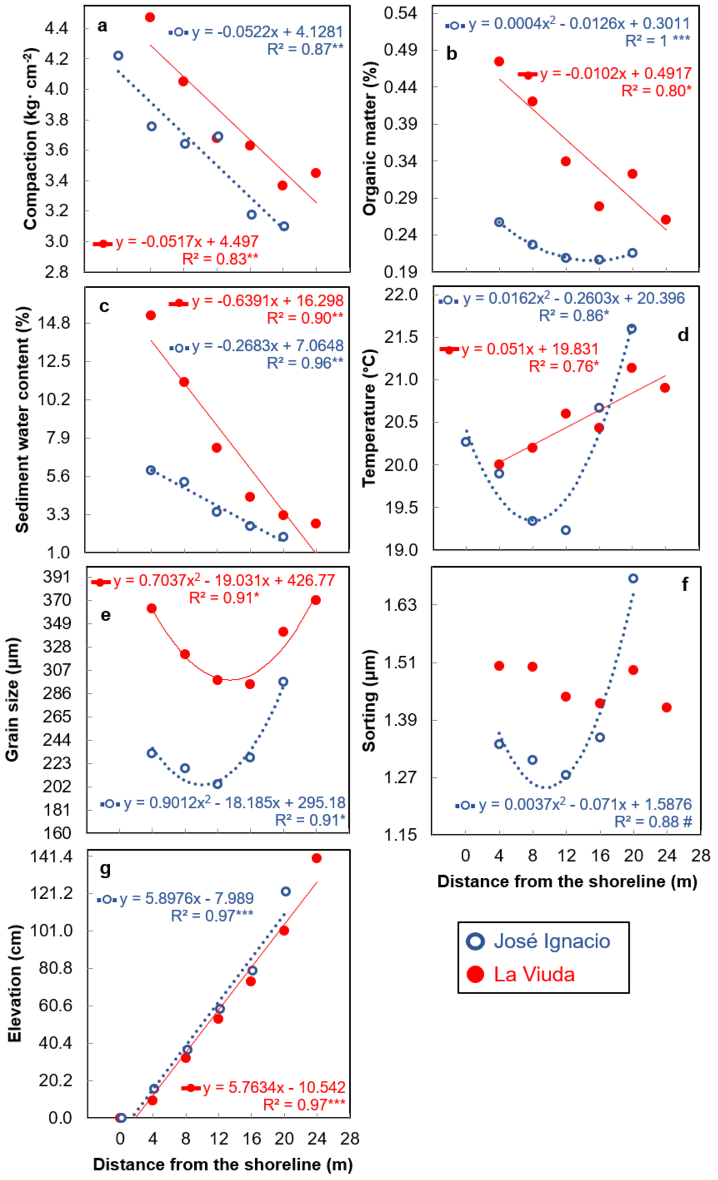


Figure 21. Autumn across-shore variations in environmental characteristics on José Ignacio (○) and La Viuda (●): a, compaction; b, organic matter; c, sediment water content; d, temperature; e, grain size; f, sorting; g, elevation. The best models fitted between mean values per sampling level and the distance from the shoreline are shown: ***$p<0.001$, **$p<0.01$, *$p<0.05$ and # $0.05<p<0.10$.

In spring on José Ignacio, sandhoppers were found only at station 8, while on La Viuda the highest density value was found at station 24 (Fig. 22a). In autumn, on José Ignacio beach the highest density value was found at station 16, while on La Viuda the highest density was found at station 20 (Fig. 22b).

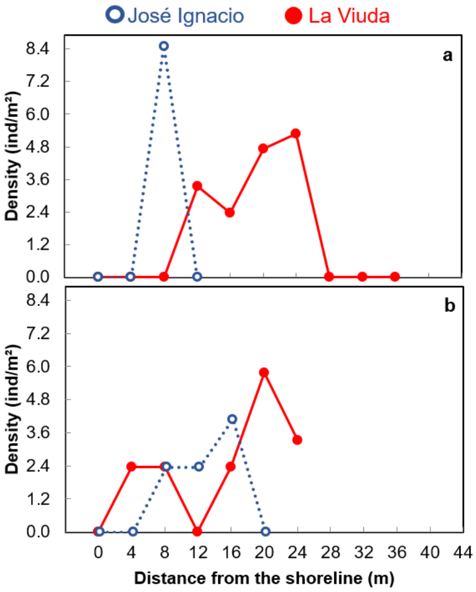


Figure 22. Across-shore density variations of *Atlantorchestoidea brasiliensis* of José Ignacio (○) and La Viuda (●) beaches: a, spring; b, autumn.

On La Viuda, in spring, density decreased linearly with increasing organic matter content (Fig. 23a): sandhoppers were found between stations 12 and 24, corresponding to 0.19% and 0.31% of organic matter content, while at higher values of this variable, no sandhoppers were found (Figs. 22 and 23a). Although almost significant (p=0.06), the correlation between density and temperature was explained by a quadratic model (Fig. 23b): it can be noticed that between the stations where sandhoppers were found, the density values increased with temperature, presenting the highest density value (square-root ind/m^2 equal to 5.27) at 25.73°C, corresponding to station 24 (Figs. 22 and 23b). Density decreased linearly with increasing grain size values: the four stations with sandhoppers corresponded to a range of grain size of 247.9-289.54 µm (Fig. 23c). A quadratic model explained the correlation between density and elevation: sandhoppers were found at intermediate values of elevation, increasing between 32 cm and 75 cm of elevation (Fig. 23d).

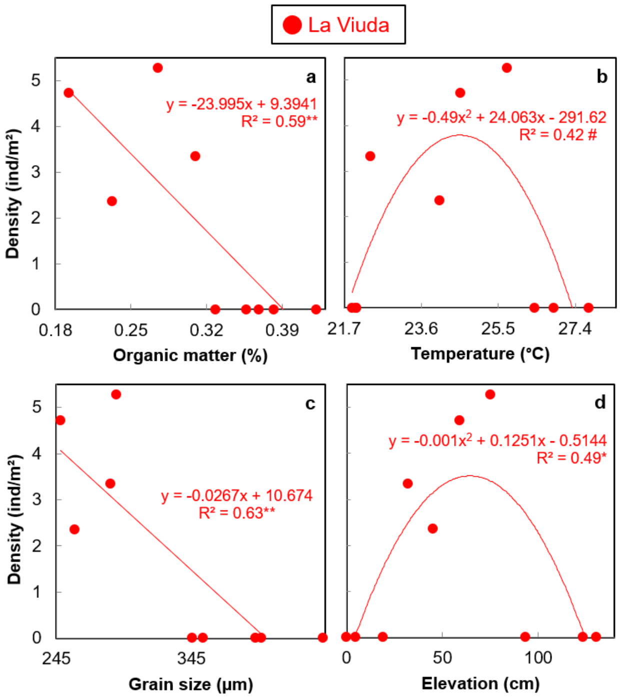


Figure 23. Spring density variations of *Atlantorchestoidea brasiliensis* on La Viuda (●) beach as a function of: a, sediment organic matter content; b, temperature; c, grain size; d, elevation. The best models fitted between density values per sampling level and the environmental variables are shown: **p<0.01, *p<0.05 and # 0.05<p<0.10.

In spring on José Ignacio beach, the population structure presented all the four classes at station 8, with higher relative percentages of juveniles with respect to each class of adults, while on La Viuda beach, the four classes were present all together with similar relative percentages at station 24, immature females and juveniles only were found at station 12, males at station 16 and juveniles and mature females at station 20 (Fig. 24). In autumn on José Ignacio, only mature females were found at station 8, only juveniles at station 12, and similar relative percentages of juveniles, males and immature females at station 16, while on La Viuda beach, only juveniles were found at stations 4 and 8, only mature females at station 16 and similar relative percentages of adults and juveniles at stations 20 and 24, with males present at station 20 only and no immature females found on the whole beach (Fig. 24).

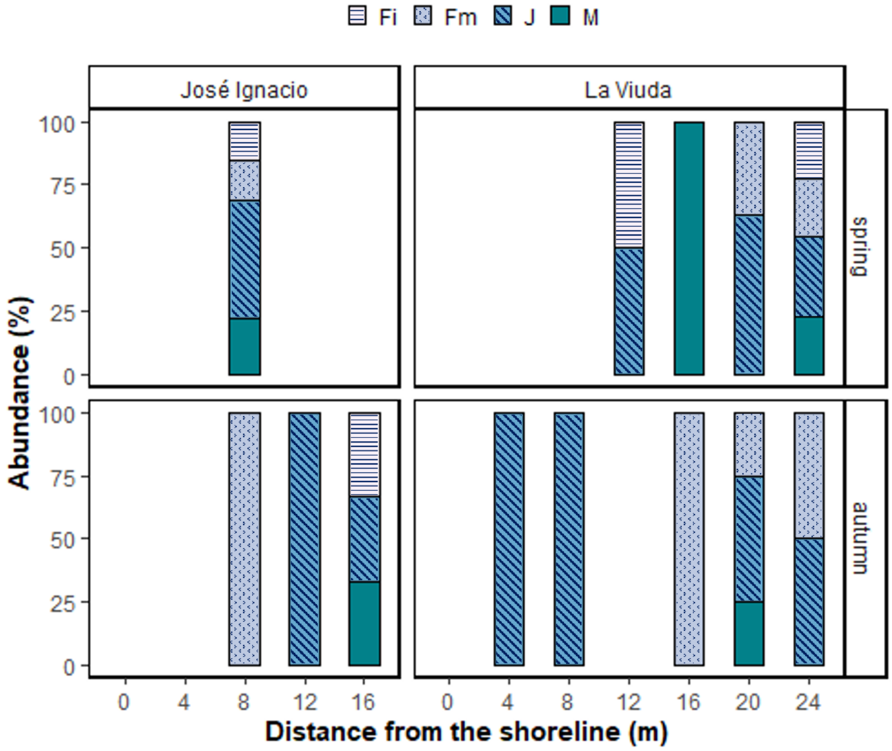


Figure 24. Across-shore population structure of *Atlantorchestoidea brasiliensis* in spring and autumn, on José Ignacio and La Viuda beaches. Fi, immature females; Fm, mature females; J, juveniles; M, males.

Regarding the mean population variables and their distribution in the four classes, larger numbers of sandhoppers were collected for the orientation tests, thus more detailed descriptions and comparisons were made in section 3.6.2. The proportion of the four classes collected in the zonation sampling differed between beaches and seasons (Tables 7). In both seasons and beaches, juveniles presented the highest proportion among classes; similar proportions were found for males and females, except for La Viuda in autumn, where mature females had higher proportion than males (Table 7).

The across-shore distributions of cephalic length and antennal segments reflect the distribution of the classes on the two beaches shown in Fig. 24 (Figs. 25 and 26).

Table 7. Population variables of *Atlantorchestoidea brasiliensis* for José Ignacio and La Viuda beaches, in spring and autumn.

	José Ignacio	**J**	**Fi**	**Fm**	**M**
spring	Number of individuals	9	1	1	2
tot=13	Proportion: ind/tot	0.69	0.08	0.08	0.15
	Cephalic length (mm): mean ± se	0.58 ± 0.02	0.83	1	0.72 ± 0.15
	Number of antennal segments: mean ± se	8.33 ± 0.16	12	13	12.5 ± 1.5
	Sex ratio [M/(M+F)]	0.5			
autumn	Number of individuals	2	1	1	1
tot=5	Proportion: ind/tot	0.4	0.2	0.2	0.2
	Cephalic length (mm): mean ± se	0.67 ± 0.02	0.95	1.13	1.3
	Number of antennal segments: mean ± se	9.5 ± 0.5	12	15	18
	Sex ratio [M/(M+F)]	0.33			
	La Viuda	**J**	**Fi**	**Fm**	**M**
spring	Number of individuals	6	2	2	2
tot=12	Proportion: ind/tot	0.5	0.17	0.17	0.17
	Cephalic length (mm): mean ± se	0.68 ± 0.06	0.87 ± 0.03	1.25 ± 0.2	0.9 ± 0.2
	Number of antennal segments: mean ± se	10.33± 0.49	12.5 ± 0.5	14 ± 3	14.5 ± 2.5
	Sex ratio [M/(M+F)]	0.33			
autumn	Number of individuals	7	0	3	1
tot=11	Proportion: ind/tot	0.64	0	0.27	0.09
	Cephalic length (mm): mean ± se	0.69 ± 0.02	-	1.09 ± 0.03	0.95
	Number of antennal segments: mean ± se	10.71 ± 0.42	-	15.33 ± 0.33	14
	Sex ratio [M/(M+F)]	0.25			

		José Ignacio	**La Viuda**
spring	Mean cephalic length ± se (min - max)	0.65 ± 0.04 mm (0.53 - 1)	0.84 ± 0.08 mm (0.53 - 1.45)
	Mean number of antennal segments ± se (min - max)	9.62 ± 0.59 (8 - 14)	12 ± 0.76 (8 - 17)
autumn	Mean cephalic length ± se (min - max)	0.94 ± 0.13 mm (0.65 - 1.30)	0.82 ± 0.06 mm (0.63 - 1.13)
	Mean number of antennal segments ± se (min - max)	12.8 ± 1.66 (9 - 18)	12.27 ± 0.71 (9 - 16)

tot = total of captured individuals; se, standard error; M, males; F, females; J, juveniles; Fi, immature females; Fm, mature females

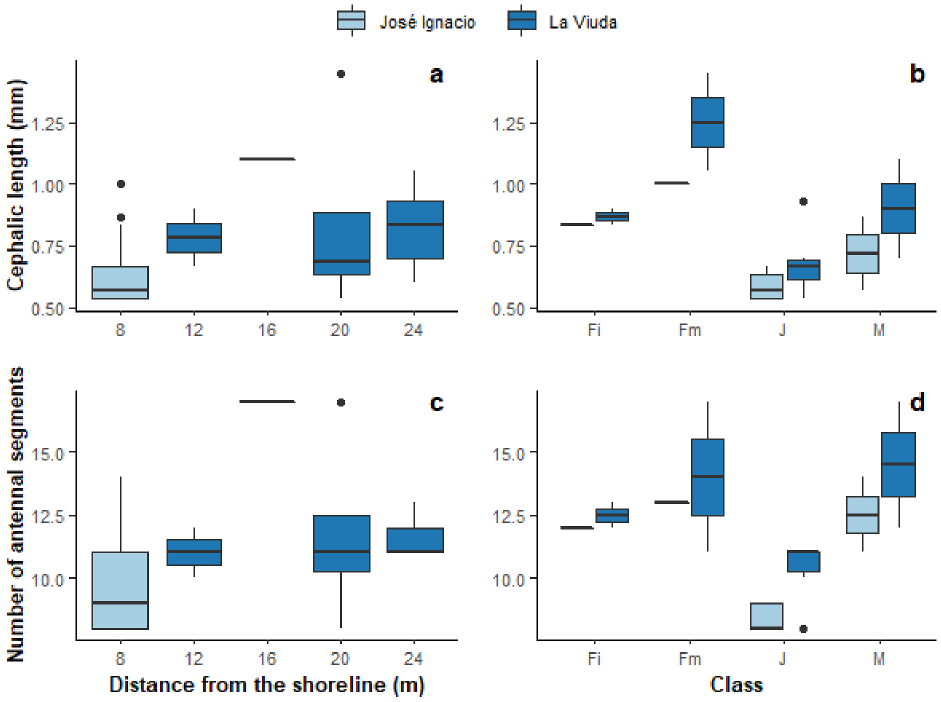


Figure 25. Across-shore (a and c) and among classes (b and d) distributions of population variables of *Atlantorchestoidea brasiliensis* of José Ignacio and La Viuda beaches in spring. a and b, cephalic length; c and d, number of antennal segments. Fi, immature females; Fm, mature females; J, juveniles; M, males.

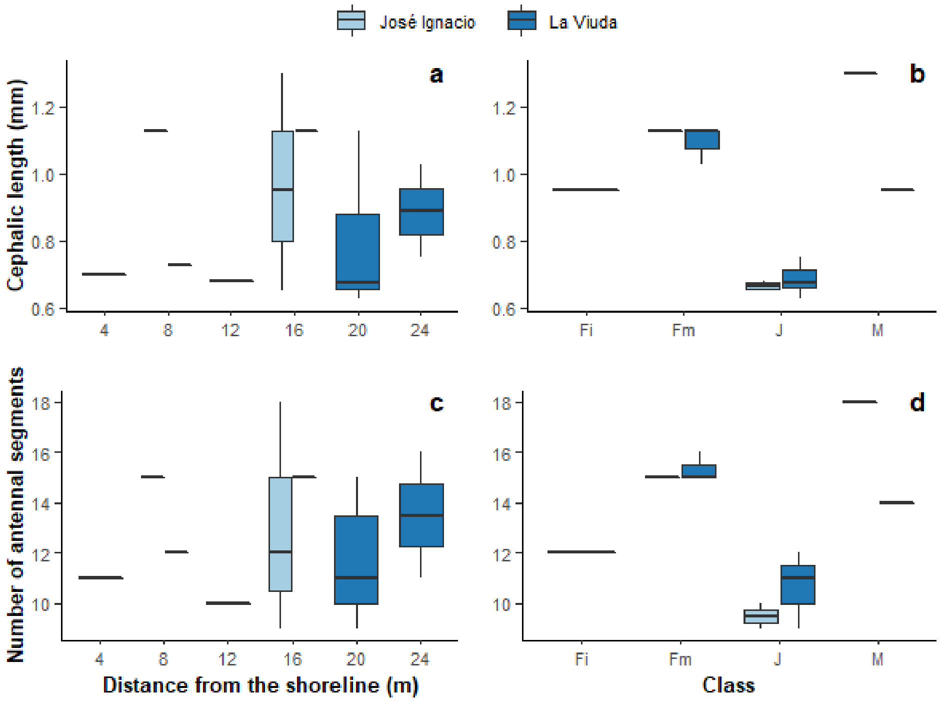


Figure 26. Across-shore (a and c) and among classes (b and d) distributions of population variables of *Atlantorchestoidea brasiliensis* of José Ignacio and La Viuda beaches in autumn. a and b, cephalic length; c and d, number of antennal segments. Fi, immature females; Fm, mature females; J, juveniles; M, males.

3.4. Collelungo and Macchiatonda: orientation behaviour

3.4.1. Environmental conditions during the orientation tests

Both on Collelungo and Macchiatonda beaches, air temperature, air relative humidity and solar radiation were significantly higher in autumn (ANOVA: *p*<0.001), except for air relative humidity on Macchiatonda beach, for which no differences between seasons were found (Table 8). In spring, on Collelungo, air temperature and solar radiation were significantly higher and air relative humidity lower than on Macchiatonda, while in autumn only the temperatures differed significantly, being higher on Macchiatonda (Table 8).

Table 8. Environmental variables measured during the orientation experiments on Collelungo and Macchiatonda beaches in spring and autumn. Comparisons between the two beaches, ANOVA: ****p*<0.001 and *ns* = non-significant.

		Collelungo	**Macchiatonda**	*p*
Spring	Sea water pH	8.18	8.12	
	Salinity	36.90	37.23	
	Cloudiness (% of observations)	0/8 (28.03); 1/8 (18.18); 2/8 (26.52); 7/8 (27.27)	0/8 (88.75); 7/8 (11.25)	
	Sun visibility (% of observations)	vis (72.73); vel (27.27)	vis (88.75); vel (11.25)	
	Mean air temperature ± se (min - max): °C	28.57 ± 0.25 (24.7 - 36.5)	24.51 ± 0.15 (20.2 - 29.4)	***
	Mean air relative humidity ± se (min - max): %	39.21 ± 0.36 (29 - 45)	52.54 ± 0.43 (39 - 65)	***
	Mean solar radiation ± se (min - max): klx	34.42 ± 1.15 (16.28 - 59.40)	25.34 ± 0.99 (17.8 - 65.2)	***
	Tidal range (m)	0 - 0.2	0.2 - 0.1	
Autumn	Sea water pH	8.05	8.04	
	Salinity	36.40	36.37	
	Sea water temperature (°C)	27.20	27.97	
	Cloudiness (% of observations)	1/8 (27.1); 2/8 (24.52); 3/8 (48.39)	1/8 (43.88); 2/8 (6.47); 5/8 (17.99); 6/8 (31.65)	
	Sun visibility (% of observations)	vis (100)	vis (38.13); vel (61.87)	
	Mean air temperature ± se (min - max): °C	29.93 ± 0.07 (28.8 - 32.4)	31.30 ± 0.2 (27.2 - 35.5)	***
	Mean air relative humidity ± se (min - max): %	52.6 ± 0.28 (44 - 57)	52.18 ± 0.36 (43 - 59)	*ns*
	Mean solar radiation ± se (min - max): klx	95.32 ± 1.21 (69.5 - 122.6)	95 ± 1.79 (55 - 124.5)	*ns*
	Tidal range (m)	0.1 - 0.3	0.2 - 0.3	

percentages of observations = number of observations under each condition/total of observations; se = standard error; vis = visible; vel = veiled

3.4.2. Population variables and structure of the tested samples

Non-significant differences were detected for size and age proxies between seasons or beaches (ANOVA: *p*>0.05). In both seasons and beaches, juveniles presented the highest proportions with respect to adults; sex ratio was female biased on Collelungo and male biased on Macchiatonda (Table 9).

Table 9. Population variables of *Talitrus saltator* of Collelungo and Macchiatonda beaches, tested for orientation in spring and autumn. Comparisons between the two beaches, ANOVA: *ns* = non-significant.

	Collelungo	**J**	**Fi**	**Fm**	**M**
spring	Number of individuals	57	18	22	35
tot=132	Proportion: ind/tot	0.43	0.14	0.17	0.27
	Cephalic length (mm): mean ± se	0.49 ± 0.01	1.07 ± 0.02	1.31 ± 0.02	1.20 ± 0.04
	Number of antennal segments: mean ± se	8.09 ± 0.19	19.1 ± 0.5	25.59 ± 0.59	25.2 ± 0.84
	Sex ratio [M/(M+F)]	0.46			
autumn	Number of individuals	71	14	38	32
tot=155	Proportion: ind/tot	0.46	0.09	0.25	0.21
	Cephalic length (mm): mean ± se	0.49 ± 0.01	1.04 ± 0.03	1.24 ± 0.01	1.06 ± 0.03
	Number of antennal segments: mean ± se	8.28 ± 1.17	20.64 ± 0.55	23.37 ± 0.3	23.72 ± 0.98
	Sex ratio [M/(M+F)]	0.38			
	Macchiatonda	**J**	**Fi**	**Fm**	**M**
spring	Number of individuals	70	9	30	51
tot=160	Proportion: ind/tot	0.44	0.06	0.19	0.32
	Cephalic length (mm): mean ± se	0.49 ± 0.01	0.83 ± 0.08	1.32 ± 0.02	1.22 ± 0.04
	Number of antennal segments: mean ± se	9.51 ± 0.16	15.33 ± 1.51	22.9 ± 0.37	23.75 ± 0.81
	Sex ratio [M/(M+F)]	0.57			
autumn	Number of individuals	53	14	23	49
tot=139	Proportion: ind/tot	0.38	0.1	0.17	0.35
	Cephalic length (mm): mean ± se	0.48 ± 0.01	0.94 ± 0.04	1.29 ± 0.02	1.17 ± 0.03
	Number of antennal segments: mean ± se	7.68 ± 0.27	17.43 ± 0.89	23 ± 0.27	26.2 ± 0.73
	Sex ratio [M/(M+F)]	0.57			

		Collelungo	**Macchiatonda**	*p*
spring	Mean cephalic length ± se (min - max)	0.89 ± 0.03 mm (0.26 - 1.65)	0.90 ± 0.03 mm (0.35 - 1.71)	*ns*
	Mean number of antennal segments ± se (min - max)	16.71 ± 0.73 (5 - 32)	16.89 ± 0.61 (7 - 33)	*ns*
autumn	Mean cephalic length ± se (min - max)	0.84 ± 0.03 mm (0.28 - 1.44)	0.90 ± 0.03 mm (0.24 - 1.48)	*ns*
	Mean number of antennal segments ± se (min - max)	16.28 ± 0.64 (5 - 29)	17.73 ± 0.76 (5 - 32)	*ns*

tot = total of captured individuals; se = standard error; M, males; F, females; J, juveniles; Fi, immature females; Fm, mature females

Mature females presented the highest values of cephalic length and juveniles the lowest. Regarding the numbers of antennal segments, mature females and males always presented similar values, except for Macchiatonda in autumn, where males had the highest values; juveniles always presented the lowest values (Table 9; Fig. 27).

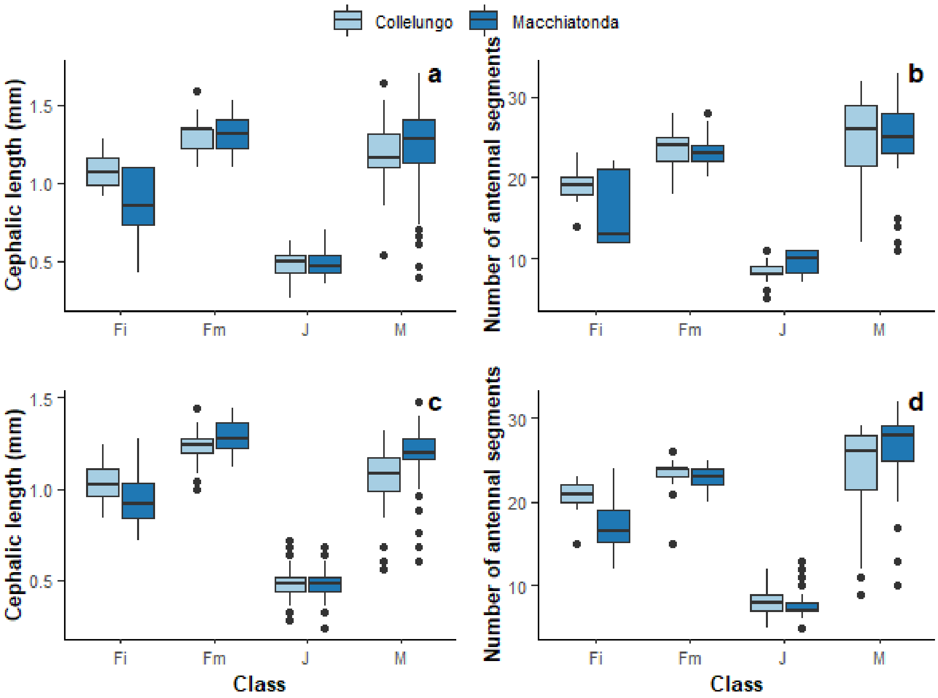


Figure 27. Cephalic length and number of antennal segments of *Talitrus saltator* by population component in Collelungo and Macchiatonda beaches in spring (a and b) and autumn (c and d). Fi, immature females; Fm, mature females; J, juveniles; M, males.

3.4.3. Orientation tests: circular statistics

On both beaches in spring, sandhoppers were seawards directed, and the Rayleigh tests showed that both distributions were significantly different from uniformity (p<0.001, Table 10); the mean vector length r (*i.e.* the concentration of the samples around the mean direction) was lower on Macchiatonda beach, however the mean vector was oriented to the TED, while a slight south deviation from the TED was observed on Collelungo (Table 10, Fig. 28). On Collelungo beach, the seawards orientation was only slightly affected by the presence of the screen that prevented landscape view: the vector length decreased, the circular dispersion increased, and the direction slightly deviated from the TED (Table 10, Fig. 28). On Macchiatonda beach, sandhoppers presented a high dispersion when the landscape was not visible, and the circular distribution was uniform with the individuals randomly oriented (Rayleigh test: p>0.05; Table 10, Fig. 28).

Table 10. Statistical results of the circular distributions of *Talitrus saltator* on Collelungo and Macchiatonda beaches in spring: observed mean direction ± 95% confidence interval. Rayleigh test for randomness: ***p<0.001.

		Collelungo TED=211°	**Macchiatonda TED=185°**
Total population	Observed direction ± CI (°)	199.95 ± 8.98	182.31 ± 21.04
	Mean vector length (r)	0.67***	0.30***
	Circular dispersion	0.84	5.37
	Sample size (n)	132	160
Landscape visible	Observed direction ± CI (°)	202.35 ± 12.63	173.57 ± 20.27
	Mean vector length (r)	0.70***	0.43***
	Circular dispersion	0.75	2.59
	Sample size (n)	60	83
Landscape not visible	Observed direction ± CI (°)	197.80 ± 12.76	204.70 ± 57.42
	Mean vector length (r)	0.64***	0.18
	Circular dispersion	0.91	14.23
	Sample size (n)	72	77
Juveniles	Observed direction ± CI (°)	208.26 ± 14.64	179.42 ± 18.49
	Mean vector length (r)	0.61***	0.49***
	Circular dispersion	0.95	1.83
	Sample size (n)	57	70
Immature females	Observed direction ± CI (°)	199.52 ± 24.50	145.47
	Mean vector length (r)	0.70***	0.34
	Circular dispersion	0.81	3.18
	Sample size (n)	18	9
Mature females	Observed direction ± CI (°)	206.03 ± 19.61	197.67
	Mean vector length (r)	0.75***	0.07
	Circular dispersion	0.64	79.67
	Sample size (n)	22	30
Males	Observed direction ± CI (°)	185.14 ± 15.67	200.03 ± 72.05
	Mean vector length (r)	0.74***	0.19
	Circular dispersion	0.66	12.02
	Sample size (n)	35	51

CI = confidence interval

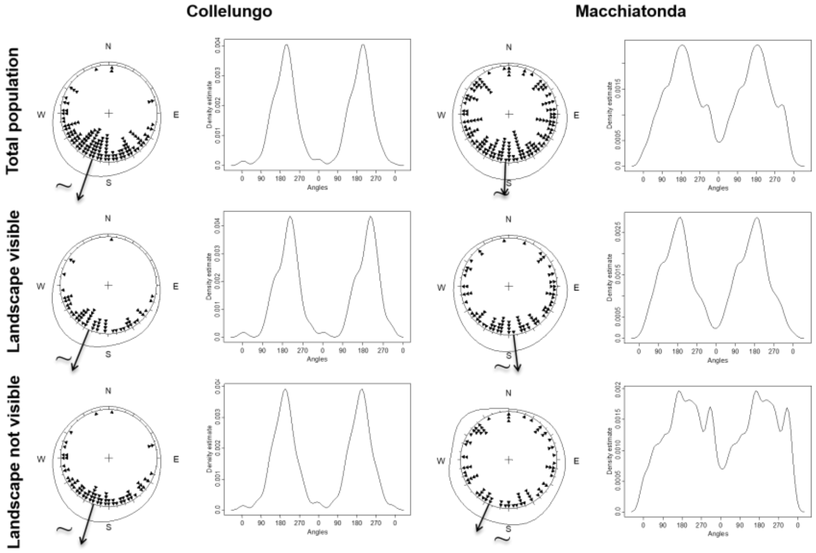


Figure 28. Spring angular distributions from the orientation tests on *Talitrus saltator* on Collelungo (on the left) and Macchiatonda (on the right) beaches: total population (top), visible (middle) and screened (bottom) landscape. Circular plots of the orientation angles (left); density estimates (kernel method) double plotted on Cartesian graphs (right); ~, sea direction (TED); arrows, mean vectors, with lengths (r) proportional to concentrations. Circular statistics results are shown in Table 10.

