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Structural and functional responses of macrobenthic communities to climate variability: a comparison between distinct climate scenarios

Anthony Martins de Abreu

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Dissertação apresentada à Universidade de Coimbra para cumprimento dos requisitos necessários à obtenção do grau de Mestre em Ecologia, realizada sob a orientação científica do Professor Doutor Miguel Pardal (Universidade de Coimbra) e da Doutora Patrícia Cardoso (investigadora auxiliar IMAR-CMA)

Anthony Martins de Abreu

2013

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Resumo

Os ecossistemas estuarinos, à semelhança de muitos outros a nível mundial, estão sujeitos a constantes e elevadas pressões, não só antropogénicas, mas também de ordem climática. Estas reflectem-se de duas formas distintas: através de eventos extremos, tais como cheias, secas e ondas de calor, ou através de variabilidade climática, traduzida em anos chuvosos ou secos. Os eventos extremos caracterizam-se pela sua elevada intensidade e curta duração, enquanto a variabilidade climática reflecte eventos de baixa intensidade, mas com uma duração mais prolongada.

As comunidades macrobentónicas são muito importantes devido à sua relevância na cadeia alimentar dos ecossistemas estuarinos e também por serem considerados como bioindicadores. De forma a uma melhor compreensão de como estas comunidades são afectadas pela variabilidade climática, foram realizadas diferentes análises (diversidade de espécies, diversidade funcional e produção secundária). Foram abordadas duas questões no estudo realizado: Será que a estrutura da comunidade macrobentónica varia em relação às diferentes condições climáticas (anos normais, chuvosos e secos)? Será que ocorrem mudanças nos atributos biológicos das comunidades para diferentes habitats e anos?

Para a realização do estudo foram escolhidos dois locais no estuário do Mondego, com características diferentes (pradarias de *Zostera noltii* e área arenosa), cujas comunidades macrobentónicas foram comparadas em diversos tipos de anos (normais, chuvosos e secos). Para a análise da diversidade funcional foram considerados quatro atributos biológicos: desenvolvimento larvar, posição bentónica, locomoção e tipo de alimentação.

Os resultados obtidos não demonstraram diferenças significativas na estutura das comunidades em relação aos diferentes tipos de anos para as várias análises desenvolvidas. No entanto, foi possível observar uma clara separação entre os anos normais e os anos com variabilidade climática (chuvosos e secos). Estes últimos apresentaram os valores mais elevados de biodiversidade, densidade dos atributos biológicos e de produtividade, podendo estes resultados serem explicados com base na Hipótese da Perturbação Intermédia. Além disso, também se observou uma clara distinção entre as comunidades macrobentónicas das duas áreas de estudo.

Palavras-chave: Variabilidade climática; Comunidade macrobentónica; atributos biológicos; Hipótese da Perturbação Intermédia.

Abstract

Estuarine ecosystems, like many other habitats worldwide are being frequently subject to high pressures, not only anthropogenic but also by climate change. Climate change is reflected in two different ways through the extreme events such as floods, drought and heat waves which are short but very intensive episodes and through the climate variability corresponding to events of low intensity but of long duration such as rainy or dry years.

Macrobenthic communities are very important due to its high relevance in food webs of estuarine ecosystems being also considered as bio-indicators. In order to better understand how macrobenthic communities would be affected by climate variability, different analyses (i.e. species diversity, functional diversity and secondary production) were performed. Two main questions were addressed in the present work: Does the macrobenthos community structure differ in relation to different years (normal, rainy and dry)? Does community´s wide biological trait shifts occur for different habitats and years?

Two sites were chosen for this study in the Mondego estuary with different characteristics (*Zostera noltii* beds and Sandflat), whose communities were compared for distinct years (normal, rainy and dry). For the functional diversity analysis were considered four biological traits: larval development mode, living position, mobility and feeding guilds.

The results showed no significant differences in community structure between years for the different analyses. But it was possible to observe a clear separation between normal years and those with climate variability (rainy and dry). These “different” years presented the highest values in terms of biodiversity, density of different traits and productivity. These results could be explained based on the Intermediate Disturbance Hypothesis. In addition, a clear distinction between macrobenthic communities of the two areas was reaffirmed.

Keywords: Climate variability; Macrobenthic community; Biological traits; Intermediate Disturbance Hypothesis.

Chapter 1

Introduction

1.1 - Importance of estuarine ecosystems and their main threats

One of the first definitions of estuary was written by Pritchard (1967), which was "an estuary is a semi-enclosed coastal body of water, which has a free connection with the open sea, and within which seawater is measurably diluted with freshwater derived from land drainage". But this definition excludes coastal lagoons and brackish seas. In other words, in a simplified way, estuaries are aquatic systems where occurs the mixing of freshwater from rivers or continental drainage systems with saltwater from the sea. These systems are highly affected by tidal action, thus constituting highly dynamic natural systems regarding their physicochemical, biological, and geological features (McLusky & Elliott, 2002; 2005). All this complexity and interaction between environmental processes and biological components make estuaries the most productive and valuable ecosystems on Earth, along with the tropical rainforest and coral reefs (Costanza et al., 1997; Barbier et al., 2011). This high production and biodiversity is supported by the input of energy from sunlight and high content of organic matter brought by tides, rivers and land adjacent to the estuary (McLusky & Elliott, 2005). These features allow a strong growth and development of primary producers such as phytoplankton and macrophytes (seaweeds, seagrasses and saltmarsh plants). The plants' growth is controlled mainly by the sunlight availability because this is essential in achieving the photosynthesis, and by nutrients availability, especially nitrogen and phosphorus (McLusky & Elliott, 2005; Leston et al., 2008). These primary producers offer ideal conditions for the establishment of a wide range of fauna since they provide protection and are nutrition sources for several species of fishes, waders and macroinvertebrates (including detritivores, herbivores and also omnivores). These conditions make those habitats as nursery areas for larvae and juveniles of many fishes (Martinho et al., 2007; Wasseran & Strydom, 2011; Primo et al., 2012) and invertebrates' species (Cardoso et al., 2010; Dolbeth et al., 2011; Grilo et al., 2011). Lastly, estuaries can attract top predators such as many fishes and birds' species (mainly waders), which can be resident or migratory, making these habitats important stopping points on their migratory routes (Terörde & Turpie, 2013).

All these characteristics make the estuaries very important both economically and ecologically. Economically, from very early, these sites attracted the person's attention due to the fact that they are used as centers of maritime transportation; this fact means that in some estuaries there was a loss of area upper to half of the original because of the construction of ports, industries, houses and the use of land for agriculture (Smith, 2000). More recently, these habitats have been intensively explored for aquaculture, tourism and other industries to our own benefit (McLusky & Elliott, 2005; Forest et al., 2009). Since these activities are developed for human profit, very often, the aquaculture and/or the industrial owners usually take options that are sometimes highly prejudicial to the ecosystem. Due to all of these activities, estuaries are subjected to a great number of anthropogenic impacts, namely contaminants' discharges such as heavy metals, pesticides, excess of nutrients and antibiotics (Birch & Taylor, 1999; Marques et al., 2003; Azevedo et al., 2010). Another activity that is associated to these ecosystems is the fishery that when carried out excessively may endanger the continuity of many fishes or bivalve (e.g. cockles) species and the ecosystem functioning (Baeta et al., 2005; Crespo et al., 2010).

Besides the numerous anthropogenic impacts associated to estuaries, their goods and services are actually endangered by another stress factor, the climate change. In the last decades, the increase of extreme events, both in intensity and frequency associated to the climate variability constitutes a matter of great concern (Constanza et al., 1997).

1.2 - Climate change

Nowadays, climate change is one of the most discussed subjects worldwide, affecting the planet as a whole in many ways. Estuaries are not an exception and can be affected by climate change through the occurrence of extreme events and climate variability.

