Universidade de Aveiro
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Characterization of insect communities in Ria de Aveiro saltmarshes

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Dissertação apresentada à Universidade de Aveiro para cumprimento dos requisitos necessários à obtenção do grau de Mestre em Ecologia Aplicada, realizada sob a orientação científica da Doutora Olga Maria Correia Chitas Ameixa, Investigadora Auxiliar do Centro de Estudos do Ambiente e do Mar (CESAM) e Doutora Catarina Barros de Prado e Castro, Investigadora Auxiliar do Centro de Estudos do Ambiente e do Mar (CESAM)

Financial support from Project SUShI - Sustainable use of insect protein in aquaculture feed (CENTRO-01-0145-FEDER-030818), co-funded by Centro 2020 program, Portugal 2020, through the European Regional Development Fund (02/SAICT/2017), through the National Science Foundation (FCT/MCTES).
Support was also given by PORBIOTA—Portuguese E-Infrastructure for Information and Research on Biodiversity (POCI-01-0145-FEDER-022127), financed by FCT through PIDAC national funds and co-funded by the FEDER

“Nor must we forget that in science there are no final truths.”

-Claude Lévi-Strauss

o júri

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agradecimentos

Agradeço em primeiro lugar as minhas orientadoras, Doutora Olga Ameixa e Doutora Catarina Castro por terem aceitado a laboriosa tarefa de me orientar até este momento e pela preciosa ajuda. Por todo o acompanhamento ao longo dos anos, das gargalhadas e das discussões. Por, não me terem dito que caminho seguir, mas orientando-me pelo caminho que escolhi. Um grande obrigado.

Aos meus pais, por desde sempre me permitirem desenvolver esta tarefa e por me sempre incutirem um espírito critico, abrir horizontes e mostrando que o saber não ocupa lugar.

A todos os amigos que conheci durante este percurso académico que fizeram destes anos os melhores e menos memoráveis da minha vida.

palavras-chave Sapais, Biodiversidade, Zonas Húmidas, Salinidade, Invertebrados.

resumo

Os sapais são habitats amplamente distribuídos por todo globo, com comunidades especializadas de flora e fauna. Estes habitats, exibem zonação para a sua flora, que vai desde bancos de vasa até as dunas ou mar, proporcionando diferentes habitats para um conjunto de invertebrados especializados à submersão regular pela água e a resultante elevada salinidade do solo. Globalmente, este habitat tem vindo a desaparecer rapidamente nas últimas décadas. Isto é motivo de preocupação visto que este ecossistema é um dos mais produtivos no mundo, e proporciona serviços ecológicos importantes, por exemplo, proteção da costa, controlo da erosão, purificação da água, apreensão de carbono, matéria bruta e comida e contribui para várias atividades recreativas. Os sapais, são também essenciais para a cadeia alimentar na zona costeira, proporcionando áreas de proteção para os peixes, crustáceos e aves. A Ria de Aveiro é a maior área de sapal contígua em Portugal e uma das maiores da Europa. É atualmente um sítio de Investigação socio-ecológica de longo-termo (Long-Term Socio-Ecological Research - LTsER) e está classificado na rede natura 2000, onde se inclui uma Área de Proteção Especial. Mesmo sendo considerada um laboratório vivo e alvo de muitos estudos, não existe muita informação sobre as espécies de insetos que aqui ocorrem, o que constitui uma grande lacuna no conhecimento deste táxon, que fornece vários Serviços dos ecossistemas. O objetivo deste trabalho foi caraterizar as comunidades de insetos das áreas de sapal da Ria de Aveiro. Para atingir este objetivo, foram recolhidos insetos da vegetação halófita dominante em setembro 2020 em sete locais, com o auxílio de redes entomológicas. Os insetos foram posteriormente identificados até ao nível máximo possível de resolução taxonómica. Em cada local, também foram retiradas amostras de sedimento para obtenção de parâmetros físico-químicos de cada local (salinidade, condutividade, pH e matéria orgânica). Foram capturados um total de 2816 indivíduos, pertencendo a 11 ordens e 80 famílias. As ordens mais abundantes foram as ordens Diptera e Hemiptera. Neste trabalho foram identificadas 17 novas espécies para Portugal (1 pertencendo à ordem Hemiptera e 16 à ordem Diptera). Apesar dos esforços realizados, as curvas de acumulação de espécies indicam que não foram recolhidas todas as espécies que ocorrem nos locais amostrados, sugerindo que será necessário um maior esforço de amostragem para melhor compreender as comunidades de insetos da Rai de Aveiro.

keywords Saltmarshes, Biodiversity, Wetlands, Salinity, Invertebrates.

abstract

Saltmarshes are widely distributed around the globe, with specialized communities of flora and fauna. These ecotones typically exhibit a spatial zonation of their vegetation from its mudflats to the seawall or dunes, providing different habitats to specific invertebrate assemblages adapted to regular submergence by seawater, and the resulting high soil salinity. Worldwide these ecotones have been declining at a rapid pace in the last few decades. This is a serious problem since these ecosystems are some of the most productive in the world and provide important ecosystem services, such as coastal protection, erosion control, water purification, carbon sequestration, raw materials and food and contribute to recreational activities. Also, they are essential to support coastal food webs, providing nursery areas for fish, crustaceans, and birds. Ria de Aveiro is the largest contiguous salt marsh area in Portugal and one of the largest in Europe. It is currently a Long-Term Socio-Ecological Research site (LTsER), and it is classified under the Natura 2000 network, encompassing a Special Protection Area (SPA). Despite being considered a living lab and being the target of many studies there is little information on insect species that occur here. This is a major knowledge gap since this taxon provides several important ecosystem services. In this work, we aimed to characterize Ria de Aveiro saltmarsh insect communities in this ecotone. To achieve this goal, insects were collected by sweep-netting the dominating halophyte vegetation in September 2020, in seven locations of Ria de Aveiro saltmarshes areas and later identified to the maximum possible level of taxonomic resolution. In each site, soil samples were collected to access physicochemical soil parameters of each site (salinity, conductivity, pH, and organic matter). A total of 2816 individuals belonging to 11 orders and 80 families were identified. The most abundant orders were the Diptera and Hemiptera. In this work, 17 new species were identified for Portugal (1 belonging to Hemiptera and 16 to Diptera order). Despite these efforts, the species accumulation curves indicate that not all species were collected in all seven sites indicating that further studies are necessary to fully understand insect communities of Ria de Aveiro saltmarshes.

TABLE OF CONTENTS

List of figures

1. Introduction

1.1 Saltmarshes

Laffoley & Grimsditch (2009) define saltmarshes as intertidal ecosystems strongly dominated by vascular plants. These can be found in the sub-artic to tropical climatic conditions and between coastal, right after dunes ridges, and estuarine, where is protected from strong wave activity and storms (Adam *et al*., 2002; Rickert *et al*., 2012). These are some of the rarest ecosystems, contributing to 0.01% of the Earth's surface (Desender & Maelfait *et al*., 1999; Georges *et al*., 2011). Saltmarshes in Europe, have been declining at a rapid pace in the last few decades (Bakker *et al*., 2002; Georges *et al*., 2011). Their characteristics allow very specialized communities of semi-terrestrial flora and fauna, as they are under constant tidal inundation (Beeftink *et al*., 1992; Rickert *et al*., 2012), and under different salinities (Adam *et al*., 1990).

Saltmarshes are crucial ecosystems, being found mostly on estuarine areas, but they can also be found associated with barrier islands, spits, embayment's, and open shores with low wave activity (Allen *et al*., 2000) and as fringing coastal lagoons (Adam *et al*., 2002). So they act as a link between land and sea, and also the saltwater with freshwater, creating an ecosystem that serves as a refuge from predators, a place to feed, for protection and reproduction (Mcowen, 2017). Other services that the saltmarshes can provide include shoreline protection, storm buffering, sediment retention, water quality maintenance, nutrient recycling, preservation of biodiversity, provision of natural environmental amenities, climate regulation, carbon sequestration, as well as cultural heritage and spiritual benefits (Brander *et al*., 2006; Blankespoor *et al*., 2014; Mcowen *et al*., 2017).

The flora found in these habitats is typically constituted by vascular plants, more precisely halophytes, meaning that they can withstand a wide range of salinities and different periods of flooding (Adam *et al*., 1993; Ameixa & Sousa *et al*., 2020). Their range is as higher as the seawater can go in the highest tide, being the upper limit, and its lower limit being the lowest neap tide (Ameixa & Sousa *et al*., 2020). As we go upstream the lower section is also marked with the end of the seawards and the beginning of vascular plants (Adam, 1990). So, as we cross the saltmarshes to inland we can see, a formation of several plants communities over a softly incline, being this incline relative to the mean high-water level also affecting the flooding and duration, thereby controlling the wetland productivity and species distribution, thus creating three types zonation's distributed throughout this area, lower, middle and high saltmarshes (Ameixa & Sousa, 2020; Anthony, 2008), These three areas face harsh conditions, such as tidal flooding, salt stress, desiccation, and competitive pressure from other species (Zedler & Kercher, 2004; Pennings, 2005; Anthony, 2008).

In temperate areas, the flora found in the lower areas of the saltmarshes belongs to genus such as *Salicornia* sp., *Suaeda* sp., *Aster* sp. and *Spartina* sp. (Doody *et al*., 1992; Perillo *et al*., 2019). Since they are the first plants to colonize these areas they are called pioneers, as they set the conditions for other species by reducing the erosion and biding the sediments with their roots (Friess *et al*., 2012; Short *et al*., 2016), increasing the area horizontally, and also vertically, creating a middle saltmarsh area that allows species such as *Sacocornia* sp (A.J. Scott)., *Halimione portulacoides* (L.) and *Limonium* sp. (Mill) to become established (Ameixa & Sousa, 2020). At higher saltmarsh areas, we start to see less salt-tolerant plants, such as *Juncus maritimus* (Lam.) (Sousa *et al*., 2017; Ameixa & Sousa, 2020).

In tropical and subtropical areas, the saltmarshes are replaced with mangroves. The plant's species found here are also salt-tolerant, but instead of being mainly vascular plants, they are mainly arboreal (Laffoley & Grimsditch, 2009). Unfortunately, these areas are also in danger due to anthropogenic reasons, such as over-harvesting timber, salt pond constructions and many others (Laffoley & Grimsditch *et al.*, 2009).