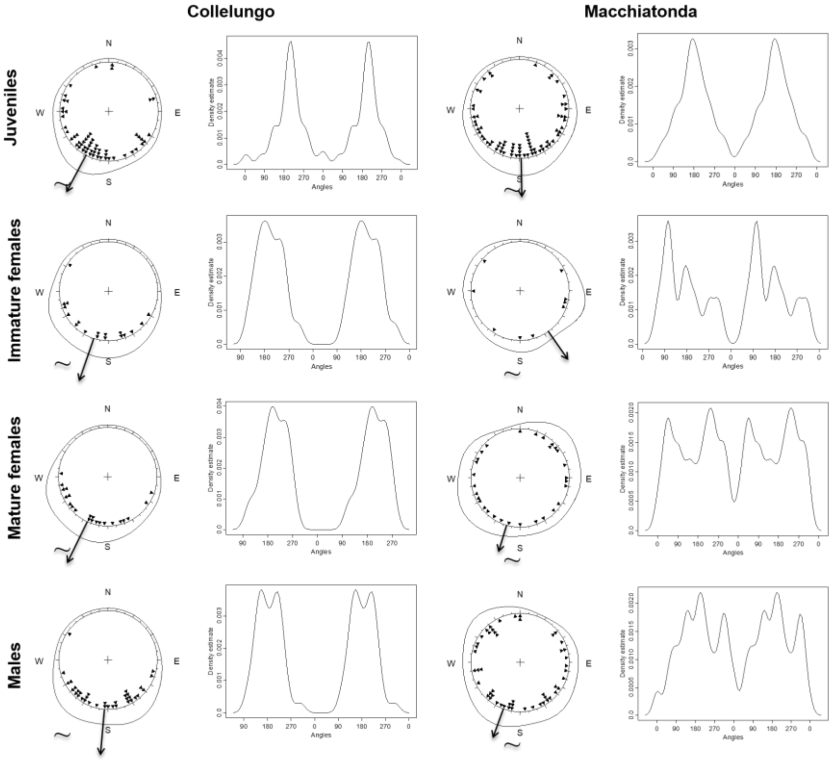


Figure 29. Spring angular distributions from the orientation tests on *Talitrus saltator* on Collelungo and Macchiatonda beaches: the four classes separated. Circular plots of the orientation angles (left); density estimates (kernel method) double plotted on Cartesian graphs (right); ~, sea direction (TED); arrows, mean vectors, with lengths (r) proportional to concentrations. Circular statistics results are shown in Table 10.

Focusing on the four classes, juveniles presented the best seawards orientation on both beaches, being more concentrated seawards on Collelungo with respect to Macchiatonda (compare the vectors in Fig. 29); adults were well oriented on Collelungo (mature females had the highest concentration seawards and males showed two small peaks, one seaward and one longshore), while on Macchiatonda they presented random distributions (Table 10, Fig. 29).

The orientation of sandhoppers in autumn was significantly seawards oriented on both beaches, a little more dispersed on Collelungo and slightly deviated from the TED on Macchiatonda (compare the directions, with their CI, the length r of the vectors and circular dispersion, Table 11, Fig. 30). When the landscape was visible, the orientation of sandhoppers on Collelungo was significantly seawards oriented, while when the landscape was screened off, the deviation from the TED was higher, and a small landwards peak appeared (Table 11, Fig 30). On Macchiatonda, sandhoppers presented a slightly better seawards orientation when the landscape was visible with respect to the orientation without the landscape view (Table 11, Fig 30), being less dispersed with respect to the sandhoppers of Collelungo in both cases (compare circular dispersion in Table 11).

On Collelungo, the orientation of juveniles was seawards but showed some scatter, with several specimens oriented towards the inland, while on Macchiatonda beach juveniles were well seawards concentrated (Table 11, Fig. 31). Immature females were randomly oriented on Collelungo beach, while on Macchiatonda they were significantly oriented, but deviated to east with respect to the seaward direction (Table 11, Fig. 31). On Collelungo, mature females showed some scatter, but their orientation significantly differed from randomness, while on Macchiatonda they were seawards oriented. Males presented a highly significant seawards orientation on both beaches, being slightly more concentrated on Macchiatonda with respect to Collelungo (Table 11, Fig 31).

Table 11. Statistical results of the circular distributions of *Talitrus saltator* on Collelungo and Macchiatonda beaches in autumn: observed mean direction ± 95% confidence interval. Rayleigh test for randomness: ***p<0.001 and **p<0.01.

		Collelungo TED=219°	**Macchiatonda TED=185°**
Total population	Observed direction ± CI (°)	212.64 ± 11.03	173.49 ± 7.11
	Mean vector length (r)	0.49***	0.71***
	Circular dispersion	1.48	0.56
	Sample size (n)	155	139
Landscape visible	Observed direction ± CI (°)	214.62 ± 11.35	173.81 ± 8.70
	Mean vector length (r)	0.64***	0.74***
	Circular dispersion	0.77	0.43
	Sample size (n)	76	72
Landscape not visible	Observed direction ± CI (°)	209.13 ± 23.30	173.12 ± 11.62
	Mean vector length (r)	0.35***	0.68***
	Circular dispersion	3.22	0.71
	Sample size (n)	79	67
Juveniles	Observed direction ± CI (°)	212.46 ± 12.66	177.94 ± 9.65
	Mean vector length (r)	0.55***	0.77***
	Circular dispersion	0.89	0.39
	Sample size (n)	71	53
Immature females	Observed direction ± CI (°)	188.53 ± 78.36	150.57 ± 26.90
	Mean vector length (r)	0.36	0.58**
	Circular dispersion	3.50	0.75
	Sample size (n)	14	14
Mature females	Observed direction ± CI (°)	218.64 ± 31.14	178.33 ± 21.95
	Mean vector length (r)	0.41**	0.64***
	Circular dispersion	2.65	0.84
	Sample size (n)	38	23
Males	Observed direction ± CI (°)	214.48 ± 24.10	171.50 ± 11.70
	Mean vector length (r)	0.53***	0.73***
	Circular dispersion	1.39	0.52
	Sample size (n)	32	49

CI = confidence interval

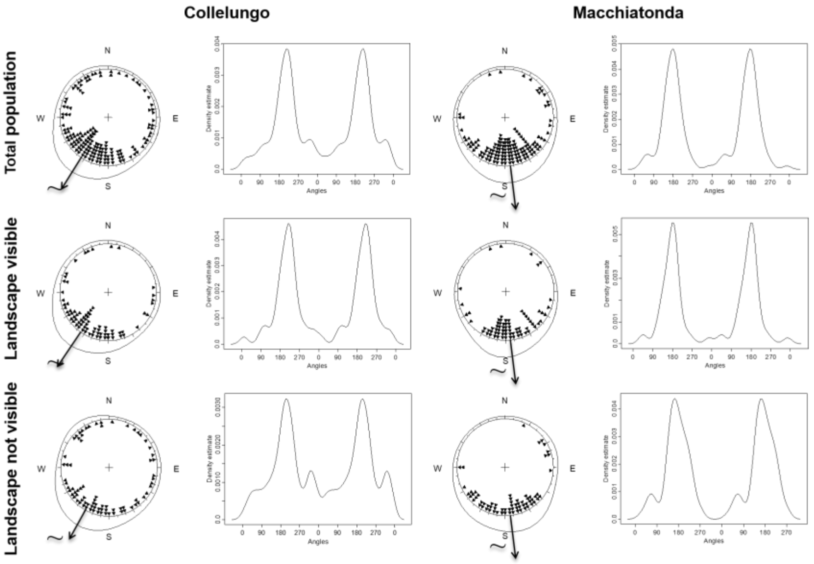


Figure 30. Autumn angular distributions from the orientation tests on *Talitrus saltator* on Collelungo (left) and Macchiatonda (right) beaches: total population (top), visible (middle) and screened (bottom) landscape. Circular plots of the orientation angles (left); density estimates (kernel method) double plotted on Cartesian graphs (right); ~, sea direction (TED); arrows, mean vector, with lengths (r) proportional to concentrations. Circular statistics results are shown in Table 11.

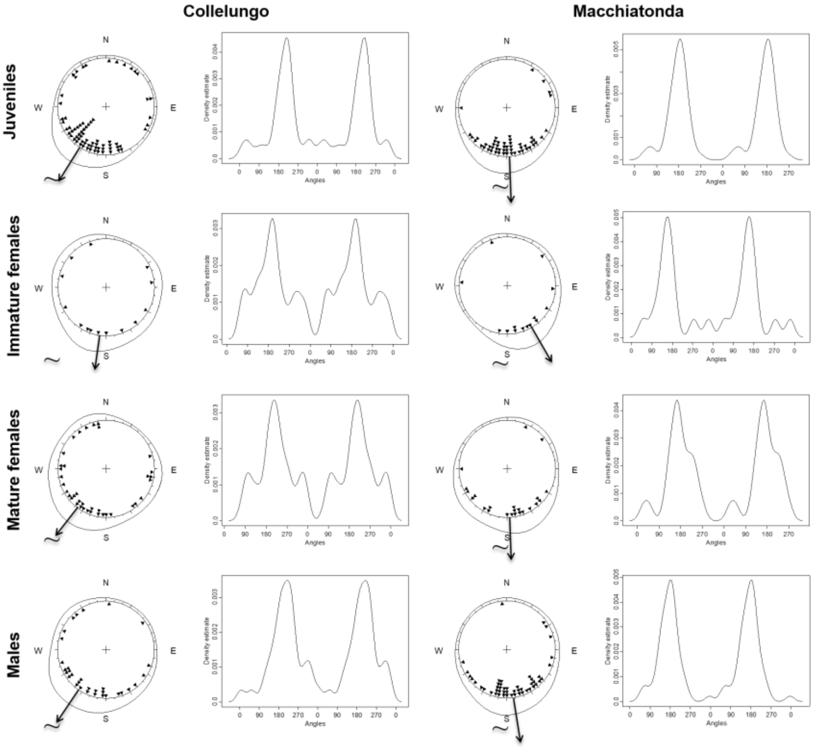


Figure 31. Autumn angular distributions from the orientation tests on *Talitrus saltator* on Collelungo and Macchiatonda beaches: the four classes separated. Circular plots of the orientation angles (left); density estimates (kernel method) double plotted on Cartesian graphs (right); ~, sea direction (TED); arrows, mean vectors, with lengths (r) proportional to concentrations. Circular statistics results are shown in Table 11.

3.4.4 Multiple regression of orientation angles with intrinsic and environmental variables and factors

The best additive model developed from the whole dataset of Collelungo and Macchiatonda in both seasons highlighted the effect on distributions of some environmental and intrinsic variables and factors (Table 12). The LRT showed that some variables and factors were more significant than others, but also not significant ones improved the AIC of the best model, thus they were not dropped from the model; the variables and factors not included in the best model did not improve the AIC.

Table 12. Multiple regression models (SPLM) developed for orientation on Collelungo and Macchiatonda beaches. Likelihood Ratio Test: ****p*<0.001, ***p*<0.01, **p*<0.05. Bold values = lowest AIC.

	Collelungo and Macchiatonda: spring and autumn	
Model name	**Model description**	**SPLM statistics**
M0: ***initial additive model***	angle ~ beach + season + cloudiness + sun visibility + air temperature + air relative humidity + solar radiation + tide cycle + landscape visibility + morning/afternoon + azimuth + general class + cephalic length + number of antennal segments	Likelihood = 1695.0587, AIC = 1759.0587, df = 554
M1: ***best additive model***	angle ~ cloudiness*** + landscape visibility*** + morning/afternoon* + azimuth** + general class**	Likelihood = 1710.8765, AIC = 1738.8765, df = 572
M2: ***best model with interaction with factor "beach"***	angle ~ beach*** (cloudiness** + air temperature*** + air relative humidity*** + tide cycle*** + azimuth*) + solar radiation* + landscape visibility*** + number of antennal segments*	Likelihood = 1623.6089, AIC = 1683.6089, df = 556
M3: ***best model with interaction with factor "season"***	angle ~ season*** (beach*** + cloudiness*** + sun visibility* + air temperature*** + solar radiation* + landscape visibility*** + morning/afternoon + azimuth*) + number of antennal segments*	Likelihood = 1596.5019, AIC = **1676.5019**, df = 546
	Collelungo and Macchiatonda: spring	
M4: ***initial additive model***	angle ~ beach + cloudiness + sun visibility + air temperature + air relative humidity + solar radiation + tide cycle + landscape visibility + morning/afternoon + azimuth + general class + cephalic length + number of antennal segments	Likelihood = 850.5918, AIC = 910.5918, df = 262
M5: ***best additive model***	angle ~ beach*** + cloudiness* + sun visibility* + air relative humidity* + landscape visibility*** + morning/afternoon + azimuth*	Likelihood = 865.7633, AIC = 897.7633, df = 276
M6: ***best model with interaction with factor "beach"***	angle ~ beach*** (cloudiness*** + air temperature** + air relative humidity*** + landscape visibility** + morning/afternoon** + azimuth** + general class** + number of antennal segments*) + sun visibility*** + cephalic length	Likelihood = 791.8259, AIC = **879.8259**, df = 248
	Collelungo and Macchiatonda: autumn	
M7: ***initial additive model***	angle ~ beach + cloudiness + sun visibility + air temperature + air relative humidity + solar radiation + tide cycle + landscape visibility + morning/afternoon + azimuth + general class + cephalic length + number of antennal segments	Likelihood = 724.2938, AIC = 784.2938, df = 264
M8: ***best additive model***	angle ~ beach*** + cloudiness* + sun visibility + air temperature*** + solar radiation** + landscape visibility* + morning/afternoon + azimuth* + cephalic length*	Likelihood = 731.1936, AIC = **771.1936**, df = 274

M9: *best model with interaction with factor “beach”*	angle ~ beach*** (cloudiness** + air temperature*** + solar radiation + landscape visibility* + morning/afternoon* + azimuth*) + sun visibility* + class* + cephalic length**	Likelihood = 698.1833, AIC = 774.1833, df = 256
	Collelungo	
M10: *initial additive model*	angle ~ season + cloudiness + sun visibility + air temperature + air relative humidity + solar radiation + tide cycle + landscape visibility + morning/afternoon + azimuth + general class + cephalic length + number of antennal segments	Likelihood = 770.5535, AIC = 830.5535, df = 257
M11: *best additive model*	angle ~ season** + sun visibility** + air relative humidity*** + tide cycle* + landscape visibility** + azimuth*	Likelihood = 783.0675, AIC = 811.0675, df = 273
M12: *best model with interaction with factor “season”*	angle ~ season*** (cloudiness*** + air temperature*** + air relative humidity** + solar radiation** + landscape visibility* + morning/afternoon** + azimuth** + general class** + number of antennal segments**) + sun visibility***	Likelihood = 692.2339, AIC = **784.2339**, df = 241
	Macchiatonda	
M13: *initial additive model*	angle ~ season + cloudiness + sun visibility + air temperature + air relative humidity + solar radiation + tide cycle + landscape visibility + morning/afternoon + azimuth + general class + cephalic length + number of antennal segments	Likelihood = 820.7994, AIC = 880.7994, df = 269
M14: *best additive model*	angle ~ cloudiness** + air temperature*** + air relative humidity*** + solar radiation* + tide cycle*** + landscape visibility*** + azimuth* + cephalic length*	Likelihood = 829.9968, AIC = 865.9968, df = 281
M15: *best model with interaction with factor “season”*	angle ~ season*** (solar radiation***+ azimuth***) + air relative humidity** + landscape visibility*** + number of antennal segments*	Likelihood = 825.5569, AIC = **861.5569**, df = 281

AIC = Akaike Information Criterion; df = degrees of freedom

In M1, the factors cloudiness and landscape visibility showed the highest significance (Table 12). A lower AIC value was found including the interaction of the factor “beach” with other variables and factors; the interaction with factor “beach” resulted highly significant ($p<0.001$), showing that the two populations followed different models (M2, Table 12). The influence of hourly factors like sun azimuth highlighted the use of the sun as a cue, while the high significance of landscape visibility confirmed the use of landscape view independently from the beach. The meteorological variables (cloudiness, air temperature and relative humidity, see Table 8) also resulted highly significant, as well as the tide cycle (ebbing/rising). The presence of intrinsic variables in the models revealed a role of intrinsic (individual) characteristics in the orientation. The highly significant interaction of the factor “season” with other variables and factors highlighted that cloudiness, air temperature and landscape view were highly significant factors possibly discriminating the orientation among beaches and seasons, the number of antennal segments (age proxy) being significant independently from the season, as from the beach, and highlighting the importance of age on the orientation (M3, Table 12).

By separating the two beaches in spring and autumn, and then the two seasons in each beach, the analyses confirmed that sandhoppers oriented differently between beaches and seasons, as was shown by the summary statistics of the circular distributions (Tables 10 and 11, Table 12 from M4 to M15). In addition to landscape view, a significant factor for both beaches, in particular for Macchiatonda in spring as compared with Collelungo, the meteorological variables (cloudiness, air temperature and relative humidity) resulted important factors likely discriminating orientation among beaches and seasons (Table 12).

Summarizing, the models highlighted differences both between beaches and seasons (M2, M3, Table 12). Meteorological, hourly and orienting factors were significant in the overall best model, discriminating by season (M3, Table 12). Intrinsic factors were significant for both beaches, influencing the orientation and confirming the dependence of intrinsic factors on meteorological, hourly and orienting factors. Collelungo best model (M12, Table 12) was more complex (with more factors) with respect to the Macchiatonda best model (M15, Table 12), the latter having only hourly factors discriminating for season, being cloudiness and sun visibility the discriminating factors between beaches (compare M12 and M15, Table 12).

3.5. Bocca di Serchio and Buca del Mare: orientation behaviour

3.5.1. Environmental conditions during the orientation tests

Both on Bocca di Serchio and Buca del Mare beaches, air temperature, air relative humidity and solar radiation significantly differed between seasons (ANOVA: $p<0.001$). The three variables were also significantly different between beaches in both seasons (Table 13).

In spring, it rained lightly during the second release of the afternoon on Buca del Mare, and in autumn it started raining during the last release of the morning on Bocca di Serchio, and stopped raining before the afternoon tests.

Table 13. Environmental variables measured during the orientation experiments on Bocca di Serchio and Buca del Mare beaches in spring and autumn. Comparisons between the two beaches, ANOVA: ***$p<0.001$ and **$p<0.01$.

		Bocca di Serchio	**Buca del Mare**	*p*
spring	Sea water pH	-	8.13	
	Salinity	-	34.63	
	Sea water temperature (°C)	-	23.5	
	Cloudiness (% of observations)	2/8 (50); 6/8 (11.80); 7/8 (14.04); 8/8 (24.16)	0/8 (55.56); 2/8 (21.64); 3/8 (12.28); 5/8 (5.85); 7/8 (4.68)	
	Sun visibility (% of observations)	vis (50); vel (19.10); nvis (30.90)	vis (89.47); shp (5.85); nvis (4.68)	
	Mean air temperature ± se (min - max): °C	29.10 ± 0.28 (23.3 - 39.3)	27.85 ± 0.27 (21 - 34.6)	**
	Mean air relative humidity ± se (min - max): %	41.42 ± 0.65 (21 - 52)	49.24 ± 1.16 (27 - 71)	***
	Mean solar radiation ± se (min - max): klx	105.05 ± 3.30 (29 - 180.3)	116.20 ± 1.90 (35.4 - 143.2)	***
	Tidal range (m)	-0.1 - 0.3 m	0 - 0.3 m	
autumn	Sea water pH	8.35	8.24	
	Salinity	34.83	35.83	
	Sea water temperature (°C)	23.33	27.1	
	Cloudiness (% of observations)	3/8 (17.47); 4/8 (6.63); 5/8 (6.02); 6/8 (9.64); 7/8 (41.57); 8/8 (18.67)	1/8 (70.69); 2/8 (11.49); 3/8 (17.82)	
	Sun visibility (% of observations)	vis (39.76); vel (4.82); nvis (55.42)	vis (100)	
	Mean air temperature ± se (min - max): °C	24.84 ± 0.16 (22 - 28.6)	31.50 ± 0.23 (26.4 - 38.1)	***
	Mean air relative humidity ± se (min - max): %	72.42 ± 0.69 (57 - 88)	39.29 ± 0.57 (28 - 54)	***
	Mean solar radiation ± se (min - max): klx	50.01 ± 2.15 (10.7 - 100)	87.63 ± 1.40 (50.4 - 120.1)	***
	Tidal range (m)	0.1 - 0.2 m	0.1 - 0.2 m	

percentages of observations = number of observations under each condition/total of observations; se = standard error; vis = visible; vel = veiled; shp = solar disk; nvis = not visible; (-) not measured

3.5.2. Population variables and structure of the tested samples

The population variables did not differ between seasons neither between beaches (ANOVA: $p>0.05$), except for Buca del Mare, in which the number of antennal segments slightly differed between seasons (ANOVA: $p=0.07$). Males always presented the highest proportions and mature females the lowest; sex ratio was always male biased except for Buca del Mare in spring where was female biased; juveniles always were more represented than immature females, except for Bocca di Serchio in spring (Table 14).

Table 14. Population variables of *Talitrus saltator* of Bocca di Serchio and Buca del Mare beaches, tested for orientation in spring and autumn. Comparisons between the two beaches, ANOVA: *ns* = non-significant.

	Bocca di Serchio	**J**	**Fi**	**Fm**	**M**
spring	Number of individuals	28	39	18	93
tot=178	Proportion: ind/tot	0.16	0.22	0.10	0.52
	Cephalic length (mm): mean ± se	0.64 ± 0.01	0.99 ± 0.03	1.14 ± 0.02	0.91 ± 0.02
	Number of antennal segments: mean ± se	10.11 ± 0.29	18.03 ± 0.59	21.89 ± 0.39	19.20 ± 0.67
	Sex ratio [M/(M+F)]	0.62			
autumn	Number of individuals	36	18	12	100
tot=166	Proportion: ind/tot	0.22	0.11	0.07	0.60
	Cephalic length (mm): mean ± se	0.58 ± 0.01	0.95 ± 0.04	1.10 ± 0.03	0.96 ± 0.02
	Number of antennal segments: mean ± se	9.5 ± 0.26	18.39 ± 0.44	22.5 ± 0.36	21.6 ± 0.49
	Sex ratio [M/(M+F)]	0.77			
	Buca del Mare	**J**	**Fi**	**Fm**	**M**
spring	Number of individuals	50	46	22	53
tot=171	Proportion: ind/tot	0.29	0.27	0.13	0.31
	Cephalic length (mm): mean ± se	0.59 ± 0.01	0.95 ± 0.02	1.22 ± 0.02	0.98 ± 0.03
	Number of antennal segments: mean ± se	9.52 ± 0.23	17.13 ± 0.43	22.23 ± 0.37	21.47 ± 0.92
	Sex ratio [M/(M+F)]	0.44			
autumn	Number of individuals	41	27	24	82
tot=174	Proportion: ind/tot	0.24	0.16	0.14	0.47
	Cephalic length (mm): mean ± se	0.60 ± 0.01	0.88 ± 0.03	1.18 ± 0.02	0.96 ± 0.02
	Number of antennal segments: mean ± se	10.20 ± 0.27	16.78 ± 0.50	23.13 ± 0.24	21.20 ± 0.64
	Sex ratio [M/(M+F)]	0.62			

		Bocca di Serchio	**Buca del Mare**	*p*
spring	Mean cephalic length ± se (min - max)	0.91 ± 0.02 mm (0.52 - 1.40)	0.89 ± 0.02 mm (0.28 - 1.40)	*ns*
	Mean number of antennal segments ± se (min - max)	17.79 ± 0.46 (8 - 33)	16.91 ± 0.50 (5 - 31)	*ns*
autumn	Mean cephalic length ± se (min - max)	0.89 ± 0.02 mm (0.48 - 1.40)	0.89 ± 0.02 mm (0.40 - 1.36)	*ns*
	Mean number of antennal segments ± se (min - max)	18.69 ± 0.49 (7 - 32)	18.18 ± 0.48 (5 - 30)	*ns*

tot = total of captured individuals; se = standard error; M, males; F, females; J, juveniles; Fi, immature females; Fm, mature females

Mature females presented the highest values of cephalic length and antennal segments, and juveniles the lowest ones (Table 14; Fig. 32).

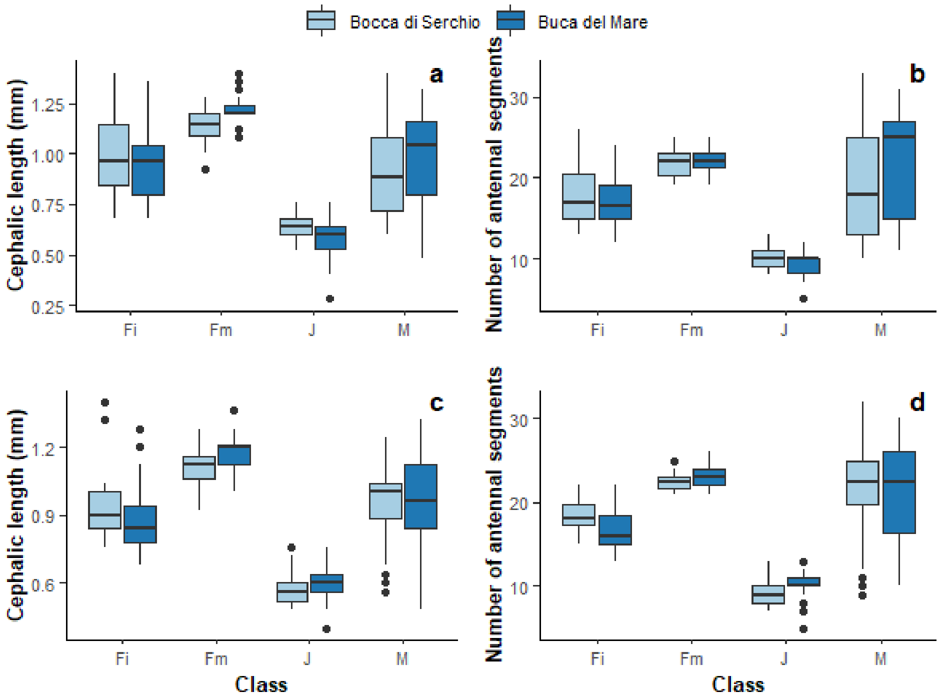


Figure 32 Cephalic length and number of antennal segments of *Talitrus saltator* by population component in Bocca di Serchio and Buca del Mare beaches in spring (a and b) and autumn (c and d). Fi, immature females; Fm, mature females; J, juveniles; M, males.

3.5.3. Orientation tests: circular statistics

In spring, on Bocca di Serchio beach sandhoppers presented a distribution significantly different from randomness, and the orientation significantly deviated to north with respect to the seawards direction (see CI in Table 15); on Buca del Mare, the vector length was higher and circular dispersion lower with respect to Bocca di Serchio and only slightly deviated from the TED (Rayleigh test: p<0.001), with two peaks to the south and to the west, in the longshore direction (Table 15, Fig. 33). The screening of the landscape affected the orientation on Bocca di Serchio beach, where sandhoppers were randomly oriented when landscape was visible, and were slightly more concentrated when the screen was present, but had a direction deviated to the north (longshore); on Buca del Mare, the concentration of the orientation was slightly higher when the landscape was visible (one peak seawards and one longshore); the direction was more precisely seawards when the landscape was screened off, but two peaks were present, mostly longshore (Table 15, Fig. 33).

Among the four classes, mature females and males presented a random orientation on Bocca di Serchio (Rayleigh test: p>0.05); also, immature females and juveniles were quite scattered, but their orientation was significantly different from randomness, the former oriented landwards and the latter longshore (Table 15, Fig. 34). On Buca del Mare beach, the directions of juveniles, immature females and males were south-west, with two major peaks longshore: juveniles were significantly seawards oriented, immature females orientation was almost significant (Rayleigh test, *p*=0.07), mature females presented a high concentration (Rayleigh test significant), but the direction was south-south-east (longshore), deviating from the TED (Table 15, Fig 34).

Table 15. Statistical results of the circular distributions of *Talitrus saltator* on Bocca di Serchio and Buca del Mare beaches in spring: observed mean direction ± 95% confidence interval. Rayleigh test for randomness: ****p*<0.001, ***p*<0.01, **p*<0.05 and # 0.05<*p*<0.10.

		Bocca di Serchio TED=252°	**Buca del Mare TED=248°**
Total population	Observed direction ± CI (°)	318.65 ± 34.97	225.19 ± 16.79
	Mean vector length (r)	0.18**	0.36***
	Circular dispersion	15.22	3.71
	Sample size (n)	178	171
Landscape visible	Observed direction ± CI (°)	278.78	211.16 ± 20.82
	Mean vector length (r)	0.11	0.41***
	Circular dispersion	39.31	2.90
	Sample size (n)	86	88
Landscape not visible	Observed direction ± CI (°)	332.52 ± 29.31	242.85 ± 25.23
	Mean vector length (r)	0.27**	0.35***
	Circular dispersion	5.74	3.93
	Sample size (n)	92	83
Juveniles	Observed direction ± CI (°)	316.70 ± 40.72	240.06 ± 18.86
	Mean vector length (r)	0.36*	0.58***
	Circular dispersion	3.10	1.36
	Sample size (n)	28	50
Immature females	Observed direction ± CI (°)	15.46 ± 39.99	223.68 ± 57.81
	Mean vector length (r)	0.34**	0.24 #
	Circular dispersion	4.19	8.58
	Sample size (n)	39	46
Mature females	Observed direction ± CI (°)	277.09	156.84 ± 32.91
	Mean vector length (r)	0.18	0.46**
	Circular dispersion	12.48	1.69
	Sample size (n)	18	22
Males	Observed direction ± CI (°)	283.35 ± 61.41	231.87 ± 30.33
	Mean vector length (r)	0.16	0.37***
	Circular dispersion	18.66	3.52
	Sample size (n)	93	53

CI = confidence interval

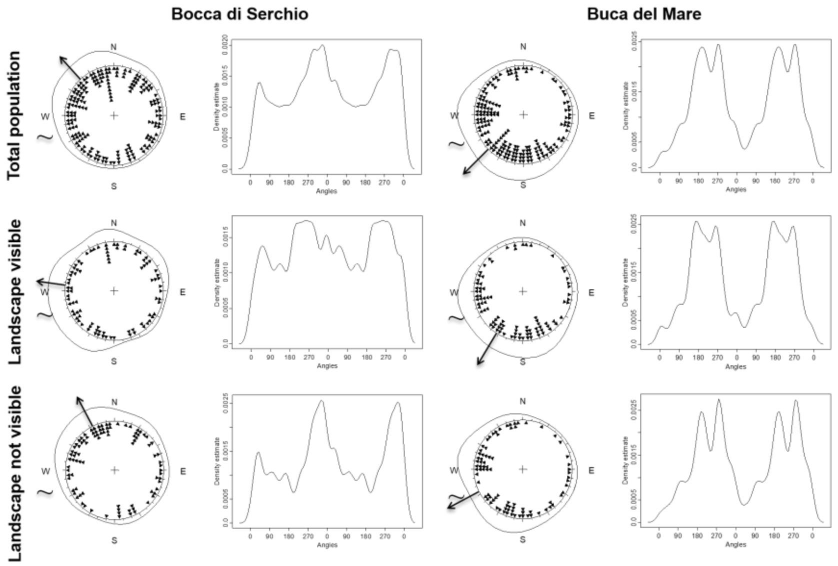


Figure 33. Spring angular distributions from the orientation tests on *Talitrus saltator* on Bocca di Serchio and Buca del Mare beaches: total population, visible and screened landscape. Circular plots of the orientation angles (left); density estimates (kernel method) double plotted on Cartesian graphs (right); ~, sea direction (TED); arrows, mean vectors, with lengths (r) proportional to concentrations. Circular statistics results are shown in Table 15.