Extreme events (e.g. floods, heat waves, droughts) are episodes with a short duration, but with extreme weather impacts mainly in precipitation, wind and atmospheric temperature. These events tend to become more intense and more frequent, affecting not only the place where they happen, but also affecting global processes such as ocean and atmospheric currents (IPCC 2012). Previous studies concerning this problematic, specifically in the Mondego estuary have shown that these extreme events may have a direct impact in environmental variables such as freshwater runoff, salinity and water temperature. Modifications in these variables might lead to negative consequences at the structure (density and biomass, species richness, evenness), and functioning (productivity and trophic diversity) of planktonic, macrobenthic and fish communities (Martinho et al., 2007; Marques et al., 2007; Cardoso et al., 2008). But not every community is affected in the same way by various extreme events. There are species that do not have much resistance to certain changes caused by extreme events and others that may not even be affected (Grilo et al., 2011). An example is the bivalve *Scrobicularia plana* that is affected by heat waves since it has a lethal temperature of 27.5ºC, which can be exceeded in intertidal pools during heat waves (Wilson, 1981).

On the other hand climate variability is characterized by episodes with lower intensity but with a longer duration. For example, a dry year is characterized by an annual precipitation lower than normal, but without the occurrence of droughts. These occurrences have actually been more frequent (IPCC 2012), which can affect communities not only because of the changes that originate in climate (less intense) but also due to the long exposure they are subjected to. This type of occurrence may not cause immediate death of many individuals, but can affect vital processes such as reproduction, growth and life cycle (Struyft et al., 2004; Harley et al., 2006).

1.3 - Macrobenthic community

Macrobenthic estuarine communities have an important role in estuarine ecosystems due to their position in food webs, being most species primary consumers, like, *Hydrobia ulvae*, *Cyathura carinata* and *Hediste diversicolor* (Elliott & Mclusky, 2005; Grilo et al., 2011). These are the base of the feeding of many fish species and even birds (McLusky & Elliott, 2005). Most of these macrobenthic species are small organisms but reaching high densities/biomasses having an important role in the trophic web (McLusky & Elliott, 2005). Another important characteristic of the macrobenthic communities is that they are considered bio-indicators, because they respond in a predictable and relatively fast way to a variety of natural and anthropogenic stressors. This is explained by the fact that they are relatively sedentary individuals being exposed to constant physicochemical changes (Calabretta & Oviatt, 2008; Wildsmith et al., 2011).

In marine benthic ecosystems, taxonomic community composition-derived diversity measurements such as species richness and diversity indexes (e.g. evenness) have traditionally been used to describe diversity in relation to different or changing environments or stress (Dolbeth et al., 2007; Van Colen et al., 2010; Grilo et al., 2011). Species specific ecological or functional characteristics (e.g. feeding habit, life habit) have often, subsequently, been linked in order to determine indirectly the processes that underpin the observed diversity patterns (Grilo et al., 2009).

In order to better understand how macrobenthic communities react to stressors is necessary to understand a very important concept which is the functional diversity (FD) that allows exploring the species coexistence and biodiversity effects on ecosystem functioning. FD refers to the functional component of biodiversity, usually measured through species traits (Violle et al., 2007; Dolbeth et al., 2013). Some of these traits are generally chosen based for example on feeding type, mobility, and life cycle. Since species assemblages are expected to be structured by the ability of species to cope with stressors, analysis of assemblage-wide shifts in biological traits face to climate variability is therefore essential to unravel the driving processes of the diversity-stress response (Cheung et al., 2008). An additional relevant issue that provides a better perception of ecosystem changes induced by climate variability is the secondary production. It is a functional characteristic that represents a direct measure of food provision delivered by an ecosystem, with a socio-economic value assigned (Dolbeth et al., 2011).

In the present study, the macrobenthic communities of two distinct habitats (*Zostera noltii* bed versus Sandflat area) will be used to evaluate how diversity, biological traits and secondary production vary in relation to the climate variability. Here, this climate variability will be measured in terms of annual precipitation variability, comparing rainy, dry and normal years. Specifically, some questions will be addressed in this work: Does the macrobenthos community structure differ in relation to different years? Do community wide biological trait shifts occur for different habitats and years?

Chapter 2

Materials and methods

2.1 - Study area

The Mondego estuary is a temperate coastal system of only 8.6Km^2, located on the Atlantic coast of Portugal (40º08'N; 8º50'W). It comprises, in its terminal part, two distinct arms, north and south, each with distinct morphological and hydrological characteristics and separated by the Murraceira Island (Fig.1). The northern arm is deeper (4–8 m during high tide, tidal range 1–3 m), highly hydrodynamic and where is located the port of Figueira da Foz. While the southern arm is shallower (2–4 m during high tide, tidal range 1–3 m), being characterized by large areas of exposed intertidal flats during low tide (Pardal et al., 2004).

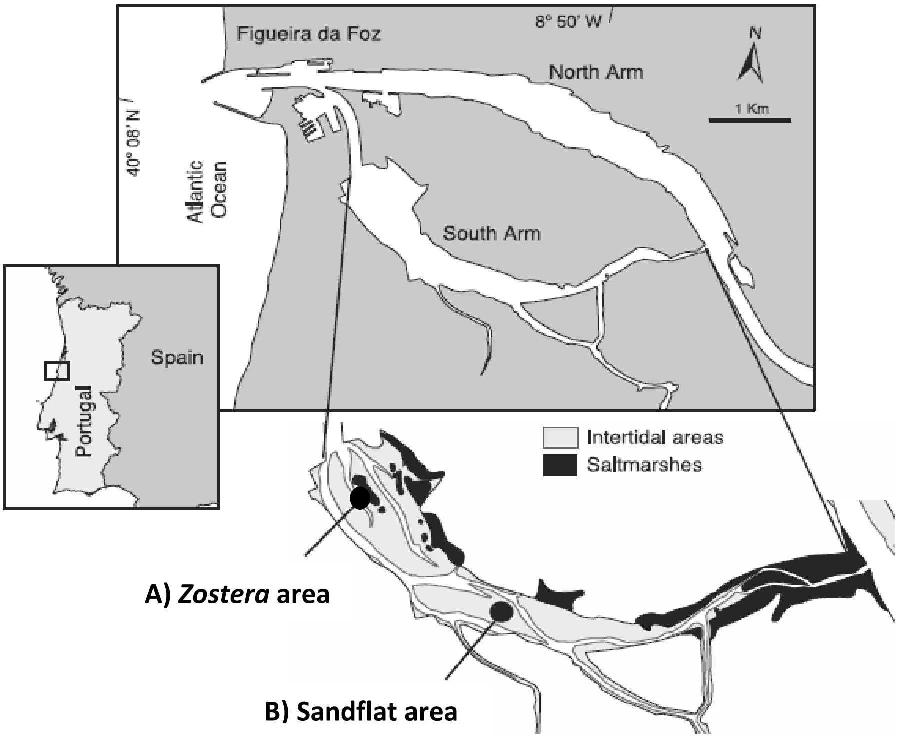

Figure 1 - Location of the Mondego estuary and sampling areas.

The macrobenthic samples were collected in two sampling areas along the south arm:

1. The zone A (*Zostera* area), located downstream, is characterized by the presence of the macrophyte *Zostera noltii* and muddy sediments. It is characterized by higher salinity (20–30) and higher organic matter content 6.8 ± 0.99% (±SD);
2. The zone B (Sandflat area), located upstream in the inner part of the estuary is constituted by sandflat. It is characterized by lower salinity (15–25) and a mean organic matter content of 3.7 ± 1.0% (±SD).

2.2 - Sampling programme and biological material processing

The macrobenthic assemblages were monitored from January 1993 to September 1995 and again from February 1999 to December 2010. In the first 18 months, samples were collected fortnightly, after which were collected monthly. At each sampling site, a set of randomly selected replicates per site (5-10) were collected with a core of 141cm^2 surface to a depth of about 20 cm. Samples were washed *in situ* with estuarine water through a mesh sieve of 500 µm. The collected material (sediment, rooted macrophytes, algae and fauna) was preserved in buffered formalin (4%). The environmental parameters (temperature, oxygen, pH and salinity) were measured *in situ* in intertidal pools and sediment samples were collected to quantify the organic matter content.

Later, animals were separated and transferred to 70% ethanol, identified to the lowest possible taxon and counted. Seagrass and macroalgal biomasses were determined as ash free dry weight (AFDW) after oven drying at 60 ^{o}C for 72 h and combustion at 450 ^{o}C for 8 h.