Saltmarshes are essential for food web support associated with natural processes of transforming and retaining nutrients (Oliveira *et al*., 2012), primary productivity, and nitrogen removal. Saltmarshes also provide ecological services for humans, such as carbon sequestration, soil and water quality filtration, flood prevention, climate change mitigation, and many others (Chabreck, 1988; Keddy *et al*., 2000, Richardson *et al*., 2001; McKee 2012 Čížková *et al*., 2013). It also provides economic values of wetlands include the supply of natural goods (Quintana-Alcantara, 2014) and recreational offer recreational, cultural, and educational opportunities for visitors and local communities (Mitsch and Gosselink *et al*., 2000, LePage *et al*., 2011, Zedler *et al*., 2012). Being in a way a unique ecosystem that attracts a wide variety of birds and other animals, which can be used as recreational or commercial ecosystem services (Barbier, 2011; Mcowen, 2017), providing recreational and economic services for humans.

The service provided by saltmarshes such as carbon sequestration helps to mitigate the increase of the atmospheric CO_2, by accumulating with the biomass and sediment of the existing vegetation. With this process, it can regulate the climate at local and global scales (Sousa *et al*., 2017)

1.2 Threats to saltmarshes

At present, wetlands, which also include saltmarshes, are losing 1-2 per cent of area per year (Bridgham *et al*., 2006), and according to Barbier (2011), about 50% of saltmarshes areas were already lost throughout the world.

One of the threats to saltmarshes is eutrophication, which influences nutrient cycling, storage, and sustainability (Ameixa & Sousa, *et al* 2020). This is a problem that affects all the saltmarshes around the world, and are one of the causes of its loss, since it increases the above-ground leaf biomass, decreases the dense below-ground biomass of bank-stabilizing roots, and increases microbial decomposition of organic matter (Deegan *et al*., 2012; Ameixa & Sousa, *et al*, 2020). With this increase, the geomorphic stability will be affected, causing creek-bank collapse with significant areas of creek-bank marsh converted to unvegetated mudflats (Deegan *et al*., 2012).

Since most saltmarshes are located in coastal areas, they are also vulnerable to sea-level rise (SLR). This will affect their stability and sustainability, influencing one of the roles of the saltmarshes, which is sedimentation affecting the vertical accretion, ability to migrate inland, plant biomass, and decomposition rates, thus controlling the wetland submergence potential (Butzeck *et al*., 2015; Ameixa & Sousa, 2020). The vulnerability of saltmarshes to multiple stressors from either natural or anthropogenic origins will influence their ability for carbon sequestration (blue carbon) and nutrient stock, (Sousa *et al*., 2017; Ameixa & Sousa, 2020).

The SLR can lead to the so-called “coastal squeeze”, which occurs when “intertidal habitats are lost due to the high-water mark are fixed by a defence or structure and the low water mark migrates landwards in response to SLR”. However, this coastal habitat loss will also happen due to anthropogenic reasons, such as seawalls, which will not allow the saltmarshes to progress inland (Pontee *et al*., 2013; Ameixa & Sousa *et al*., 2020).

As in many other ecosystems, one of the major threats to saltmarshes are biological invasions, which can affect saltmarshes as a whole. The invasive saltmarsh plants are well adapted to being submerged and are also able to quickly reproduce and additionally, can live under anoxic conditions (Ameixa & Sousa, 2020). One of the most common examples is the genus (e.g. *Spartina patens* (Aiton) Muhl), in which some species can behave as invasive and display a great capacity to transform the habitats (Bertacchi & Lombardi, 2014).

1.3 Insects communities

Saltmarshes insect communities are considered to be the richest faunas, compared to the other marine environments according to Foster and Treherne (1978) and Cheng (1976). They also have conquered food chains and food webs, with their abundance. Since they have diverse food sources *e.g*., they can be scavengers, carnivores, herbivores, among many other food sources, and they are able to live under and/or above water (Cranston, 2014).

Insects were the first group of animals to dominate flight (Dickinson, 2012) and their success in both terrestrial and freshwater environments is unrivalled (Williams and Williams, 1998). They also have a great capability to survive under extreme conditions. The perfect example is the pelagic water strider *Halobates sericeus,* which was able to conquer the ocean (Cheng, 1976).

Several insect species have adaptations to thrive in saltmarsh habitats. One of such adaptations is the one from the aphid *Staticobium staticis*, which can live underwater for some time by covering the spiracles with tuberculate stigma plates (Hille *et al*., 1939).

Another example of such adaptation is the cuticle from the exoskeleton that protects the insect from water loss, and for this reason, being a vital adaptation to survive on land (Cranston, 2014, Cheng, 1976, cap. 1). An additional important adaption is their respiratory system, in which insects produce a hypertonic excretory fluid, allowing them to osmoregulate, this is mainly produced by marine insects (Cheng, 2009). Others trap air between the hydrofuge hairs that cover the body, allowing them to breathe underwater (Cheng, 1976)

Several insect species are declining, and it is also predicted a great invertebrate extinction (Hadfield *et al*., 1993; Thomas *et al*., 2004; Ameixa *et al*., 2018). This is mainly due to numerous threats, mostly, anthropogenic reasons such as deforestation, habitat loss, etc). However, contrary to mammals, birds or reptiles for which land for "nature" is put aside, conservation of insect species is not usually a priority (Dirzo, 2014), since there is a general negative attitude towards them. Although there are some initiatives to preserve insects, these are usually restricted to butterflies and certain charismatic beetles (Dirzo, 2014).

1.4 Objectives

Since there is little information on insect communities in Portugal and even less information on saltmarsh areas this thesis aimed to provide an overview of insect communities in Ria de Aveiro saltmarshes. This work had as specific objectives:

1- To Identify and quantify insect species and characterize insect communities collected in halophyte vegetation.
2- To evaluate how insect abundance and diversity is influenced by physicochemical soil parameters.

2. Material and methods

2.1 Study area

The study was conducted in Baixo Vouga Lagunar (BVL), an area with 3362km^2, where the Vouga river estuary, meets with Ria de Aveiro (Figure - 1A and 1B), occupying 3000ha. It is a unique natural habitat of land and water, also exploited by man, namely in activities such as fishing, bait digging or other recreational activities. This area is characterized by low depth water channels, freshwater wetlands, and saltmarshes. According to Natura 2000 network, it was classified as a Site of Community Importance with an area of 2769ha (Lillebø *et al*. 2015).

Ria de Aveiro is located on the northwest coast of Portugal, connected with the Atlantic Ocean by a single inlet. This area belongs to the Baixo Vouga Lagunar with approximately 45km long by 10km wide and is characterized by four main channels with their respective branches, forming islands, inner basins, and mudflats. From the South, there are two narrow and elongated channels, Mira and Ílhavo, with 25km and 15km long, respectively. Towards the centre is the Espinheiro channel with 17km. In the North, there is the São Jacinto-Ovar channel with 29km (Lillebø *et al*., 2015).

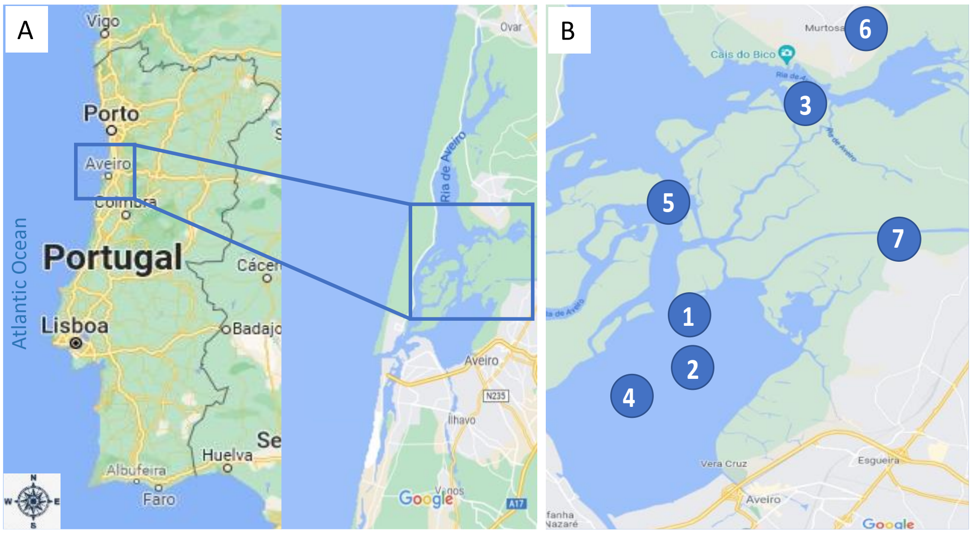

Figure 1 – A: Location of Ria de Aveiro; B: Sampled area. Map was generated using Google maps.

2.2 Experimental design

Insects were captured in the end of Summer of 2020 (September) with sweep-nets, in days with clear sky and during low tide periods. For each host plant, three samples were collected, each corresponding to 100 sweeps, a total of 300 sweeps per plant host. Sweeps were carried on *Atriplex patula* L, *Halimione portucaloides* L., *Juncus maritimus* (Lam), *Limonium vulgare* Mill. *Spartina maritima* (Curtis) Fernald., *Spartina patens* (Aiton), and *Tamarix africana* Poir.

The content of the sweep-net was collected with the help of an insect aspirator and placed into plastic flasks (Figure 2). The flasks were identified with the location, name of the host plant, date, and the number of the replicate. Each sample was then placed in a thermal container at -4°C and transported to the laboratory. In the laboratory, the samples were preserved in a fridge at -20°C until identification.

Figure 2 - Sampled sites: 1- Foz do Rio Novo (40°40'50.8"N 8°40'18.6"W), dominated by Spartina maritima (Curtis) Fernald; 2- Salinas (40°40'35.0"N 8°40'20.0"W), dominated by Halimione portucaloides (L.), Juncus maritimus (Lam) and Sarcocornia perennis (Mill.) A.J. Scott, Phragmites australis (Cav.) Trin ex. Steud.; 3- Bico (40°43'33.0"N 8°38'46.3"W) dominated by H. portucaloides L. and J. maritimus Lam.; 4- Reboxo (40°39'42.9"N 8°41'39.9"W) dominated by H. portucaloides L., S. perennis (Mill.) A.J. Scott, S. maritima (Curtis) and Limonium vulgare Mill. 5- Farinha (40°42'19.3'N 8°40'33.6'W), dominated by J. maritimus Lam and H. portucaloides L.; 6- Cambeia (40°44'08.0"N 8°37'40.7"W) dominated by H. portucaloides L., Salicornia ramosissima J. Woods, and Tamarix africana Poir.; 7- Cacia (40°41'44.2"N 8°37'31.4"W) dominated by J. maritimus Lam, H. portucaloides L. and Atriplex patula L.