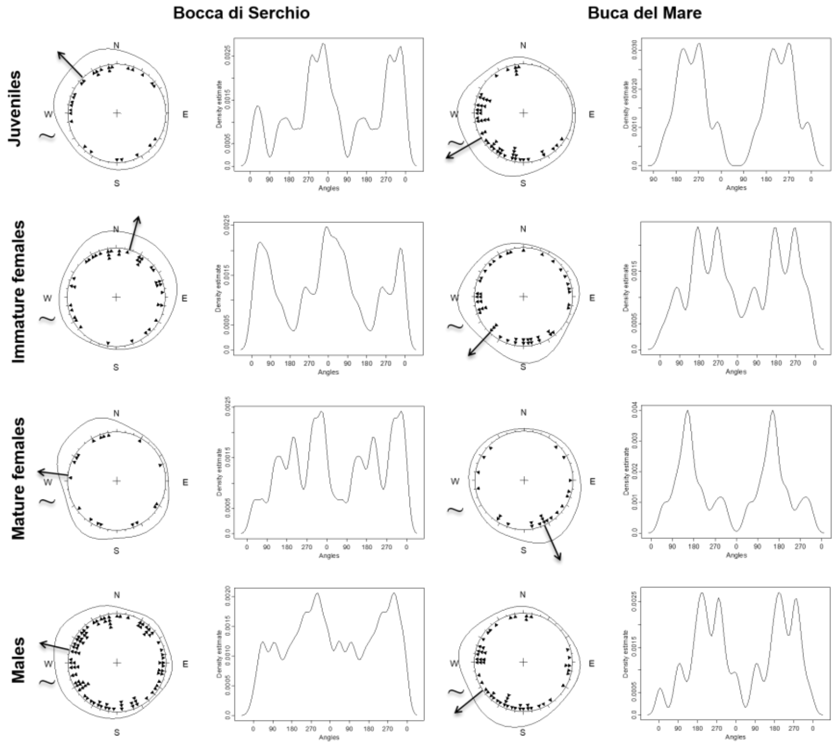


Figure 34. Spring angular distributions from the orientation tests on *Talitrus saltator* on Bocca di Serchio and Buca del Mare beaches: the four classes separated. Circular plots of the orientation angles (left); density estimates (kernel method) double plotted on Cartesian graphs (right); ~, sea direction (TED); arrows, mean vectors, with lengths (r) proportional to concentrations. Circular statistics results are shown in Table 15.

Table 16. Statistical results of the circular distributions of *Talitrus saltator* on Bocca di Serchio and Buca del Mare beaches in autumn: observed mean direction ± 95% confidence interval. Rayleigh test for randomness: ***$p<0.001$, **$p<0.01$ and *$p<0.05$.

		Bocca di Serchio TED=262°	**Buca del Mare TED=260°**
Total population	Observed direction ± CI (°)	123.34 ± 22.29	144.84
	Mean vector length (r)	0.28***	0.1
	Circular dispersion	6.22	47.61
	Sample size (n)	166	174
Landscape visible	Observed direction ± CI (°)	118.16 ± 25.84	196.54
	Mean vector length (r)	0.33***	0.05
	Circular dispersion	4.25	166.30
	Sample size (n)	86	88
Landscape not visible	Observed direction ± CI (°)	131.79 ± 42.54	131.38 ± 59
	Mean vector length (r)	0.22*	0.17
	Circular dispersion	9.52	16.45
	Sample size (n)	80	86
Juveniles	Observed direction ± CI (°)	149.46 ± 46.02	237.88 ± 22.69
	Mean vector length (r)	0.31*	0.53***
	Circular dispersion	4.85	1.59
	Sample size (n)	36	41
Immature females	Observed direction ± CI (°)	137.09	114.72 ± 66.30
	Mean vector length (r)	0.21	0.27
	Circular dispersion	8.04	5.89
	Sample size (n)	18	27
Mature females	Observed direction ± CI (°)	123 ± 27.83	66.18 ± 26.01
	Mean vector length (r)	0.65**	0.49**
	Circular dispersion	0.68	1.20
	Sample size (n)	12	24
Males	Observed direction ± CI (°)	109.94 ± 32.66	109.08
	Mean vector length (r)	0.25**	0.14
	Circular dispersion	7.58	24.61
	Sample size (n)	100	82

CI = confidence interval

In autumn, on Bocca di Serchio the orientation was highly significantly towards a longshore/landwards direction, while on Buca del Mare the distribution was random (Table 16, Fig. 35). A bimodal distribution was evident for Buca del Mare, with two peaks, south-west and north-east: the analysis for axial orientation was applied and an improvement resulted of r length, however not significant (from 0.095 to 0.135; Rayleigh test: $p>0.05$). On Bocca di Serchio beach, the sandhoppers were significantly south-east oriented both with and without the screen, but the concentration was higher when the landscape was visible (Rayleigh test: $p<0.001$); on Buca del Mare beach, random distributions were observed both with and without the screen (Table 16, Fig. 35), but a significant axial orientation was found with landscape visible, with an improvement in r length (r length in the second trigonometric moment =0.22; Rayleigh test: $p<0.05$).

On Bocca di Serchio, juveniles presented a significant south-east orientation, while immature females were randomly oriented; mature females' and males' distributions were more deviated to east (*i.e.* landwards) with respect to the other classes composed by smaller individuals: juveniles and immature females, the former being the most concentrated (compare CI, r and circular dispersion; Table 16, Fig. 36). On Buca del Mare, immature females and males presented random distributions, while juveniles were the most seawards directed among classes, and mature females were significantly landwards directed (Table 16, Fig. 36).

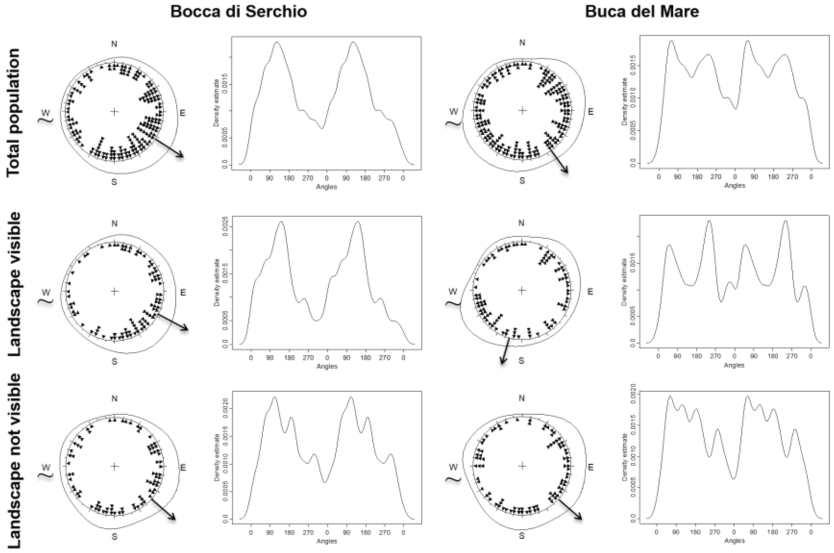


Figure 35. Autumn angular distributions from the orientation tests on *Talitrus saltator* on Bocca di Serchio and Buca del Mare beaches: total population, visible and screened landscape. Circular plots of the orientation angles (left); density estimates (kernel method) double plotted on Cartesian graphs (right); ~, sea direction (TED); arrows, mean vectors, with lengths (r) proportional to concentrations. Circular statistics results are shown in Table 16.

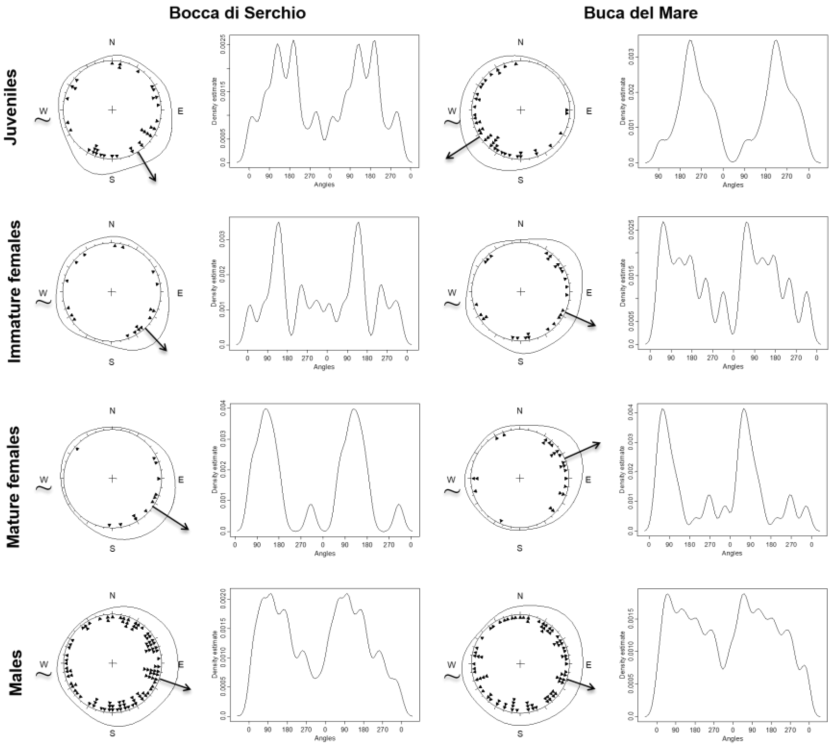


Figure 36. Autumn angular distributions from the orientation tests on *Talitrus saltator* on Bocca di Serchio and Buca del Mare beaches: the four classes separated. Circular plots of the orientation angles (left); density estimates (kernel method) double plotted on Cartesian graphs (right); ~, sea direction (TED); arrows, mean vectors, with lengths (r) proportional to concentrations. Circular statistics results are shown in Table 16.

3.5.4 Multiple regression of orientation angles with intrinsic and environmental variables and factors

The results of SPLM analyses and LRT are shown in Table 17. The best additive model developed from the whole dataset of Bocca di Serchio and Buca del Mare highlighted that season, cloudiness, the time of the day (morning/afternoon) and cephalic length were highly significant variables and factors (M1, Table 17). Both the interactions with the factor "beach" (M2) and with the factor "season" (M3) were highly significant and included the time of the day and cephalic length as highly significant factors, likely discriminating the orientation between beaches and seasons, while sun visibility was significant independently from the beach, and air temperature independently from the season; cloudiness was important to discriminate beaches, and air relative humidity to discriminate seasons (M2, M3, Table 17).

Table 17. Multiple regression models (SPLM) developed for orientation on Bocca di Serchio and Buca del Mare beaches. Likelihood Ratio Test: ***$p<0.001$, **$p<0.01$, *$p<0.05$ and # $0.05<p<0.10$. Bold values = lowest AIC.

Bocca di Serchio and Buca del Mare: spring and autumn		
Model name	**Model description**	**SPLM statistics**
M0: ***initial additive model***	angle ~ beach + season + cloudiness + sun visibility + air temperature + air relative humidity + solar radiation + tide cycle + landscape visibility + morning/afternoon + azimuth + general class + cephalic length + number of antennal segments	Likelihood = 2335.0045, AIC = 2407.0045, df = 653
M1: ***best additive model***	angle ~ season*** + cloudiness*** + air temperature + air relative humidity** + landscape visibility + morning/afternoon*** + azimuth* + cephalic length***	Likelihood = 2356.874, AIC = 2392.874, df = 671
M2: ***best model with interaction with factor "beach"***	angle ~ beach*** (season*** + cloudiness*** + morning/afternoon** + cephalic length***) + sun visibility*** + azimuth	Likelihood = 2302.9465, AIC = 2358.9465, df = 661
M3: ***best model with interaction with factor "season"***	angle ~ season*** (beach*** + air relative humidity*** + landscape visibility # + morning/afternoon*** + cephalic length***) + air temperature**	Likelihood = 2306.5843, AIC = **2358.5843**, df = 663
Bocca di Serchio and Buca del Mare: spring		
M4: ***initial additive model***	angle ~ beach + cloudiness + sun visibility + air temperature + air relative humidity + solar radiation + tide cycle + landscape visibility + morning/afternoon + azimuth + general class + cephalic length + number of antennal segments	Likelihood = 1126.1286, AIC = 1194.1286, df = 315
M5: ***best additive model***	angle ~ beach*** + air temperature* + air relative humidity*** + landscape visibility* + morning/afternoon*** + general class*	Likelihood = 1147.7922, AIC = 1179.7922, df = 333
M6: ***best model with interaction with factor "beach"***	angle ~ beach*** (air relative humidity** + solar radiation** + landscape visibility + morning/afternoon*** + azimuth* + cephalic length**) + cloudiness + sun visibility**	Likelihood = 1104.2983, AIC = **1176.2983**, df = 313
Bocca di Serchio and Buca del Mare: autumn		
M7: ***initial additive model***	angle ~ beach + cloudiness + sun visibility + air temperature + air relative humidity + solar radiation + tide cycle + landscape visibility + morning/afternoon + azimuth + general class + cephalic length + number of antennal segments	Likelihood = 1135.2725, AIC = 1199.2725, df = 308
M8: ***best additive model***	angle ~ beach* + morning/afternoon*** + cephalic length***	Likelihood = 1156.0622, AIC = 1172.0622, df = 332
M9: ***best model with interaction with factor "beach"***	angle ~ beach*** (cephalic length***) + morning/afternoon***	Likelihood = 1130.356, AIC = **1150.356**, df = 330
Bocca di Serchio		
M10: ***initial additive model***	angle ~ season + cloudiness + sun visibility + air temperature + air relative humidity + solar radiation + tide cycle + landscape visibility + morning/afternoon + azimuth + general class + cephalic length + number of antennal segments	Likelihood = 1165.7047, AIC = 1229.7047, df = 312

M11: ***best additive model***	angle ~ cloudiness** + air temperature** + air relative humidity*** + solar radiation* + morning/afternoon*** + azimuth*	Likelihood = 1183.405, AIC = 1211.405, df = 330
M12: ***best model with interaction with factor "season"***	angle ~ season*** (cloudiness*** + air temperature*** + solar radiation** + tide cycle # + morning/afternoon**) + air relative humidity*	Likelihood = 1138.0234, AIC = **1190.0234**, df = 318
	Buca del Mare	
M13: ***initial additive model***	angle ~ season + cloudiness + sun visibility + air temperature + air relative humidity + solar radiation + tide cycle + landscape visibility + morning/afternoon + azimuth + general class + cephalic length + number of antennal segments	Likelihood = 1108.8998, AIC = 1172.8998, df = 313
M14: ***best additive model***	angle ~ sun visibility** + air relative humidity* + solar radiation* + tide cycle + morning/afternoon* + azimuth + cephalic length***	Likelihood = 1120.2323, AIC = 1156.2323, df = 327
M15: ***best model with interaction with factor "season"***	angle ~ season*** (azimuth* + cephalic length***) + sun visibility**	Likelihood = 1105.82, AIC = **1137.82**, df = 329

AIC = Akaike Information Criterion; df = degrees of freedom

The time of the day was highly significant in spring and autumn, resulting an important factor mostly for Bocca di Serchio beach, as air temperature and relative humidity, which have seasonal and daily changes (M5-M12, Table 17, see also section 3.7). Another significant variable was the cephalic length in both seasons, particularly for Buca del Mare beach (M6-M9, M14 and M15, Table 17), highlighting the importance of intrinsic variables in the orientation of sandhoppers in this beach, particularly size. On Buca del Mare beach in autumn, juveniles oriented seawards, whereas the other classes tended to orient landwards (Fig. 36).

Summarizing, meteorological and hourly factors influenced the overall best model, discriminating for seasons, landscape visibility being almost significant (M3, Table 17), while intrinsic factors (age and size) significantly influenced Buca del Mare models only (M14, M15, Table 17). The major difference between beaches depended on the size of sandhoppers, being influenced by meteorological and hourly factors in a different way on the two beaches in both seasons, independently from landscape cues.

3.6. José Ignacio and La Viuda: orientation behaviour

3.6.1. Environmental conditions during the orientation tests

On José Ignacio beach, air temperature and solar radiation were higher in spring than in autumn (ANOVA: p<0.001), while air relative humidity did not differ between seasons. On La Viuda beach, air temperature (ANOVA: p=0.06) and solar radiation were higher and air relative humidity lower in spring than in autumn (ANOVA: *p*<0.001). The three variables significantly differed between beaches in both seasons (Table 18): on La Viuda, solar radiation in spring and air temperature in autumn were higher with respect to José Ignacio.

Table 18. Environmental variables measured during the orientation experiments on José Ignacio and La Viuda beaches in spring and autumn. Comparisons between the two beaches, ANOVA: ****p*<0.001.

		José Ignacio	**La Viuda**	*p*
spring	Sea water pH	-	8.16	
	Salinity	30	25.5	
	Sea water temperature (°C)	19.8	21	
	Cloudiness (% of observations)	0/8 (50); 4/8 (22.94); 5/8 (9.41); 6/8 (7.65); 8/8 (10)	0/8 (100)	
	Sun visibility (% of observations)	vis (57.65); vel (24.70); shp (17.65)	vis (100)	
	Mean air temperature ± se (min - max): °C	25.92 ± 0.18 (22.4 - 30.4)	24.44 ± 0.25 (19.3 - 28.9)	***
	Mean air relative humidity ± se (min - max): %	65.37 ± 0.72 (49 - 83)	41.75 ± 0.31 (36 - 51)	***
	Mean solar radiation ± se (min - max): klx	131.34 ± 2.23 (60.4 - 173.8)	140.46 ± 1.33 (113.3 - 163.8)	***
	Tidal range (m)	0.4 - 0.9 m	0.4 - 1.1 m	
autumn	Sea water pH	7.6	7.14	
	Salinity	21.8	29.1	
	Sea water temperature (°C)	19.4	19.9	
	Cloudiness (% of observations)	3/8 (25.34); 4/8 (38.36); 5/8 (36.30)	0/8 (100)	
	Sun visibility (% of observations)	vis (69.18); vel (30.82)	vis (100)	
	Mean air temperature ± se (min - max): °C	21.89 ± 0.09 (20.4 - 24)	23.77 ± 0.18 (19 - 26.4)	***
	Mean air relative humidity ± se (min - max): %	63.79 ± 0.65 (49 - 73)	52.46 ± 0.75 (43 - 70)	***
	Mean solar radiation ± se (min - max): klx	76.21 ± 1.69 (30.2 - 97.2)	85.58 ± 1.09 (56.8 - 109.2)	***
	Tidal range (m)	0.5 - 0.9 m	0.5 - 1.1 m	

percentages of observations = number of observations under each condition/total of observations; se = standard error; vis = visible; vel = veiled; shp = solar disk; *ns* = not significant; (-) not measured

3.6.2. Population variables and structure of the tested samples

On José Ignacio beach, the cephalic length and antennal segments differed between seasons (ANOVA: *p*<0.001), the value in spring being smaller than in autumn. On La Viuda beach, the two variables did not differ between seasons (ANOVA: *p*>0.05). No differences were detected between beaches in spring, while in autumn, the two variables were significantly higher on José Ignacio than on La Viuda (Table 19).

On José Ignacio beach, males in spring and mature females in autumn presented the highest proportion among classes; immature females had a quite high proportion in spring and low proportion in autumn, as juveniles (Table 19).

Table 19. Population variables of *Atlantorchestoidea brasiliensis* for José Ignacio and La Viuda beaches, in spring and autumn. ANOVA: ***$p<0.001$ and *ns* = non-significant.

	José Ignacio	**J**	**Fi**	**Fm**	**M**
spring	Number of individuals	52	39	20	59
tot=170	Proportion: ind/tot	0.31	0.23	0.12	0.35
	Cephalic length (mm): mean ± se	0.65 ± 0.01	0.84 ± 0.01	1.05 ± 0.02	0.86 ± 0.01
	Number of antennal segments: mean ± se	9.37 ± 0.14	11.71 ± 0.16	13.85 ± 0.21	12.29 ± 0.16
	Sex ratio [M/(M+F)]	0.5			
autumn	Number of individuals	14	18	67	47
tot=146	Proportion: ind/tot	0.10	0.12	0.46	0.32
	Cephalic length (mm): mean ± se	0.68 ± 0.02	0.91 ± 0.02	1.11 ± 0.01	1.02 ± 0.01
	Number of antennal segments: mean ± se	9.43 ± 0.14	12.28 ± 0.3	14.94 ± 0.12	14.74 ± 0.23
	Sex ratio [M/(M+F)]	0.36			
	La Viuda	**J**	**Fi**	**Fm**	**M**
spring	Number of individuals	58	18	24	37
tot=137	Proportion: ind/tot	0.42	0.13	0.18	0.27
	Cephalic length (mm): mean ± se	0.59 ± 0.01	0.83 ± 0.03	1.20 ± 0.1	0.95 ± 0.3
	Number of antennal segments: mean ± se	8.95 ± 0.14	11.78 ± 0.24	15.42 ± 0.16	14.22 ± 0.49
	Sex ratio [M/(M+F)]	0.47			
autumn	Number of individuals	75	13	45	21
tot=154	Proportion: ind/tot	0.49	0.08	0.29	0.14
	Cephalic length (mm): mean ± se	0.65 ± 0.01	0.92 ± 0.04	1.09 ± 0.01	0.95 ± 0.03
	Number of antennal segments: mean ± se	9.53 ± 0.13	12.54 ± 0.24	14.47 ± 0.14	13.67 ± 0.45
	Sex ratio [M/(M+F)]	0.27			

		José Ignacio	**La Viuda**	*p*
spring	Mean cephalic length ± se (min - max)	0.81 ± 0.01 mm (0.47 - 1.17)	0.83 ± 0.02 mm (0.47 - 1.3)	*ns*
	Mean number of antennal segments ± se (min - max)	11.45 ± 0.14 (8 - 16)	11.88 ± 0.28 (7 - 20)	*ns*
autumn	Mean cephalic length ± se (min - max)	1.01 ± 0.01 mm (0.6 - 1.33)	0.84 ± 0.02 mm (0.33 - 1.3)	***
	Mean number of antennal segments ± se (min - max)	14.02 ± 0.17 (9 - 17)	11.79 ± 0.21 (8 - 18)	***

tot = total of captured individuals; se, standard error; M, males; F, females; J, Juveniles; Fi, immature females; Fm, mature females

On La Viuda beach, juveniles presented the highest proportion in both seasons, and immature females the lowest; mature females had a low proportion in spring and quite high in autumn, opposite with respect to males (Table 19). Sex ratio was always female biased, except for José Ignacio in spring, where it was balanced, and quite balanced on La Viuda in spring (Table 19).

Mature females always presented the highest cephalic length, while juveniles the lowest; immature females and males presented similar values, males being slightly larger (Table 19; Figs. 37a, 37c). Regarding the number of antennal segments, the values decreased from mature females to males, immature females, and juveniles (Table 19; Figs 37b, 37d).

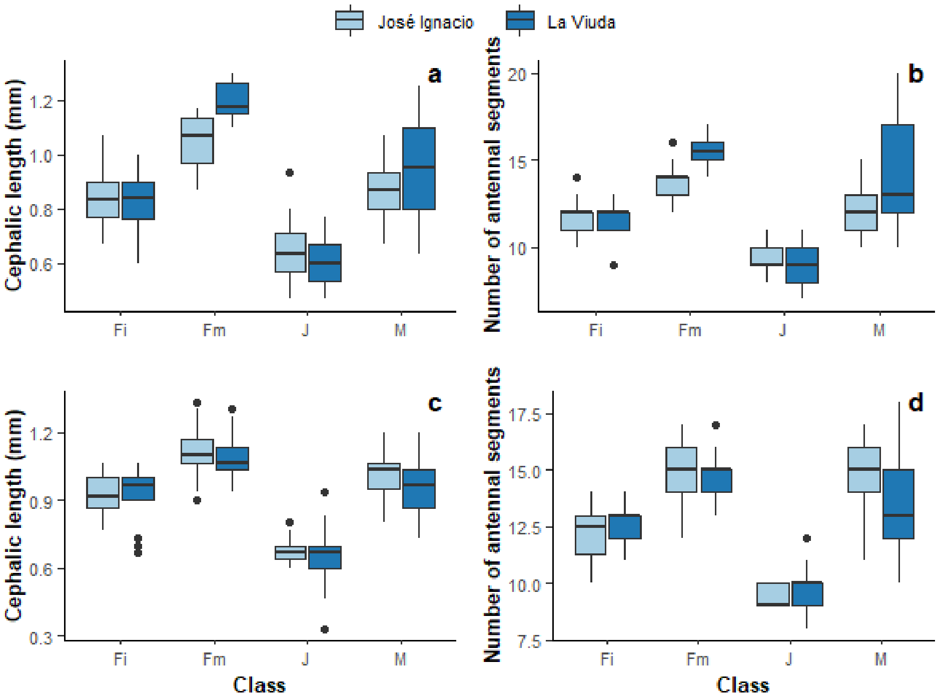


Figure 37. Cephalic length and number of antennal segments of *Atlantorchestoidea brasiliensis* by population component in José Ignacio and La Viuda beaches in spring (a and b) and autumn (c and d). Fi, immature females; Fm, mature females; J, juveniles; M, males.

3.6.3. Orientation tests: circular statistics

In spring, on both beaches, sandhoppers were seawards directed and well concentrated; the orientation was significantly different from randomness both with and without the screen for landscape view, highlighting the use both of environmental and solar cues; on both beaches, the orientation seawards improved when the landscape was visible (compare CI, r length and circular dispersion in Table 20; Fig. 38).

Table 20. Statistical results of the circular distributions of *Atlantorchestoidea brasiliensis* on José Ignacio and La Viuda beaches in spring: observed mean direction ± 95% confidence interval. Rayleigh test for randomness: ***p<0.001.

		José Ignacio TED=127°	**La Viuda TED=151°**
Total population	Observed direction ± CI (°)	134.92 ± 7.13	157.76 ± 8.17
	Mean vector length (r)	0.69***	0.66***
	Circular dispersion	0.68	0.72
	Sample size (n)	170	137
Landscape visible	Observed direction ± CI (°)	133.22 ± 8.69	156.24 ± 8.94
	Mean vector length (r)	0.80***	0.78***
	Circular dispersion	0.43	0.41
	Sample size (n)	72	65
Landscape not visible	Observed direction ± CI (°)	136.58 ± 11.19	159.71 ± 14.61
	Mean vector length (r)	0.61***	0.55***
	Circular dispersion	0.96	1.19
	Sample size (n)	98	72
Juveniles	Observed direction ± CI (°)	137.79 ± 12.06	157.46 ± 12.73
	Mean vector length (r)	0.73***	0.68***
	Circular dispersion	0.59	0.73
	Sample size (n)	52	58
Immature females	Observed direction ± CI (°)	127.83 ± 15.34	163.86 ± 17.57
	Mean vector length (r)	0.63***	0.82***
	Circular dispersion	0.71	0.43
	Sample size (n)	39	18
Mature females	Observed direction ± CI (°)	143.75 ± 27.96	157.41 ± 20.12
	Mean vector length (r)	0.60***	0.58***
	Circular dispersion	1.15	0.74
	Sample size (n)	20	24
Males	Observed direction ± CI (°)	133.99 ± 11.26	154.61 ± 17
	Mean vector length (r)	0.73***	0.62***
	Circular dispersion	0.59	0.82
	Sample size (n)	59	37

CI = confidence interval

On both beaches each of the four classes presented a highly significant seawards direction, mature females resulting slightly less concentrated with respect to the other classes (Table 20, Fig. 39).

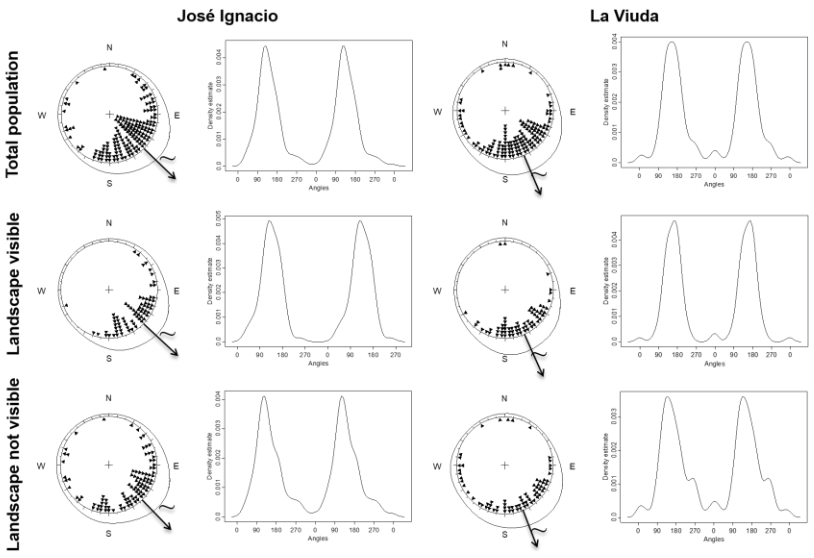


Figure 38. Spring angular distributions from the orientation tests on *Atlantorchestoidea brasiliensis* on José Ignacio and La Viuda beaches: total population, visible and screened landscape. Circular plots of the orientation angles (left); density estimates (kernel method) double plotted on Cartesian graphs (right); ~, ocean direction (TED); arrows, mean vectors, with lengths (r) proportional to concentrations. Circular statistics results are shown in Table 20.

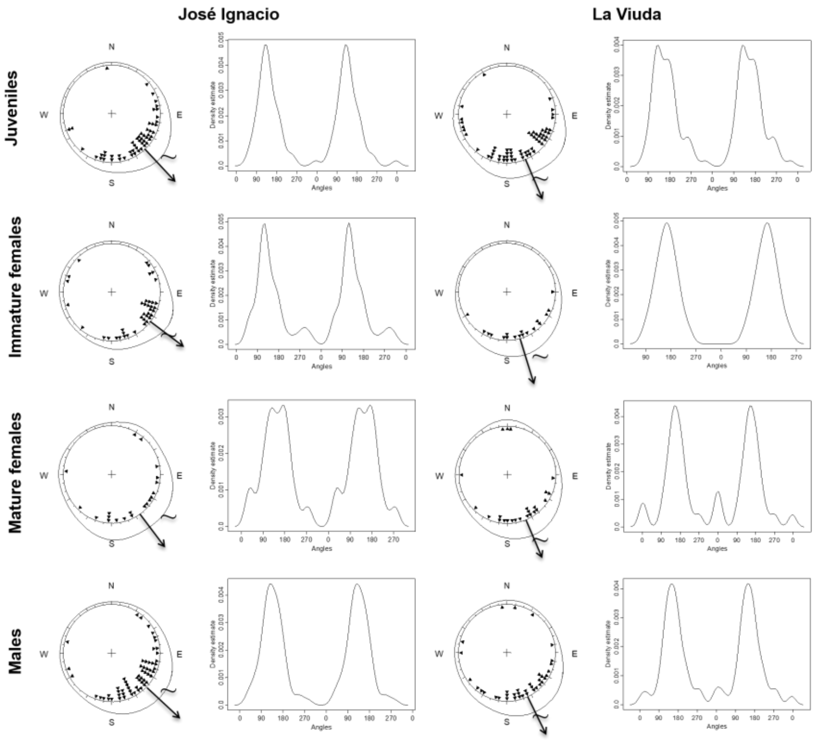


Figure 39. Spring angular distributions from the orientation tests on *Atlantorchestoidea brasiliensis* on José Ingacio and La Viuda beaches: the four classes separated. Circular plots of the orientation angles (left); density estimates (kernel method) double plotted on Cartesian graphs (right); ~, ocean direction (TED); arrows, mean vectors, with lengths (r) proportional to concentrations. Circular statistics results are shown in Table 20.

Table 21. Statistical results of the circular distributions of *Atlantorchestoidea brasiliensis* on José Ignacio and La Viuda beaches in autumn: observed mean direction ± 95% confidence interval. Rayleigh test for randomness: ***p<0.001, **p<0.01 and *p<0.05.

		José Ignacio TED=131°	**La Viuda TED=144°**
Total population	Observed direction ± CI (°)	172.02 ± 17.45	155.98 ± 67.48
	Mean vector length (r)	0.36***	0.12
	Circular dispersion	3.42	34.21
	Sample size (n)	146	154
Landscape visible	Observed direction ± CI (°)	163.39 ± 22.89	133.20 ± 39.31
	Mean vector length (r)	0.37***	0.24*
	Circular dispersion	2.84	8.67
	Sample size (n)	72	83
Landscape not visible	Observed direction ± CI (°)	180.78 ± 25.60	247.92
	Mean vector length (r)	0.36***	0.11
	Circular dispersion	3.60	37.33
	Sample size (n)	74	71
Juveniles	Observed direction ± CI (°)	136.17 ± 61.74	113.13 ± 45.79
	Mean vector length (r)	0.40	0.21
	Circular dispersion	2.83	10.03
	Sample size (n)	14	75
Immature females	Observed direction ± CI (°)	144.88 ± 28.53	305.85
	Mean vector length (r)	0.54**	0.27
	Circular dispersion	1.07	4.55
	Sample size (n)	18	13
Mature females	Observed direction ± CI (°)	189.39 ± 21.94	205.27
	Mean vector length (r)	0.41***	0.19
	Circular dispersion	2.43	13.23
	Sample size (n)	67	45
Males	Observed direction ± CI (°)	169.45 ± 41.35	186.93
	Mean vector length (r)	0.28*	0.23
	Circular dispersion	5.34	8.33
	Sample size (n)	47	21

CI = confidence interval

In autumn, on José Ignacio beach sandhopper distribution was significantly oriented, however south deviated with respect to the TED, showing two peaks, a larger one seawards and a smaller one longshore; when the landscape was not visible, the direction was south oriented and a smaller peak appeared to the south-west direction (longshore) and a larger one seawards, with a slightly higher circular dispersion with respect to the orientation obtained with the landscape vision (compare CI and r length in Table 21; Fig. 40).