2.3 – Climate data

Precipitation and runoff data were obtained from the website of the Portuguese Institute of Ocean and Atmosphere (http://www.ipma.pt/pt/) and National Information System of Water Resources (http://snirh.pt/), respectively. The runoff values were obtained from the station called Açude ponte de Coimbra and the precipitation values were obtained from the station named Cernache.

An analysis was also made of the available information on drought conditions, by constructing a drought index, based on a Decis - classification (http://http//www.meteo.pt/pt/clima/clima_seca3.html) (Cardoso et al., 2008). It consists in the division of rainfall data in 10 equal parts (each part corresponds to the difference between the highest annual value of precipitation and the lowest, divided by 10), delimited by 1st decil (which is the precipitation value of the year with the lowest rainfall value), 2nd decil, and so on until the 10th decil, to provide the following classification:

INTER-DECIS INTERVAL	QUALITATIVE DESIGNATION
1	**Extremely dry**
2	**Very dry**
3,4	**Dry**
5,6	**Normal**
7,8	**Rainy**
9	**Very Rainy**
10	**Extremely Rainy**

2.4 – Macrofauna diversity

For the diversity study of the macrobenthic communities in the two areas (*Zostera* and Sandflat) was used the species richness (simple count of number of species recognized) and the Pielou's evenness measures (Krebs, 1999).

For the diversity analysis, as well as for the traits and secondary production, calculations were done in the presence and absence of the small gastropod *Hydrobia ulvae*. Since this is the most abundant species and its presence can mask the pattern of the remaining community.

2.5 – Traits

Four body traits that best reflected the environmental changes caused by the different precipitation conditions were chosen. Data on traits were obtained from established databases, including BIOTIC – Biological Traits Information Catalogue (www.marlin.ac.uk/biotic/) and WoRMS – world register of marine species (www.marinespecies.org) and previously published articles (Grilo et al., 2011; Dolbeth et al., 2013).

The four traits chosen were (1) Larval development mode (present/absent); (2) living position (infauna/epifauna); (3) mobility (crawler, burrower, swimmer and drifter); (4) and finally, feeding guilds (carnivores (C), herbivores (H), omnivores (O) and detritivores (D)). Since detritivores are the main trophic group, they were subdivided into surface-deposit feeders (SDF), subsurface-deposit feeders (SsDF) and suspension feeders (SuF) in order to better identify which group would be more affected (Grilo et al., 2011).

For this study were used 25 species that accounted for more than 98% of density, biomass and secondary production from the intertidal flats of the Mondego estuary which were scored for traits according to Dolbeth et al., (2013).

2.6 - Secondary production

Secondary production of the macrobenthic communities was calculated by the empirical method of Brey (2001) since in the 25 species chosen was not possible to register cohorts in all species (Dolbeth et al., 2005). The empirical methods are based on good correlations found between population (e.g., life span, maximum individual weight, mean individual weight, mean biomass) or environmental (e.g., temperature, depth) characteristics and secondary production or $P/\bar{B}$ ratio (Medernach & Grémare, 1997; Brey, 2001). This method is considered the best choice of empirical methods, presenting results similar to the increment summation method (Dolbeth et al., 2005). For this calculation, was used the worksheet provided in Brey (2001) 4.04 http://www.thoma-brey.de/science/virtualhandbook/navlog/index.html.

2.7 – Statistical analysis

Univariate analysis: One-way ANOVAs were performed to seek for statistical differences in environmental parameters, secondary production and biodiversity between the years (normal, rainy and dry). Two-way ANOVAs were applied to search for statistical differences between different body traits, and different years. All data were previously subjected to the Kolmogorov-Smirnov test for normality and the Levene test for homogeneity of variances (Zar, 1996). These analyses were performed using the statistical program STATISTICA^{TM}software (StatSoft Inc., 2005, version 7.0).

Multivariate approach: Firstly, a detrended correspondence analysis (DCA) with the biological data (body traits density) was used to evaluate which ordination method, linear or unimodal, was suitable to apply. Due the result of DCA to be a linear response, then was carried out a redundancy analysis (RDA) with the biological and environmental parameters in order to study the relationship between them. Initially, six environmental parameters were tested: oxygen, temperature, salinity, pH, precipitation and runoff. Co-linearity between environmental parameters was checked (Draftsman plot and variation inflation factors) and the model forward selection with Monte Carlo permutation tests was used to identify the minimal significant subset of environmental variables ($P<0.05$) needed to explain the observed patterns of community change (Ter Braak & similaeur, 1998). To the achievement of these statistical tests were used following programs, v.5 PRIMER and CANOCO 4.5 (Ter Braak & Similaeur, 1998).

Chapter 3

Results

3.1 Environmental data

For each year designation (normal, dry and rainy) according to the drought index, were considered data from three different years. Therefore, normal years included data from 1999, 2003 and 2006, respectively with 778 mm, 885 and 900mm of total annual precipitation. Dry years included information from 2002, 2004 and 2007 with an annual precipitation of 711, 653 and 705 mm, respectively. Finally the rainy years included data from 2000, 2009 and 2010 with an annual precipitation of 1122, 960 and 928mm, respectively.

An environmental characterization of both study areas is expressed in tables 1 and 2. Regarding annual runoff, as expected, the highest values were recorded in rainy years, while the lowest ones were recorded in dry years (Tables 1 and 2), but no significant differences were observed (one-way ANOVA, F_2=1.38, P>0.05) between years.

Concerning water temperature, oxygen, salinity and pH, for each of the study areas no significant differences were observed between years (one-way ANOVA, *Zostera* area, temperature: F_2=0.93, P>0.05, oxygen: F_2=0.65, P>0.05, salinity: F_2=1.46, P>0.05, pH: F_2=0.10, P>0.05; Sandflat area, temperature: F_2=0.81, P>0.05, oxygen: F_2=0.33, P>0.05, salinity: F_2=0.74, P>0.05, pH: F_2=3.59, P>0.05). For the dissolved oxygen, slightly higher values were detected in rainy years, for both sites. On the contrary, salinity values were slightly higher in dry years. The pH values were very similar both between areas and years (Table 1 and 2).

Regarding the annual precipitation, for both sites, significant higher values were observed in rainy years, followed by normal and dry years (one-way ANOVA, $F_2=15.37$, $P< 0.05$) (Table 1 and 2).

Table 1 - Environmental Parameters (Mean ± SE) in the *Zostera* area.

	Normal	**Dry**	**Rainy**
Runoff (dam^3)	2 443 319 ± 742 448	1 466 295 ± 234 943	2 562 728 ± 424 293
Temperature (ºC)	18.2 ± 0.4	18 ± 0.5	18.4 ± 0.4
Oxygen (mg.L^{-1})	11.2 ± 0.8	11.8 ± 1.3	15.2 ± 4.4
Salinity	26 ± 1.2	29.4 ± 0.7	26.4 ± 2.2
pH	8.5 ± 0.1	8.6 ± 0.1	8.5 ± 0.0
Precipitation (mm)	854 ± 38	689 ± 18	1013 ± 57

Table 2 - Environmental Parameters (Mean ± SE) in the Sandflat area.

	Normal	**Dry**	**Rainy**
Runoff (dam^3)	2 443 319 ± 742 448	1 466 295 ± 234 943	2 562 728 ± 424 293
Temperature (ºC)	20.7 ± 0.4	20.5 ± 0.3	19.7 ± 0.9
Oxygen (mg.L^{-1})	11.8 ± 1.4	12.7 ± 0.6	13.9 ± 2.8
Salinity	24.6 ± 1.3	26.6 ± 0.2	24.1 ± 2.3
pH	8.5 ± 0.1	8.6 ± 0.1	8.4 ± 0.1
Precipitation (mm)	854 ± 38	689 ± 18	1013 ± 57

3.2 - Macrofauna diversity

Species richness was slightly higher in the *Zostera* area than in the Sandflat area (Fig. 2), especially for the normal years, while for dry and rainy years were similar. For both sites, years with climate variability presented slightly higher values than normal years. However both in the *Zostera* area with *Hydrobia ulvae* (one-way ANOVA, F_2=0.56, P>0.05) and without *Hydrobia ulvae* (one way ANOVA, F_2=0.56, P>0.05) and in the Sandflat area with *Hydrobia ulvae* (one-way ANOVA, F_2=3.37, P>0.05) and without *Hydrobia ulvae* (one-way ANOVA, F_2=3.29, P>0.05), there were no significant differences between years.