2.3 Identification of the insects

Captured insects were identified with the help of a stereomicroscope Leica S8APO (Figure 3), using dichotomous keys. These identifications were complemented with specialist discussion forums whenever keys were not available or specific doubts needed clarification.

Figure 3- Material used for identification

For identifications Barnard (2011), was used for most orders found in this study, as well as Mikes Insect Keys – History website (2021) (https://sites.google.com/view/mikes-insect-keys/mikes-insect-keys). For the Hymenoptera order, Goulet and Huber (1993) keys for families were used. For the order, Diptera Oosterbroek (2006) key was used to identify families, as well as with the aid of experts in several families to identify specimens up to species.

When species identification was not carried (e.g., lack of keys or expertise) these were identified with different numbers to have a better perspective on diversity. After identification, the insects were preserved in 70% ethanol, in Eppendorf's tubes.

2.4 Diversity estimators

The communities in each site were characterized by the Shannon-Wiener index (1) and Shannon's evenness (2). The sampling effort for each site was evaluated with species accumulation curves (3) and species richness was estimated by using the non-parametric estimators: Jack Knife 1 and 2 (4) and Chaos 1 and 2 (5).

1. The Shannon-Wiener diversity index (H'), is a measure of the diversity of the species present, was calculated using the formula:

$$H' = -\sum_{i=1}^{s} p_i \log p_i$$

Where pi represents the proportion of individuals of species among all individuals sampled and S is the number of species.

2. The Shannon's evenness (EH), when the proportions of all species are equal:

$$E_{\mathrm{H}} = H'/\ln(S)$$

Shannon-Wiener values were then compared with a one-way ANOVA.

3. Simpson D, measures the diversity of species in a community.

$$D = \sum_{i=1}^{S} p_i^2$$

4. Species accumulation curves S(est): plots the cumulative number of species as a function of sampling effort (Magurran *et al*., 2004)

$$S(n) = \frac{S_{\max} n}{B + n}$$

Where S(n) is the number of species observed in n samples; S_{max} is the total of number of species in the assemblage; and B is the effort required to detect 50% S_{max} (Magurran *et al*., 2004).

5. Non-parametric estimators Jack Knife 1 and 2:

Jack Knife 1 estimate the total richness using the exact number of just one sample (uniques). While Jack Knife 2 uses the uniques and the number of individuals that occur in the two samples (duplicates) (Magurran *et al*., 2004).

Jack Knife 1 formula:

$$S_j = s + Q_1 \frac{n-1}{n}$$

Sj is the estimated richest is used to measure the richness, s is the observed richness, Q1 is the number that occurs in j samples and n is the total of samples (Magurran *et al*., 2004).

Jack Knife 2 formula:

$$S_j = s + \frac{Q_1(2n-3)}{n} - \frac{Q_2(n-2)^2}{n(n-1)}$$

6. Non-parametric estimators Chaos 1 and 2: Chaos 1 is an estimator for the absolute number of species in an assemblage Chaos 2 is an adaptation using the presence/absence taking into account the species distribution in the samples. In other words, we only need to know the species present in one sample (uniques) and the number of species found in exactly two (duplicates) (Magurran *et alet al*., 2004).

In their classic form, the Chao1 and Chao2 estimators are:

Chao 1 formula:

$$S_1 = S_{obs} + \frac{F_1^2}{2F_2}$$

Chao 2 formula:

$$S_2 = S_{obs} + \frac{Q_1^2}{2Q_2}$$

Fi is the number of individuals that have exactly i individuals in each sample.

2.5 Soil sampling and soil preparation

Soil samples were collected on the same day as insects, with the help of a small shovel, retrieving a sample of soil (with 3 replicates) from each site.

Samples were stored in a thermal container and transported to the laboratory, where they were weighted to obtain the fresh weight. After which they were placed in a climatic chamber at 40°C until they were dried. When the samples were completely dried, they were weighed again to obtain the dry weight. Soil samples were then prepared for analysis. These were passed through a 2mm sieve, to have a homogeneous granulometry and the proper soil particle size for physicochemical determinations of pH, salinity, conductivity, and organic matter.

2.5.1 pH, Salinity and Conductivity of the soil samples

Values of pH, salinity, and conductivity were determined using a ratio of 1:5 (soil: water). For these determinations, 5g of soil were weighted and 25ml of distilled water were added to the containers. For each soil sample, three analytical replicates were prepared, for a total of 30 samples. Each sample was placed in a Witeg Wisd Orbital Shaker SHO (WITEG Biotechnic) for 30min, with a rotation of 135rpm. After 30min the samples were left to rest for 2h, after which the parameters were measured with a Multiparameter Waterproof Meter HANNA INSTRUMENTS® HI-98194.

Figure 4- Precision scale

2.5.2 Determination of dry weight, and organic matter

The porcelain crucibles were numbered and dried at 500°C for 3h in a muffle furnace, after this period they were removed into an exicator and weighted in a precision scale (Figure 4). Dried soil samples were added to the crucibles and weighted, to obtain the soil dry weight. The samples were placed in the muffle furnace at 500°C for 6 hours, to burn all the organic matter contained in the soil samples. After cooling off, each sample was weighed again and the difference between the dry weight and the weight obtained after burning the organic matter corresponds to the percentage of organic matter that was lost on ignition (LOI %).

2.6 Data analysis

To study the relation between soil parameters and sites, one-way variance analysis (one-way ANOVA) was performed. The independent variables on soil characteristics were pH, salinity, conductivity, and organic matter content. To address these questions, the following null hypothesis was tested: H0=there are no differences in soil parameters between sites.

Comparisons of the number of species, abundance, and all the diversity estimators between sites were made also with a one-way variance analysis (one-way ANOVA). To address these questions, the following null hypothesis was tested: H0=there are no differences on these parameters between sites.

First, the assumptions of ANOVA were tested with a normality test (Shapiro-Wilk) and an Equal Variance Test (Brown-Forsythe). The Dunnett test was used as a *post hoc* multiple comparison procedure to distinguish which soil characteristics significantly differed.

Tukey test was used as a post-hoc comparison procedure to see which parameter significantly differed. The P-value for these tests were 0.05 and the tests were conducted using SPSS version 25.

The species richness estimators evaluated were the nonparametric Chao 1, Chao 2, first-order jack-knife, second-order jack-knife. These were calculated using the software EstimateS, version 9.1.0, Copyright R. K. Colwell. To better perceive how soil parameters relate to the abundance and number of insect species, a Principal Components Analyses (PCA) was plotted using the software PAST version 4.

3. Results

3.1 Diversity estimators

We collected a total of 2816 individuals from 187 species, distributed by 11 insect orders (see Annex I). The most abundant order was the Diptera with 1176 individuals divided into 62 species. The most common species found from this order was *Machaerium maritimae* (Haliday, 1832) (Diptera, Dolichopodidae) (Figure 5).

Figure 5 - *Macherium maritimae* on the left. Photo by Rui Andrande; *Prokelisia marginata* on the right. Photo by Mark Dunkling

The second most abundant order was Hemiptera with 757 specimens, represented by 32 species, being the non-native *Prokelisia marginata* (Van Duzee, 1897) (Hemiptera, Delphacidae) the most common. Several new species for Portuguese territory were found, during this survey. One belonged to the Hemiptera order, *Teratocoris antennatus* (Boheman, 1852) from the Miridae family was identified for the first time for Portuguese territory (Figure 6) and a short note is under preparation (Santos *et al*. in preparation). In the Diptera order, on the Ephydridae family except for *Psilopa nitidula* (Fallen, 1813) and *Diasemocera maritima* (Perris, 1847), all the other 16 species, are new records for Portugal (Carles-Tolrá *et al*., 2002). Preliminary results were presented in a poster the XIX Iberian Congress of Entomology, held in Coimbra on 21st September 2021 (Annex II).

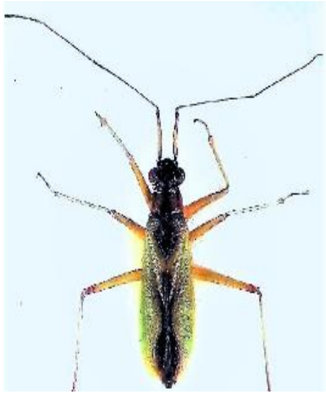

Figure 6 - *Teratocoris antennatus* (male specimen) (Boheman, 1852). Photo Paride Dioli.

Table 1 Characterization of insect communities collected in different sampling sites (Averages, n=3). Different letters represent statistically significant differences between the means recorded in the different sampling plots for the Simpson index. Shannon-Weiner index (H'), Shannon Evenness (E) and Simpson index (D).

	Abundance	Nº of species	H'	E	D
Site 1	54^{a}	272^{a}	4.25^{a}	1.79^{a}	0.91^{a}
Site 2	52^{a}	201^{a}	3.52^{a}	1.52^{a}	0.96^{b}
Site 3	36^{a}	153^{a}	5.97^{a}	2.93^{a}	0.94^{c}
Site 4	54^{a}	561^{a}	6.65^{a}	2.44^{a}	0.9^{d}
Site 5	24^{a}	160^{a}	4.72^{a}	2.61^{a}	0.91^{e}
Site 6	100^{a}	797^{a}	5.4^{a}	1.81^{a}	0.83^{f}
Site 7	79^{a}	591^{a}	3.2^{a}	1.07^{a}	0.93^{g}

Regarding insect diversity, Table 1 shows that the diversity and the equitability from each site, did not have any statistical difference (P=0.058; P=0.069, respectively) between them and as for the dominance there was a statistical difference between sites.

Site 6 had the highest abundance and number of species (Table 1), but where the Shannon index was higher, was on site 4 (6.65), followed by site 3 (5.97). Site 3 had a more even distribution. Sites 6 and 7 had the highest Simpson index (D) meaning that in these sites there were few dominating species less evenly distributed.

The dendrogram of similarities in the species composition recorded at the collections sites is shown in Figure 7. Site 5 appears as an outlier in terms of insect species composition and then two main clusters are formed by sites 6, 7 and 3 and by sites 4, 1 and 2. Sites 6 and 7 were the most similar followed by sites 1 and 4.