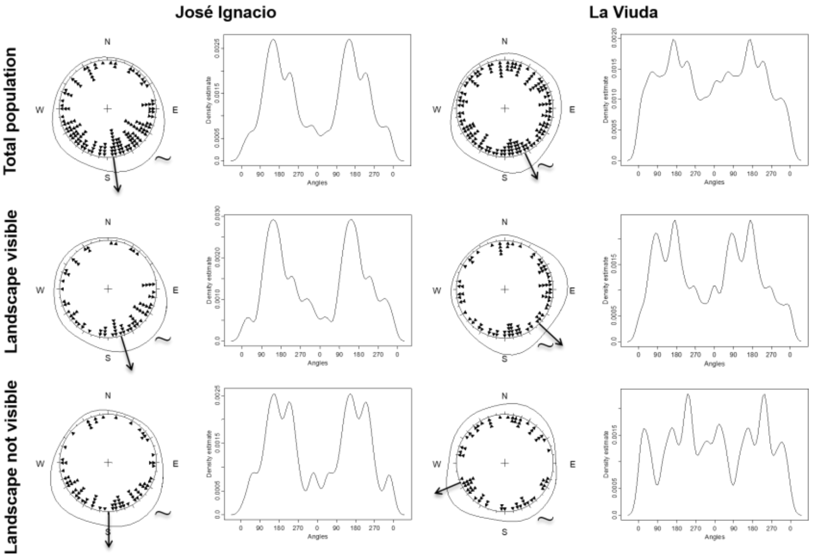


Figure 40. Autumn angular distributions from the orientation tests on *Atlantorchestoidea brasiliensis* on José Ignacio and La Viuda beaches: total population, visible and screened landscape. Circular plots of the orientation angles (left); density estimates (kernel method) double plotted on Cartesian graphs (right); ~, ocean direction (TED); arrows, mean vectors, with lengths (r) proportional to concentrations. Circular statistics results are shown in Table 21.

On la Viuda beach, sandhoppers were randomly oriented, but when the landscape was visible, the direction was significantly seawards and the distribution presented two peaks to east and south (*i.e.* longshore); the random orientation observed in autumn when the landscape was screened off, under clear sky and visible sun, can be explained considering the effect of intrinsic or climatic variables in the SPLM analysis in autumn (Table 21, Fig. 40, for SPLM see 3.6.4 and Table 22 below).

On José Ignacio, juveniles were randomly oriented, while immature females were seawards oriented, and mature females and males were south oriented (*i.e.* longshore), but all the adults presented a low concentration around the mean direction (Table 21, Fig. 41). The random orientation of each of the four classes, when considered separately on La Viuda beach, reflects the scatter of the entire population (Table 21, Fig. 41).

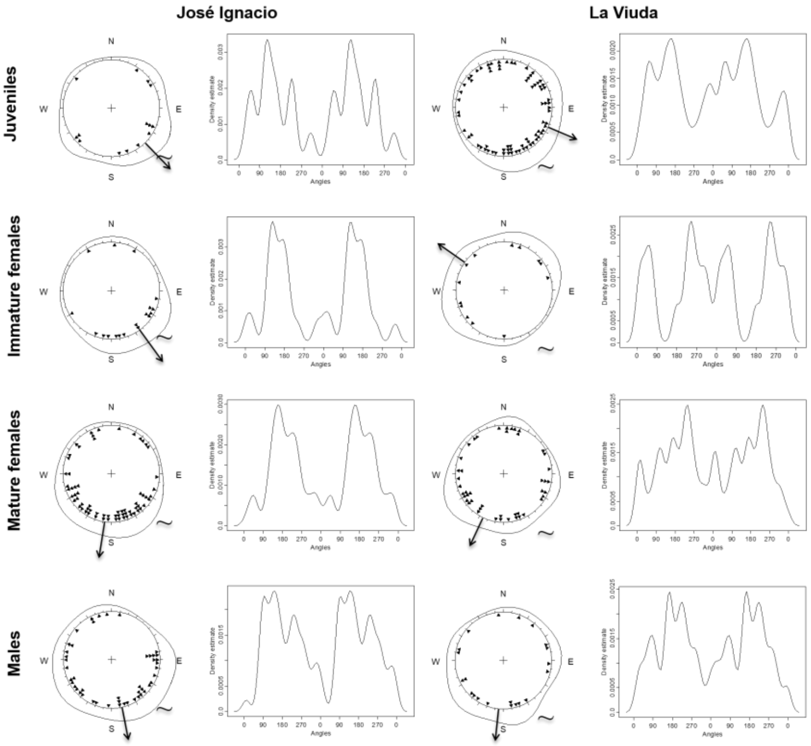


Figure 41. Autumn angular distributions from the orientation tests on *Atlantorchestoidea brasiliensis* on José Ingacio and La Viuda beaches: the four classes separated. Circular plots of the orientation angles (left); density estimates (kernel method) double plotted on Cartesian graphs (right); ~, ocean direction (TED); arrows, mean vectors, with lengths (r) proportional to concentrations. Circular statistics results are shown in Table 21.

3.6.4 Multiple regression of orientation angles with intrinsic and environmental variables and factors

The best additive model developed from the whole dataset of José Ignacio and La Viuda, highlighted the importance of the factors: beach, season, tide cycle, meteorological and hourly variables (cloudiness, air temperature, solar radiation) on the orientation of the two populations in both seasons (M1, Table 22). Both the interactions of beach and season were highly significant, including within both interactions air temperature, relative humidity and sun azimuth; tide cycle and the time of the day (morning/afternoon) were significant factors, likely discriminating the orientation between beaches; solar radiation and landscape view were significant factors, discriminating between seasons, while sun visibility and cephalic length were significant independently from the season (M2, M3, Table 22).

Table 22. Multiple regression models (SPLM) developed for orientation on José Ignacio and La Viuda beaches. Likelihood Ratio Test: ***p<0.001, **p<0.01, *p<0.05 and # 0.05<p<0.10. Bold values = lowest AIC.

José Ignacio and La Viuda: spring and autumn		
Model name	**Model description**	**SPLM statistics**
M0: ***initial additive model***	angle ~ beach + season + cloudiness + sun visibility + air temperature + air relative humidity + solar radiation + tide cycle + landscape visibility + morning/afternoon + azimuth + general class + cephalic length + number of antennal segments	Likelihood = 1783.2835, AIC = 1851.2835, df = 573
M1: ***best additive model***	angle ~ beach*** + season*** + cloudiness** + air temperature*** + air relative humidity + solar radiation*** + tide cycle*** + landscape visibility** + number of antennal segments	Likelihood = 1787.2004, AIC = 1831.2004, df = 585
M2: ***best model with interaction with factor “beach”***	angle ~ beach*** (air temperature*** + air relative humidity*** + tide cycle*** + landscape visibility* + morning/afternoon*** + azimuth***) + sun visibility	Likelihood = 1775.8757, AIC = 1839.8757, df = 575
M3: ***best model with interaction with factor “season”***	angle ~ season*** (air temperature*** + air relative humidity*** + solar radiation*** + landscape visibility*** + azimuth***) + sun visibility*** + cephalic length***	Likelihood = 1768.298, AIC = **1828.298**, df = 577
José Ignacio and La Viuda: spring		
M4: ***initial additive model***	angle ~ beach + cloudiness + sun visibility + air temperature + air relative humidity + solar radiation + landscape visibility + morning/afternoon + azimuth + general class + cephalic length + number of antennal segments	Likelihood = 738.5438, AIC = 798.5438, df = 277
M5: ***best additive model***	angle ~ sun visibility** + air temperature* + solar radiation*** + landscape visibility** + azimuth*	Likelihood = 743.9276, AIC = **771.9276**, df = 293
M6: ***best model with interaction with factor “beach”***	angle ~ beach** (air temperature* + air relative humidity # + landscape visibility # + morning/afternoon*)	Likelihood = 744.6055, AIC = 784.6055, df = 287
José Ignacio and La Viuda: autumn		
M7: ***initial additive model***	angle ~ beach + cloudiness + sun visibility + air temperature + air relative humidity + solar radiation + tide cycle + landscape visibility + morning/afternoon + azimuth + general class + cephalic length + number of antennal segments	Likelihood = 994.608, AIC = 1054.608, df = 270
M8: ***best additive model***	angle ~ beach*** + cloudiness** + sun visibility* + air temperature* + solar radiation** + tide cycle* + morning/afternoon* + azimuth* + cephalic length	Likelihood = 1005.9239, AIC = 1045.9239, df = 280
M9: ***best model with interaction with factor “beach”***	angle ~ beach*** (air temperature*** + air relative humidity*** + solar radiation*** + azimuth*) + sun visibility*** + landscape visibility + cephalic length	Likelihood = 986.0842, AIC = **1038.0842**, df = 274
José Ignacio		
M10: ***initial additive model***	angle ~ season + cloudiness + sun visibility + air temperature + air relative humidity + solar radiation + tide cycle + landscape visibility + morning/afternoon + azimuth + general class + cephalic length + number of antennal segments	Likelihood = 872.7488, AIC = 936.7488, df = 284

M11: ***best additive model***	angle ~ season*** + cloudiness + sun visibility*** + solar radiation* + morning/afternoon + azimuth*	Likelihood = 880.2026, AIC = 912.2026, df = 300
M12: ***best model with interaction with factor "season"***	angle ~ season*** (solar radiation*** + azimuth***)	Likelihood = 888.0149, AIC = **912.0149**, df = 304
	La Viuda	
M13: ***initial additive model***	angle ~ season + air temperature + air relative humidity + solar radiation + tide cycle + landscape visibility + morning/afternoon + azimuth + general class + cephalic length + number of antennal segments	Likelihood = 872.725, AIC = 924.725, df = 265
M14: ***best additive model***	angle ~ air temperature*** + air relative humidity*** + tide cycle*** + landscape visibility** + morning/afternoon*** + azimuth***	Likelihood = 884.9013, AIC = **912.9013**, df = 277
M15: ***best model with interaction with factor "season"***	angle ~ season*** (air temperature* + air relative humidity*** + solar radiation* + landscape visibility* + morning/afternoon* + azimuth*) + cephalic length	Likelihood = 857.834, AIC = 917.834, df = 261

AIC = Akaike Information Criterion; df = degrees of freedom

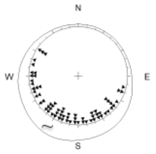
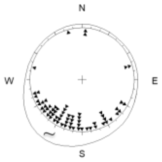
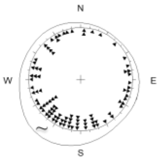
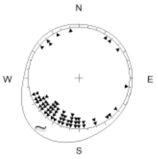
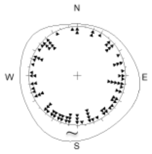
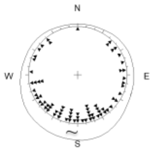
Factors such as sun azimuth and solar radiation (hourly factors, which changed throughout the experiments) were significant in almost all the models developed separating the two beaches in spring and autumn, and then the two seasons in each beach, except for the model with the interaction with beach in spring (M6, for both factors, Table 22) and the additive model for La Viuda beach (M14, Table 22), highlighting the importance of these factors on the orientation of both populations. Sun visibility was confirmed as an important orienting factor, particularly for José Ignacio beach (M11, Table 22), but we must consider that on La Viuda beach the sun was always visible and cloudiness always equal to 0 (thus these factors did not vary during the orientation experiments) (Table 18). Air temperature was important in all models except for José Ignacio beach (M11, M12, Table 22), and air relative humidity was highly significant in the model with the interaction with beach in autumn (discriminating for beaches) and for La Viuda beach (M9, M14, M15, Table 22), highlighting the importance of climatic variables, mostly for la Viuda beach. Intrinsic variables where not significant and only present in the models with the interaction with beach in autumn and with the interaction with season for La Viuda beach (M9, M15, Table 22).

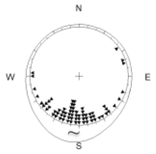
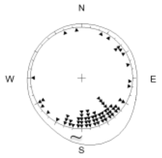
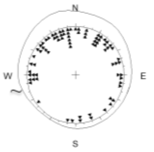
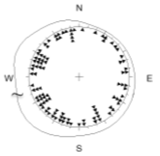
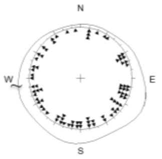
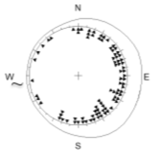
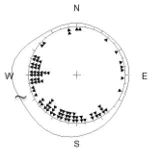
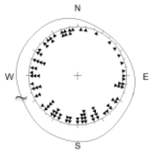
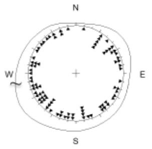
Summarizing, meteorological factors were significant for both beaches, discriminating for season (compare M11, M12 and M14, Table 22), whereas landscape view was a significant factor for La Viuda only (M14, Table 22). Intrinsic factors, namely age and size, significantly influenced the overall best model (M3, Table 22), confirming the dependence of size on meteorological factors, particularly air humidity. Thus, the major differences between the two beaches depended on the response to landscape cues.

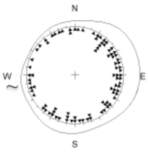
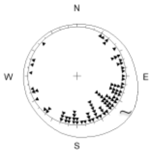
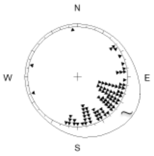
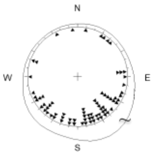
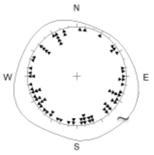
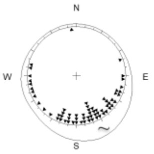
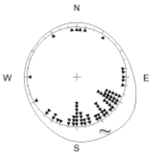
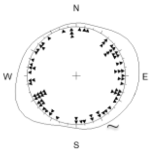
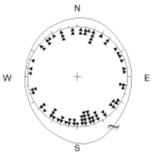
3.7. Influence of meteorological factors, tide cycle and time of the day

A summarizing table was constructed with the circular distributions of the six beaches according to the tide cycle, (ebbing and rising tide); when the numbers of observations did not allow to compare the two tide cycles, comparisons were made according to the time of the day (morning/afternoon); comparisons were also conducted for the differences between air temperature, air relative humidity and solar radiation (Table 23).

Table 23. Summary of the morning (upper line) and afternoon (lower line) conditions of each beach in the two seasons. The angular distributions shown correspond to the tide cycle; the distributions highlighted with (*) correspond to the time of the day (morning/afternoon). Significant differences of air temperature, air relative humidity and solar radiation between rising/ebbing tide or morning/afternoon are highlighted with symbols (ANOVA: *p*<0.05). Empty spaces represent not significant differences.

Beach	Season	Cloud	Sun	T	Humid	Lux	Tide	Orientation
Collelungo	Spring	☁ ⛅	◐ ☼	⊖	⊕	⊕	↑	seawards (*) 
		0 ⛅	☼	⊕	⊖	⊖	↑	seawards (*) 
	Autumn	⛅	☼	⊖		⊕	↓	seawards 
		⛅	☼	⊕		⊖	↑	seawards 
Macchiatonda	Spring	0 ☁	☼ ◐	⊖		⊕	↑	scattered 
		0	☼	⊕		⊖	↓	seawards 

	Autumn	☁	☼ ◑	⊖	➕		↓	seawards 
		☁	◑	➕	⊖		↑	longshore 
Bocca di Serchio	Spring	**☁**	● ◑	⊖	⊖	⊖	↑	longshore/landwards 
		☁	☼	➕	➕	➕	↓	seawards/longshore 
	Autumn	**☁**	● ◑	⊖	➕	⊖	↑	scattered (*) 
		☁ **☁**	☼ ●	➕	⊖	➕	↑	landwards (*) 
Buca del Mare	Spring	0	☼	➕	⊖	➕	↑	two peaks longshore 
		☁ **☁**	● ◌ ☼	⊖	➕	⊖	↓	longshore 
	Autumn	☁	☼	➕	⊖	➕	↓	scattered (two peaks seawards/landwards) 

		☁	☼	⊖	⊕	⊖	↑	landwards 
José Ignacio	Spring	☁ ☁	◐ ○ ☼	⊕		⊖	↓	seawards 
		0	☼	⊖		⊕	↑	seawards 
	Autumn	☁ ☁	◐ ☼	⊖	⊕	⊖	↑	longshore 
		☁ ☁	◐ ☼	⊕	⊖	⊕	↓	scattered 
La Viuda	Spring	0	☼	⊖			↓	seawards 
		0	☼	⊕			↑	seawards/longshore 
	Autumn	0	☼	⊖	⊕	⊖	↑	scattered (axial) (*) 
		0	☼	⊕	⊖	⊕	↑	scattered (axial) (*) 

Cloud, cloudiness; 0, no clouds; (☁), cloudiness equal to 1-4/8; (☁), cloudiness equal to 5-8/8; Sun, sun visibility; (☼), visible sun; (◐), veiled sun; (○), solar disk; (●), not visible sun; T, air temperature; Humid, air relative humidity; Lux, solar radiation; (⊕), significantly higher; (⊖), significantly lower; (↑), rising tide; (↓), ebbing tide

In spring, on Macchiatonda beach, a difference was observed in orientation with rising and ebbing tide (tide cycle resulted an important factor also in the SPLM, see 3.4.4, Table 12 above): with rising tide (from the morning to the first afternoon release), the orientation was scattered (mean direction ± CI: 175.83 ± 53.63; r length=0.18; circular dispersion=15.02; n=89; Rayleigh test: *p*>0.05), while with ebbing tide the orientation was significantly seawards (mean direction ± CI: 185.42 ± 19.68; r=0.46; circular dispersion=2.10; n=71; Rayleigh test: *p*<0.001). Considering the time of the day, the orientation similarly changed as with the tide cycle (being scattered in the morning and seawards in the afternoon). On Collelungo beach the tide was rising during all experimental sessions; the orientation was seawards oriented, slightly more concentrated in the afternoon (mean direction ± CI: 199.07 ± 10.93; r=0.69; circular dispersion=0.61; n=65; Rayleigh test: *p*<0.001) than in the morning (mean direction ± CI: 200.87 ± 14.39; r=0.65; circular dispersion=1.08; n=67; Rayleigh test: *p*<0.001).

In autumn, on Macchiatonda, during the morning the tide was ebbing and the orientation was seawards (mean direction ± CI: 186.62 ± 8.38; r length=0.80; circular dispersion=0.39; n=70; Rayleigh test: *p*<0.001), while during the afternoon the tide was rising and the orientation was south-east displaced, *i.e.* longshore (mean direction ± CI: 157.68 ± 10.58; r length=0.67; circular dispersion=0.61; n=69; Rayleigh test: *p*<0.001). On Collelungo sandhoppers were seawards oriented both in the morning (mean direction ± CI: 221.17 ± 29.49; r length=0.31; circular dispersion=4.92; n=78) and in the afternoon (mean direction ± CI: 208.76 ± 9.24; r length=0.68; circular dispersion=0.52; n=77; Rayleigh tests: *p*<0.001). The tide was ebbing during the morning and the first release of the afternoon, then it was rising, orientation being similar to morning/afternoon releases.

On Bocca di Serchio beach, in spring, during the morning tests, sandhopper orientation was quite concentrated northwards; during the afternoon tests, sandhopper orientation resulted less concentrated than in the morning tests (compare the vector lengths and circular dispersion), but significantly oriented to the south-west, more seawards with respect to the morning (Fig. 42). Similar results were found considering the tide cycle: tide was rising during the morning until release 7 (mean direction ± CI: 359.38 ± 21.31; r length=0.41; circular dispersion=2.68; n=78; Rayleigh test: *p*<0.001), then tide was ebbing, and sandhoppers concentration decreased (mean direction ± CI: 248.72 ± 37.41; r length=0.23; circular dispersion=9.61; n=100; Rayleigh test: *p*>0.01). On Buca del Mare beach, in spring, during the morning sandhopper orientation was quite concentrated to south-west (with two peaks longshore); during the afternoon experiments the tide was ebbing, sandhopper orientation resulted more scattered than in the morning (compare the vector lengths and circular dispersions) however significantly oriented to south-south-west: the second release was performed under light raining condition (Fig. 42).

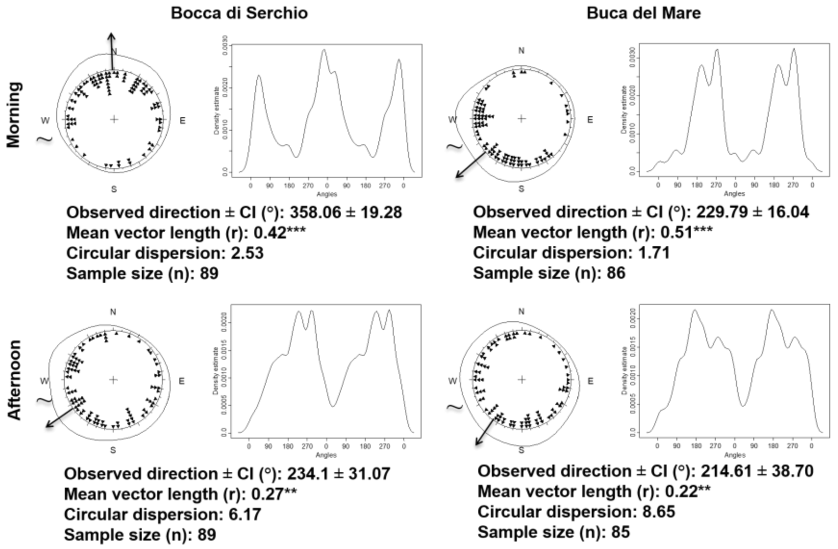


Figure 42. Spring angular distributions from the orientation tests on *Talitrus saltator* on Bocca di Serchio and Buca del Mare beaches: morning and afternoon. Circular plots of the orientation angles (left); density estimates (kernel method) double plotted on Cartesian graphs (right); ~, sea direction (TED); arrows, mean vectors, with lengths (r) proportional to concentrations. Circular statistics results are shown below each distribution. Rayleigh test: ****p*<0.001 and ***p*<0.01.

On Bocca di Serchio, in autumn, during the morning tests sandhoppers were scattered (mean direction ± CI: 170.17 ± 49.70; r length=0.19; circular dispersion=12.11; n=80; Rayleigh test: *p*>0.05), while during the afternoon sandhoppers were landward directed (mean direction ± CI: 105.49 ± 19.86; r length=0.43; circular dispersion=2.59; n=86; Rayleigh test: *p*<0.001). Tide was mostly rising during all day, and a landward orientation, slightly scattered, was observed; only the first release of the morning and the last of the afternoon were during ebbing tide, and a random orientation was observed. On Buca del Mare, during the morning tests, the tide was ebbing, and sandhoppers were scattered, but one peak seaward and one landward were observed (mean direction ± CI: 219.19 ± 56.36; r length=0.16; circular dispersion=15.52; n=86; Rayleigh test: *p*>0.05); during the afternoon, the tide was rising, and sandhoppers were landwards oriented (mean direction ± CI: 97.97 ± 41.02; r length=0.21; circular dispersion=9.87; n=88; Rayleigh test: *p*<0.05).

In spring, on José Ignacio the orientation was seawards both during the morning, with ebbing tide (mean direction ± CI: 139.99 ± 12.86; r length=0.59; circular dispersion=1.10;

n=85; Rayleigh test: $p<0.001$), and the afternoon, with rising tide (mean direction ± CI: 131.10 ± 7.91; r length=0.79; circular dispersion=0.42; n=85; Rayleigh test: $p<0.001$). On La Viuda sandhoppers were seawards both in the morning, with ebbing tide (mean direction ± CI: 168.16 ± 12.27; r length=0.67; circular dispersion=0.75; n=74; Rayleigh test: $p<0.001$), and in the afternoon, with rising tide (with a peak longshore, mean direction ± CI: 148.62 ± 10.67; r length=0.67; circular dispersion=0.65; n=73; Rayleigh test: $p<0.001$).

In autumn, on José Ignacio beach during the morning, with rising tide, sandhoppers were longshore oriented (mean direction ± CI: 164.14 ± 15.62; r length=0.54; circular dispersion=1.38; n=73; Rayleigh test: $p<0.001$); during the afternoon, with ebbing tide, sandhoppers were dispersed (mean direction ± CI: 193.76 ± 50.33; r length=0.20; circular dispersion=11.26; n=73; Rayleigh test: $p>0.05$). On La Viuda, the tide was rising both during the morning and afternoon experiments, and the orientation was scattered but appeared axial both in the morning (two peaks longshore; mean direction: 143.23; r length=0.07; circular dispersion=89.76; n=77; Rayleigh test: $p>0.05$; axial orientation: r length=0.22, Rayleigh test: $p<0.05$) and in the afternoon (two peaks longshore and landwards; mean direction ± CI: 160.90 ± 50.79; r length=0.17; circular dispersion=12.73; n=77; Rayleigh test: $p>0.05$; axial orientation: r length=0.31, Rayleigh test: $p<0.001$).

3.8. Comparisons among the six beaches

3.8.1. Environment

A cluster analysis was made including the environmental variables registered at the different stations of the six beaches. When all the environmental data are included in the cluster analysis, a group of stations "0" (*i.e.*, at the shoreline) was formed, while when the elevation values were excluded, these samples were redistributed to other groups (Fig. 43); likely the elevation equal to 0 was highly important in grouping the shoreline samples. The Euclidean distance equal to 3 highlighted four groups (Fig. 43): (A) Collelungo samples, (B) Macchiatonda, Bocca di Serchio and Buca del Mare samples, (C) and (D) Uruguayan beaches, considering the exclusion of missing values, and station 0 of Macchiatonda in spring, which was included in group (D). Collelungo had similar environmental characteristics in both seasons (group A), showing a higher similarity within the same beach instead of similarities of the same stations (*i.e.* distance from shoreline) in different beaches; an Euclidean distance equal to 2 subdivided the samples in three groups: two groups formed by the upper stations in autumn (8-18) and in spring (6-20), and one group of lower stations in spring (0-4) and autumn (0-6), showing minimal seasonal differences in the environmental characteristics between the same zones of Collelungo beach. The three beaches Macchiatonda, Bocca di Serchio and Buca del Mare formed another group (B), and subgroups were formed by the same stations (*i.e.*

similarities between the same zone in different beaches or seasons, see Bocca di Serchio and Buca del Mare samples), or samples of the same beach (see Macchiatonda samples); interestingly, station 2 and 4 of Macchiatonda in spring formed a subgroup separated from all the other samples of group (B), having the greatest compaction and sediment water content and lowest temperature values within the samples of the same beach, excluding stations 0. Groups (C) and (D) subdivided the Uruguayan beaches in spring samples (group C, with the exception of station 20 of José Ignacio in autumn) and autumn samples (group D), and also subdividing spring samples of La Viuda in upper stations (24-40 in group C) and lower stations (12-20 in group D) (Fig. 43). Station 20 of José Ignacio in autumn had the greatest sediment temperature and sorting and lowest water content values within the same beach. Station 0 of Macchiatonda in spring was included in group (D), within the subgroup of lower stations of La Viuda in both seasons, having similar sediment compaction, organic matter, water content and temperature of stations 4 and 8 of La Viuda in autumn (Fig. 43).

The Principal component analysis (PCA) was performed on environmental data excluding elevation. The first two principal components of the PCA explained the 62.9% of the variation (Fig. 44). The first principal component (34% of the variation explained) mostly subdivided the Italian beaches from the Uruguayan ones: the former presented greater values of sediment organic matter content and lower values of sorting and compaction (on the right of the plot), while the latter had the opposite values (on the centre and the left of the plot). The second principal component (28.9% of the variation explained) subdivided the samples as follows: samples with higher sediment water content and lower temperature and grain size, including samples of Collelungo (except for the upper stations in autumn), José Ignacio in autumn (except for station 20) and the lower stations of La Viuda in both seasons; samples with opposite values (lower sediment water content and greater grain size and temperature), including Macchiatonda, Bocca di Serchio and Buca del Mare in both seasons, José Ignacio in spring (and station 20 of autumn samples) and the upper stations of La Viuda, in both seasons.

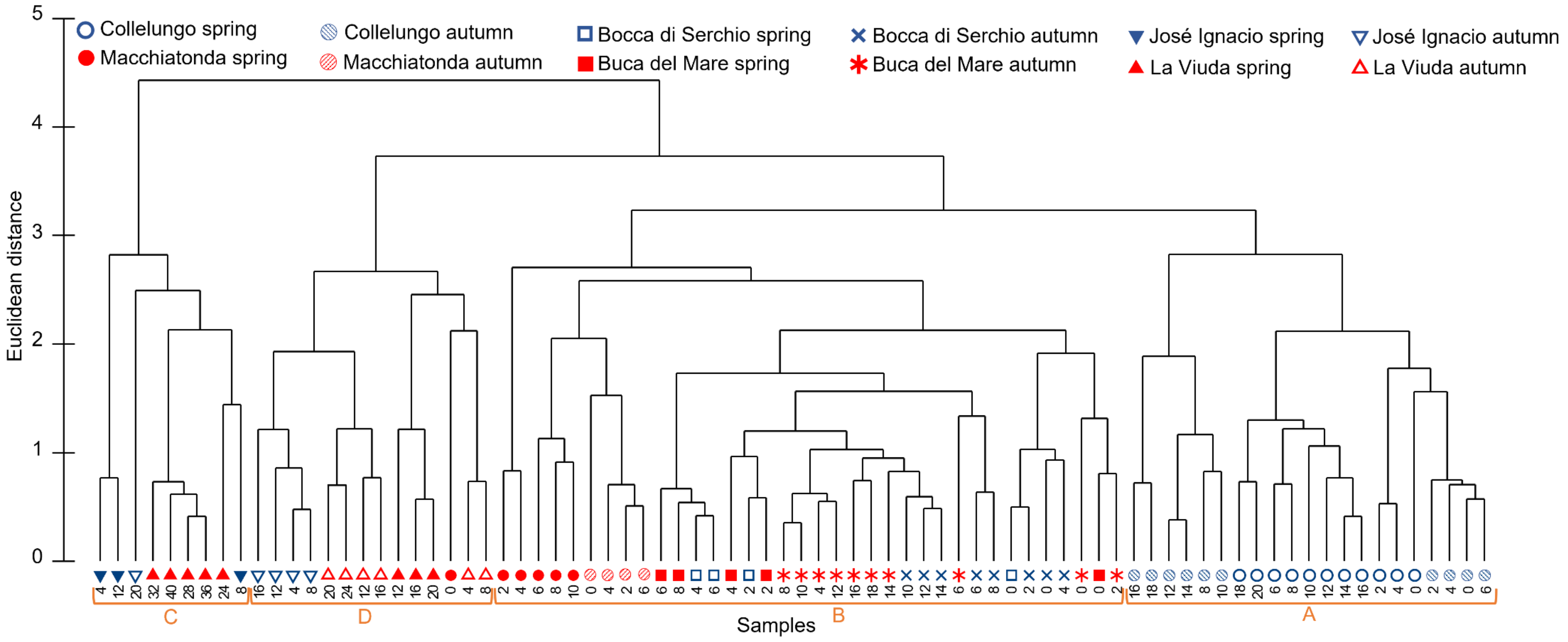


Figure 43. Cluster analysis of environmental data. The samples numbers indicate the distance (m) from the shoreline. The Euclidean distance of 3 is highlighted with capital letters A-D. Elevation values were excluded from the analyses. Station 0 of José Ingacio and La Viuda in both seasons and stations 4 and 8 of La Viuda in spring were excluded from the analysis (missing values).