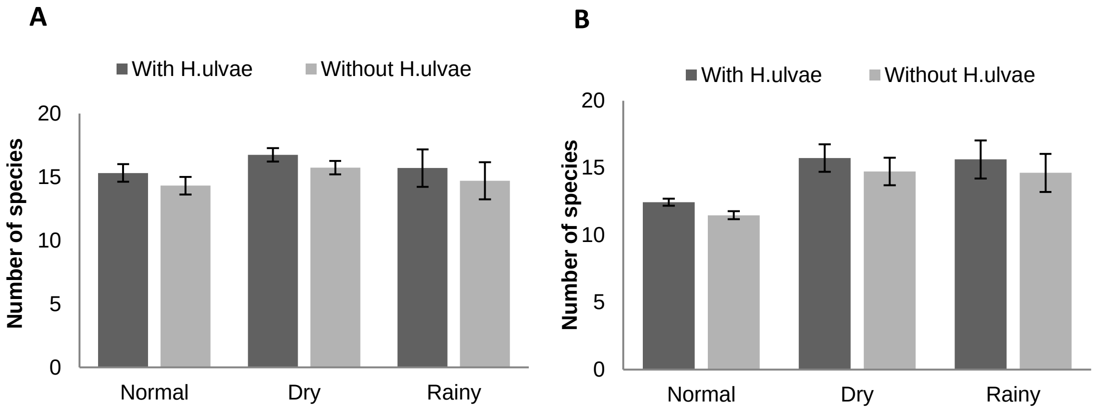

Figure. 2 – Species richness in *Zostera* area (A) and Sanflat area (B). Error bars correspond to standard errors.

Regarding evenness, lower values in the *Zostera* area (with *H. ulvae*) were observed compared to the Sandflat area (Fig. 3A), but no significant differences between years were detected (one-way ANOVA, F_2=0.33, P>0.05). In the absence of *Hydrobia ulvae* evenness was higher (Fig. 3A) but also similar between years (one-way ANOVA, F_2=0.24, P>0.05). In the Sandflat area, the evenness was just a little bit lower in rainy years than other years (Fig. 3B), but without significant differences both with *Hydrobia ulvae* (one-way ANOVA, F_2=2.91, P>0.05) and without *Hydrobia ulvae* (one-way ANOVA, F_2=2.91, P>0.05).

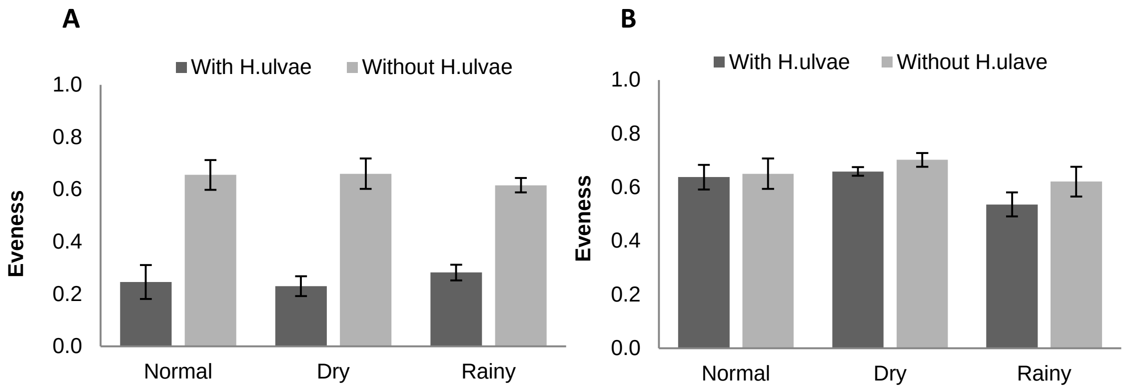

Figure. 3 – Eveness in *Zostera* area (A) and Sanflat area (B). Error bars correspond to standard errors.

3.3 – Traits

For the larval development mode trait (present / absent) no significant differences were found between the different years in any situations (two-way ANOVA, Fig. 4A: F_2=0.64, P>0.05; B: F_2=2.71, P>0.05; C: F_2=0.24, P>0.05; D: F_2=2.68 P>0.05) despite there was a tendency for the occurrence of higher values in dry and rainy years. In the presence of *Hydrobia ulvae* both in the *Zostera* and in the Sandflat area, there was a higher density of individuals with larval stage present than absent. In addition, normal years presented lower densities than the others (Fig. 4A, B). In the absence of *Hydrobia ulvae*, mainly in the *Zostera* area, the results were the opposite since more individuals without larval stage were observed (Fig. 4C). In the Sandflat area this did not occur but the number of individuals with or without larval stage was closer (Fig. 4D).

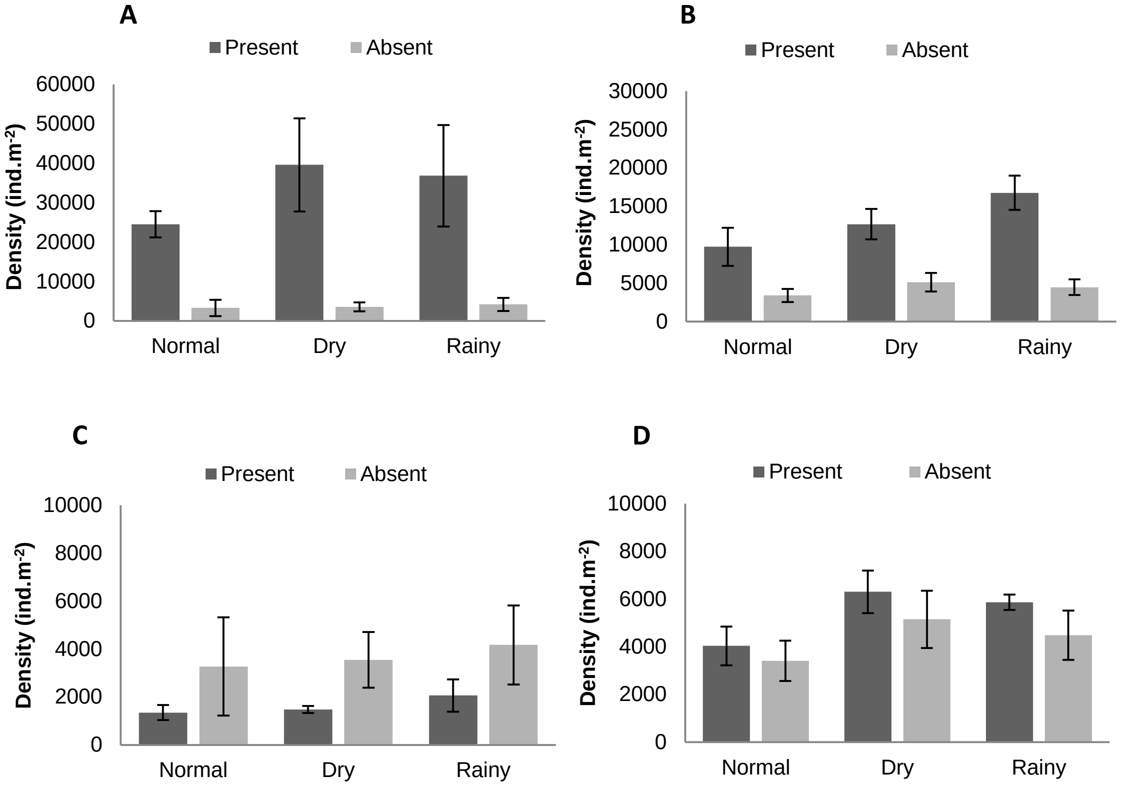

Figure. 4 – Density of individuals with Larval development mode present or absent with *Hydrobia ulvae* in *Zostera* area (A) and Sanflat area (B) and Without *Hydrobia ulvae* in *Zostera* area (C) and Sanflat area (D). Error bars correspond to standard errors.