The analysis of species accumulation curves (Figure 8) shows that the sampling (three replicates per host plant per site), did not reach a plateau in all the collection sites. This means that it would be necessary to increase the sampling effort to capture most insect diversity. But it also seems that in some sites such as 4, 5, 6, 7 they probably are very close to a plateau.

According to different non-parametric methods (Figure 9), the estimated total species richness can be calculated in 261 (by Chao1), 244 (by Jack-knife1) and 238 (by Chao 2), and 271 (by Jack-Knife 2). It is also possible to observe that Chao2 has reached a plateau, and it is also possible to see that Jack-Knife 2, is almost reaching a plateau. As for Chao 1 and Jack-Knife 1, both did not reach a plateau.

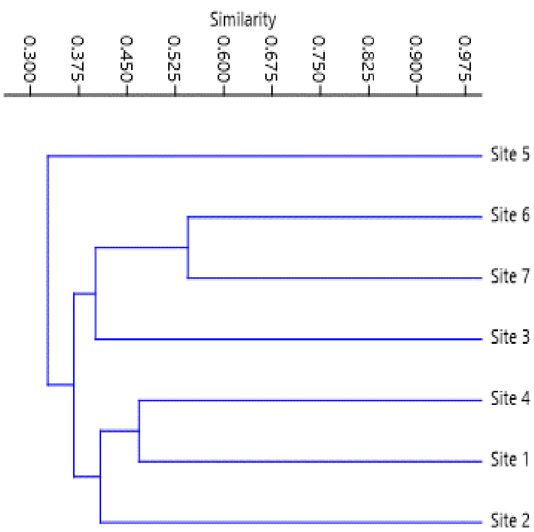

Figure 7 - Dendrogram of the Sørensen–Dice index showing the similarities of the sites and the matrix of similarity of each site.

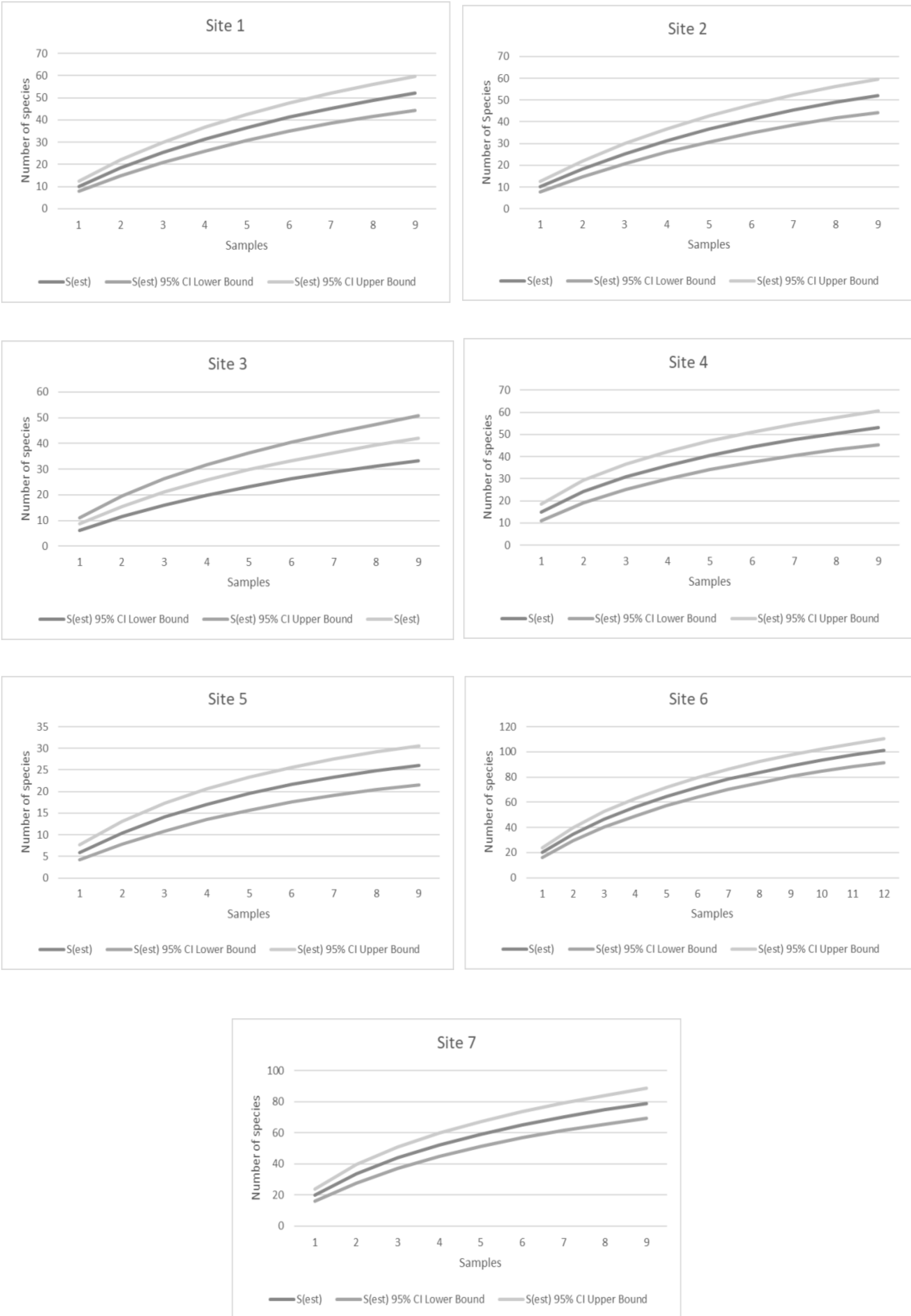

Figure 8 - Species richness estimates (with 95% confidence intervals) for the estimators S(est) (analytical) based on 100 randomized samples for the total data of entomofauna sampled in Ria de Aveiro.

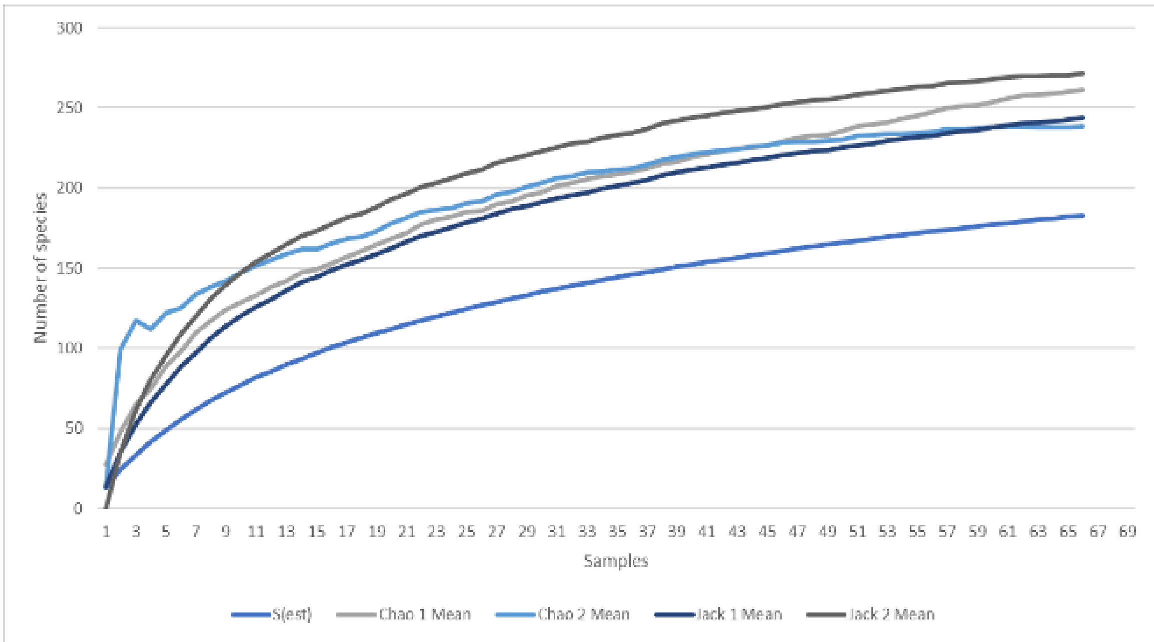

Figure 9 - Diversity estimators, Collector's curve, Chaos 1 and 2, Jack-Knife 1 and 2, based on 100 randomized samples for the total data of the insects caught in Ria de Aveiro.

3.2 Soils

3.2.1 pH, Conductivity, Salinity and Organic matter

There were significant differences in the salinity measurements between all collection sites (F= 95.67, P< 0.001) (Figure 10). Sites 2, 3 and 7 had lower salinities and sites 3 and 5 had the highest salinity values.

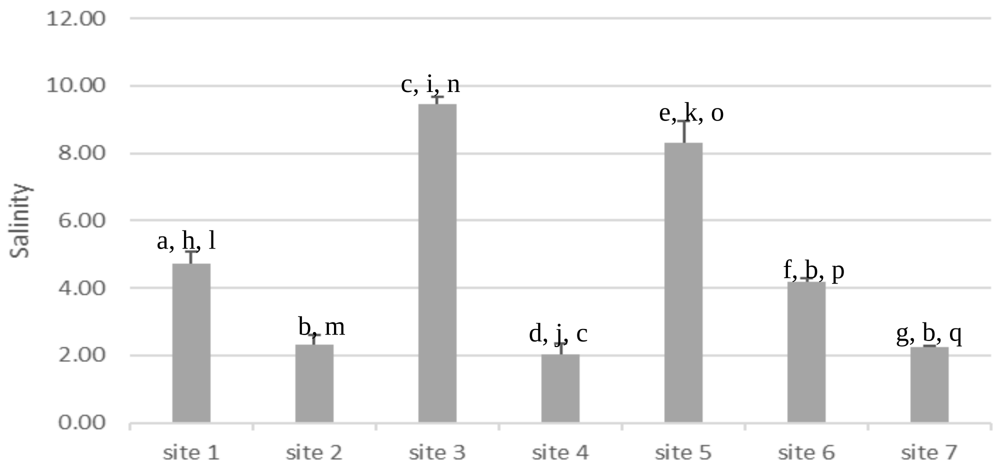

Figure 10 - Mean salinity values (± SD; n=3) of sampling sites. Different letters represent statistically significant differences (P<0.05) between the means.

In conductivity (Figure 11), a statistical difference was found between the seven sites (F= 140.71, P< 0.001). Sites 3 and 5 had the highest values and sites 2, 4 and 7 have the lowest.