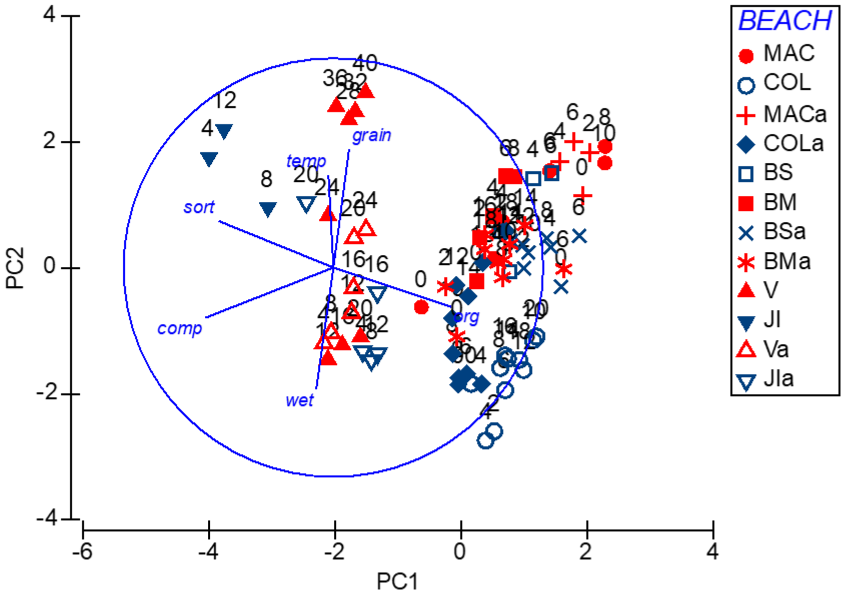


Figure 44. Principal component analysis of environmental data. The samples numbers indicate the distance (m) from the shoreline. Station 0 of José Ingacio and La Viuda in both seasons and stations 4 and 8 of La Viuda in spring were excluded from the analysis (missing values). Comp, compaction; org, organic matter; wet, sediment water content; temp, sediment temperature; grain, grain size, sort, sorting; COL, Collelungo; MAC, Macchiatonda; BS, Bocca di Serchio, BM, Buca del Mare; V, La Viuda; JI, José Ignacio; a=autumn samples.

3.8.2. Density and zonation

The density variations in autumn had generally a wider extension with respect to spring (*i.e.* distribution landwards), except for La Viuda, which had the same across-shore extension of density in both seasons, and Macchiatonda, where sandhoppers in autumn were found at station 0 only (Fig. 45a-f). The Uruguayan beaches presented a distribution of the density more landward dislocated with respect to the Italian beaches, mostly in spring (Fig. 45a and b). Juveniles presented higher densities near the shoreline, mostly in spring, while adults had lower densities and were more landwards burrowed with respect to juveniles (Fig. 45c-f).

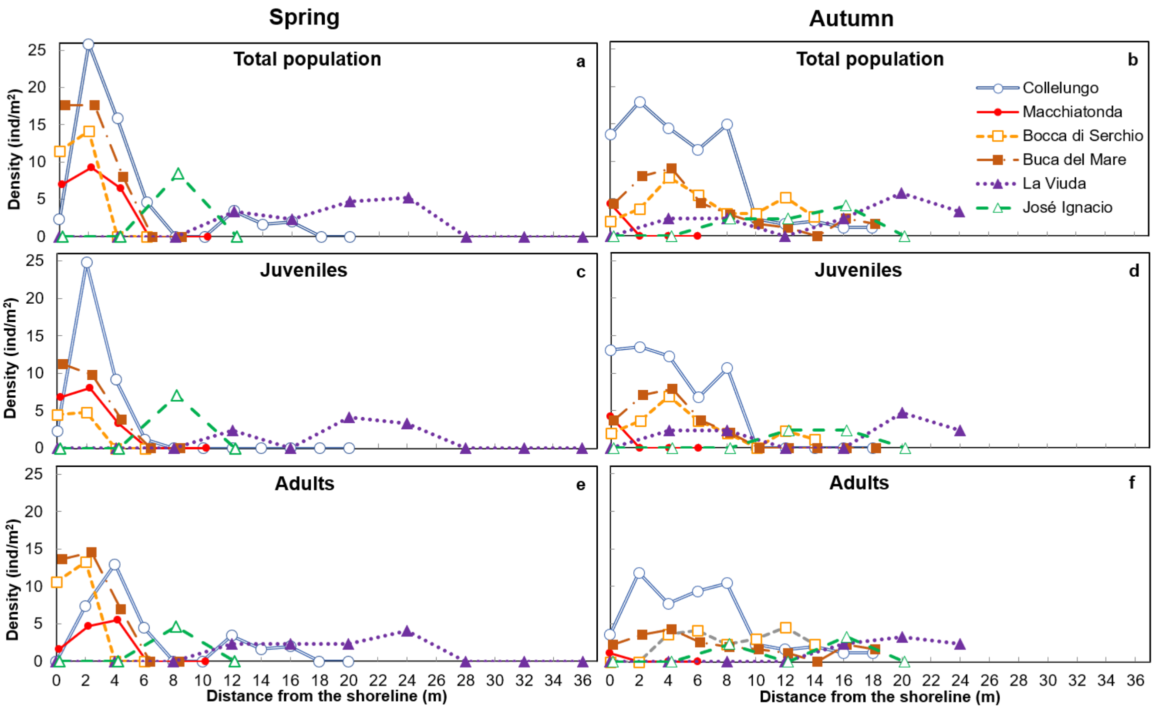


Figure 45. Across-shore density variations on the six beaches in spring (left) and autumn (right): a-b, total population; c-d, juveniles; e-f, adults.

Mean zonation was significantly closer to the shoreline on Italian beaches (Mediterranean) than on Uruguayan ones (Atlantic) (Fig. 46; ANOVA: *p*<0.01). A Tukey post-hoc test revealed that sandhoppers presented a higher mean zonation on Uruguayan beaches in both seasons than on Mediterranean beaches (12.20 m in spring; 12.13 m in autumn; *p*<0.05), while no differences between seasons within the same beach were found, even if a higher mean zonation in autumn was evident (Fig. 46).

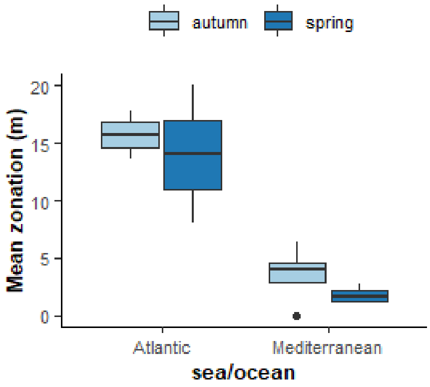


Figure 46. Mean zonation (m from the shoreline) of sandhopper populations on Uruguayan (Atlantic) and Italian (Mediterranean) beaches in spring and autumn.

Density increased linearly in beaches with greater organic matter content, excluding Macchiatonda in autumn, which presented high organic matter content (1.37%) but a small density value (2.16 ind/m^2; Fig. 47a). The mean zonation linearly increased with compaction, the four highest values corresponding to the Uruguayan beaches (Fig. 47b); the mean zonation decreased with greater organic matter content (Fig. 47c) and increased with sorting, if the value of José Ignacio in spring was excluded (8 m, 1.89 µm; Fig. 47d).

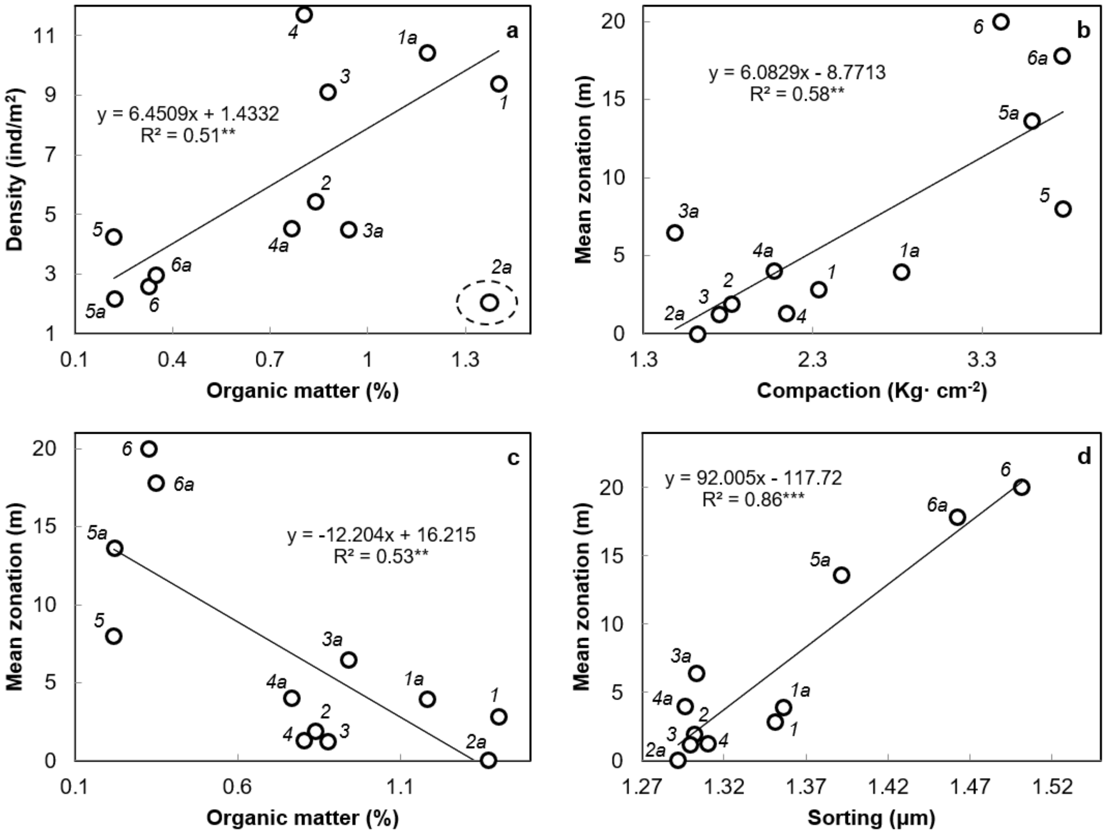


Figure 47. Variation of density and mean zonation of the six beaches in both seasons: a, density variation as a function of organic matter content; b, c and d, mean zonation as a function of compaction (b), organic matter content (c) and sorting (d), respectively. *1*, Collelungo; *2*, Macchiatonda; *3*, Bocca di Serchio; *4* Buca del Mare; *5*, José Ignacio; *6,* La Viuda; *a*=autumn samples. The outlier excluded from model fitting in (a) is encircled (dashed line); in (d), the value of José Ignacio in spring is excluded.

3.8.3. Orientation

The concentration of the orientation (r) decreased linearly with higher elevation of the beach (Fig. 48). The highest concentration was observed in spring on José Ignacio, Collelungo and La Viuda and in autumn on Macchiatonda and Collelungo.

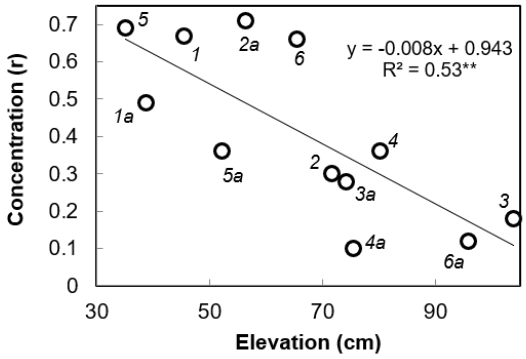


Figure 48. Variation of concentration of orientation in relation to the elevation of the beach: *1*, Collelungo; *2*, Macchiatonda; *3*, Bocca di Serchio; *4* Buca del Mare; *5*, José Ignacio; *6,* La Viuda; *a*=autumn samples.

Even if the difference among classes was not significant (ANOVA: *p*>0.05), it can be noticed that juveniles had the highest concentration among classes, followed by mature females, immature females and males, that presented the wider variation (Fig. 49a). Sandhoppers inhabiting the Atlantic beaches presented higher concentrations with respect to the Mediterranean populations, although the difference was not significant (ANOVA: *p*>0.05; Fig. 49b).

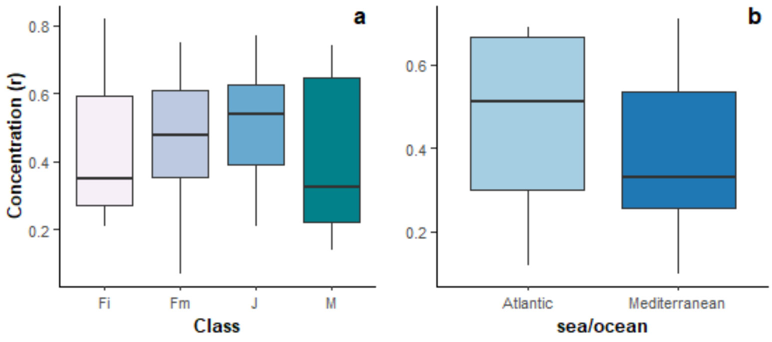


Figure 49. Concentration of orientation of the four classes in the six beaches (a) and the populations of the Mediterranean and Atlantic beaches (b). Fi, Immature females; Fm, mature females; J, juveniles; M, males.

4. *Discussion*

4.1 Relation between the physical environment, abundance and zonation

4.1.1 Substrate characteristics and sandhopper density

Considering all the six beaches, a general comparison among the physical environment, density, zonation and orientation can be made, even if not all the differences analysed were significant. Firstly, the Cluster analysis (Fig. 43) and PCA (Fig. 44) highlighted that grouping samples for similarities in the environmental characteristics can follow different rules. Collelungo samples had the smallest grain size and the highest sediment water content in both seasons, and these were probably the most important factors that separated this beach from the others. Macchiatonda samples were divided for season and were similar to Bocca di Serchio and Buca del Mare samples, while La Viuda and José Ignacio had the highest compaction and sorting values among the six beaches, and these factors probably separated them from the other beaches and formed two groups mostly divided for season, except for the lowest stations of La Viuda in spring, which grouped with autumn values, having higher compaction and sediment water content with respect to the other stations in spring. In general, a separation between Italian and Uruguayan beaches is clear, and a grouping of the stations closest to the shoreline and the ones more landwards can be noticed (Fig. 43).

Prediction 1 of larger populations on beaches with coarser sand and higher elevation was not confirmed by this study. The relationships between density and environmental variables varied among beaches and seasons. In general, it is not always possible to find biological and environmental correlations using field data, considering also that here the interactions with other species, which can influence density and zonation, were not studied (Bouslama et al., 2009). The highest densities were found on Buca del Mare in spring and Collelungo in autumn, while the highest elevations were on Bocca di Serchio in spring and La Viuda in autumn, and the coarsest sand was on Macchiatonda beach.

The higher substrate compaction on Collelungo and Buca del Mare with respect to the other two Italian beaches can be associated with the higher abundance (and density) (Tables 2 and 4), except for Buca del Mare in autumn, but on the Uruguayan beaches a relation between compaction and density was not observed (Table 6). On eight San Rossore beaches subjected to beach nourishment and separated by groynes, Fanini et al. (2007) observed higher abundance with lower penetrability values. In our study, sandhoppers were found between a compaction equal to 1.1 $kg\cdot cm^{-2}$ (station 0 of Bocca di Serchio in autumn) and 4.61 $kg\cdot cm^{-2}$ (station 12 of La Viuda in spring). On La Viuda beach in spring there was a sediment distribution different from expectation, with coarser sand and debris near the dunes: probably a storm in the previous days had brought landwards sand and debris, which are normally located near the shoreline (Omar Defeo, personal communication; see Fig. 20e). In addition, a worse sorting (higher values) of the same samples was observed at stations where normally a better sorting is expected (Fig. 20f), considering that, generally, in the dunes a well sorted sand is found (Pranzini, 2004). Regarding sediment temperature, the negative correlation with density found on Collelungo beach showed that higher density values can be found at lower temperatures associated with higher sediment water content (Figs. 6b, 6c, 7a, 7b); this relation was particularly evident in autumn (Fig. 7b). We must consider that Collelungo beach had the lowest elevation among the studied beaches, particularly in autumn, and this can favour wet conditions going landwards, also considering the small grain size, usually capable of retaining more water (Colombini et al., 2013). On this beach, the number of sandhoppers was high both in spring and in autumn. A salty sand crust was observed on this beach in spring, formed by dried water pools, from the shoreline to the base of the dunes, due to the low elevation and high level of the water table (Colombini et al., 2006). Thus, the size of population did not depend on the greater height and the larger grain size, but more likely on water presence in the sediment interstices. It can be noticed that sandhoppers were never found in sand with water content below 2.6%, in neither of the six beaches studied (Fig. 50): this can indicate a minimum value of suitable wet condition for sandhoppers both on Italian and Uruguayan beaches. On British shores, Williams (1983) found *T. saltator* burrowed in sand with a water content between 2-4%, irrespective of the temperature profile.

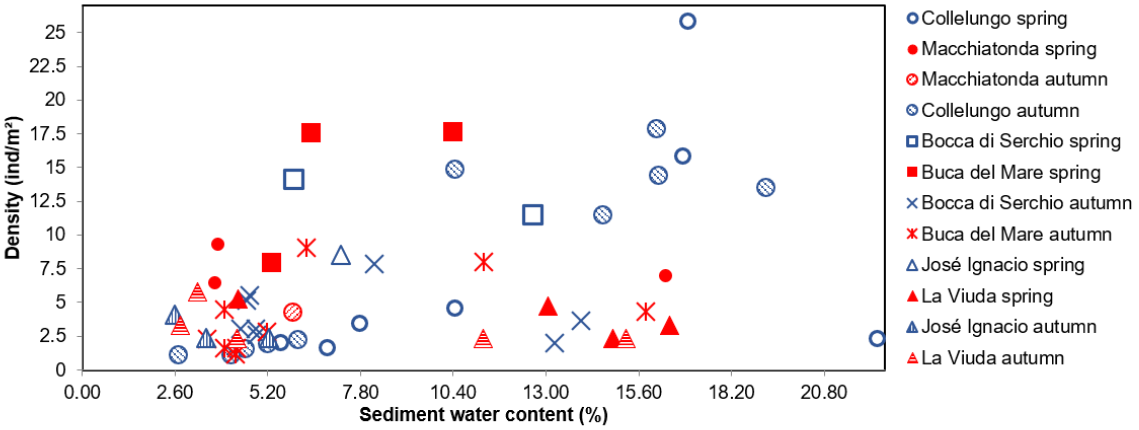


Figure 50. Density distribution of all the six beaches as a function of sediment water content in both seasons.

4.1.2 Substrate characteristics and sandhopper zonation

The prediction 2 of a sandhopper zonation closer to the shoreline in beaches with coarser and less compact sand and greater elevation was verified on Macchiatonda beach, where the zonation was limited to the first stations near the shoreline (Figs. 5, 8), **while if compaction only is considered, the tendency of a sandhopper zonation closer to the shoreline with less compact sand was generally verified** (Fig. 47b). The distribution of sandhoppers on Macchiatonda, which was seawards concentrated despite the width of the beach in both seasons (a constant width of this beach was registered also by Scapini et al., 1992), can be explained considering various factors, such as the abrupt change of elevation in spring from station 6 (*i.e.*, the beach surface rapidly separates from the water table, Fig. 2; notice that sandhopper zonation stopped at station 4, Fig. 8), the coarser sand and related lower values of sediment water content that tended to decrease sharply going inland, in both seasons (Figs. 3c, 4c); the even more restricted zonation in autumn (at station 0 only, Fig. 8) with few captures, although the elevation increased less steeply (Fig. 2), can be related to the less sediment water content and higher air temperature in autumn (at similar air relative humidity conditions) with respect to spring, which can lead sandhoppers to move towards the wet sand near the shoreline (Fig. 8, Table 8), as was found on the same beach by Fallaci et al. (2003) and in Tunisia by Bouslama et al. (2009); in these conditions, orientation would be more concentrated seawards (see below, paragraph 4.2). Furthermore, the low number of females in spring and their absence in autumn suggests their refuge landwards, at higher levels of the beach up the dunes and/or deeper in the sand with respect to males and juveniles (Fig. 8, Table 3). Scapini et al. (1992) and Colombini et al. (2013) also observed seasonal differences in zonation on these beaches, with a tendency of females to recover more landwards in autumn and winter. On an Irish sea beach (western Atlantic) Williams (1995) highlighted that *T. saltator* burrowing activity was driven by the desiccation stress more than to avoid wave inundation, and this stress was higher for juveniles and lower for ovigerous females, showing deeper and more landwards burrows of adults with respect to juveniles, with an up- or down-shore displacement and a reduction of the depth according to the season. The adults' random orientation in spring and seawards or longshore in autumn on Macchiatonda is discussed below (paragraph 4.2). We must consider that this beach was subjected to coastal protection interventions (submerged geocontainers and nourishment) in 2010 (Enzo Pranzini, personal communication). On Collelungo beach, the distribution of sandhoppers from the shoreline to the dunes in both seasons can be related to the low elevation of the entire beach and the percentages of sediment water content sufficiently high on almost the whole beach, as discussed above for density (Figs. 3c, 4c, 5, 8). On Bocca di Serchio and Buca del Mare beaches, zonation similarly varied between the two seasons, being more seawards concentrated in spring, and distributed from the shoreline up the dunes in autumn (Figs. 14, 16): considering the intermediate grain size values and quite high elevations, sediment water content rapidly decreased landwards but maintained sufficient high percentages in autumn allowing the presence of sandhoppers also on higher beach levels (Figs. 12c, 13c). The autumn distribution on Bocca di Serchio can be explained considering the higher air relative humidity and lower air temperature with respect to spring, which permits the avoidance of desiccation stress also going landward, but on Buca del Mare these variables were reversed (Table 13). Both beaches present vegetated dunes, higher dunes on Bocca di Serchio and trees just behind the dunes on Buca del Mare. Mezzetti et al. (2010) discussed the capability of *T. saltator* to orient landwards to forage during the night, thanks to the eye structure and physiology, which enhances visual sensitivity at night allowing to catch low levels of environmental light. We must consider also the high behavioural plasticity documented in this species, which permits to respond in different ways to changing conditions, caused both by nature and human activities (Bouslama et al., 2009). It was interesting the more inland displacement of sandhoppers on the Uruguayan beaches with respect to the Italian ones (even if the number of captures in Uruguay was lower than in Italy): despite the greater distance between the stations in Uruguay, which may have skipped some areas where the population was present, especially near the shoreline, the shift of the sandhopper populations towards the dunes was evident (Figs. 22, 24, 45). In Brazil, Veloso et al. (2010) found adults of *A. brasiliensis* in the midlittoral and juveniles near the swash line and travelling along the beach; Cardoso (2002) found a more restricted zonation during the day with respect to night zonation, near the upper limit of the swash zone, and juveniles closer to the shoreline than adults; Innocenti Degli et al. (2021; Chapter 3) found *A. brasiliensis* some metres more inland with respect to the upper swash level both on La Viuda and Barra del Chuy (Uruguay) in autumn. The different distribution between sandhopper populations in Italy and Uruguay could be due to a greater tidal excursion on the oceanic beaches compared to the Mediterranean ones (even if all the six beaches were classified as microtidal), or the greater energy of waves acting on the shoreline, *i.e.* higher wind force (tidal range, Tables 8, 13 and 18). Even in the absence of a circatidal clock (Cardoso, 2002; Naylor, 2010), the zonation and orientation of sandhoppers, which mainly take shelter in the wet sand near the shoreline during the day, may be influenced by the shift of the waterline resulting from the tidal excursion, even on microtidal beaches. On a Brazilian beach, Cardoso (2002) described a zonation of *A. brasiliensis* more seawards during neap tide with respect to spring tide and a more seaward zonation of juveniles with respect to adults, while in this study adults and juveniles of *A. brasiliensis* were found together even more landward than sandhoppers in Italy (Figs. 22 and 45). Grain size values were intermediate on both the Uruguayan beaches in spring, finer on José Ignacio in autumn, and sediment water content was sufficiently high (≥2.6%) going inland (Figs. 20c, 20e, 21c, 21e). The concentrated distribution of sandhoppers on José Ignacio beach can be due to the narrowness of the area between the shoreline and the dunes, also considering the more landwards sandhopper distribution on the Uruguayan beaches (Fig. 45, Chapter 3, Innocenti Degli et al., 2021). José Ignacio beach had also a higher air relative humidity with respect to La Viuda beach in both seasons (Table 18). In Uruguay, air temperature values were generally lower than in Italy in both seasons, and air relative humidity was higher (Tables 8, 13 and 18), probably reducing the dehydration risk going landwards.

Prediction 3 of juveniles' distribution closer to the shoreline with respect to adults was verified for all beaches (Fig. 45 c-f), confirming previous studies (Bouslama et al., 2009; Colombini et al., 2013). In autumn, the distributions of juveniles were more landward on almost all beaches, in particular on Bocca di Serchio (from the shoreline to 14 m inland) in relation to the environmental characteristics, and also on La Viuda, as found by Fallaci et al. (1999) on a French Atlantic coast, maybe related to the higher wave energy acting on that shoreline, which may influence the zonation of oceanic beaches.

The sandhoppers sampled for the zonation study were mostly smaller with respect to sandhoppers collected for the orientation tests, except for La Viuda in spring (compare the population variables means in Tables 3 and 9, 5 and 14, 7 and 19). These differences can be due to the higher proportions of juveniles out of the total number of captures in the zonation data, which had smaller dimensions.

4.1.3 Sex ratio

The prediction regarding sex ratio for amphipod populations would be to find balanced numbers of males and females on an annual basis. In this study, we observed mostly male biased sex ratio in Italy and female biased in Uruguay, with some exceptions. The sex ratio observed in orientation samples of Collelungo was female biased in both seasons (Table 9); the sex ratio calculated from orientation samples of Macchiatonda was slightly male biased, in both seasons, and we must consider the absence of females captured in the autumn zonation samples, as well as the low capture rates in the same season (Tables 3 and 9). On Buca del Mare in spring, the sex ratio from orientation samples was female biased, and the sex ratio calculated from zonation samples was almost balanced (Tables 5 and 14). On José Ignacio, the sex ratio was balanced in spring both from zonation and orientation samples, while in autumn and on La Viuda in all samples, the sex ratio was female biased (Tables 7 and 19). In previous annual or seasonal studies on the same beaches, and also on other beaches, collecting animals with pitfall traps (*i.e.* active individuals), sex ratios were male biased (Fallaci et al., 2003; Scapini et al., 2005; Fanini et al., 2007) or balanced (Colombini et al., 2013); sampling with quadrats/corers (*i.e.* capturing burrowed individuals), sex ratios were balanced (Gómez and Defeo, 1999; Fallaci et al., 2003) or female biased (Cardoso and Veloso, 1996; Fanini et al., 2017); by collecting animals manually (*e.g.* for orientation tests), female biased sex ratios were found (Scapini et al., 1999; Bessa et al., 2013; Nourisson et al., 2014; Nourisson and Scapini, 2015; Bessa et al., 2017). The female biased sex ratio was mostly explained with female longer life span; the bias towards males found in Italy in this study may be due to the chosen seasons (characterized by the presence of juveniles), which may be the peak period of males that may decrease during the other months (Marques et al., 2003). However, a male biased sex ratio may also be due to the sampling method, if we consider the increase of captured females on Collelungo, Macchiatonda and Buca del Mare when the samples were manually searched on the shoreline or few metres inland, *i.e.* the optimal zone, instead of capturing burrowed individuals across the beach, from the shoreline to the base of the dune. This is in contrast with the idea of females' migration landwards discussed for Macchiatonda in autumn, where no females were found in the zonation study, but were instead found by searching for specimens for orientation; however, an increased capture of females near the shoreline is in agreement with the zonation found on Macchiatonda in autumn, concentrated at station 0, as discussed above.

4.2. Orientation behaviour

The prediction 4 of a more concentrated orientation in beaches with coarser sand and higher elevation was not verified by this study (Fig. 48), probably because in the considered beaches not always these characteristics were related to a more suitable environment for sandhoppers, or because the populations may face (or have to prepare for) overriding stress, like winter storms. Collelungo beach, with small grain size and low elevation, presented a sandhopper population highly concentrated seaward: this beach may currently be in a state of dynamic equilibrium, which has allowed the population to refine their orientation in accordance with the direction of the shoreline, in addition to the small tidal range and probably the little influence of winter and spring storms on this coast. In contrast, Bocca di Serchio and Buca del Mare had greater grain sizes and higher elevations, but a worst orientation was observed, probably due to the more unstable shoreline and the higher risk related to storminess. Sandhoppers on Macchiatonda beach had a high concentration in autumn, mainly for the stability of the beach (this beach was nourished years ago and is actually under erosion, which has not changed the shoreline direction), while in spring it is plausible that the risk of storms, without the possibility of migrating inland due to the hostility of the upper part of the beach, leads sandhoppers to move longshore; this could be combined with previous beach cleaning actions that may have disturbed the animals. On José Ignacio and La Viuda, it is possible that the worsening of the weather conditions and the increased risk of storms in autumn were the main causes of the observed poor orientation of sandhoppers, as nourishment or coastal engineering actions are absent on these beaches.

Prediction 5 that juveniles tend to orient more seawards was true in most of the studied populations. In fact, juveniles are more subjected to dehydration risk and therefore use the sun to orient seawards while adults may find a protection also landwards under stranded material or digging into soft sand. Exceptions were Bocca di Serchio, where juveniles oriented longshore (north-west in spring, south-east in autumn, Figs. 34 and 36); this can be explained by the wet conditions that can be found along that shore due to the stranded vegetal material, representing a suitable environment. Even if immature females had smaller dimensions than mature females and males, being subjected to the same risks of juveniles, in this study they had lower concentration with respect to the other two classes of adults (Fig. 49), and were mostly displaced with respect to the seaward direction (Figs. 29, 31, 34, 36, 39 and 41).

Intrinsic factors (*i.e.* size and age proxies and class) significantly influenced the multiple regression models for Collelungo and Macchiatonda, differently between beaches in spring: on Macchiatonda, only juveniles were significantly seawards oriented (see M6 Table 12, Fig. 29). The scattered orientation of adults in spring on Macchiatonda could be explained by their higher resistance to dehydration and higher ability to burrow deeply into the sand with respect to juveniles, being able to seek shelter also in other directions, but adult sandhoppers can also be more sensitive and respond more promptly to natural or artificial disturbances (storms, beach cleaning). On Collelungo, the lower concentration of adults with respect to juveniles can similarly depend on a lower dehydration risk. Looking at the orientation distributions, it is important to notice that sometimes the mean direction was in the middle of two peaks: in spring, on Collelungo mature females had a seaward mean direction, while in the observed distribution the angles were displaced to the south and to the west (longshore), similarly to the immature females (more south displaced); males had a mean direction southward, but the observed orientation angles were south-west and south-east displaced, *i.e.* seawards and longshore (Fig. 29). On Bocca di Serchio and Buca del Mare intrinsic factors influenced the models, discriminating both for beach and season (M2, M3, M6, M9 Table 17), but did not seem to influence the orientation on Bocca di Serchio (M11 and M12, Table 17): on this beach, even if the significance of orientation was different among classes (see Figs. 34 and 36), only meteorological and hourly factors influenced the orientation. Together with the absence of influence of landscape cues, and the differences considering the time of the day (discussed below), this could suggest a recently renewed population, or it could be a consequence of the instability of the beach (discussed below), which may cause a worst orientation of the population, unable to adapt to sudden changes. In the population of Bocca di Serchio (compare CI and *r*, Tables 15 and 16), the concentration of orientation improved in autumn compared to the previous spring, even if the orientation was landwards, as if the population learned to orient according to the experienced environment by avoiding the shoreline (confirmed by the better orientation with the vision of the landscape, Table 16, Fig. 36); it is interesting that juveniles in autumn had the same direction of adults, but were more displaced longshore, while adults were landwards oriented (see the south-south-west peak of juveniles, Fig. 36). On Buca del Mare the opposite situation was observed, the total population in autumn being scattered when the use of landscape cues was not possible (Tables 15 and 16), and showing a seaward orientation in spring (considering the two peaks longshore). This can be explained by the introduction of a disturbing event between the two seasons, considering the spring population also present in autumn and hypothetically acclimatized. A possible disturbance can be the fact that the park staff is allowed to use this beach during the summer and cleans the beach before summer. It is interesting to notice that across the beach there were three "groups" of organic matter content (Fig. 13b), corresponding to three stripes of decreasing organic matter beginning from station 0, 6 and 14 going inland. Similarly, three groups starting from these three stations can be visualized also for compaction, grain size and sorting (Fig. 13a, 13e, 13f), as the sand had been subjected to a remixing in stripes. Anyway, San Rossore Park has strict rules to avoid mechanical removal of stranded material, and the low disturb of the limited bathers suggests that the instability of the shoreline (*e.g.* erosion) represents the strongest disturbance for sandhopper population. In spring, no models of José Ignacio and La Viuda included intrinsic factors (M5, M6, Table 22), being all the four classes seawards directed (Fig. 39). Fanini et al. (2009) found an effect of "sex" on La Viuda beach in response to a visual pattern (artificially introduced to analyse the influence of scototaxis), with males more scattered than females, probably because of their exploratory behaviour.