Considering the living position trait (infauna / epifauna) no significant differences between years were recorded in any situations (two-way ANOVA, Fig. 5A: F_2=0.67, P>0.05; B: F_2=2.82, P>0.05; C: F_2=0.23, P>0.05; D: F_2=1.77 P>0.05). In the *Zostera* area (with *H. ulvae*) it was possible to observe that much more epifauna individuals were observed than infauna ones, especially for dry and rainy years (Fig. 5A). In the Sandflat area densities of both epifauna and infauna were quite closer, with slightly higher values for the dry and rainy years (Fig. 5B). Without *Hydrobia ulvae* in both areas, the pattern was the opposite, with higher densities of infauna than epifauna (Fig.5C, D). In all cases, normal years presented the lowest densities (Fig. 5).

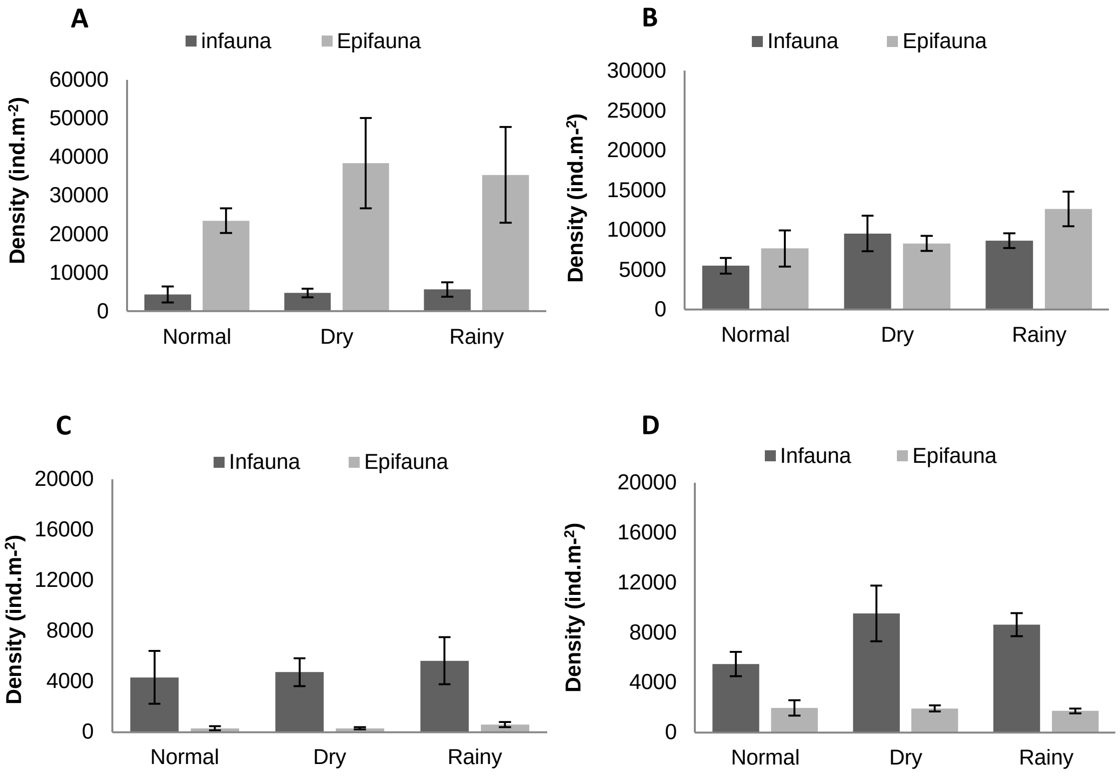

Figure. 5 – Density of individuals in relation to living position (Infauna/ Epifauna) with *Hydrobia ulvae* in Zostera area (A) and Sanflat area (B) and Without *Hydrobia ulvae* in Zostera area (C) and Sanflat area (D). Error bars correspond to standard errors.

Considering the type of movement, for the *Zostera* area (with *H. ulvae*) higher densities of the different categories were observed compared to the Sandflat area. Also, the burrower category was the dominant one for all the scenarios. In addition, generally, normal years presented lower values than dry and rainy years (Fig. 6C, D). However, significant differences between years were only detected for the Sandflat area (with *H. ulvae*) (two-way ANOVA, F_2=4.80, P<0.05) (Fig. 6B), more specifically between the normal and rainy years (P<0.05). While in other cases there were not significant differences between years (two-way ANOVA, A: F_2= 2.57, P>0.05; C: F_2=0.24, P>0.05; D: F_2=2.56, P>0.05).

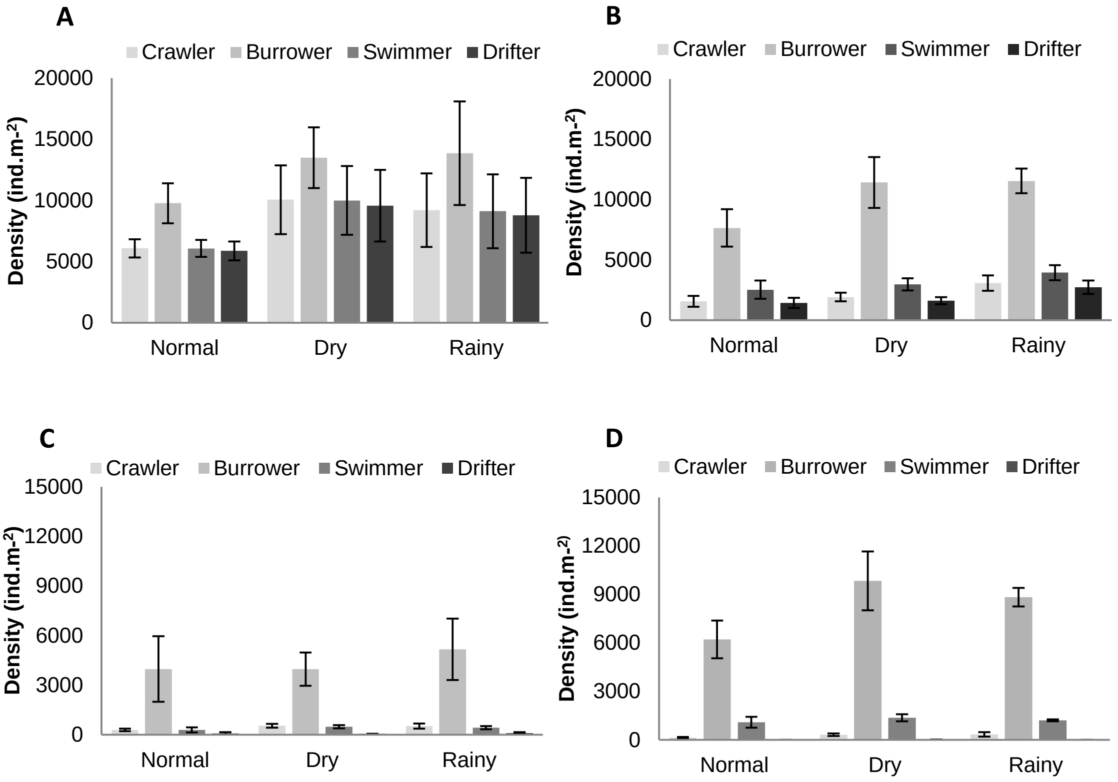

Figure. 6 – Density of individuals in relation to mobility (Crawler, Burrower, Swimmer and Drifter) with *Hydrobia ulvae* in Zostera area (A) and Sanflat area (B) and Without *Hydrobia ulvae* in Zostera area (C) and Sanflat area (D). Error bars correspond to standard errors.

Regarding the feeding guilds, also a similar pattern was observed, with the *Zostera* area presenting higher densities of the different categories and in general, normal years presenting lower densities than dry or rainy years. However, no significant differences between the years in any situations, (two-way ANOVA, Fig. 7A: F_2=1.26, P>0.05; B: F_2=3.35, P>0.05; C: F_2=0.23, P>0.05; D: F_2=2.35, P>0.05) were observed. In all cases, herbivores and detritivores were the most abundant groups (Fig. 7A).