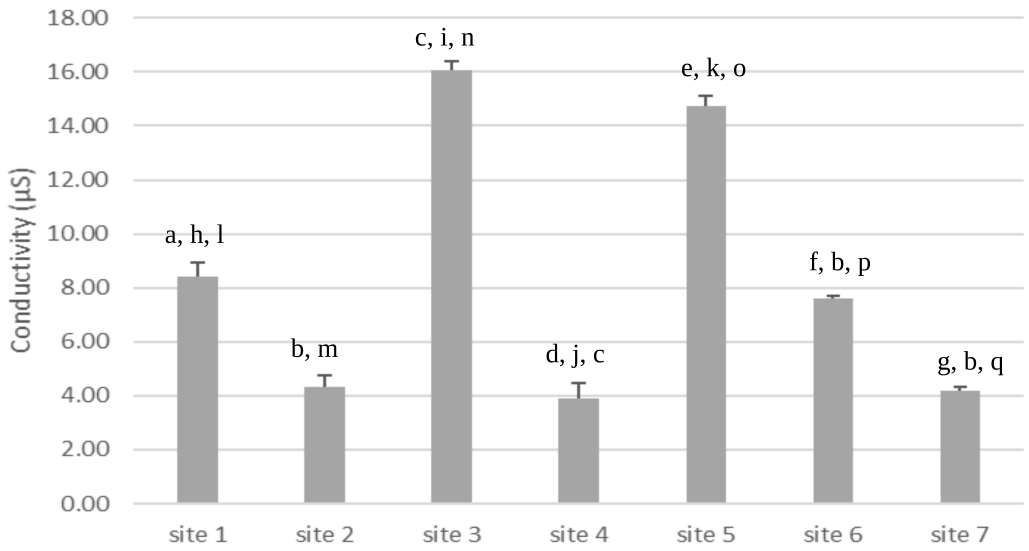

Figure 11- Mean conductivity values (± SD; n=3) of sampling sites. Different letters represent statistically significant differences (P<0.05) between the means.

In Figure 12 is shown the pH values at the seven sampled sites. There was a statistical difference between them (F= 78.06, P< 0.001). The sites had all similar pH values, except for site 4 (3.53).

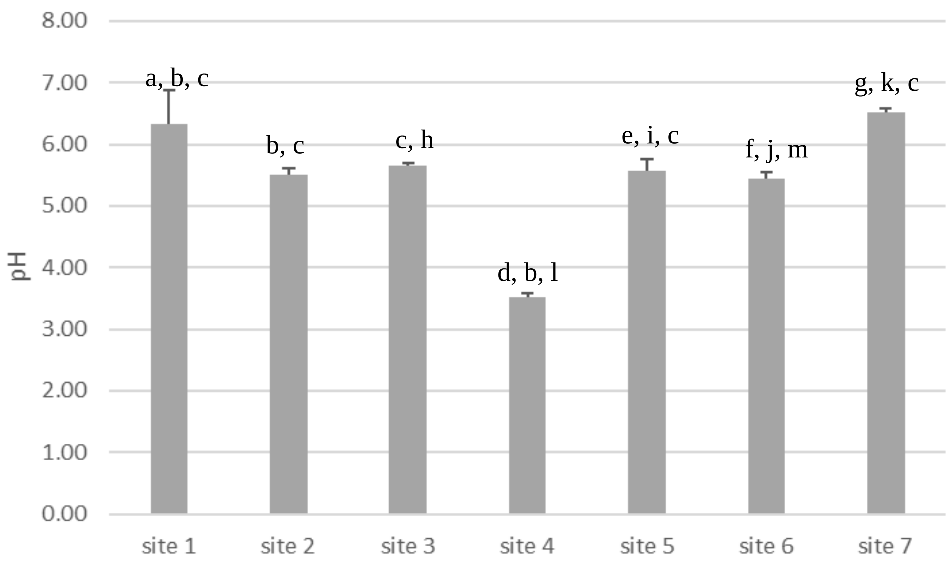

Figure 12 – Mean pH values (± SD; n=3) of sampling sites. Different letters represent statistically significant differences between the means recorded in the different sampling plots.

There were no significant differences between organic matter (F=3.49, P= 0.055). content between the sites (Figure 13).

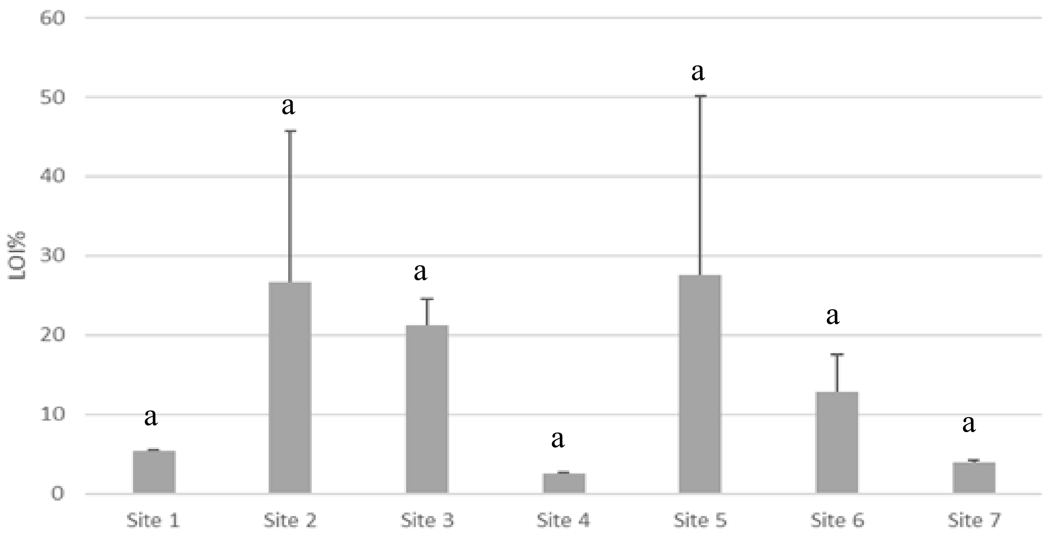

Figure 13 - Mean organic matter content (± SD; n=3) of sampling sites determined by Loss on ignition (LOI (%). Different letters represent statistically significant differences between the means.

3.3 Principal Components Analyses

In figure 14, the two-axis explain 99.62% of the total variability, with the first axis (PC1) explaining 99.08%, having a correlation with Salinity and Conductivity. PC2 explained 0.54 % of the observed variability.

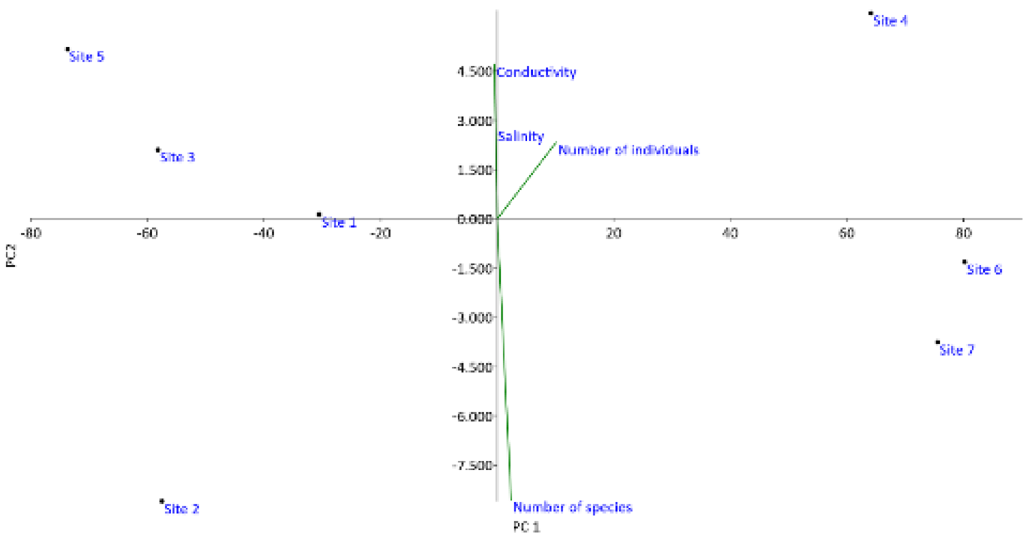

Figure 14 - Principal Components Analyses (PCA) showing the variation of each site variables are salinity, number of species, number of individuals, conductivity. PC1 explaining 99.08%, PC2 explains 0.54%, for a total of 99.08%.

4. Discussion

The objective of this study was to characterize the entomofauna in Ria de Aveiro saltmarshes. This is a relevant study since these ecotones face several threats, including their complete loss due to sea-level rise. A total of 187 species were identified. Several species remained to be identified mostly due to the lack of information and dichotomic keys, and to the fact that the existing ones are outdated or do not include information on the insect diversity of Portugal. This was more frequently for Hymenoptera order, more specifically in the suborder Apocrita-Parasitica and in some less abundant Diptera families.

The insect communities found in Ria de Aveiro varied along with the sites, but always with a predominance of the Diptera order, more specifically the species *Machaerium maritimae,* whose lipid profile from flies collected at different sites in Ria de Aveiro was recently characterized (Duarte et al., 2021). In site 1, Hemiptera order was the most abundant, with a predominance of the *Prokelisia marginata* in this site, most likely due to the high abundance of plant host *Spartina maritima*. On site 6, there was the highest abundance for the main orders, such as Diptera, Hemiptera and Coleoptera. One of the reasons for this may be because of the diversity of plants found here (Harvey et al., 2010). Comparing the floristic diversity, on sites 1, 2, 3, 4, 5 it is possible to see that *J. maritimus* and *H. portulacoides* are the most abundant in these sites, occurring also in less amount, *S. patens, S. maritima* and *Limonium vulgare*. However, in sites 6 and 7 it is possible to see less salinity tolerant species, such as *Tamarix africana* on site 6, and the *Atriplex patula* on site 7. With this addition, we see an increased abundance in these sites. One of the possible reasons for this is that sites experiencing periodical flooding, end up controlling the salinity, and determine the plants species occurring here One of the most important results from this thesis are the addition of 16 new Ephydridae (Diptera) species from Portugal contributing to enlarge the knowledge regarding the distribution of these species. Like many other Diptera families, Ephydridae remains a poorly studied group in Portugal, mainly due to the lack of expertise in this family.