Prediction 6 of a higher seaward concentration of the Mediterranean populations with respect to the Atlantic ones was not verified (Fig. 49b), confirming that the meteorological conditions are not sufficient to explain different behavioural adaptations, as was stressed by Scapini et al. (2019a) in their meta-analysis of orientation of a large dataset of orientation of European and North-African populations. The low seawards concentration observed in the Mediterranean populations in this study, with the exception of Collelungo in both seasons and Macchiatonda in autumn (Tables 10-11, 14-15), may be due to environmental stress caused by management actions on beaches, such as mechanical cleaning, coastal engineering interventions and nourishment actions in recent times (years to decades), which may have caused habitat loss and consequent bottle necks in sandhopper populations. On the other hand, in Uruguayan beaches, populations appeared well oriented (adapted to their shoreline) in spring, but less well oriented in autumn when the highest risks may derive from oceanic storms and strong winds, which are rare on Italian coasts. The more frequent landward displacement of sandhoppers on these beaches could be an adaptation to harsher weather conditions (see also mean zonation in Fig. 46).

A novelty of this study was to find an influence of tides on sandhoppers inhabiting microtidal beaches. The time of the day and/or tide cycle were important factors significantly influencing the orientation on all the six beaches (see M11, M12, M14, M15 of Tables 12, 17 and 22). On Collelungo, the observed difference in orientation between morning and afternoon (both with rising tide) in spring and between ebbing and rising tide in autumn was the precision in seaward direction, being in both cases higher with rising tide, during the afternoon. On Macchiatonda beach, the deconstruction of the orientation according to the tidal cycle showed a general seaward orientation when the tide was ebbing and scatter or longshore direction (displaced to the south-east) when the tide was rising. These results suggest an influence of the tide on orientation even on microtidal beaches, as discussed above for zonation (paragraph 4.1.2). Anyway, similar results were obtained considering the time of the day: differences between morning and afternoon can suggest an influence of other hourly factors, such as solar radiation and sun azimuth (M11, M12, M14 and M15, Table 12) as well as circadian rhythms (not tested in this study, but well known from the literature: Rossano et al., 2008, 2017). Also a better sun visibility and lower cloudiness positively influenced the seaward direction, confirming the sun as an important orientation cue (sun compass), but also a direct influence (phototaxis) of sun light: sandhoppers on Macchiatonda may have a difficulty in compensating the azimuth movement (as in a perfect sun compass), but may show a phototactic deviation orienting to the sun according to the different sun position in different seasons (Pardi and Papi, 1953; Papi and Pardi, 1953). Hence, on Collelungo the stable and protected environment of the beach ensured the use of the same behaviour (*i.e.* seaward orientation through sun compass) in both seasons and during the all day, while on Macchiatonda with rising tide and poor sun visibility, sandhoppers tended to avoid the TED, searching for shelter in the wet sand longshore, mostly in spring. On Bocca di Serchio and Buca del Mare, a higher variability was observed both considering the time of the day and tide cycle. On Bocca di Serchio in spring, during the afternoon tests, with ebbing tide, sandhoppers performed a seawards orientation, while during the morning tests, with mostly rising tide, they were oriented northwards. Such changes in orientation from the morning to the afternoon suggest a phototactic behaviour, following the apparent movement of the sun towards the south-west direction, which may represent a not perfect adaptation to the inhabited shoreline (phototaxis is a simpler and basic behaviour than sun compass that is adapted to a specific beach) and can be explained by considering an inexperienced population, like a renewed one, or an highly changing environment, which does not allow the evolution and fixation of sun compass adapted to the shoreline (Scapini et al., 2005). On Bocca di Serchio in autumn, with tide mostly rising, sandhoppers oriented landwards during the afternoon, when the sun was visible, while they were scattered during the morning, with not visible sun. This confirmed the sun as orienting cue, but the population oriented opposite to the TED: Scapini et al. (2002) related the landward orientation of *T. saltator* in Tunisia to an avoidance behaviour, being sandhoppers influenced by a change in air pressure (registered) due to an incoming storm (observed). During our experiments in autumn, it was raining between the morning and the afternoon, but it stopped before the starting of the afternoon session, and there was no storm after the experiments. On Buca del Mare in spring, with rising tide, the morning distribution presented two peaks longshore: the higher precision of orientation in the morning with respect to the afternoon (longshore directional tendency, with ebbing tide) can be related to the higher dehydration risk with lower air relative humidity and higher temperature. On Buca del Mare in autumn, the scattered orientation observed in the morning during ebbing tide, and the landward direction observed in the afternoon during rising tide, were both under visible sun conditions; thus, also on this beach the landward direction was not correlated to an incoming storm. As for Macchiatonda, also in these two beaches sandhoppers appeared to avoid the seawards direction with rising tide, being scattered or directing landwards or longshore; we must consider that also on Bocca di Serchio in autumn there was rising tide during all day. Therefore, an explanation for the changing orientation under different weather conditions can be a low stability of the shoreline (discussed above), a high exposure of the beach and a landward environment suitable enough to host sandhoppers, as was observed also for zonation, mostly in autumn; moreover, comparing these beaches with Collelungo, which presented a similar beach width, smaller elevation and a suitable landward environment, however, a population orienting seawards in both seasons and throughout the day, beach stability appears the most important driver for a constant orientation behaviour, as was suggested by Scapini et al. (1995). For both the Uruguayan beaches, the highest differences between morning and afternoon were observed in autumn, indicating a worsening of the orientation from spring to autumn, being scattered (with ebbing tide during the afternoon) or longshore directed (with rising tide during the morning) on José Ignacio, or axially oriented on La Viuda (with rising tide all day), and in all cases avoiding the seawards direction; in spring, considering the time of the day and tide cycle, the difference between the distributions was the better concentration seawards during the afternoon on José Ignacio (with rising tide) and during the morning on La Viuda (with ebbing tide), thus the influence of the time of the day and/or tide cycle was not evident.

In general, the influence of water movement above the beach surface may represent a recent stressor for species inhabiting microtidal beaches, as a consequence of sea level rise and the impossibility of the beaches to migrate inland (*i.e.* coastal squeeze, Fig. 1 in Defeo et al., 2021). Under climate change impacts, such as increasing sea level, onshore winds, storminess and rainfall, a more variable orientation is expected, as well as changes in direction and timing, and the substitution of complex mechanisms (*i.e.* sun compass) with simpler taxes (chapter 2; Scapini et al., 2019b).

Orienting factors (sun and landscape visibility) usually improve orientation when visible; in this study, exceptions were Bocca di Serchio in spring (Table 15) and Buca del Mare in autumn (Table 16), where the orientation with the visible landscape was scattered. This was evident from the SPLM, where the factor landscape visibility was not significant in the models (M1-M3 Table 17). Interesting is the fact that, without the landscape view, sandhoppers of Bocca di Serchio in spring oriented north-west, as if the orientation with the solar compass was along the coast instead of seawards to south-west (Fig. 33); in autumn, both with and without the landscape visibility the orientation was to the south-east, approximately landwards (Fig. 35). We must consider that both these beaches presented stranded material and debris at the base of the dunes, which might be used as shelter.

Beach stability/instability appear to be important factors in explaining sandhopper orientation. The north-western Tuscan coast was subjected to modifications in the past: after erosive events in the delta of the Arno River, interventions of coastal protection were carried out starting from the ‘60s, which allowed the accretion of the shoreline in some parts of the San Rossore Park, but also the erosion of some other coastal sections, where Bocca di Serchio and Buca del Mare beaches are located (Cipriani et al. 2010, Casarosa, 2016). The instability of these two beaches can explain the orientation deviated from the TED, as discussed above, which confirms the previous findings by Ugolini and Scapini (1988) on the same location. On Collelungo, the orientation was more concentrated seawards with the landscape view, but the difference with the orientation without landscape view in spring was very small (compare CI and r in Table 10 and Fig. 28): this can be explained by a high stability of the beach, and a good adaptation of the population to the shoreline direction (using the sun compass), as discussed above. It was interesting to observe an increase of the stability of this beach with respect to the period 2011-2012, when the orientation of sandhoppers was less concentrated because of an accretion of this sector of the beach (compare site 6000 in Nourisson and Scapini, 2015); in this study we observed an improvement of the adaptation of the inhabiting sandhoppers as predicted by Scapini et al. (2005) and Ketmaier et al. (2010). The same explanation of the dependence of orientation on beach stability can be suggested for Macchiatonda in autumn; in spring the scattered orientation without the landscape view may indicate the need of using landscape cues, because of the more stressing conditions in this season: considering that this beach is more or less constant in width, the stability of the beach suggests that the scattered orientation in spring can be related to the high exposure of the beach, the impact of winter and spring storms creating harsh conditions on the beach face. Related to exposure, in spring it can be noticed an increase of grain size and organic matter going landwards (the opposite of expectations, Fig. 3e and 3b), which can be the result of landwards dislocation of sand and organic debris from the shoreline by waves; however, sand dislocation can also be a result of human actions, such as mechanical cleaning operations on the beach before the tourist season (personal observations during preliminary tests in April 2018). Collelungo and Bocca di Serchio beaches, which are located inside natural parks, are more preserved from the impact of tourists and related activities, even during the summer period. On a Tunisian beach, Scapini et al. (2002) also found an enhanced concentration with landscape view both for *T. saltator* and *Talorchestia brito*. The *A. brasiliensis* populations of José Ignacio and La Viuda had a high seaward concentration in spring, similar between beaches, slightly less concentrated without the landscape view, particularly on la Viuda (Table 20, Fig. 38). In autumn, both populations showed a worse orientation: on La Viuda the population was scattered, except for the orientation with the landscape view, which presented a seaward mean direction and two longshore peaks, while on José Ignacio the orientation was displaced towards the south direction, longshore, however it was slightly better seawards concentrated with landscape view (Table 21, Fig. 40). An opposite result was found by Fanini et al. (2009), who observed a higher precision of orientation without landscape view on La Viuda beach ("Punta del Diablo" in their study). However, a higher precision in the orientation of *A. brasiliensis* was observed also on Brazilian beaches when the landscape was visible (Bessa et al., 2017). These results confirmed the landscape as important orientation cue also for sandhoppers inhabiting Uruguayan beaches. Moreover, Fanini et al. (2009) compared the orientation on La Viuda beach with a population of Barra del Chuy: this beach, classified as dissipative beach (with smaller grain size and elevation and higher sediment water content and compaction with respect to the reflective beach La Viuda) was excluded from our beach selection due to the absence of an adequate number of sandhoppers. The perturbations on the macroinvertebrate community on that beach after the 2015-2016 El Niño, consequently to climate change increasing impacts, were analysed by Jorge-Romero et al. (2021), highlighting a reduction of the abundance of several species on Barra del Chuy beach (compare Chapter 3, Innocenti Degli et al., 2021, where observations made the day before El Niño 2016 are reported).

In summary, this study reinforced the understanding of the species - environment relationships on different beaches and provided useful insights to reinforce the theoretical framework about how behavioural adaptations act to enhance the survival of sandhoppers facing different stressors. Dehydration risk is the major danger for sandhoppers, and they adopt different survival strategies depending on external and internal variables, confirming the high plasticity of their behavioural adaptations. However, the increase in the sources of stress over time and space can lead to a decrease in the resilience capacity of these and other species dwelling sandy beaches: the need to manage these environments with an ecosystem-oriented vision is mandatory to avoid a further loss of biodiversity (Defeo et al., 2021).

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Chapter 5 – Overall conclusions

1. *Highlights of the study*

This thesis work deals with behavioural adaptations of sandy beach macrofauna in relation to environmental variables and their responses to changes on sandy beaches, subjected to various conditions.

The first chapter introduced the subject and explained the structure of the thesis.

In the second chapter, the main environmental and biological drivers potentially affecting sandy beaches and inhabiting macrofauna populations were analysed and hypotheses suggested of their effects on behavioural adaptations, on the light of published evidence on a large geographical scale. Behavioural adaptations of sandy beach macrofauna were proposed as bioindicator of environmental changes, which may contribute to predict future scenarios and ecosystems resilience/resistance/fragility under climate change stress (Scapini, 2014; McLachlan and Defeo, 2018). The importance of behavioural plasticity was shown particularly in cases of gradual environmental changes, but the high risk of population loss was also highlighted in case of strictly adapted endemic sandy beach fauna (Scapini et al., 2019). The adaptation potential of sandy beach macrofauna under climate change impacts is worthy of exploration, by the means of wide geographic comparisons, which may contribute to understand survival limits and conservative mechanism preventing species and habitat loss. Moreover, a worldwide reorganization of the management of sandy beaches is mandatory to limit additional stress in addition to those of climate change on the macrofauna living in these narrow environments.

In the third chapter, a field study was conducted on two exposed sandy beaches with contrasting morphodynamics in Uruguay, to verify the Habitat Safety Hypothesis (HSH) that predicts higher abundance and species richness on reflective beaches with respect to dissipative ones (Defeo and Gómez, 2005; Defeo and McLachlan, 2011). The results confirmed the HSH for abundance, however different patterns in the across-shore distributions, diversity and abundance emerged among taxa through deconstructive analyses and between beach zones (backshore and dunes). These results showed that the distribution of the same species may present different patterns on beaches with contrasting morphodynamics, and highlighted the importance of studying single species in relation with their habitat and of the comparison of beaches on the same coast differing in environmental characteristics (Innocenti Degli et al., 2021). Furthermore, the knowledge of species-habitat relationships is not static but constantly changing, especially under ongoing climate change. It is therefore important to promote ecological and behavioral studies that make it possible to keep updated the knowledge of the sandy beach ecosystems in a changing context.

In the fourth chapter, the case study of sandhopper behavioural adaptations (zonation and orientation) was analysed as bioindicator of environmental changes (Scapini, 2006), and different beaches were compared on a large geographical scale, Mediterranean and Atlantic, in southern Europe (Italy) and southern America (Uruguay). Predictions had been developed on the basis of previous research, including that of the second and third chapters, regarding density, zonation and orientation in relation with intrinsic characteristics of the animals (size, age and sex) and the environmental ones (substrate variables, beach elevation and meteorological conditions). Significant relations resulted between the zonation of sandhoppers and substrate compaction; juveniles were located closer to the shoreline with respect to adults; the orientation of juveniles was more seaward concentrated than the adults’ one. On the other hand, densities were not related with grain size and elevation; the concentration of orientation was not related with grain size and decreased with elevation. The concentration seawards or to other directions on Mediterranean and Atlantic beaches depended on the season, confirming the plasticity of this behavioural adaptation (Scapini et al., 2005). Both Mediterranean and Atlantic populations changed the directional tendency according to the environmental context, which may provide higher protection landwards or longshore, when conditions are suitable, offering protection and humidity. An orientation displaced with respect to the TED seawards may be considered an immediate adaptation to a changing situation, both meteorological and environmental: if the beach presents suitable habitats for survival also in other directions besides the shoreline, the adaptation may be successful, as can occur on a beach that can naturally migrate inland, but if we consider the human-made impacts on land (e.g., seaward encroachment by recreational, urban and industrial development) squeezing the beach, this adaptation may be unsuccessful because of the disappearance of suitable habitats (Scapini et al., 2019).

Overall, this thesis confirmed that the plasticity of behavior is the key for population survival in case of gradual environmental changes, unless their habitat is destroyed. The great dynamism of sandy beaches has led to a specialization of the inhabiting macrofauna to face changing natural conditions; the increase in human-induced stressors over a relatively short period of time puts a strain on these habitats. A novelty of this study was to find an influence of tides on sandhoppers inhabiting microtidal beaches (chapter 4). The coastal squeeze may have consequences on behaviour by introducing new risk factors, such as being swept away by waves on beaches where previously this stressor only rarely occurred, because of the increasing of storminess, sea level and erosion. An increase in behavioural variation and the substitution of complex mechanisms, such as sun compass, with simpler taxes (as predicted in chapter 2, paragraph 2) may represent an early warning signal. Updating knowledge of adaptive strategies and devising a management system that takes into account the responses of macrofauna and environment to ongoing changes, can be useful in preventing the complete disappearance of habitats and species.

2. *Limitations of the study*

The second chapter of the thesis collected previous behavioural studies on a large geographical scale, searching for a common strategy to address the challenges of a changing environment such as sandy beaches, which nowadays also face the additional stress of climate change. The main difficulty in studying climate change impacts is gathering long-term information. Behavioural adaptations of sandy beach macrofauna are considered an immediate response to environmental changes; however, they do not necessarily provide information on long-term changes. Adapting to a new environment can take a long time, especially in terms of evolutionary strategies (natural selection) that allow the survival of populations in changing environments. Nonetheless, the timing of climate change is not the same as that of evolutionary change, so addressing stresses on a shorter time scale than the evolutionary one can be done by modifying behaviour (*e.g.* individual learning during the life span) and rapidly adapting to novelty. Collecting information that gives a current picture of how animals can cope with daily difficulties in their environment may be useful to understand how to best manage the growing impacts of climate change on sandy beaches.

The third chapter analysed the relationships between sandy beach macrofauna on two Uruguayan beaches and the characteristics of the environment in which they live. The snapshot research (one day-night sampling in autumn on two beaches) failed to give seasonal information, and did not consider the environmental and behavioural changes that can occur throughout a year, or even in a few days with changing weather conditions. However, the full coverage of backshore and the dunes, the 24-h sampling (which covered the night and day change and tidal range) and the synchronicity of data collection on the two beaches, provided reliable information on actual species interactions with the environment on beaches with contrasting morphodynamics.

The fourth chapter analysed six beaches in different localities, regions and countries in spring and autumn. As for the study of chapter three, seasonal changes throughout a year were not taken into account, and the single days of field work may have influenced samplings and orientation experiments. However, two seasons (spring and autumn) were chosen when the populations were likely well structured, according to sandhopper biology, providing information on size/class strategies under different meteorological conditions, and before and after the period of greatest use of the beaches by tourists. The focus on sandhoppers only may be a limit if we consider the relationships between different species in an ecosystem. However, the choice of a single bioindicator, which is common, abundant and widespread on sandy beaches, has maximized the effort by studying the adaptation strategies in the species habitat, without endangering protected and rare species. To compare the results of orientation tests, experiments should be conducted using the same tools; in general, the analyses of orientation experiments made under natural conditions are not easy to interpret, and multiple regression analyses adapted to angular data have high statistical power if datasets are large enough; sandhopper populations are widely distributed, and it is possible to collect sufficient numbers of specimens for the experiments without a great effort of time and people. The standard instrumentation to conduct the orientation experiments can be easily built, the collection of environmental variables is carried out with field instruments that are normally available in ecology laboratories, the protocols are clear and easily executable. Behavioural adaptations may give information on changes in a specific environment in the short term (life span), allowing comparisons even at a large geographical scale.

3. *Lessons learned for management*

Sandy beaches attract humans for their amenity and aesthetic value, and have been exploited for living, commercial activities and tourism (Fanini et al., 2020). The high plasticity expressed by macrofauna in adapting to changing habitats such as sandy beaches allowed them to survive, by coping with natural stresses (chapter 2; Scapini et al., 2019). The human pressure represented by increased beach uses can be an additional stressor, that may modify the macrofauna adaptation not always in a successful way. It is mandatory for beach managers to consider the fact that changes in beach stability (variation in geomorphological and ecological characteristics) may lead to a genetic depletion of macrofauna populations that may become mal-adapted to the changed environment, considering that genetic changes require longer time-scale with respect to behavioural ones (Ketmaier et al., 2010). Beach stability can be naturally or artificially disrupted, which adds to climate change impacts; it is therefore important to consider minimizing disturbances on sandy beaches, by placing limitations on hard engineering actions (*e.g.* armouring, construction) and overexploitation of these environments (Defeo et al., 2009). The tourists' use of sandy beaches must not be eliminated, but it can be controlled, also considering that many species have the ability to adapt to changes thanks to behavioural plasticity, expressed by individuals during their life-span. Although a sandy beach could be wide enough to permit landwards migration of mobile macrofauna, the suitable habitat for survival could be only a narrow strip. In natural parks, the whole beach, from the dunes to the sea, is protected with access limits for tourists, and sometimes there are limits in the operations of stabilization of the shore; in areas that are not included in natural parks, in many cases, only the dunes are considered an important section of the beach to be protected, while the beach that develops in front of the dunes (*i.e.* the backshore) is not considered a habitat to be protected (Fanini et al., 2020). Sandy beaches are not clearly included in either terrestrial or marine environments, and poor understanding of these ecosystems leads to weak protection and management (Defeo et al., 2021). Sometimes, hard engineering is also used within protected areas because the main concern is to preserve the beach if it is under erosion, which leads to a decline of the attractiveness for tourism, or the disappearance of habitats that are necessary for “umbrella” species. Nevertheless, hard engineering actions are highly impacting the macrofauna that inhabits sandy beaches, namely crustaceans and insects (Costa et al., 2020; Fanini et al., 2020).

Gradual or limited-in-space actions of beach stabilization should be favoured, so that populations can adapt to or recolonize the new environment. Beach nourishment, even if it is considered soft engineering and therefore less negative for beach systems, is always risky due to the strong dependence of the macrofauna populations on the characteristics of the sediment, which was shown by the results of my research in chapters 3 and 4 (see also Costa et al., 2020, and references therein). Mechanical beach cleaning should be avoided, because it eliminates the possibility for many species to shelter under or forage on the stranded material; this material may also be a loophole in order to face an immediate change, *i.e.* avoidance of dehydration risk if the zone of recovery is not reachable. Urban, recreational or industrial developments in the Littoral Active Zone should be limited or prohibited, since this increases the risk of coastal squeeze, reducing the possibility for the beach to migrate inland in case of erosion: since this possibility is now lost in many cases of beaches close to inhabited areas, actions to raise awareness on a more sustainable use of the beaches should be encouraged (Fanini et al., 2019). Weak governance (*e.g.* open access system) should be avoided, reinforcing limits to the exploitation of sandy beaches to prevent the collapse of these socio-ecological systems (Defeo et al., 2021). The life cycle and zonation of macrofauna across the beach should be taken into consideration, as they may have reproductive peaks before the summer season, when beaches are often cleaned up to prepare them for the tourist season. The involvement of local people in the management of the environment in which they live may lead to an increase in attention to sustainability; deepening the knowledge of the impacts on sandy beaches, which lead to economic losses, may awaken the interest of the local institutions; coordinated actions between near localities, to then arrive at common strategies on a large scale, are to be favoured (Defeo et al., 2021).

4. Prospects

The choice of sandhoppers as bioindicators has proven valid, due to their abundance, wide distribution, and the similar adaptations between different species living on sandy beaches. It is necessary to broaden the geographical scale of the analyses and comparisons, using the same methods to study beaches in different geographic areas and compare the behavioural strategies in relation to the environmental variables. It is also important to develop long-term studies, and include at least an annual sampling with seasonal sampling periodicity to capture all the characteristics of the populations. Large geographical scale studies are important, but the local scale must be always taken into account, otherwise the relations between species and those between animals and their habitat may be overlooked.

Finally, an interesting development would be to study the genetics of plasticity, to better understand the mechanisms that regulate the inheritance of plasticity across generations, which can be important to understand the possible successful adaptation strategies in view of climate change negative impacts on coastal environments.

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Appendix – Ecological differentiation of *Carex* species coexisting in a wet meadow: Comparison of pot and field experiments

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Abstract

Competitive exclusion is to be expected between phylogenetically similar species that share traits and resources. However, species may overcome this, either through differentiation of their responses to biotic and abiotic conditions, or by trait differentiation, thus enabling their coexistence. We identified differences in phenotypic traits between seven coexisting *Carex* species and their responses to competition and fertilization in pot experiments, before using long-term field experiments to generate responses of the *Carex* species to fertilization and mowing and to illustrate temporal variability between species. Finally, we assessed how effective the results of the pot experiment were at predicting

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species responses in the field. In pot experiments, we found that species responded more to competition than to fertilization. Notably, all species showed similar responses to these factors in the pot experiments. Fertilization decreased the root:shoot ratio, whilst competition decreased growth-related characteristics such as total biomass, irrespective of the species. Differences among species were only found in their clonal response to competition, namely rhizome production and generation rate of new ramets. These findings support the idea that different clonal growth strategies may facilitate niche partitioning of *Carex* species. Species responses measured from pot experiments were poor predictors of their responses in the field experiment. Nevertheless, we confirmed the prediction that, over time, *Carex* species with lower growth rates in pot experiments showed more stable biomass production than in the field. We suggest that differences in clonal traits and temporal dynamics support the ability of *Carex* species to avoid competitive exclusion, enabling their coexistence.

Keywords: *Carex*, coexistence, clonality, competitive exclusion, nutrients, limiting similarity.

1. *Introduction*

Several mechanisms have been proposed to explain how similar species coexist, yet this question remains central to ecology (Palmer 1994; Vellend 2016). Following Hutchinson (1959), MacArthur and Levins (1967) introduced the concept of limiting similarity. This predicts that, for similar species to coexist, there should be some minimum difference in species resource utilization. For example, coexisting species should not completely overlap in the niches they occupy or in their responses to biotic and abiotic conditions. Plants use a very limited number of resources, and yet, at small spatial scales, species richness can be high, with several similar species competing for the same resources (Wilson et al., 2012; Chytrý et al., 2015). Whereas we can expect a high degree of overlap among resource utilization of similar species, we may also expect some differences in resource use, and particularly resource utilization curves (see below) that would enable species coexistence (Palmer 1994).

Phylogenetic conservatism would also predict that closely related taxa, such as congeneric species, share similar life-history traits and therefore resource-use capacities (Prinzing et al., 2001; Davies et al., 2013). Indeed, (Darwin, 1859, p. 111) stated that closely related species are similar in their morphology and ecological requirements, increasing the intensity of competition among them. In an apparent breakdown of the predictions of competitive exclusion, how are closely related species, sharing similar resources, able to coexist?

The genus *Carex* consists of a high number of species that occupy a diversity of habitats, ranging from open grassland to forests (Waterway et al., 2009). In a species-rich wet meadow in Ohrazení (Lepš 2014) where long-term experimental plots are located, several *Carex* species are regularly found coexisting at very fine spatial scales. Niche segregation is not uncommon among closely related coexisting wetland species. However, this usually occurs along environmental gradients such as soil moisture or acidity (Waterway et al., 2009). During intensive sampling campaigns of field plots in Ohrazení, up to four *Carex* species were found to coexist in a single 10 × 10 cm grid cell (i.e. small enough to be environmentally homogenous); 2–8 *Carex* species were regularly found in 1 m2 plots (which, in the mown unfertilized variant, contained up to 40 vascular plant species), while a total of 10 *Carex* species were found in the 1 ha site among more than 100 vascular plant species (Lepš 2014). Similarly, in Laelatu wooded meadow (Estonia, one of the most species rich meadows in the world, Wilson et al., 2012), five *Carex* species were found in a 20 × 20 cm grid cell (Kull and Zobel 1991). Previous studies from the Ohrazení site demonstrated that the number of species, including *Carex*, decreased with fertilization (Lepš, 1999). Application of fertilizer almost immediately increased total biomass of the community, which in turn increased competition for light, suppressing weaker competitors (Lepš 2014). Although this held true for most *Carex* species, other members of this genus displayed a range of responses to fertilization (Lepš 2014), suggesting that even small differences in responses to competition and fertilization can contribute to coexistence among *Carex*.

Resource utilization curves describing variation in the growth of species along resource gradients are used to define limiting similarity (MacArthur and Levins 1967). When unknown, as is more often the case, resource utilization curves have to be determined experimentally. A greater range of variation in the response of closely related species along such gradients implies lower similarity of resource use, offering a potential explanation for their coexistence. For example, where there is low heterogeneity of biotic and abiotic conditions within a meadow community, differences in the traits associated to timing and type of clonal growth could enable species coexistence. *Carex* species display a variety of clonal traits and strategies (Krahulec, 1994). Some species can respond to environmental heterogeneity by varying the amount of rhizome branching and rhizome length (de Kroon and Hutchings 1995). This so called “foraging behavior” enables these species to avoid unfavorable patches of soil while positioning their rhizomes or stolons in areas of high nutrient availability. For example, *Carex flacca* may overcome fine-scale variation in resource availability by transferring resources between connected rhizomes (de Kroon et al., 1998).

Another potential coexistence mechanism is the storage effect which states that no species can thrive under all conditions and that different species use a range of coping strategies under changing conditions to ameliorate against bad years (Cáceres, 1997; Angert et al., 2009). For example, the temporal coefficient of variation (CV) in biomass of a species with a high relative growth rate (RGR) is likely to fluctuate more across favorable and unfavorable years, profiting from ‘good’ years and declining more in ‘bad’ years. Alternatively, populations of species with lower RGR tend to be less sensitive to annual fluctuations in conditions (have lower temporal CV), showing buffered population growth (Chesson and Warner, 1981, Kelly and Bowler, 2002). It is therefore possible that the coexistence of congeneric species, such as *Carex*, could be supported by differing RGRs, each predicting different patterns of temporal stability (Májeková et al., 2014).

In an attempt to improve our understanding of coexistence of closely related species we used the example of *Carex* to ask the following questions: (i) Do phenotypic responses of coexisting *Carex* species to nutrient availability and competition vary among species in a short-term pot experiment? Since the findings of pot experiments may not provide a realistic prediction of how *Carex* species respond in field experiments, we also ask (ii) whether the differences in species traits and responses found in pot experiments predict the long-term performance and temporal fluctuations of *Carex* species in a long-term field experiment. We compared the results of the pot experiments to the first four years of data from our long-term experiment which provided values for the initial responses to treatments (Lepš, 1999). The long-term responses to treatments were characterized by species abundances 20 years after the start of the experiment (Lepš, 2014) and also by species temporal variability (Májeková et al., 2014).

2. *Materials and methods*

2.1. Study material

The genus *Carex* (Cyperaceae) is a species rich genus in Central Europe (and in temperate flora in general). The following *Carex* species were used in this study: *C. demissa* Hornem, *C. hartmanii* A. Cajander, *C. pallescens* L., *C. panicea* L., *C. pilulifera* L., *C. pulicaris* L. and *C. umbrosa* Host. Other *Carex* species present in the locality are: *C. leporina* L., *C. echinata* Murray and *C. nigra* (L.) Reichard. These species were not included in this study because we were not able to collect a sufficient number of their ramets. *C. leporina* and *C. echinata* are rare at the locality and *C. nigra* is very difficult to identify in early April, when the ramets were collected (see below). *Carex* rhizomes typically branch sympodially. Among our focal species, *C. hartmanii* and *C. panicea* are able to produce numerous long horizontally creeping rhizomes (particularly in *C. hartmanii* where the length of the rhizome branch can reach about 0.5 m – Appendix 1). *C. demissa*, *C. pallescens* and *C. umbrosa* possess only very short rhizome branches. *C. pilulifera* and *C. pulicaris* form frequent but rather short rhizome branches. To characterize the type and extent of rhizomes of our focal *Carex* species, we excavated one rhizome system per species from our experimental site in Ohrazení, mapping the position of individual ramets and length of spacers (see the schematic in Appendix 1 for further detail).