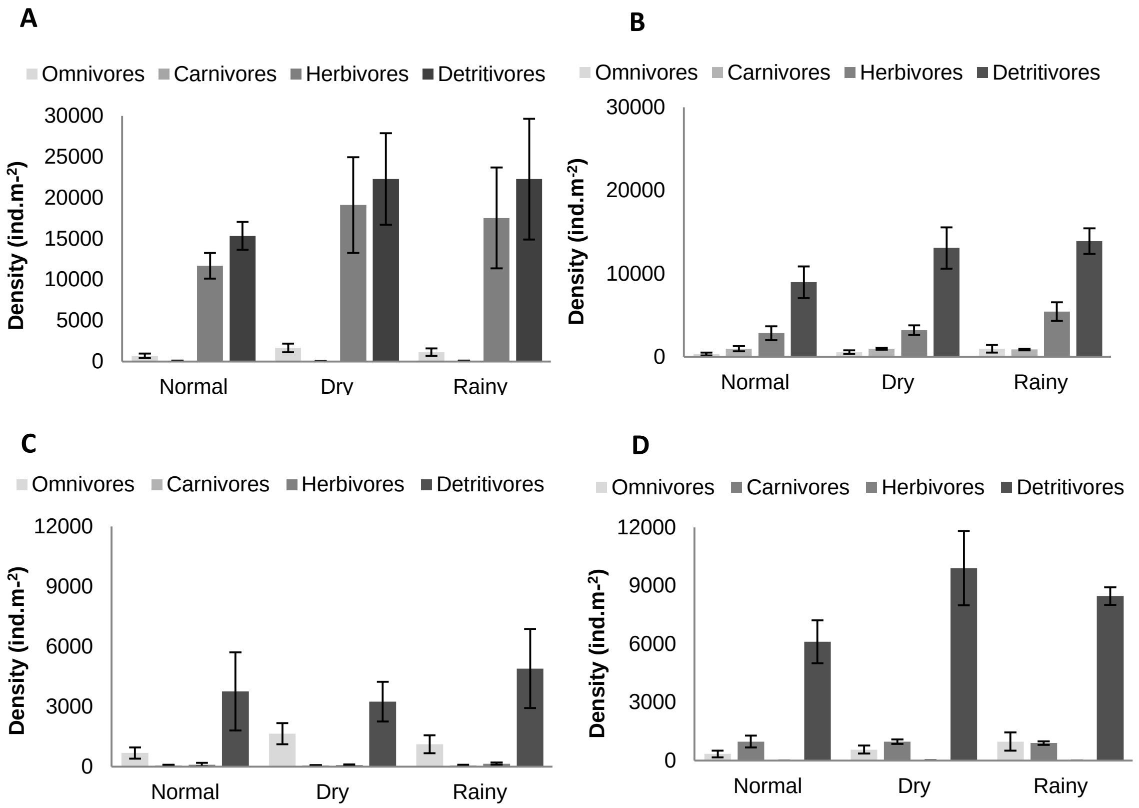

Figure. 7 – Density of individuals in relation to feeding guilds (carnivores (C), herbivores (H), omnivores (O) and detritivores (D)) with *Hydrobia ulvae* in *Zostera* area (A) and Sanflat area (B) and Without *Hydrobia ulvae* in *Zostera* area (C) and Sanflat area (D). Error bars correspond to standard errors.

In addition, the detritivores group was explored more in detail, considering the sub-divisions into surface-deposit feeders (SDF), subsurface-deposit feeders (SsDF) and suspension feeders (SuF). In the presence of *H. ulvae*, surface-deposit feeders were the dominant group (Fig. 8A, B) for both areas, while in its absence subsurface-deposit feeders dominated (Fig. 8C, D). Once again, normal years presented lower densities than dry or rainy years. But no significant differences were observed between them (two-way ANOVA, A: F_2=0.58, P>0.05; B: F_2=2.80, P>0.05; C: F_2=0.34, P>0.05; D: F_2=3.27, P>0.05).

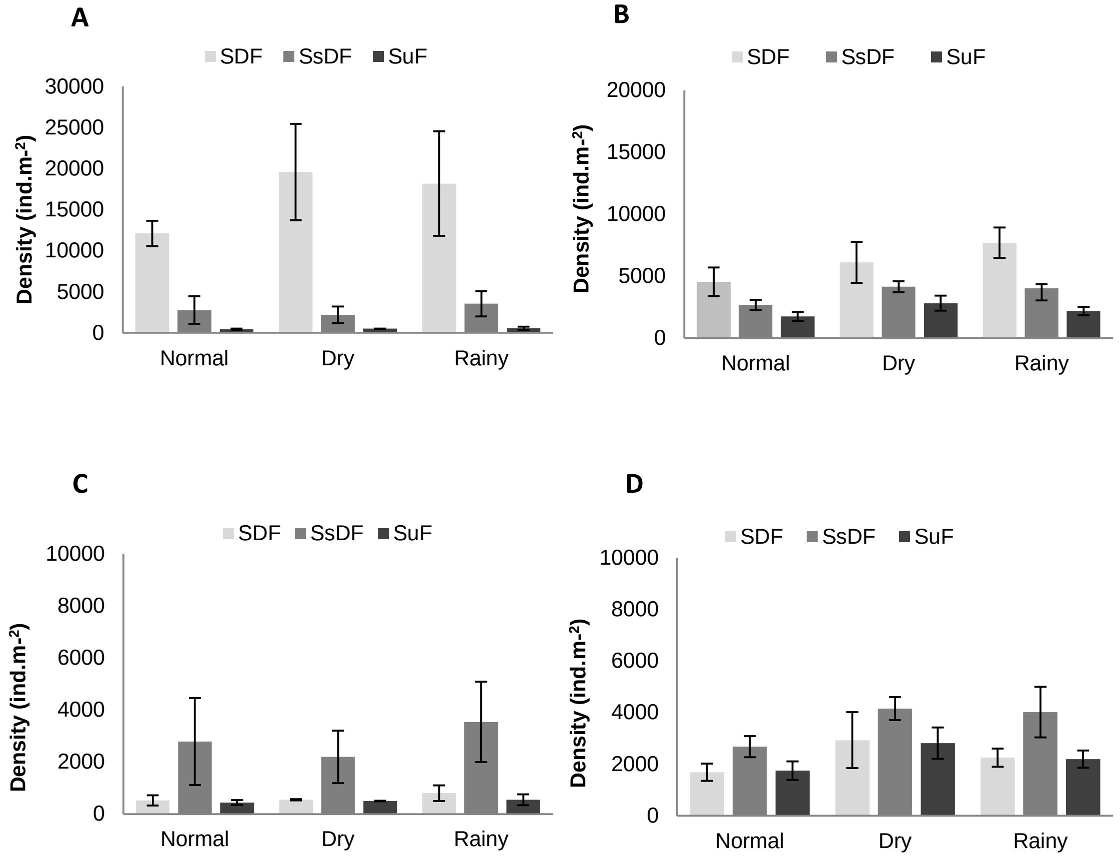

Figure. 8 – Density of individuals of detritivores surface-deposit feeders (SDF), subsurface-deposit feeders (SsDF) and suspension feeders (SuF) with *Hydrobia ulvae* in *Zostera* area (A) and Sanflat area (B) and Without *Hydrobia ulvae* in *Zostera* area (C) and Sanflat area (D). Error bars correspond to standard errors.

3.4 – Secundary production

Concerning the secondary production, higher values were recorded in the *Zostera* area (with *H. ulvae*) compared to the Sandflat area (Fig. 8A, B). In the absence of *Hydrobia ulvae*, the growth production in both areas was quite similar for the different years (Fig. 8C, D). Comparing distinct years, no significant differences between years were detected for the four scenarios (one-way ANOVA, A: F_2=0.27, P>0.05; B: F_2=0.62, P>0.05; C: F_2=2.61, P>0.05; D: F_2=0.49, P>0.05).