Another important contribution of this thesis was the record of *Teratocoris antennatus* (Hemiptera: Miridae), which was found on site 3, on *J. maritimus*, being one of its host plants (Dioli and Salvetti *et al*., 2016). This species is strongly correlated to wetlands and can be extremely rare it is included in the “Red Lists” of Germany and Austria due to the loss of wetland areas and their associated vegetation in these countries (Rabitsch, 2012).

Over these collections, more exactly on site 2 on *Halimione portucaloides,* some non-native species were collected. One of these species was the invasive ant species *Linepithema humile* (Mayr, 1868) (Hymenoptera, Formicidae) native to South America. This species is known to have negative effects on native ants species, other invertebrates, vertebrates, and plants (Roura-Pascual *et al*., 2004). Carpintero (2005) studied the impact of the *L. humile* on an arboreal ant community and observed that it harmed the native ants, suggesting that there was a possibility that the *L. humile* could lead the native ants to extinction, probably due to its superior mobility and recruitment ability, as well as its aggressiveness and omnivore diet (Touyama *et al*., 2003).

Another non-native species collected was *Prokelisia marginata*, native from North America having *Spartina alterniflora* (Loisel).as its typical hostplant in its native range, but in Europe, it can be found on *Spartina anglica* (C.E. Hubb.). This species can also be found on *Spartina maritima* (Ouvrard & Soulier-Perkins, 2012), which is a highly abundant pioneer plant species in Ria de Aveiro saltmarshes. Some authors refer that *P. marginata* can have important negative impacts on this plant species (Seljak, 2004; Harkin, 2016; Endrestol & Almedal, 2019). This planthopper is also referred to have negative effects on *Spartina patens* (Harkin & Stewart, 2020), an invasive plant species in several European saltmarshes including Ria de Aveiro, where it has spread in the last few years (Ameixa, O. personal communication). *P. marginata* may represent in this way an opportunity for S. *patens* biological control. Although there are no studies available on its impact on other saltmarshes plants (exception for *Spartina maritima*), in this study, this species was collected in *Atriplex patula, Limonium vulgare, Spartina maritima* and *Spartina patens*.

Insect communities varied among the sites. However, for most sites, Diptera was the dominant order, exceptions occurred in site 4 in which the Hemiptera order dominated, followed by Coleoptera and Hymenoptera. There was an increase in species abundance in sites 6 and 7, probably due to a more rich floristic composition (Harvey *et al*., 2010), another reason may be the presence of invasive plants since they can change the diversity and their abundance (Adam *et al*., 2002; Wu *et al*., 2009). Borer (2012) also report that if there is an unbalance from extinction or invasion due to the presence of other plant species, this can affect biomass and energy flux to control diversity, production and stability of both plant and consumer communities. The presence of *Spartina patens* in some locations (sites 1, 2, 3 and 6), can potentially induce a decrease in the biodiversity in these sites. Sites 1 to 5 are influenced by flooding, which limits the plant species which may thrive in these conditions. More inland sites 6 and 7, are not affected by flooding, for this reason, have more heterogenic vegetation (Moreira *et al*., 2016).

The species accumulation curves show that the sampling effort was not sufficient to reach a plateau, meaning that it would be necessary to increase the effort to unravel all the insect diversity in Ria de Aveiro, but it is also possible to observe that, sites 4, 5, 6 and 7 are, apparently close to reaching a stable asymptote. Comparing the accumulation curve from all the samples from each site with the diversity estimators is possible to see that Chaos 2 reached a plateau at 238 species, and Jack-Knife 2 was also close to reaching a plateau (271). This means that both these estimators are correlated with the number of species that are found in only one sample (uniques) or in two samples (duplicates) (Colwell & Coddington *et al*, 1994; Gotelli & Colwell *et al*, 2011; Yurkov *et al*., 2011; Paller *et al*., 2018).

Regarding salinity data, sites 3 and 5 had the higher values, which may be because both these sites have inconstant flooding regimes since they are in more elevated locations, thus being exposed to dryer conditions (Dítě *et al*., 2019), having a higher salt concentration. Lower salinities were found in sites 1, 2 and 4, which are subjected to frequent flooding. According to Adam (1990), sites in constant periodical flooding can maintain lower salinities.

Watson (2016), found that the lower areas of the saltmarshes had a higher concentration of salts which led to a lower percentage of organic matter in the soils, which corresponds to the results of organic matter obtained in this study (except for site 2). The reason attributed to this phenomenon is the tidal regime, which decreases the organic matter but increases the sulphide and salinity. More inland and elevated sites (6 and 7), can also have a freshwater input, thus preventing the accumulation of salts being less affected by tidal flooding (Thibodeau *et al*., 1998).

The conductivity decreased with the increase of saltmarsh elevation (Vallés *et al*., 2015; Contreras-Cruzado *et al*., 2017), which corresponds with values recorded in sites 6 and 7. Sites 1, 2, and 4 showed the lowest values, probably since they were able to maintain a low salinity level since these two abiotic conditions are correlated. Sites 3 and 5, are more elevated and exposed to dryer conditions, which may be due to higher evapotranspiration (Sánchez *et al*., 1998).

The pH values, surpass the value of 4 in most sites, contributing to the increase in the capability of saltmarshes to support halophyte plants since the acidic soils tend to have low levels of the necessary nutrients for plant survival (Craft *et al*., 1988; Hikouei *et al*., 2021). Some of these nutrients originate from the organic matter brought by the tides, influencing the livelihood of the plants in the saltmarshes. There was also a negative correlation between salinity and organic matter, which decreases the pH values, by interfering with the denitrification process (Zhou *et al*., 2017). A sites 3 and 5, were recorded the highest values of salinity were recorded, and the reasons may be due to various environmental factors, such as the dilution of salts by rainfall, or the salinity increase due to high temperatures (Araoye *et al*., 2009; Contreras-Cruzado *et al*., 2017). For sites 6 and 7, the pH values were below 7, however, according to Vallés (2015), these values should have been alkaline, which may be because organic matter negatively interferes with the pH values or any other environmental factor. On site 4 we can see that the amount of organic matter was very low (2.5), probably due to the acidity input from organic matter decomposition (Araoye *et al*., 2009; Contreras-Cruzado *et al*., 2017).

In the PCA, salinity and conductivity are correlated with the number of individuals, which may explain, why site 5 had such low diversity. Additionally, salinity and conductivity were negatively correlated with the number of species, explaining the higher diversity found in site 4. However, the number of species and number of individuals do not correlate. In sites 6 and 7, it is possible to observe that, the higher the number of individuals, the lower the abundance on the site, whereas on sites that are under periodical flooding their abundance, was lower.

5. Conclusion

Abiotic factors and flora zonation contributed to the heterogeneity of habitats which influence the abundance and diversity of insects, and further studies need to be done to understand and better protect the entomofauna living in these wetlands. Despite the difficulties with the identification of the insect species, this study contributed to the uncovered 17 new species for Portuguese insect fauna. To improve our knowledge of the existing entomofauna, and to protect this fragile ecosystem, it is necessary a better support of taxonomist expertise.

This study took a single snapshot of insect communities in Ria de Aveiro saltmarshes, to have a full knowledge of these communities it would be necessary to collect more samples throughout the year or at least at the end of spring/beginning of summer when several insect species are more active.

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Annex I

Table 1 - Systematic list of all the collected specimens, number of individuals and their relative abundance at each site in Ria de Aveiro