On April 2, 2001 and April 4, 2002, ramets of each species were taken from the Ohrazení site for use in the pot experiments, where growth responses to nutrient availability and competition respectively were tested. Each ramet consisted of a young vegetative rosette with several young roots. Initial individual size was recorded in order to calculate their responses.

2.2. Fertilization pot experiment

In the fertilization experiment, plants were grown in pots (upper diameter 16 cm, lower diameter 10 cm, height 15 cm, volume 2 L) containing substrates with low, medium and high mineral nutrient levels. The basic (low nutrient) substrate consisted of a mixture of commercially sold peat and sand (in 1:2 ratio). The medium and high nutrient substrates were created by adding 1 g and 4 g of commercial NPK fertilizer (19% N, 6% P, 12% K) respectively, to the basic substrate in each pot. There were five pots of each substrate type, containing one ramet per pot of each species, totalling 105 pots. Potted plants were grown for 96 days in a greenhouse at the University of South Bohemia, Czechia. The final design was not fully balanced due to some mortality and preliminary misidentifications of ramets in early spring that were later corrected when individuals were more developed.

Before planting, the fresh weight of ramets was recorded. At the end of the experiment, all plants were harvested, and we counted the number of individual ramets and rhizomes, recorded the fresh and dry biomass (accuracy 0.01 g) after drying for 24 h at 80 ◦C, separated above ground parts into leaves and flowering stems and belowground parts into roots and rhizomes. Based on the regression of the dry weight on the fresh weight recorded at the end of the experiment, we estimated the dry weight of each ramet at the beginning (separate regression for each species). Then we used this value to calculate the relative growth rate (RGR) as

$$\text{RGR} = \frac{\ln(DW_t)-\ln(DW_0)}{t}$$

where *DWt* is the dry weight of the whole ramet at the end of the experiment, i.e. in time t (i.e. time of duration of the experiment, i.e. 96 days), and *DW0* is the dry weight at the start of the experiment. The values of RGR are thus in [days^{-1}]. Individuals that were established but died (in the competition experiment), or decreased their weight, were assigned an RGR of 0. From the above- and belowground parts of the final biomass we calculated the root:shoot ratio as the dry weight biomass of roots (i.e. belowground resources acquisition structure, thus excluding the rhizomes) divided by the dry weight of leaves (i.e. photosynthetic structure). Dry biomass weight of a dead individual was recorded as zero, and the height and root:shoot ratio were considered as missing values.

2.3. Competition pot experiment

For the competition pot experiment, another set of ramets was prepared as above, and planted into low nutrient substrate. The treatments of no-, moderate, or high intensity of interspecific competition were achieved by using a single *Carex* ramet for no-competition, by sowing 15 seeds of *Holcus lanatus* L. with the ramet for moderate competition, and 45 seeds of *H. lanatus* to achieve high competition. Following germination, the seedlings of *H. lanatus* were thinned to 5 and 15 for the moderate and high competition treatments respectively. Again, for each combination of species and competition level, five replicates were used, totaling 105 pots. Some individuals subsequently died (various species, two in low, three in medium, and two in high competition), leading to a slightly unbalanced design. Plants were allowed to grow for 98 days. *H. lanatus* was selected as a competitor, because the species is common at the site and thus is an important competitor in the field. Based on experience from our previous experiments, *H. lanatus* germinates easily and grows quickly (including clonal spread), which is important in a relatively short-term experiment.

Following the previous experiment, fresh biomass of each ramet was weighed before the experiment. During the experiment, the number of ramets was counted at 28, 52, 78 and 98 days. At the end of the experiment, plants were harvested allowing measurement of fresh and dry, as well as above- and belowground biomass. RGR and root:shoot ratio were calculated as detailed above and the numbers of belowground rhizomes were counted.

2.4. Field experiment

As discussed in the introduction we used data from a field experiment of Lepš (1999, 2014) and Májeková et al. (2014). The study site is located near Ohrazení, 10 km South-East of České Budějovice (48° 57′ N, 14° 36′ E, 510 m a.s.l.), with 7.8°C mean annual temperature and 620 mm mean annual rainfall (local meteorological station). The site is a species-rich oligotrophic wet meadow, traditionally managed by mowing, once or twice a year. Species composition corresponds to Molinion caeruleae, with some transitions to Violion caninae. None of the *Carex* species can be considered dominant species in the study area, but *C. hartmanii* and *C. panicea* achieve cover above 15% in some parts of the meadow.

In 1994 a long-term experiment was established at this study site, combining the fertilization, mowing and removal of dominant, *Molinia caerulea* (L.) Moench. The experiment was set in a factorial design with each of the eight possible combinations replicated in three, 2 × 2 m plots (24 plots altogether). The results and detailed description of the design of this experiment and monitoring regimes have been previously published as follows. The results (development of species composition) against which we compare species performance in the pot experiments are published in (1) Lepš (1999) which describes the immediate response of community composition to the introduction of the treatments. These data were also analysed and used as a training set in the chapter 15 of Šmilauer and Lepš (2014) textbook on multivariate data analysis. (2) Lepš (2014) provided a summary of vegetation development during the first 15 years. (3) Májeková et al. (2014) detailed the temporal variability of biomass of individual species.

In this paper, we use three characteristics of individual *Carex* species derived from the field experiment: (1) the immediate response to mowing and fertilization at the beginning of the experiment, (2) the long-term responses based on species composition in 2014 (i.e. 20 years after the start of the experiment), and (3) the variability of biomass of individual species over a 13 year period, expressed as a coefficient of variation (CV). We only used the responses to fertilization and mowing, discarding the removal of the dominant treatment, because only species composition showed a significant response to them.

2.5. Data analysis

2.5.1. Pot experiments

We analysed the response of individual species to different levels of fertilization and competition with a two-way analysis of variance (ANOVA), considering the following response variables: relative growth rate (RGR), root:shoot ratio, height, number of ramets, total dry weight, and number of rhizomes. Most variables were measured at the end of the experiment, with the exception of total dry weight of *C. pilulifera* with No fertilization treatment (only one replicate due to mortality) for which the average value of the various individuals of *C. pilulifera* from no competition treatment (virtually identical) was assigned. The values for total dry weight were log-transformed (to cope with the zero values for individuals that died, we have used log (x+1)). In the fertilization experiment, we tested the main effect of species identity, level of fertilization and their interaction and, in the competition experiment, the effect of species identity, level of competition, and their interaction. When testing the effect of the treatments on number of rhizomes, the species that did not produce any rhizomes were excluded from the ANOVA (although they are maintained in figures for the purpose of visualization).

To measure the number of ramets in the competition experiment, we conducted 4 counts over the experiment. This data was analyzed with a repeated measurement ANOVA, using the number of ramets as a response variable. Species identity, level of competition, time, and their first- and second-order interactions, were used as explanatory variables. In all the ANOVAs, the significant interaction species by treatment signifies that the response to the treatment differs among species. In repeated measurement ANOVA, the interaction species by time means different timing of increase of the number of ramets among species, and the second order interaction (species × treatment × time) shows that species differ in the temporal dynamics of their response to the treatment.

Because the individual response variables were not independent, and because we carried out a separate ANOVA for each of them, there is a danger that some of the significant results might be just due to Type I error. Consequently, we decided to further provide a multivariate common test for all characteristics. We used Redundancy Analysis (RDA; Šmilauer and Lepš 2014), with the five characteristics available for all species as response variables (i.e. relative growth rate (RGR), root:shoot ratio, height, number of ramets, log of total dry weight), species and treatment (i.e. either nutrients, or competition level), as predictors. All the response variables were centered and standardized. Analyses corresponding to the main effects in ANOVA are partial RDAs, with one factor being the explanatory variable, and the other, the covariable. The analysis testing the interaction is obtained by partial RDA, with the interaction being the explanatory variable, and both the main effect being the covariables. The ordination diagrams also indicate correlation between individual response variables.

2.5.2. Field responses

We calculated the field responses of *Carex* species by means of multivariate analyses (RDA), using species composition, characterized by cover of individual species, estimated in the central 1 m^2 quadrats in the 2 × 2 m plots under different treatments. For the estimate of immediate response to the treatment, the data from the first four years of the experiment were used. The first year provided the baseline data, i.e. before any introduction of the treatments, thus, the interaction with time (as a quantitative variable, 0 for the baseline, and 1, 2 and 3 for subsequent years) is the best explanatory variable during the early years. Thus, the interaction: Time × Treatment Under Consideration, was the only explanatory variable, while: Plot Identity, Time, and Time × Other Treatments were the covariables in the RDA on the covariance matrix. The scores of individual species on the constrained axis equate to the characteristics of the individual species' response to the treatment under consideration. This method follows an example of multivariate counterpart of repeated measures ANOVA detailed in Šmilauer and Lepš (2014, chap. 15).

To characterize the response 20 years after the start of the experiment (when the difference among treatments had stabilized), we used the 1 m^2 cover data from 2014. The treatment under consideration was the only explanatory variable used, while the other two treatments were designated as covariables in the RDA. Scores of individual species on the single constrained axis represented how the species responded to the treatment in question. In both cases, positive values indicated a positive response to the treatment (i.e. either the species increased at different rates during the first four years, or the species was more abundant after 20 years of exposure to that treatment.

Finally, in the mown plots, we evaluated how biomass varied between species and with time (biomass is not applicable from unmown plots). Biomass was measured over the 13 years and its variability was characterized by the coefficient of variation (CV = standard deviation / mean). Both standard deviation and mean were calculated for each 0.25 m^2 plot over 13 years and averaged across the whole site. Species that appeared infrequently were excluded to avoid overestimating CV, as increased measurement error would skew apparent variability. The included species were present in at least six (out of twelve) plots and had an average biomass > 0.002 g per plot. Species with an average biomass <0.002 g were included if they were found in at least nine plots. During the first seven years, variation of biomass of individual species was governed by directional changes (Lepš et al., 2019) and so these years were omitted, thus, CV should only reflect non-directional variability (see Májeková et al., 2014 for further details).

2.5.3. Predicting field response

Finally, we attempted to predict the short and long-term responses of species to fertilization and mowing. We also attempted to predict the temporal variability of individual species using the species responses determined by pot experiments. Setting the scores of individual species along the constrained axes as response variables, we used the species response variables from the pot experiments as the explanatory variables: relative growth rate, root:shoot ratio, height, number of ramets, total dry weight and number of rhizomes. We considered, as explanatory variables, both the mean values of the traits per species, and the difference in trait values between treatment levels. The means were calculated as the averages of the trait values in control pots of both the experiments (i.e. no fertilization and no competition). Differences were calculated separately for fertilization and competition pot experiments, by subtracting the mean trait values in the respective control from the trait values in the highest treatment level (i.e. high nutrients or high competition). Therefore, we had three different explanatory variables: differences in fertilization pot experiment, differences in competition pot experiment, and trait averages. In order to explore the data, we selected the best explanatory variable (i.e. the lowest AIC) for the five characteristics mentioned above (i.e. short- and long-term response to fertilization and mowing, and variability in time), and then tested significance using linear regression.

The number of *Carex* species in our locality was limited and not all species were abundant enough to reliably estimate their responses. For this reason, we could not provide robust field data for all the species used in the pot experiments. Given the limited sample of species (n = 7), caution should be taken when considering the power of the statistical tests, which is clearly very low. Moreover, it should be noted that there were many different predictors obtained from the pot experiment, and that the best predictor was always selected for each of the responses. Therefore, caution should also be taken when considering the p-values and thus the ecological significance of our conclusions.

The univariate analyses were carried out in R (R Core Team, 2019), and the RDA in Canoco 5 (ter Braak and Šmilauer, 2012). Univariate models were validated on the basis of the distribution of residuals (Appendix 2).

3. ***Results***

3.1. Pot experiments

Species differed in all measured variables both in the fertilization and competition pot experiments (species effects, Table 1, Figs. 1 and 2). However, these differences were not markedly modified by fertilization and competition. Almost no significant interactions between species and treatments (in their response to fertilization and competition) were detected ($p > 0.05$), indicating a similar species response to these factors (Table 1). Nutrient availability had the effect of significantly decreasing the root:shoot ratio and had a close to significant positive effect on RGR. Both these patterns were similar across all species. There were no significant species-treatment interactions. Competition significantly decreased RGR, number of ramets, and total dry weight. The only significant interaction between species and competition was apparent for the number of rhizomes (Table 1B, Fig. 2), i.e. the variable used for the rhizomatous species, and not included in the RDA analyses.

In the repeated measures ANOVA, the number of ramets differed between species, changed with time, and was affected by the level of competition ($p < 0.01$ for all). There were also significant first and second order interactions (Table 2, Fig. 3). The response of *Carex hartmanii* to competition was an increase in number of ramets toward the end of experiment. In most other species, competition had the opposite effect.

In all cases, the multivariate analyses (RDA) agreed with the result of the univariate analysis of the respective characteristic. In both experiments, there were significant differences among species, but also significant differences between treatment levels (i.e. both, nutrients and competition), in all of them with $p < 0.001$. Therefore, it is unlikely that the significant effects of these two main factors in the univariate analyses (i.e. ANOVA) were a consequence of Type I errors. The amount of variability explained by competition was considerably higher than that of nutrients (Appendix 3). Notably, the species × treatment interaction was not significant in either experiment. The results also show that most variables were positively correlated, with exception of root:shoot ratio. The detailed results of the multivariate analyses are in Appendix 3.

3.2. Predicting field responses

The best predictor of short-term field response to fertilization was the difference in root:shoot ratio in response to fertilization from the pot experiment, while the best predictor for long-term response was the difference in number of rhizomes (Appendix 4, Fig. 4A and B respectively). The best predictors of field responses to mowing were the average height in the short-term (Appendix 4, Fig. 4C) and the difference in number of ramets in the long-term (although not significant; Appendix 4). Temporal variability of the biomass of species in the field was best predicted by the RGR value of that species (Appendix 4, Fig. 4D). It should be noted that, even though the best predictor was selected in each case, very few significant relationships were found.

Table 1. Influence of species and fertilization (A) and competition (B) level on six traits, tested by two-way ANOVA. For testing the effect on the number of rhizomes only four species could be used in the analysis. The bold numbers indicate significant effects ($p < 0.05$). For the fertilization experiment Error df = 65, except for number of rhizomes where Error df = 46. For the competition experiment Error df = 84, except for height and root:shoot ratio where Error df = 77, and number of rhizomes where Error df = 48.

A	df	RGR		Number of ramets		Height		Total dry weight		Root:shoot ratio		df	Number of rhizomes	
		F	*p*	*F*	*p*	*F*	*p*	*F*	*p*	*F*	*p*		*F*	*p*
Species	6	9.687	**<0.001**	8.429	**<0.001**	50.086	**<0.001**	17.922	**<0.001**	5.029	**<0.001**	4	6.957	**<0.001**
Nutrients	2	3.088	0.052	1.365	0.263	0.602	0.551	1.675	0.195	51.015	**<0.001**	2	0.455	0.637
Species:Nutrients	12	0.891	0.559	1.137	0.347	0.830	0.619	0.783	0.666	0.787	0.662	8	0.171	0.994

B	df	RGR		Number of ramets		Height		Total dry weight		Root:shoot ratio		df	Number of rhizomes	
		F	*p*	*F*	*p*	*F*	*p*	*F*	*p*	*F*	*p*		*F*	*p*
Species	6	18.215	**< 0.001**	8.487	**< 0.001**	23.846	**< 0.001**	23.502	**< 0.001**	4.845	**< 0.001**	3	22.281	**< 0.001**
Competition	2	21.821	**< 0.001**	27.547	**< 0.001**	0.336	0.715	14.125	**< 0.001**	0.158	0.854	2	3.171	0.051
Species:Competition	12	0.637	0.805	1.839	0.055	0.959	0.494	0.924	0.527	0.799	0.650	6	3.346	**<0.008**

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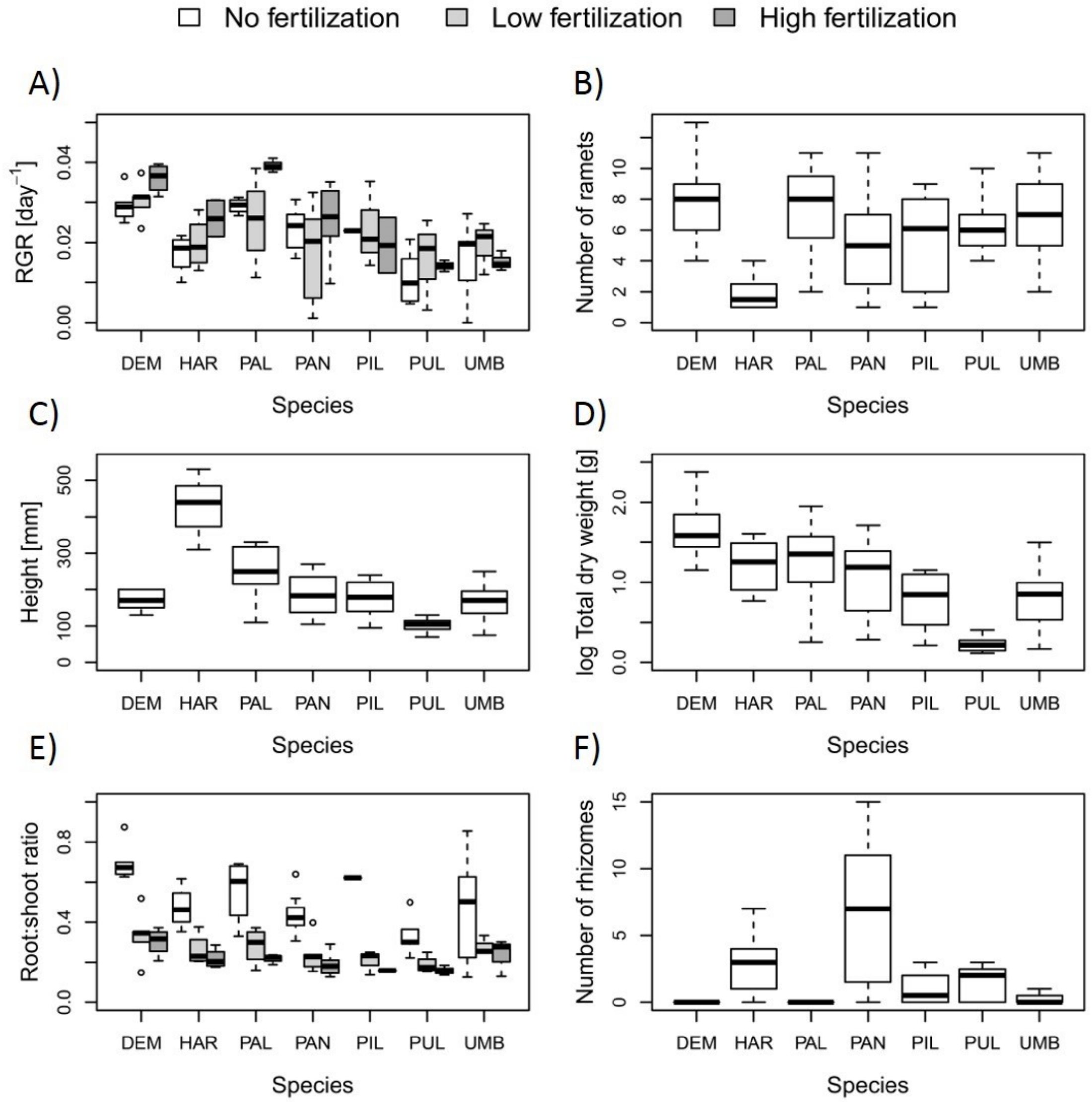

Figure 1. Response of the seven selected *Carex* species to different levels of fertilization. Graphs with a single boxplot are shown where there are no significant differences between treatments, and the average values of all treatments are presented. Multiple boxplots indicate significant (or close to significant) differences between treatments. The measured variables: (A) Relative growth rate, (B) Number of ramets, (C) Height, (D) Total dry weight in logarithmic scale, (E) Root:shoot ratio and (F) Number of rhizomes. Results of Two-Way ANOVA are shown in Table 1. (DEM – *C. demissa*, HAR – *C. hartmanii*, PAL – *C. pallescens*, PAN – *C. panicea*, PIL – *C. pilulifera*, PUL – *C. pulicaris*, UMB – *C. umbrosa*).

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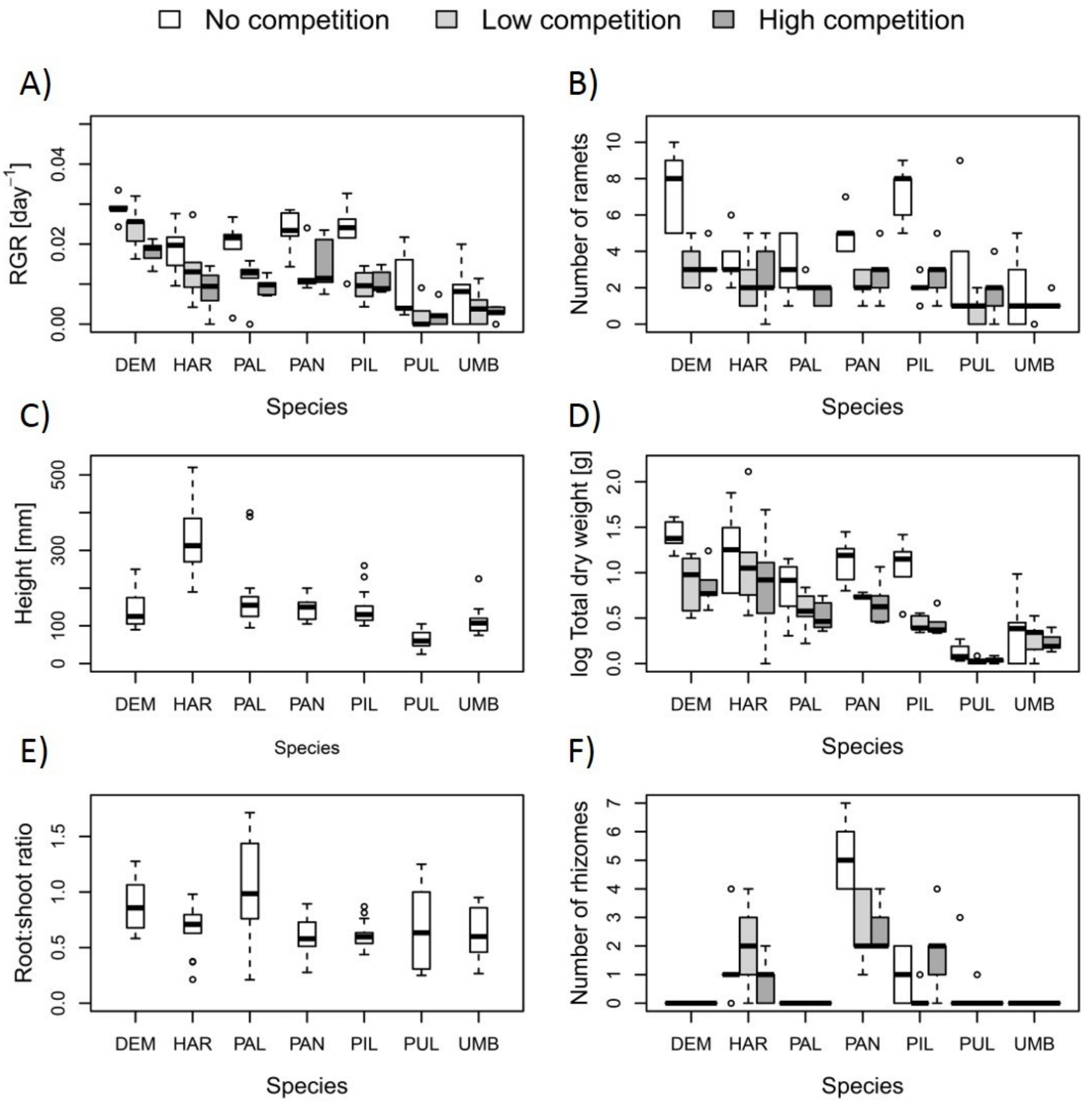

Figure 2. Response of the seven *Carex* species to different levels of competition. Graphs with a single boxplot are shown where there are no significant differences between treatments, and the average values of all treatments are presented. Multiple boxplots represent significant differences between treatments. The measured variables were (A) Relative growth rate, (B) Number of ramets, (C) Height, (D) Total dry weight in logarithmic scale, (E) Root:shoot ratio and (F) Number of rhizomes. (DEM – *C. demissa*, HAR – *C. hartmanii*, PAL – *C. pallescens*, PAN – *C. panicea*, PIL – *C. pilulifera*, PUL – *C. pulicaris*, UMB – *C. umbrosa*).

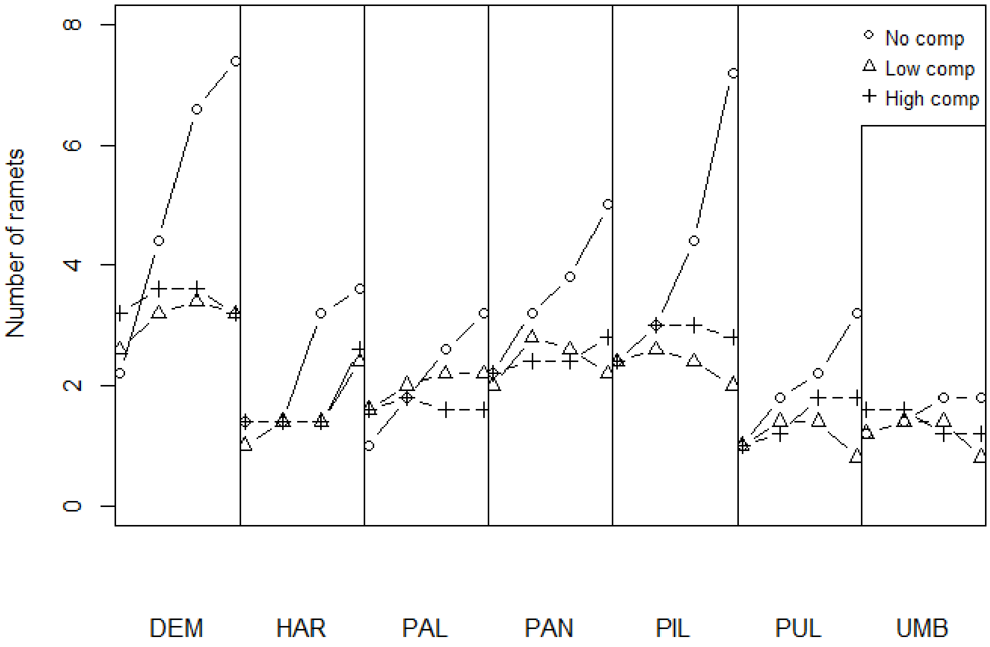

Figure 3. Ramet production over the 98 day time period in *Carex* species in control pots at high and low levels of competition. (DEM – *C. demissa*, HAR – *C. hartmanii*, PAL – *C. pallescens*, PAN – *C. panicea*, PIL – *C. pilulifera*, PUL – *C. pulicaris*, UMB – *C. umbrosa*).

Table 2. Results of repeated-measurement ANOVA for number of ramets of *Carex* species. The bold numbers indicate significant effects ($p < 0.05$).

	Error: Between (df = 84)		
	df	*F*	*p*
Species	6	14.796	**< 0.001**
Competition	2	12.948	**< 0.001**
Species:Competition	12	0.867	0.583
	Error: Within (df = 252)		
	df	*F*	*p*
Time	3	41.879	**< 0.001**
Species:Time	18	2.614	**< 0.001**
Competition:Time	6	24.535	**< 0.001**
Species:Competition:Time	36	2.003	**0.001**

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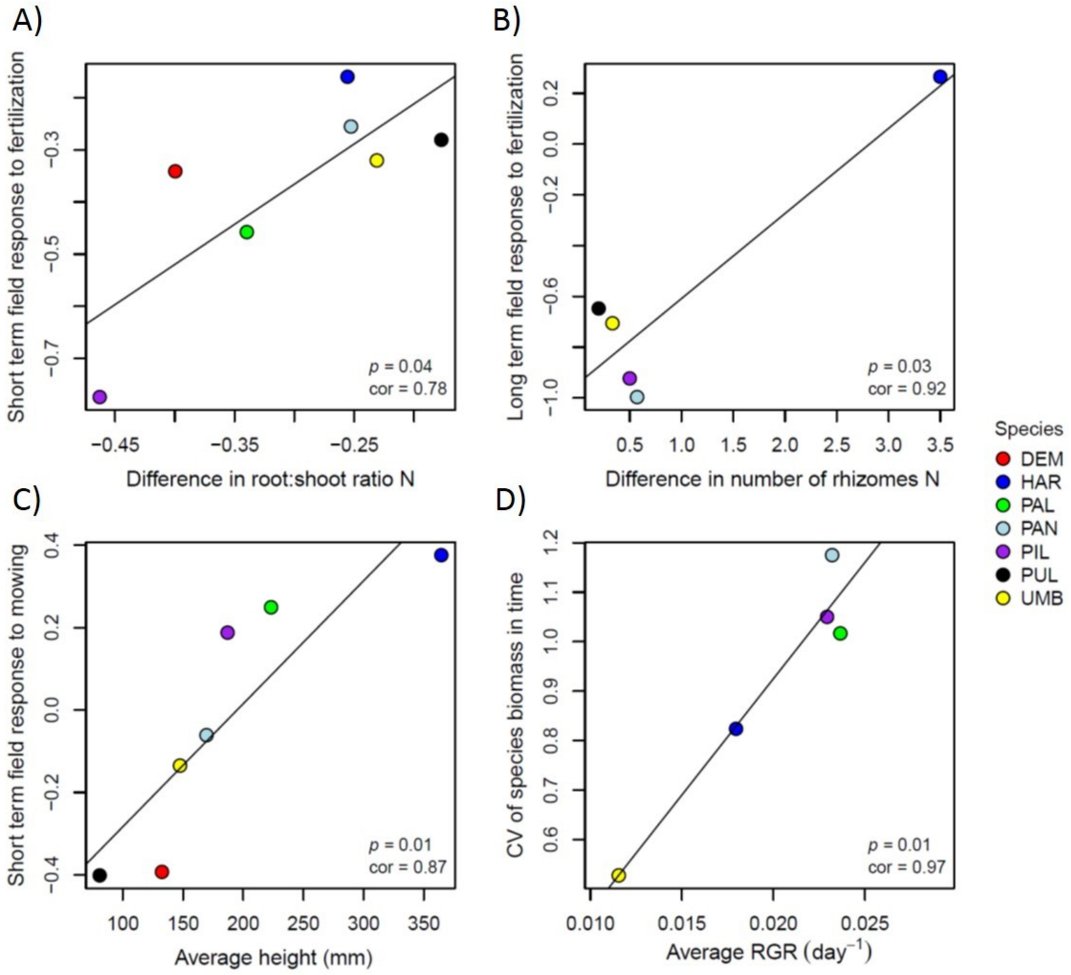

Figure 4. Relating results from the field to results of pot experiments. Lines represent linear regression, p-values of these and Pearson correlation coefficients are marked for each subfigure. (A) Short-term field response to fertilization explained by the difference in root:shoot ratio in fertilization pot experiments. (B) Long-term field response to fertilization explained by the difference in number of rhizomes in fertilization pot experiments. Note, this correlation is mainly driven by one species *C. hartmanii*. *C. demissa* and *C. pallescens* were excluded from the model because they do not produce rhizomes. (C) Short-term field response to mowing explained by the average height. (D) CV from the field explained by the average RGR. *C. demissa* and *C. pulicaris* were not included because there are no reliable estimates of CV due to species rarity. (DEM – *C. demissa*, HAR – *C. hartmanii*, PAL – *C. pallescens*, PAN – *C. panicea*, PIL – *C. pilulifera*, PUL – *C. pulicaris*, UMB – *C. umbrosa*).