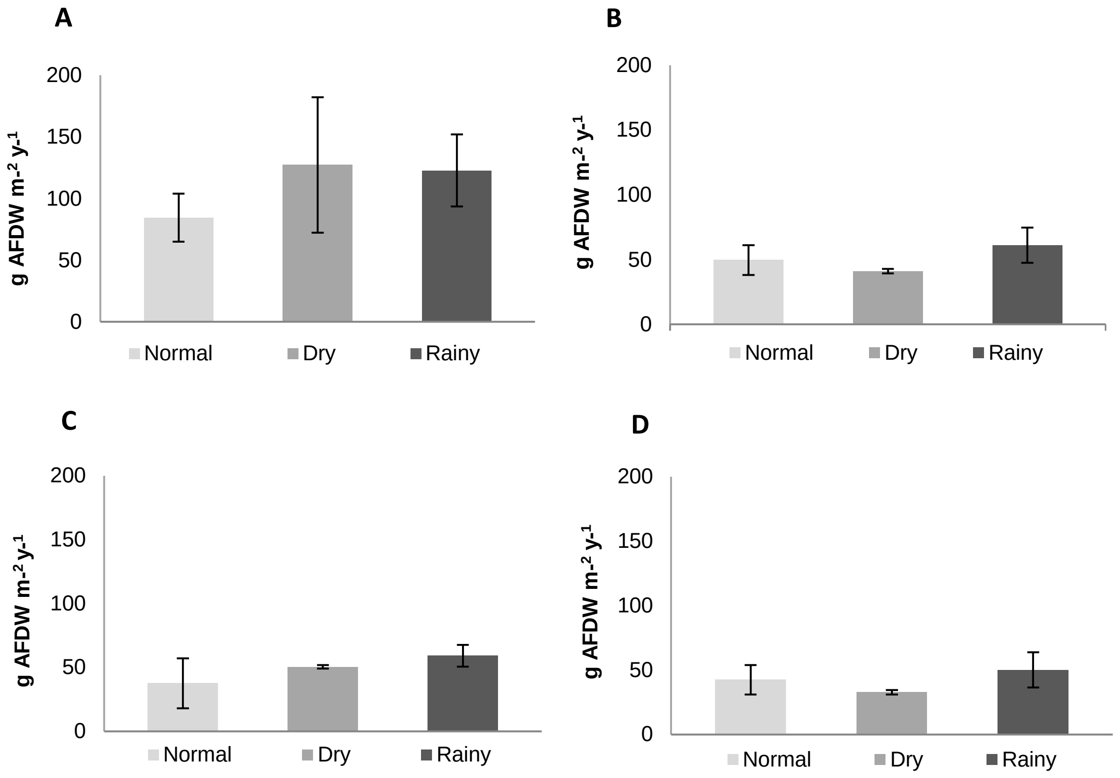

Figure. 8 – Annual secundary production with *Hydrobia ulvae* in *Zostera* area (A) and Sanflat area (B) and Without *Hydrobia ulvae* in *Zostera* area (C) and Sanflat area (D). Error bars correspond to standard errors.

3.5 – Redundancy analysis (RDA)

The relation between the biotic variables in different years and environmental parameters was evaluated through a Redundancy Analysis (RDA). Originally, six environmental parameters were tested: dissolved oxygen, water temperature, salinity, pH, runoff and precipitation, of which only three were statistically significant and selected for the analysis. They were runoff, oxygen and precipitation. In the presence of *Hydrobia ulvae* ($P>0.05$), 94.1% of data variability was explained by these environmental variables of which 95% was explained by the first axis. While without *Hydrobia ulvae* ($P<0.05$), 93.6% of data variability was explained by the same environmental variables and 93.1% by the first axis.

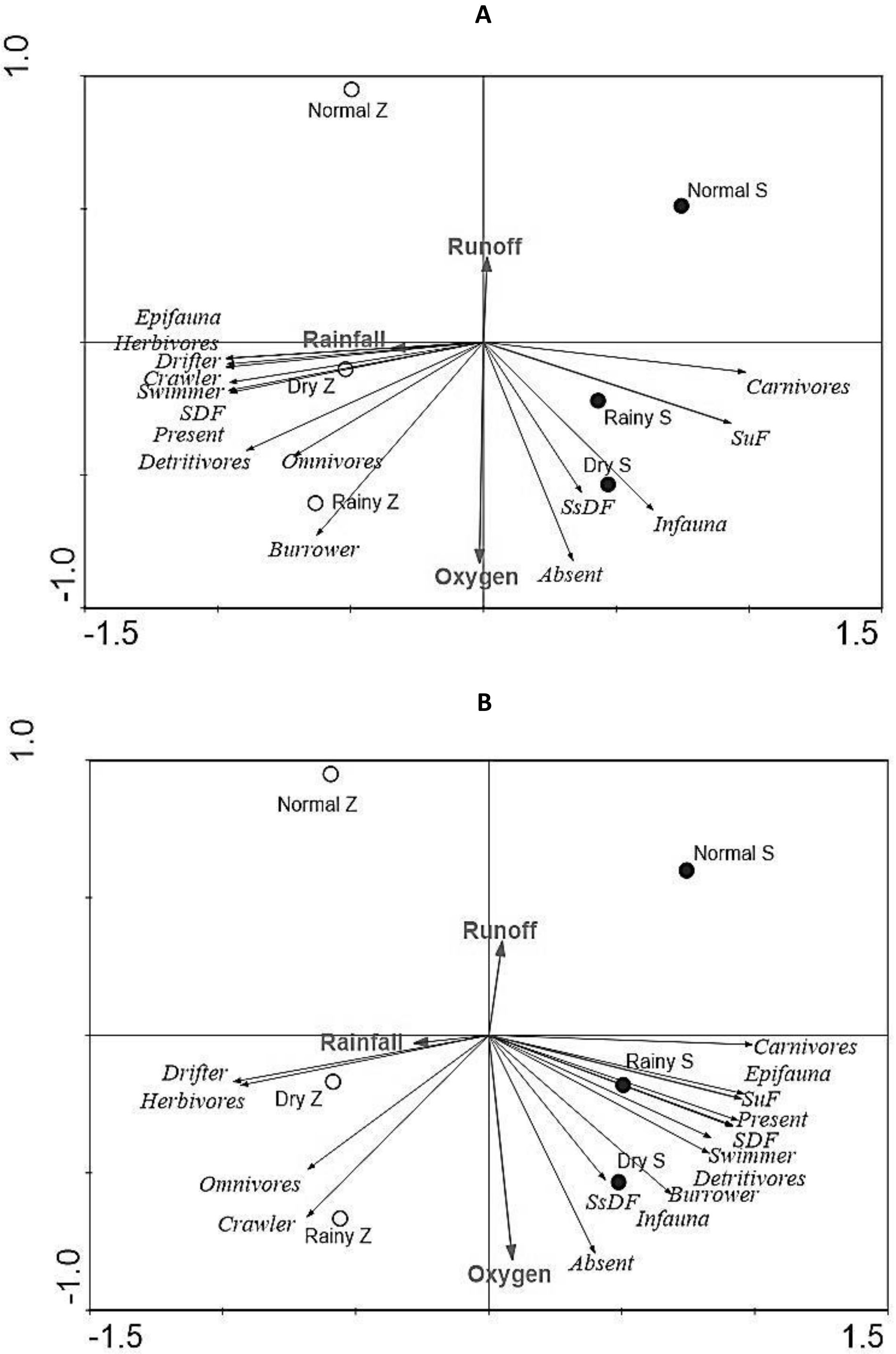

Figure. 9 – RDA ordination triplots relating years, traits and significant environmental parameters (after Monte Carlo permutation tests), with *Hydrobia ulvae* (A) and Without *Hydrobia ulvae (B).*

Through the analysis of both plots it was clear the physical separation between samples from the two sampling areas and also it was possible to observe that normal years appeared quite separated from the other two, which were more closer (Fig. 9). From the three environmental variables, the oxygen revealed to be the one with highest relevance, followed by the runoff. Finally, considering the presence or absence of *Hydrobia ulvae*, the disposition of the different biotic parameters in the space may vary considerably.

Chapter 4

Discussion

4.1 - Environmental data

Regarding environmental variables, most of them reflected the expected effects caused by precipitation. The runoff presented a direct relation to rainfall, in other words, in rainy years it was observed the highest value while in dry years lowest values were recorded, as already observed by Dolbeth et al., (2011). The salinity, has an inverse response to rainfall, the higher rainfall corresponds to a lower salinity (Habib et al., 2008) as previously registered in the Mondego estuary (Dolbeth et al., 2007). This relationship was also obtained in our data, where the highest salinity was recorded in dry years and the lowest one in rainy years. The salinity values were slightly higher in the *Zostera* area, since it is closer to the sea than the upstream Sandflat area. Dissolved oxygen is also influenced by rainfall, since under high precipitation oxygen levels are generally higher. But this could not be straightforward like the previous parameters because oxygen is primarily affected by the primary producers since it is a product of photosynthesis (Yin et al., 2004; Eyre & Ferguson, 2006; Ochieng et al., 2010). In the present data, the concentrations of dissolved oxygen were higher in years with higher precipitation. Temperature and pH are the parameters that are less influenced by precipitation.