Orders	Families	Species	Sites collected	Abundance	Abundance
Diptera	Asteiidae	Sp. 1	2, 5	6	0.21
Diptera	Agromyzidae	Sp. 2	6, 7	3	0.11
Diptera	Anthomyzidae	Sp. 3	1, 3, 4, 6	15	0.53
Diptera	Canacidae	Sp. 4	4	4	0.14
Diptera	Ceratopogonidae	Sp. 5	1, 2	2	0.07
Diptera	Chamaemyiidae	Sp. 6	4	1	0.04
Diptera	Chironomidae	Sp. 7	1, 4, 5, 7	11	0.39
Diptera	Chloropidae	Sp. 9	6, 7	11	0.39
Diptera	Chloropidae	Sp. 10	All sites	247	8.77
Diptera	Chloropidae	Sp. 11	1, 2, 3, 5, 6, 7	42	1.49
Diptera	Chloropidae	Sp. 12	3, 5, 6, 7	62	2.20
Diptera	Chloropidae	Sp. 13	7	1	0.04
Diptera	Chyromyidae	Sp. 14	2, 6, 7	50	1.78
Diptera	Culicidae	Sp. 15	1, 6, 7,	14	0.50
Diptera	Dolichopodidae	Dolichopus diadema	3	1	0.04
Diptera	Dolichopodidae	Aphrosylus mitis	4	1	0.04
Diptera	Dolichopodidae	Dolichopus nubilus	7	2	0.07
Diptera	Dolichopodidae	Hydrophorus oceanus	1, 2, 4, 5	31	1.10
Diptera	Dolichopodidae	Machaerium maritimae	All sites	174	6.18
Diptera	Dolichopodidae	Medetera nr flavipes	2	3	0.11
Diptera	Dolichopodidae	Micromorphus nr albipes	1, 4	3	0.11
Diptera	Dolichopodidae	Muscidideicus praetextatus	4	1	0.04
Diptera	Dolichopodidae	Thinophilus flavipalpis	3, 6, 7	6	0.21
Diptera	Drosophilidae	Sp. 16	3	1	0.04
Diptera	Ephydridae	Psilopa sp.	1, 2, 3, 4, 6, 7	54	1.92
Diptera	Ephydridae	Atissa	7	1	0.04
Diptera	Ephydridae	Clanoneurum cimiciforme	2, 3, 6, 7	14	0.50
Diptera	Ephydridae	Diasemocera maritima	1, 2, 4	128	4.55
Diptera	Ephydridae	Diasemocera spac. aff fratella	5	1	0.04
Diptera	Ephydridae	Diasemocera glabricula	7	18	0.64
Diptera	Ephydridae	Diasemocera nana	7	19	0.67
Diptera	Ephydridae	Glenanthe ripicola	2	9	0.32
Diptera	Ephydridae	Glenanthe sp.	4	3	0.11
Diptera	Ephydridae	Philothelma defectum	7	2	0.07
Diptera	Ephydridae	Psilopa compta	2	1	0.04
Diptera	Ephydridae	Psilopa nitidula	1, 4, 6	12	0.43
Diptera	Ephydridae	Psilopa obscuripes	4, 6	3	0.11
Diptera	Ephydridae	Psilopa rutilans	1, 2, 6, 7	15	0.53
Diptera	Ephydridae	Psilopa thora	1, 2, 4	5	0.18
Diptera	Ephydridae	Scatella ciliata	7	1	0.04
Diptera	Ephydridae	Schema acrosticale	6	1	0.04
Diptera	Hybotidae	Sp. 17	6	1	0.04
Diptera	Hybotidae	Sp. 18	6	7	0.25
Diptera	Lauxaniidae	Sp. 19	2, 6	7	0.25
Diptera	Lauxaniidae	Sp. 20	6	3	0.11
Diptera	Muscidae	Sp. 21	1, 3, 4, 6	20	0.71
Diptera	Muscidae	Sp. 22	1	1	0.04
Diptera	Nematocera	Sp. 23	1, 2, 4, 6, 7	12	0.43
Diptera	Phoridae	Sp. 24	6, 7	9	0.32
Diptera	Rhinophoridae	Sp. 25	6, 7	10	0.36
Diptera	Rhinophoridae	Sp. 26	7	1	0.04
Diptera	Scatophagidae	Sp. 27	1	1	0.04
Diptera	Scatophagidae	Sp. 28	1, 2, 3, 4, 5	28	0.99
Diptera	Scatophagidae	Sp. 29	1, 4	6	0.21
Diptera	Sciaridae	Sp. 30	2, 6	3	0.11
Diptera	Sepsidae	Sp. 31	1, 6	3	0.11
Diptera	Sepsidae	Sp. 32	3, 6	3	0.11
Diptera	Sphaeroceridae	Sp. 33	3, 4, 6, 7	28	0.99
Diptera	Sphaeroceridae	Sp. 34	4, 6	2	0.07
Diptera	Sphaeroceridae	Sp. 35	All sites	104	3.69
Diptera	Tephritidae	Sp. 36	6, 7	2	0.07
Aranaee	Thephritidae	Sp. 37	1, 2, 7	8	0.28
Aranaee	Ulidiidae	Sp. 38	3, 6, 7	15	0.53
Aranaee	Araneidae	Sp. 39	1, 2	3	0.11
Aranaee	Araneidae	Cyclosa sp.	6	8	0.28
Aranaee	Araneidae	Larinioides	1, 3	2	0.07
Aranaee	Araneidae	Larinioides cornutus	1, 5	4	0.14
Aranaee	Araneidae	sp. 40	2, 3, 6	10	0.36
Aranaee	Dyctinidae	Lathys dentichelis	4	1	0.04
Aranaee	Salticidae	Ballus sp.	6, 7	6	0.21
Aranaee	Salticidae	Heliophanus cupreus	2, 4	2	0.07
Aranaee	Salticidae	Heliophanus sp.	2	1	0.04
Aranaee	Salticidae	Neon sp.	6, 7	2	0.07
Aranaee	Salticidae	Sitticus sp.	7	1	0.04
Aranaee	Salticidae	sp. 50	7	1	0.04
Aranaee	Tetragnatidae	Tetragnata extensa	All sites	93	3.30
Aranaee	Tetragnathidae	Tetragnata montana	1, 2, 3, 4	10	0.36
Aranaee	Tetragnathidae	Tetragnatha sp.	2, 3, 6, 7	11	0.39
Aranaee	Thomisidae	Xysticus cristatus	6	4	0.14
Aranaee	Thomisidae	Runcinia grammica	3, 6, 7	17	0.60
Aranaee	Thomisidae	sp 51	6	1	0.04
Hemiptera	Thomisidae	Xysticus	3, 6, 7	8	0.28
Hemiptera		sp. 52	4	1	0.04
Hemiptera	Aphididae	Staticobium staticis	5, 7	43	1.53
Hemiptera	Cicadellidae	Conosanus obsuletus	1, 6, 7	36	1.28
Hemiptera	Cicadellidae	Zyginidia sp.	4, 6, 7	56	1.99
Hemiptera	Cicadellidae	Macrosteles horvati	4, 5, 6, 7	5	0.18
Hemiptera	Cicadellidae	Opsius stactogalus	6	25	0.89
Hemiptera	Cixiidae	Oliarus liquidus	2, 3, 4, 6, 7	18	0.64
Hemiptera	Delphacidae	Megamelodes quadrimaculatus	1	1	0.04
Hemiptera	Delphacidae	Stenocranus sp.	1	1	0.04

Hemiptera	Delphacidae	*Prokelisia marginata*	1, 2, 4, 6, 7	250	8.88
Hemiptera	Delphacidae	*Javesella pellucida*	7	1	0.04
Hemiptera	Geocoridae	*Geocoris lineola*	6	1	0.04
Hemiptera	Lygaeidae	*Cymis caviculos*	1, 3, 6, 7	30	1.07
Hemiptera	Lygaeidae	*Nysius sp.*	4, 6, 7	10	0.36
Hemiptera	Lygaeidae	*Ischnodenus sp.*	7	5	0.18
Hemiptera	Miridae	*Orthotylus sp.*	All sites	99	3.52
Hemiptera	Miridae	*sp. 53*	3	1	0.04
Hemiptera	Miridae	*Tuponia sp.*	4, 5	3	0.11
Hemiptera	Miridae	*Teratocoris antennatus*	1	2	0.07
Hemiptera	Miridae	*Trigonotylus caelutialium*	1	1	0.04
Hemiptera	Miridae	*Lygus wagneri*	7	1	0.04
Hemiptera	Nabidae	*Nabis provencalis*	1, 5, 7	14	0.50
Hemiptera	Nabidae	*Nabis pseudoferus*	6	3	0.11
Hemiptera	Nabidae	*Nabis sp.*	1, 3, 4, 5, 7	74	2.63
Hemiptera	Pentatomidae	*Eysarcoris ventralis*	6	2	0.07
Hemiptera	Pentatomidae	*Aelia acuminata*	2, 6, 7	5	0.18
Hemiptera	Pentatomidae	*Geotomus punctalatus*	7	2	0.07
Hemiptera	Pentatomidae	*Neotiglossa sp.*	2, 7	2	0.07
Hemiptera	Pentatomidae	*Neotiglossa lineolata*	2, 6, 7	10	0.36
Hemiptera	Psyllidae	*Colposcenia aliena*	6	1	0.04
Hemiptera	Rhopalidae	*Stictopleurus punctatonervoso*	7	10	0.36
Hemiptera	Saldidae	*Saldula sp.*	1, 2, 4, 6	45	1.60
Coleoptera	Anthiciidae	*Cyclodinus coniceps*	3, 6, 7	18	0.64
Coleoptera	Apionidae	*Pseudaplemonus limonii*	6	1	0.04
Coleoptera	Athicidae	*Cyclodinus sp.*	6	1	0.04
Coleoptera	Carabidae	*larva*	7	1	0.04
Coleoptera	Carabidae	*Harpalinae sp.*	2	1	0.04
Coleoptera	Carabidae	*Cymindis macularis*	2, 3,	10	0.36
Coleoptera	Carabidae	*Paradromius linearis*	6, 7	2	0.07
Coleoptera	Cerambicidae	*Corymbia macularis*	6	3	0.11
Coleoptera	Cerambycidae	*Scticoleptura sp.*	2	1	0.04
Coleoptera	Chrysomelidae	*Longitarsus brunneu*	6	4	0.14
Coleoptera	Chrysomelidae	*Neocrepidodera impressa*	1, 7	4	0.14
Coleoptera	Chrysomelidae	*Chaetocnema pelagica*	6	90	3.20
Coleoptera	Chrysomelidae	*Chaetocnema depressa*	7	1	0.04
Coleoptera	Chrysomelidae	*Chaetocnema obesa*	2	1	0.04
Coleoptera	Chrysomelidae	*Bruchos loti*	6	1	0.04
Coleoptera	Coccinelidae	*Scymnus*	2	4	0.14
Coleoptera	Coccinellidae	*Sthethorus punctilum*	1	1	0.04
Coleoptera	Coccinellidae	*Novius cardinalis*	1	1	0.04
Coleoptera	Coccinellidae	*Larva*	6	2	0.07
Coleoptera	Coccinellidae	*Thythaspis sedectimpunctata*	2, 6, 7	58	2.06
Coleoptera	Latridiidae	*Corticaria sp.*	3	1	0.04
Coleoptera	Latriidae	*Melanophathalma sp.*	1	1	0.04
Coleoptera	Latriidae	*Melanophathalma sp.*	1	1	0.04
Coleoptera	Latridiidae	*Melanophthalma suturalis*	5	2	0.07