4. Discussion

To explain the coexistence of several *Carex* species, we expected variation in species responses to nutrient availability and competition. Variable resource use would offer an explanation for this level of coexistence through niche differentiation, as predicted by limiting similarity (MacArthur and Levins 1967). However, we observed only a few instances where species responses to nutrient availability and competition varied significantly. In contrast to our expectation, we found similar species responses to the treatments with a lack of significant interactions between species and treatments (Table 1). The growth of all the species was negatively affected by competition, and the response to increased soil nutrients was weak for all species (except the root:shoot ratio, and some effect on RGR). Despite a considerable addition of nutrients, biomass only increased weakly. In the pot experiment, a 1 g dose of fertilizer (medium level) in a 16 cm diameter pot matched the dose used in the field experiment (ca 50 g of fertilizer per m^2), in line with standard meadow fertilization regimes (i.e. 95 kg of N per ha, based on the 19% of N of the fertilizer used). This result agrees with Lepš (1999) where *Carex* species did not increase in biomass following field fertilization, despite increasing total community biomass. Across our focal *Carex* species, only the clonal traits showed some differential responses to competition (rhizomes, Table 1B; production of ramets, interaction species × competition × time, Table 2), supporting the view that the response of clonal traits and their temporal variation diverge among *Carex* species in their reaction to competition. The results also show marked differences between species traits, in both pot and field experiments. The differences were particularly clear in RGR and clonal growth characteristics. Despite these differences, their predictive power for species performance in the field experiment remained weak.

4.1. Fertilization

The effect of a decreased root:shoot ratio by fertilization was shared across all species. Fertilization also seems to have some positive effect on RGR, however, the number of ramets, height, total dry weight, and number of rhizomes were not affected. Numerous other studies also show that increasing fertilization often has the effect of decreasing the root:shoot ratio in various species (for example: Aerts et al., 1992 where all *Carex* species responded similarly, with no species-fertilization interactions; and Li et al., 2010). An increase in nutrients generally promotes growth of aboveground photosynthetic tissue at the expense of root growth. Aerts et al. (1992) also demonstrated that “high-productive species” profited the most from increased nitrogen levels and increased their biomass production with lower root:shoot ratios, in contrast to “low-productive species”.

According to Bernard et al. (1988), *Carex* species are typically more capable of nutrient uptake when availability is low, an idea reinforced by the findings of our experiments. The *Carex* species concerned in this study are mostly confined to low nutrient habitats (Řepka and Grullich, 2014) with no species responding positively to fertilization in our field experiment. This probably explains why, in the pot experiment, increased nutrient availability gave little advantage, even when additional resources promoted above ground investment (indicated by lower root:shoot ratios). No significant difference in the response of *Carex* to nutrient availability was detected, thus, it seems unlikely that possible differences in soil-nutrient acquisition can facilitate *Carex* coexistence.

4.2. Competition

Where the response to nutrients was generally very weak, all species reacted significantly to competition, which caused a more marked response than fertilization. The presence of the competitor species *Holcus lanatus* significantly decreased RGR, the number of ramets, and the total dry weight. As well as competing for light, we assume *H. lanatus* also competed for below ground resources with our *Carex* species. As with fertilization, these responses were ubiquitous and, with the exception of clonal traits, showed a lack of significant species × competition interactions. We found significant variation in species where the number of rhizomes changed in response to competition. This corresponded to field results, where *C. hartmanii* produced long rhizomes (see Appendix 1), representing a typical ‘guerilla’ type growth strategy. In pot experiment, *C. hartmanii* produced most rhizomes at low competition level. With the ability to move using underground rhizomes, *C. hartmanii* is probably able to avoid competition pressure. Thus, variability in the abilities of *Carex* species to respond to competition through different clonal growth strategies, may represent an important mechanism enabling the coexisting of species through circumventing the forces of competitive exclusion (Klimešová et al., 2018).

Similarly, the number of ramets over time also changed in response to competition (Table 2 and Fig. 3). Indeed, this response had the clearest variation across species (significant triple interaction of species × competition × time, Table 2), in species with different clonal growth responses, including those with different temporal responses. This suggests it is not the differences in traits that characterize productivity, but rather the clonal characteristics that responded differently to competition. We also observed pronounced differences between individual species in their architecture of rhizome systems and thus ability to spread laterally in the field (see Appendix 1). Therefore, we can expect differences in clonal traits and their response to competition to cause differences in spatial foraging for nutrients, which might also contribute to the coexistence of otherwise similar species (He et al., 2007; Klimešová et al., 2018). Vojtko et al. (2017) also suggest that clonal traits are a significant factor enabling the coexistence of similar species, calling for further investigation into the significance that clonal traits play in overcoming competitive barriers.

That *Carex* species reacted considerably to competition in the pot experiment, yet community composition did not respond to the removal of the dominant species in the field experiment (i.e. decrease of competition pressure), is not inconsistent. In pot experiments, individuals either were, or were not under competition pressure. In the field experiment, although the removal of *Molinia* would have provided some direct relief from competition with this single species, any gains would have been negated by increased competition with the remaining species in the community. Generally, after removal, all the species struggled to occupy the new space, so the effect on individual species was not so pronounced (Lepš 1999, 2014). Moreover, all *Carex* species were suppressed by *Holcus*, suggesting they were themselves relatively weak competitors, making it likely that other competitors in the removal plots would suppress them.

4.3. Predictions of field experiment responses

Generally, only weak relationships between our pot and field experiments were detected. In this context, it is important to stress that even when we adopted a rather liberal approach to predicting field responses by pot experiments (see methods), the selection resulted in very weak predictions. No significant predictors were detected for the long-term response to mowing. For the short-term response to fertilization, the strongest relationship was also rather weak ($p = 0.036$) and for the long-term field response to fertilization, the significant results were likely driven by a single outlier (*C. hartmanii*). These results suggest very weak correspondence of experimental and field data. As the source plants used in these experiments originate from a single population, it is possible that a different population might have responded differently to competition and fertilization because of local adaption process (documented for *Carex* species by He et al., 2007; Schmidt et al., 2018). In our case, however, the experimental plants for the pot experiment were taken from the locality of the field experiment, so that both should have the same local adaptations.

The incongruence between results from the pot and field experiments might also be caused by the combined interactions with other species in the field (absent in the pot experiments). Moreover, there was little variation in the response of *Carex* species in the pot experiments. Where differences were apparent, they were mostly in underground clonal traits. Despite a recent increase in studies promoting the importance of clonality to niche segregation and the coexistence of species (for example: de Bello et al., 2011; Klimešová et al., 2016), such traits are rarely studied, due to the difficulty of measuring them. In this context, the difference in number of rhizomes in the high and no fertilization sets was the best predictor of the long-term field response to fertilization, although this evidence was mainly driven by one species (*C. hartmanii*, Fig. 4B).

The short-term response to mowing was best predicted by plant height, with taller plants showing more positive response than shorter species. This goes against our expectation that taller species would be affected more negatively by mowing (Noy-Meir et al., 1989; Opdekamp et al., 2012). At a constant mowing height, a larger proportion of the aboveground biomass would be removed. In addition, the height advantage in competition for light is also removed. However, Klimešová et al. (2008) noted that plant height, often considered the best predictor of a species' response to grassland management, is often coupled with other more relevant functional traits. Within our species, this relationship was driven mainly by the tall *C. hartmanii* which also has the most extensive rhizome system – the most distant connected ramets in the field were more than 1 m apart from each other (see Appendix 1). This might explain how *C. harmanii* is able to respond positively to mowing. Furthermore, the correlation was only positive in the short-term, suggesting *C. hartmanii* can quickly recover from mowing while resources are not limited. However, over longer time scales this response would likely change, as below ground resources are gradually depleted. For this reason, we presume an indirect relationship between height and response. *C. hartmanii* can accumulate large belowground nutrient stores in their rhizomes, which can readily be mobilized after mowing. This also illustrates the limitation of the pot experiments, where the potential of this species for clonal spreading could not be demonstrated.

The temporal stability of species in the field experiment, characterized by temporal variation in biomass (Harrison 1979; Májeková et al., 2014), was well predicted using RGR from pot experiments. Previous studies suggest that slow-growing long-lived species have more stable biomasses over time because of their reduced responsiveness to environmental change (for example Lepš et al., 1982). These patterns are usually assessed using indirect proxies such as traits linked to the leaf-economy spectrum. For example, Májeková et al. (2014) demonstrated that CV is negatively correlated with LDMC. The theory of r-K strategy (Pianka, 1970) also predicts that r-selected organisms will exhibit more pronounced abundance fluctuations in time, because of their higher population level growth rate (Southwood et al., 1974). However, this relationship has so far only been demonstrated in insects (Spitzer et al., 1984) and to the best of our knowledge, the relationship between RGR and population fluctuation has not been demonstrated in plants.

In clonal plants, population growth rate is difficult to measure because of the challenge of identifying individuals. Consequently, we have used RGR based on the biomass changes in potted plants. This is probably a fair proxy for population growth rate, and, in this case, predicted temporal variability in biomass in the field. This agrees with the findings of Májeková et al. (2014), obtained using LDMC, which is expected to negatively correlate with growth rate.

Our results suggest the main differences among the studied *Carex* species were in their clonal traits, in particular, the size of rhizome networks. The *Carex* species in this study generally have rather conservative growth strategies. However, their clonal performance proved to be highly diverse. Species, such as *C. hartmanii*, possess extensive rhizome systems that correspond to typical guerilla strategies, while others, such as *C. umbrosa*, grow in tussocks. Our study clearly shows that the main differences among these closely related species are in clonal traits (and their responses) and that the clonal behaviour of our focal *Carex* species is highly variable. This variation in clonal responses and strategies is likely to allowing them to escape competitive exclusion, thus enabling the coexistence of these closely related *Carex* species.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.actao.2020.103692

Data availability

The data for this project are provided as Appendix 5.

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6. Supplementary materials

Appendix 1. Rhizome systems of individual *Carex* species as uncovered in the field.

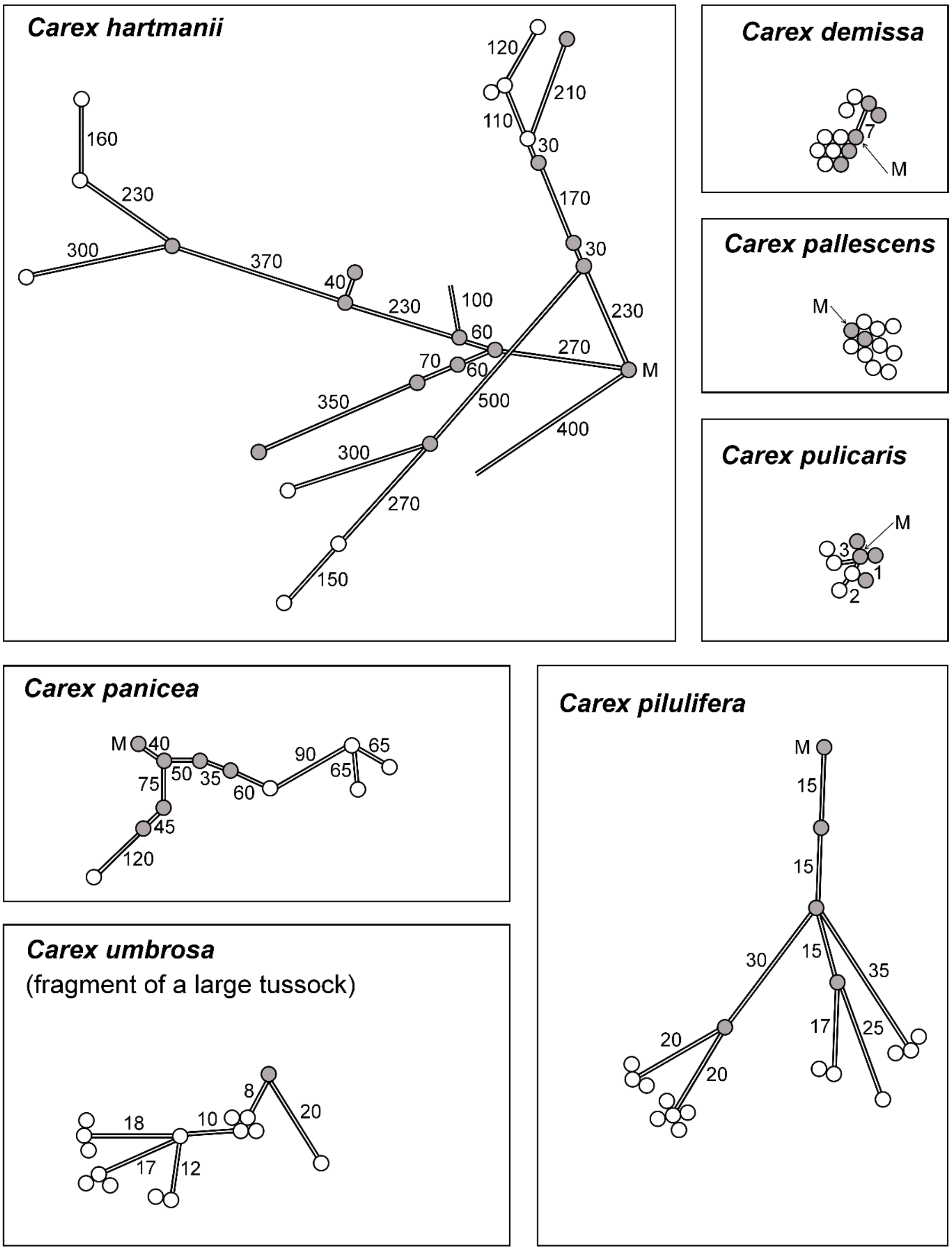

Figure S1. Rhizome systems of individual *Carex* species as uncovered in the field. All the depicted plants came from the locality Ohrazení. Their rhizome systems were uncovered in the second half of October 2001 (*Carex hartmanii*) and 2002 (the other species). *Symbols:* empty circle – ramet with living leaves; full circle – ramet without living leaves; double lines – rhizome branches; M – maternal ramet; numbers – length (mm).

Tammaru, K., et al., 2021. Ecological differentiation of *Carex* species coexisting in a wet meadow: Comparison of pot and field experiments. Acta Oecologica, 110, 103692.

Note 1: Rhizome systems of *Carex pilulifera*, *C. demissa*, *C. pallescens*, *C. pulicaris* and *C. umbrosa* are shown at 10-times greater scale than those of *C. hartmanii* and *C. panicea*. *Note 2:* Rhizome branches of *C. umbrosa* are rather ascending than horizontal. Thus, the distances between ramets in the field are somewhat smaller than depicted.

Appendix 2. Analysis of model validity

The validity of each model presented in the manuscript was tested, for example checking the normality and homoscedasticity of the residuals. In the following figures we show the distribution of the residuals of each of the models: Figure S2 - univariate analysis of variance (ANOVA) of several traits in fertilization experiment; Figure S3 - ANOVA of several traits in competition experiment; Figure S4 - linear regression between experiment and field responses. For the latter, note the low N of the model.

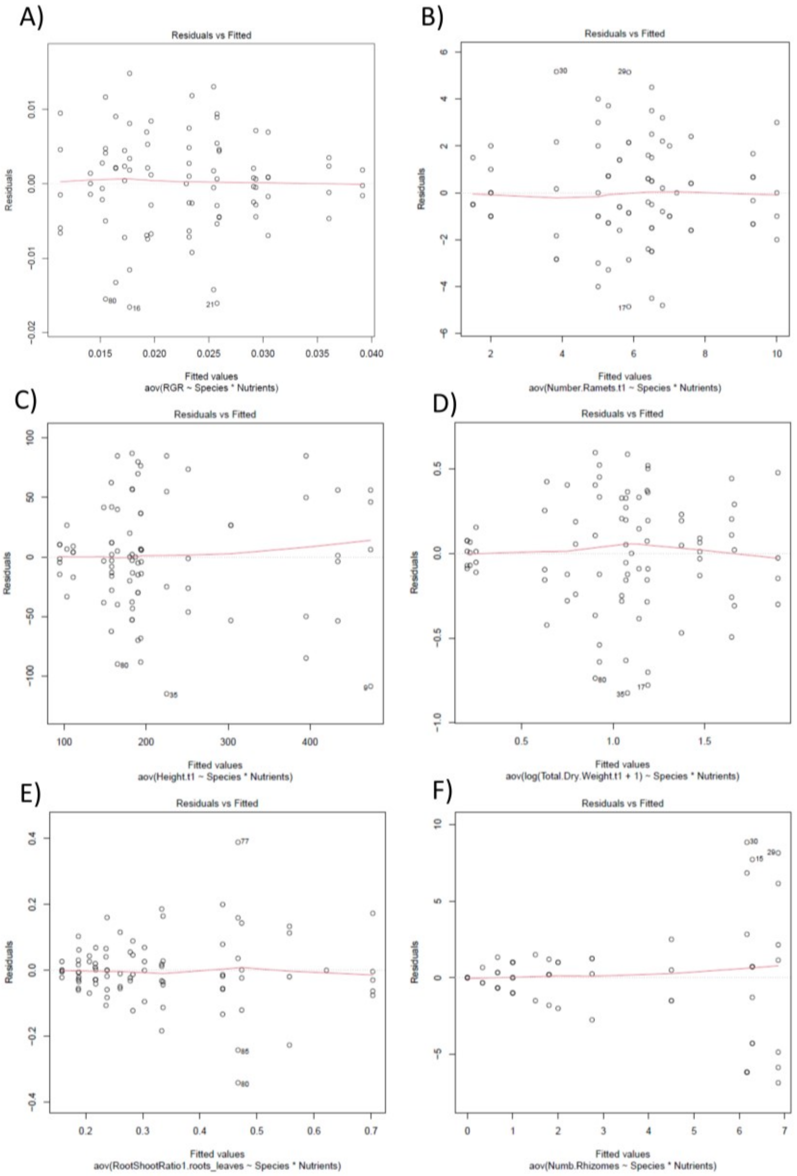

Figure S2. Distribution of the residuals of the ANOVA models for each particular trait from the fertilization experiment. A) Relative growth rate, B) Number of ramets, C) Height, D) Total dry weight in logarithmic scale, E) Root:shoot ratio and F) Number of rhizomes.

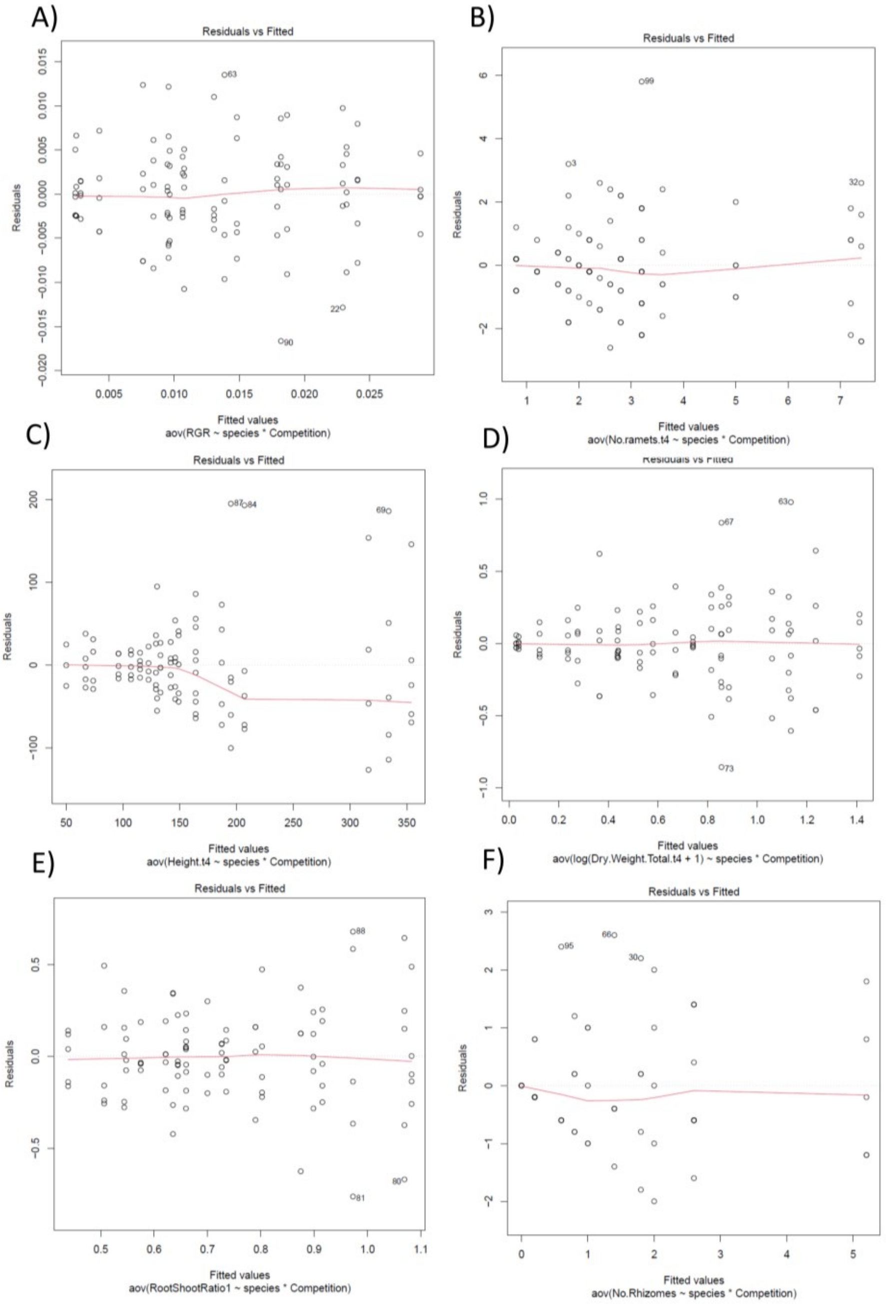

Figure S3. Distribution of the residuals of the ANOVA models for each particular trait from the competition experiment. A) Relative growth rate, B) Number of ramets, C) Height, D) Total dry weight in logarithmic scale, E) Root:shoot ratio and F) Number of rhizomes.

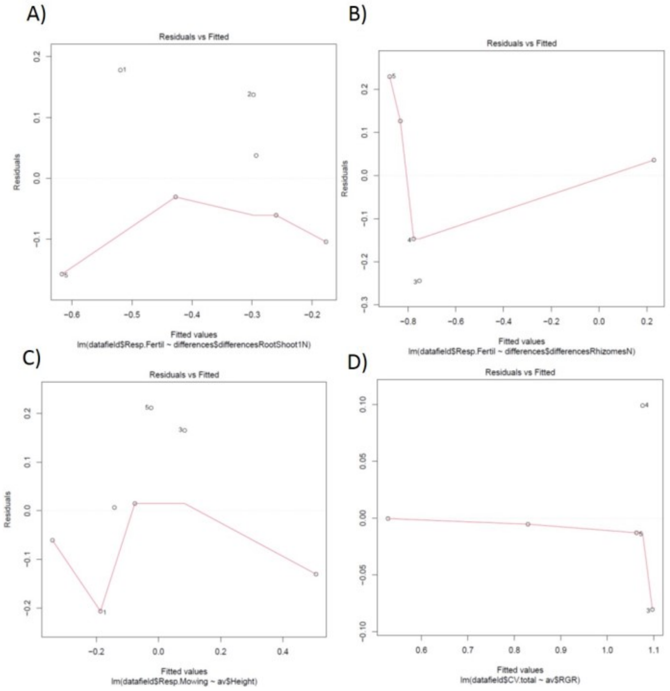

Figure S4. Distribution of the residuals of the several linear models used for the predictions of the field response by the response from the pot experiments. A) Short-term field response to fertilization explained by the difference in root:shoot ratio in fertilization pot experiments. B) Long-term field response to fertilization explained by the difference in number of rhizomes in fertilization pot experiments. C) Short-term field response to mowing explained by the average height. D) CV from the field explained by the average RGR.

Appendix 3. Multivariate analysis of the two experiments

In both experiments, we used the Redundancy Analysis (RDA; Šmilauer and Lepš 2014), with the five characteristics available for all the species as response variables (i.e. relative growth rate (RGR), root:shoot ratio, height, number of ramets, log of total dry weight), and species and treatment (i.e. either nutrients, or competition level) as predictors (both considered factors, i.e. the categorical variable). We have not used the number of rhizomes, because not all the species formed rhizomes in the experiment. The analyses were designed to correspond as much as possible to the ANOVA for the univariate response. The tests of the main effects (i.e. either treatment or species) were obtained from partial RDA, with the effect tested being the explanatory variable, and the other the covariable. The test of the interaction (treatment × species) was obtained by partial RDA, with the interaction being the explanatory variable, and both the main effects being the covariables. All the analyses provided amount of explained variability and pseudo-F statistics, which was used to test the significance by the Monte Carlo permutation test with 4999 permutations. Note that the amount of explained variability is dependent on degrees of freedom, which is quite different – for treatment, df = 2, for species, df = 6. Amount of explained variability is provided as percentage of the total variability in the response variables. The ordination diagrams also provide a lead on the correlation of individual response variables.

For the competition experiment, for few individuals, some characteristics were not available (for the individuals that died during the experiment, we were not able to provide root:shoot ratio, together × individuals). Because RDA needs complete samples, we calculated two versions, first with the complete cases only, and then with the missing values replaced by the mean of the variable. The two versions provided nearly identical results, and we present here the one with substitution of the mean. Results are summarized in Table S1.

Table S1. Summary of the results of the partial RDA with the main effects of treatment and species for the fertilization experiment and competition experiment.

Fertilization experiment	df	Explained variability	pseudo-F	*p*
Species	6	46.3	14.2	0.0002
Fertilization	2	11.6	10.6	0.0002
Species × Competition	12	6.1	0.91	0.5786

Competition experiment	df	Explained variability	pseudo-F	*p*
Species	6	40.4	13.3	0.0002
Competition	2	11.0	10.9	0.0002
Species × Competition	12	6.4	1.07	0.3654

The results show that the differences among species are still more pronounced than are differences between treatment levels: the species factor uses 6 df vs 2 df for the treatment, whereas the explained variability by species is more than three times higher, and usually, the explained variability increases with the df less than linearly, so the interspecific differences are more pronounced than the differences among treatment levels. In both experiments, the treatment × species interaction is not significant, and explains negligible amount of variability.

Further, we provide the ordination diagrams characterizing the effect of the treatments, i.e. the RDA with treatment as the explanatory, and species as covariable.

Both the ordination diagrams show significant effect of the treatment levels and the response variables. The ordination diagram for fertilization (Figure S5) shows that the root:shoot ratio is the most responsive characteristics to fertilization, with highest values in non-fertilized plots, and that weight, RGR, number of ramets are positively affected by (mainly high) fertilization.

The ordination diagram for competition (Figure S6) also shows that the main difference is between the no competition level and the competition (either low or high). Note the pronounced difference between variability explained by the first and the second axis, ascertaining that the truly different level is the no competition. It also shows that the plants without competition have higher weight, RGR, number of ramets and these three are highly correlated. The height and root:shoot ratio are not affected, which perfectly corresponds with the univariate analyses.

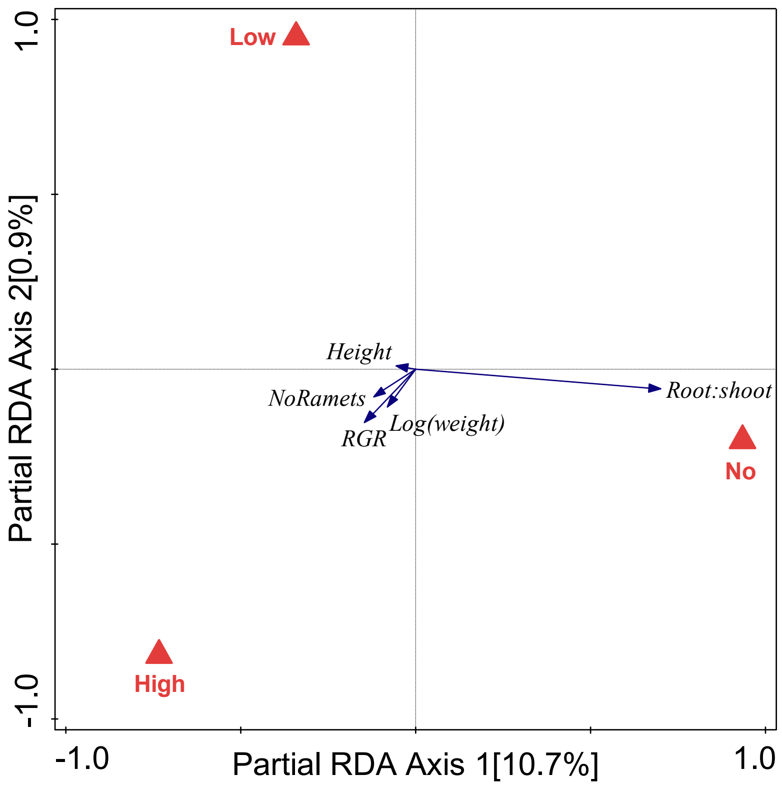

Figure S5. Effect of levels of fertilization (shown by red triangles, as centroids) on the characteristics of individuals. The values in axes labels brackets signify percentage of the total variability of the response variables explained by given axis.

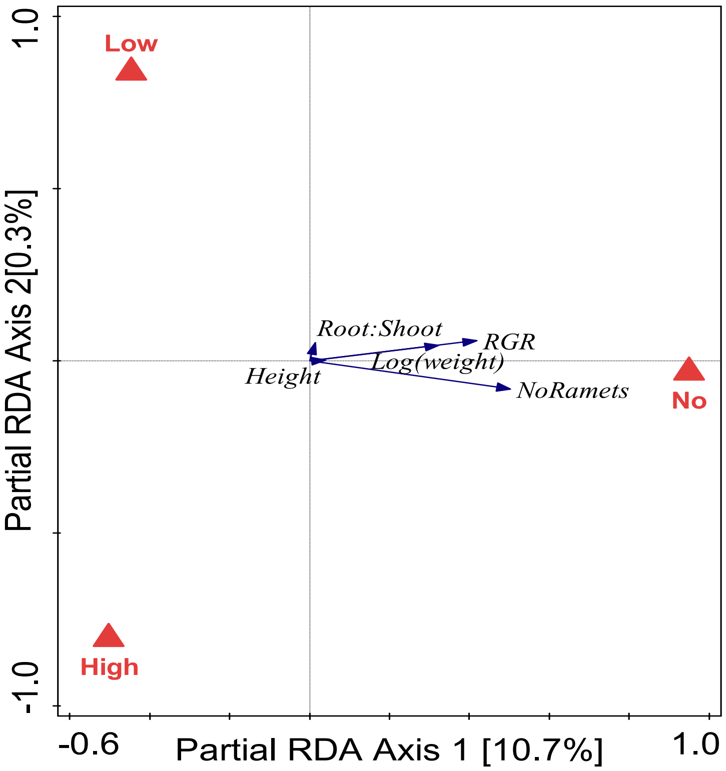

Figure S6. Effect of levels of competition (shown by red triangles, as centroids) on the characteristics of individuals. The values in axes labels brackets signify percentage of the total variability of the response variables explained by given axis.

In summary, the results of the multivariate analyses of both experiments show that the treatment has an effect on the characteristics measured and thus the significant results for individual characteristics are not solely effect of Type I error (they are not likely to be just a consequence of “statistical fishing”). Expectedly, the RDA have shown the most responsive characteristics, and these are those with the significant main effect. Nevertheless, in concert with the univariate analyses, the species × treatment interactions are not significant (both with $p > 0.3$).

Reference

Šmilauer, P., Lepš, J. 2014. *Multivariate analysis of ecological data using Canoco 5*. Cambridge university press.

Appendix 4. Predicting short and long-term responses and temporal variability of the species in the field.

Table S2. Predicting short and long-term responses and temporal variability of the species in the field. Results of the analysis of best explanatory variables for the field responses. Best predictors are selected from the response variables of the pot experiments. (N – fertilization pot experiment, C – competition pot experiment, R^2_{Adj} – Adjusted R-squared, df – residuals degrees of freedom)

Field responses	**Best predictor**	**Standardized coefficient**	**R^2_{Adj}**	***F***	***df***	***p***
Short-term field response to fertilization	differences root:Shoot N	0.78	0.54	7.97	5	0.037
Long-term field response to fertilization	differences rhizomes N	0.92	0.80	17.34	3	0.025
Short-term field response to mowing	average Height	0.87	0.72	16.08	5	0.010
Long-term field response to mowing	differences ramets C	-0.67	0.31	3.24	4	0.146
Coefficient of variation	average RGR	0.97	0.91	43.42	3	0.007

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