4.2 - Macrofauna diversity

Generally, species richness in the *Zostera* area was higher than in the Sandflat area especially in normal years. In rainy and dry years the difference between sites was minimized, possibly based on the heterogeneity caused by the climate variability, which triggered similar responses in both communities.

Differences between areas were expected and have already been observed previously (Heck et al., 1995; Lee et al., 2001). This difference is explained by the higher stability and complexity of the *Zostera* area due to the high abundance of the macrophyte *Zostera noltii* (Blanchet et al., 2004; Battley et al., 2011). Seagrass provides abundant food resources through the degradation of plant tissues and also by ensuring great organic matter retention (sedimentation), since the current in these areas is further reduced (Fonseca & Fisher, 1986). Areas covered by *Zostera noltii* are also characterized by low predation pressure due to habitat complexity that offers more refuges and greater diversity of niches, thus decreasing the occasional meetings of prey-predator (Orth et al., 1984; Polte et al., 2005). Regarding the annual variance, despite the no significant differences observed between years, it was visible a tendency of greater species richness in rainy and dry years compared to the normal ones for both sites.

Regarding evenness, no significant differences between the years were observed, too. But it was possible to see that in the Sandflat area higher evenness values were observed compared to the *Zostera* area (especially with *Hydrobia ulvae*), due to the lower dominance of the gastropod in the first area as already observed in other studies (Casagranda et al., 2005; Dolbeth et al., 2007). This difference in the gastropod abundance is due to the existence of better conditions (e.g. food availability, protection against predators) to develop in the seagrass area than in the Sandflat area.

4.3 – Traits

For the larval development mode trait no significant differences between the years were obtained. It would be expected that in rainy years the number of individuals with larval stage would be lower than in normal or even dry years, due to the effect of high turbidity and possible entrainment (McLusky & Elliott, 2005; Eyre & Ferguson, 2006). However, this effect was not observed in the present results, maybe because the precipitation was not sufficiently strong to cause such effects like in the case of an extreme flood event. A particular case of flood impact in the macrobenthic species structure was observed for the *Hydrobia ulvae* during the flood of 2000/2001 (Cardoso et al., 2008) in which was verified that a great part of the population was dragged out of the estuary. As the difference between areas in the analysis without Hydrobia ulvae, *Zostera* area has the highest density of individuals without larval stage, while in the Sandflat area individuals with the highest density are those with the larval stage. The result is contrary to what would be expected since in the *Zostera* area there is a higher protection against predators and outside factors and greater food availability (Battley et al., 2011; Lee et al., 2011). What might be happening is that *Scrobicularia plana* is influencing the analysis due to its high abundance in the Sandflat area (Dolbeth et al., 2007).

Concerning the living position trait, it would be expected to observe differences in the density of epifauna and infauna individuals according to the precipitation intensity. One of the effects originated by high rainfall in these ecosystems is an excessive sediment transport that may cause hypoxic conditions in some individuals, affecting especially the infauna individuals. Infauna may be more exposed to this pressure type due to its lower position and when they are affected may have behaviors that put them at risk of being preyed and in extreme cases, they can die from lack of oxygen (Norkko et al., 2002; Diaz & Rosenberg, 2008; Seiz et al., 2009; Tesi et al., 2013). But this effect was not observed in the present data, since there were no significant differences between years, despite years with climate variability presented higher densities than normal years. Also, there was no difference in dominance between sites, since in both areas the infauna individuals presented the highest values.

Another biological trait, movement type, was used to see if precipitation affects the locomotion type of individuals, since it can influence the water current and sedimentation, consequently affecting the density of each type of locomotion (McLusky & Elliott, 2005). The only significant difference between years occurred in the analysis without *Hydrobia ulvae* in the Sandflat area and indicated that the years with climate variability had a positive effect in the community, leading to an increase in the density of all types of locomotion.

Feeding types were closely associated to environmental variables. Physical parameters such as hydrodynamics, sediment grain size or quantity/quality of the available food have been indicated as factors determining the dominance of feeding types (García-Arberas & Rallo, 2002; Grilo et al., 2011). In the present results no significant differences between the years were observed, however, the same annual tendency was observed, with higher densities in rainy and dry years. In fact, the most abundant feeding group for all conditions was the detritivores, which is a typical characteristic of estuarine ecosystems (Gastron & Naci, 1998; García-Arberas & Rallo, 2002).

Despite different types of detritivores may be affected by currents, turbidity, organic matter, sediment size and other parameters dependent on precipitation (Norkko et al., 2002; García-Arberas & Rallo, 2002; Battley et al., 2011), no significant differences between the years were observed. Analysing macrobenthic communities of both areas with *Hydrobia ulvae* the surface-deposit feeders (SDF) were the most abundant group, which was not evident excluding the gastropod *Hydrobia ulvae*, with a subsurface-deposit feeders (SsDF) dominance. Modifications in dominance between sites would be expected due to differences in soil formation and hydrodynamics, which in turn affect the relative abundance of each type of detritivores. For example, SsDF are more related with sediments presenting high percentage of coarse elements as well as high levels of organic matter (García-Arberas & Rallo, 2002).

4.4 – Secundary production

Regarding the secondary production, as expected, the *Zostera* area presented higher values than the Sandflat area. This high production occurs due to the greater complexity and stability of the *Zostera* area (Lee et al., 2001; Dolbeth et al., 2007; Battley et al., 2011). In addition, once again, climate variability reflected higher productivity values than normal years, which is in agreement with the previous results.

Overall, despite no significant differences between years were observed for the majority of traits (with the exception of movement trait with *Hydrobia ulvae* in Sandflat area), diversity and productivity, there was a clear tendency to observe positive effects of climate variability in the macrobenthic communities structure and functioning. This means that in rainy and dry years higher community densities, as well as diversity and productivity were observed compared to normal years. These results are in accordance with the Intermediate Disturbance Hypothesis (IDH). According to Connell (1975, 1978 in Molles 1999) high biodiversity is expected in particular ecosystems subjected to changing conditions, thereby preventing accommodation. In this situation there is not also a dominance of species, allowing a better equilibrium between species (Whomersley et al., 2010; Lee et al., 2011).

In the presence of extreme environmental conditions, like floods and heat waves, an opposite response of the community could be observed, resulting in low biodiversity (Norkko et al., 2002; Salen-Pichard et al., 2003; González-Ortegón et al., 2010). These stochastic events originate mortality of most species and only the most resistant and opportunists will dominate these areas.

Another possible explanation for the higher densities observed in rainy years can be associated to the variety of conditions caused by high precipitation resulting in an increase of sediment input, which can lead to an increase of organic matter (Eyre & Ferguson, 2006). This input of organic matter means greater availability of food for a greater number of individuals, since in many cases the nutrients availability is a limitation of the community's macrobenthic growth and influence the behavior of various species.

Chapter 5

Conclusions

5.1 – Final conclusions

Overall, although we have not observed significant differences in the community structure caused by climate variability, two main conclusions could be highlighted from this work:

- An evident separation between the two studies sites; in almost all analyzes this difference was found for all the parameters: biodiversity, biological traits and secondary production;
- A clear segregation between normal years and those presenting climate variability (rainy and dry), which recorded the highest values for the different parameters evaluated. These results seem to be explained based on, the Intermediate Disturbance Hypothesis (IDH), considering episodes of climate variability as intermediate disturbances that can promote a positive response in the ecosystem.

Despite the present results could give us an idea about the effects of climate variability on the macrobenthic communities structure, it was not possible to detect changes at the distinct biological traits for the different environmental conditions, which means that in the future probably a different approach could be applied to this data. Namely, the application of other indexes, like for example the community weighted mean trait value (CWM) (Dolbeth et al 2013 and references therein) or eventually the choice of other species traits.

The high complexity of biological and environmental data in the present study meant that the justification of the results was difficult due to the high dependence to environmental factors and high diversity of biological response. For best approach to these data is necessary to conduct a more detailed study, having regarded all existing species.

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