Coleoptera	Malachiidae	*Colotes macularis*	1, 2, 3, 6, 7	83	2.95
Coleoptera	Phalacridae	*Olibrus liquidus*	3, 6	3	0.11
Coleoptera	Staphylinidae	*Aleochara*	6	1	0.04
Hymenoptera	Bethylidae	*sp. 54*	6	1	0.04
Hymenoptera	Braconidae	*Aleiodes sp.*	6	1	0.04
Hymenoptera	Braconidae	*sp, 55*	2, 4, 6, 7	12	0.43
Hymenoptera	Braconidae	*sp, 56*	4	1	0.04
Hymenoptera	Cynipidae	*sp, 57*	4	1	0.04
Hymenoptera	Cynipidae	*sp, 58*	1, 4, 7	11	0.39
Hymenoptera	Diapridae	*sp, 59*	4, 6	4	0.14
Hymenoptera	Ecoilidae	*sp, 60*	3, 4, 6	3	0.11
Hymenoptera	Eulophidae	*Euderus sp.*	1, 4, 5, 6, 7	19	0.67
Hymenoptera	Eulophidae	*Hemiptarsenus*	6	1	0.04
Hymenoptera	Eulophidae	*sp. 61*	1, 5, 6, 7	11	0.39
Hymenoptera	Formicidae	*Lasius sp.*	2	14	0.50
Hymenoptera	Formicidae	*Linepithema humile*	2	19	0.67
Hymenoptera	Formicidae	*Monomorium sp.*	6, 7	27	0.96
Hymenoptera	Formicidae	*Lasius niger*	6	4	0.14
Hymenoptera	Formicidae	*Myrmica sabuleti*	7	7	0.25
Hymenoptera	Formicidae	*Tapinona erraticum*	7	1	0.04
Hymenoptera	Halictidae	*Lasioglossum sp.*	5, 7	2	0.07
Hymenoptera	Ichneumonidae	*Neorhacodes sp.*	2, 3	3	0.11
Hymenoptera	Ichneumonidae	*sp. 62*	1, 4, 5, 6, 7	20	0.71
Hymenoptera	Mymaridae	*sp. 63*	1, 2, 4, 5, 6, 7	12	0.43
Hymenoptera	Mymaridae	*sp. 64*	6	1	0.04
Hymenoptera	Pteromalidae	*sp. 65*	All sites	35	1.24
Hymenoptera	Pteromalidae	*sp. 66*	4, 6	4	0.14
Hymenoptera	Pteromalidae	*sp. 67*	1, 4, 6	4	0.14
Hymenoptera	Pteromalidae	*sp. 68*	2, 4, 5, 7	5	0.18
Hymenoptera	Pteromalidae	*sp. 69*	4	2	0.07
Hymenoptera	Pteromalidae	*sp. 70*	4	2	0.07
Hymenoptera	Scelionidae	*sp. 71*	6	2	0.07
Hymenoptera	Scelionidae	*sp. 72*	6	1	0.04
Hymenoptera	Vespidae	*sp. 73*	6	1	0.04
Hymenoptera		*sp. 74*	6	1	0.04
Hymenoptera		*sp. 75*	6	1	0.04
Orthoptera	Acrididae	*Locusta migratoria*	1, 6	2	0.07
Orthoptera	Acrididae	*Locusta sp.*	6	1	0.04
Orthoptera	Conocephalidae	*Conocephalus fuscos*	6	1	0.04
Orthoptera	Gryllidae	*Trigonilium cicindeloides*	6, 7	12	0.43
Orthoptera		*sp. 76*	3, 6	3	0.11
Thysanoptera	Phlaeotripidae	*Haplotrips juncorum*	1, 2, 3, 6, 7	87	3.09
Psocoptera	Caeciliusidae	*Valenzuela corsicus*	1, 2, 3, 6, 7	45	1.60
Lepidoptera	Erebidae	*Eilema sp.*	1, 2, 5, 7	5	0.18
Lepidoptera	Larva	*Sp. 77*	2, 6	3	0.11
Neuroptera	Chrysomelidae	*Chrysoperla sp.*	1, 3, 4	4	0.14
Collembola	Neamuridae	*Anurida maritima*	4	4	0.14
Total		*187 species*		2816	100

Annex II

Poster presented at the XIX Iberian Congress of Entomology, held in Coimbra on 21st September 2021.

Characterization of insect communities in Ria de Aveiro saltmarshes

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Abstract

Saltmarshes are considered one of the most productive habitats in the world and exhibit a spatial zonation of its vegetation, from the intertidal flats to the seawall or dunes providing habitats to specific invertebrate assemblage adapted to regular submergence by seawater, and the resulting high soil salinity. These ecotones are essential to support coastal food webs and in providing nursery areas, for fish, crustaceans, and birds. Ria de Aveiro costal lagoon has been the focus of considerable research, but despite being part of Nature 2000 network, and being a Long-Term Ecological Research (LTER) site, insects have been systematically neglected. This is a major gap since this taxon provides important ecosystem services. In this work our aim was to characterize Ria de Aveiro saltmarsh insect communities to better understand how these are organized in these ecotones. To achieve this goal, insects were collected by sweep-netting the dominating halophyte vegetation in September 2020, in seven locations along Ria de Aveiro saltmarshes areas and later identified to the maximum possible level of taxonomic resolution. A total of 2816 individuals belonging to 11 orders and 80 families were identified so far. The most abundant orders were Diptera (with Chloropidae, Ephydridae and Dolichopodidae being the most abundant families) and Hemiptera (with Delphacidae and Miridae). Saltmarshes are especially vulnerable areas and currently face several environmental problems and threats, such as climate change and pollution. For this reason, is urgent to identify and unravel the role of insects in this ecosystem.

Introduction

Saltmarshes are wetlands mostly occurring in intertidal areas, in transitional areas of marine and terrestrial environments, connecting saline to freshwater ecosystems, being periodically flooded by saline or brackish water. Vegetation cover in these areas are have the ability to tolerate a wide range of salinities and periodic flooding (Adam 1993). Saltmarshes face many threats, either from natural or anthropogenic origin, and are among the fastest disappearing ecosystems worldwide (Bridgham et al. 2006). The vegetated saltmarshes, its mudflats, and saltpans provide a diverse range of habitats for several organisms, including insects, the most abundant and diverse animal group (Ameixa et al., 2020). For this reason, such threats can cascade into plant and animal diversity that depend on these ecosystems and their provided ecosystem services (Ameixa et al., 2018). Despite its importance, the knowledge regarding insect communities in saltmarshes is limited. This work aims to contribute to the knowledge regarding insect communities in Ria de Aveiro, Portugal saltmarsh areas.

Figure 1 – A) Studied area; B) Sampled sites: 1- Foz do Rio Novo (40° 40'50.8"N 8° 40'18.6"W), dominated by *Spartina maritima* (Curtis) Fernald; 2- Salinas (40° 40'35.0"N 8° 40'20.0"W), dominated by *H. portucaloides*, *J. maritimus* and *Sarcocornia perennis* (Mill.) A.J. Scott, *Phragmites australis* (Cav.) Trin ex.Steud.; 3- Bico (40° 43'33.0"N 8° 38'46.3"W) dominated by *Halimione portucaloides* L. and *Juncus maritimus* L.; 4- Reboxo (40° 39'42.9"N 8° 41'39.9"W) dominated by *H portucaloides*, *S. perennis*, *S. maritima* and *Limonium vulgare* Mill.; *5- Farinha (40° 42'19.3'N 8° 40'33.6'W), dominated by J. maritimus* and *H. portucaloides;* 6- Cambeia (40° 44'08.0"N 8° 37'40.7"W) dominated by *H. portucaloides*, *Salicornia ramosissima* J. Woods, and *Tamarix africana* Poir.; 7- Cacia (40° 41'44.2"N 8° 37'31.4"W) dominated by *J. maritimus*, *H. portucaloides* and *Atriplex patula* L. Map was generated using Google maps.

Material and methods

Insects were collected in September 2020 by sweeping the vegetation cover, dominated by salt tolerant plants, in 7 selected sites (see Figure 1). Our sample effort was 300 sweeps/host plant and the collected specimens were stored at -20° C until identification. The insects were identified as morphospecies using dichotomous keys, and these identifications were complemented with specialist discussion forums whenever keys were not available.

The data analysis, were carried on Microsoft Excel (2013), EstimateS, Version 9.1.0, Copyright R. K. Colwell. The insect communities collected in plant species were characterized by their abundance, number of species and diversity indexes such as, Shannon-Wiener (H'), Shannon's evenness (E) and Sørensen-Dice. Species richness was estimated with : i) Jack Knife 1, which estimates the total richness using the exact number of just one sample (uniques); ii) Jack Knife 2 which uses the uniques and the number of individuals occurring in the two samples (duplicates); and iii) Chaos 1 and 2 which estimate total richness using the number of species with just one individual (singletons) and the number of species with just two individuals (doubletons).

Results

We collected a total of 2816 individuals from 187 species, distributed by 11 insect orders. The most common species found in these habitats were *Machaerium maritimae* (Haliday, 1832) (Diptera, Dolichopodidae) and the non native *Prokelisia marginata* (Van Duzee, 1897) (Hemiptera, Delphacidae).

Table 1- Characterization of insect communities collected in different sampling sites and sediment salinities (Average±SD, n=3.

	Abundance	Nº of species	H'	E	Salinity
Site 1	283	57	4.25	1.79	4.73
Site 2	201	53	3.52	1.50	2.33
Site 3	204	42	5.97	2.93	9.76
Site 4	562	54	6.65	2.44	2.03
Site 5	162	26	4.72	2.61	8.30
Site 6	807	101	5.4	1.81	2.23
Site 7	501	79	3.2	1.07	4.21

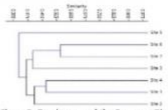

Figure 2- Dendrogram of the Sørensen-Dice index showing the similarities in species composition recorded on the different study sites

Despite the abundance and number of species being higher in site 6, the diversity index H' was higher in sites 4 and 3 (Table 1), however, the communities were more even in sites 3 and 4. Similarities in species composition recorded in the different sites is shown in Figure 2. There are two main clusters one with sites 3, 7 and 6 and another cluster with sites 4, 1 and 2. Site 5 differ the most from the other studied sites. Salinity, was higher in sites 3 and 5 (Table 1) and relatively similar in sites 2, 4 and 6. Estimates of total species number (Figure 3) showed that our observed richness values likely underestimated total richness for many groups.

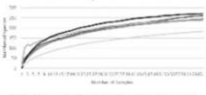

Figure 3 - Diversity estimator curves, obtained by data randomization (100 times). S(est) is the observed number of species. (EstimateS, Version 9.1.0, Copyright R. K. Colwell).

Discussion and Conclusions

- Most of the collected insects were identified to species, except Hymenoptera order and some less abundant Diptera families mostly due to the lack of dichotomous keys.
- We report the presence of *Prokelisia marginata,* a non-native planthopper introduced in Europe that can potentially negatively affect native saltmarsh vegetation.
- Species richness and abundance were higher in site 6, where the vegetation is not affected by flooding, however, H' was higher in site 4, despite the communities being more even (E) in site 3, both typical tidal saltmarsh areas.
- Sites 3 and 5 are typical low saltmarsh areas had the higher salinities, probably due to everyday tidal flow (Adam, 1990).
- Diversity estimator curves, suggest that species richness estimators sensitive to uniques and duplicates can be more adequate for the analysis of communities with uneven structure (Yurkov, Kemler and Begerow, 2011) as the ones found in heterogenic habitats, such as saltmarshes.
- To unravel how insect communities are organized we need to increase our sampling effort. Also, it will be necessary to integrate other taxonomic experts in the identification of more cryptic groups, which should increase the number of species identified in this habitat.

Acknowledgements

Thanks are due to CESAM and FCT/MCTES for the financial support to (UIDP/50017/2020+UIDB/50017/2020), through national funds and to the project SUShI - SUStainable use of Insect protein in aquaculture feed (CENTRO-01-0145-FEDER-030818) funded by FEDER, through COMPETE 2020 – Programa Operacional de Competitividade e Internacionalização (POCI), and by national funds (OE), through FCT/MCTES. O.M.C.C.A. is funded by national funds (OE), through FCT, in the scope of the framework contract foreseen in the numbers 4, 5 and 6 of the article 23, of the Decree-Law 57/2016, of August 29, changed by Law 57/2017, of July 19.

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